RESEARCH ARTICLE

Synthesis and Characterization of Co_3O_4 Spinel Nanoparticles with Antibacterial Activity

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Received: 17th March, 2023; Revised: 26th June, 2023; Accepted: 22th August, 2023; Available Online: 25th September, 2023

ABSTRACT

Nanoparticles are abundant and usable in the medical field as an antibiotic. It has been found that metal oxide nanoparticles, such as Co_3O_4, are efficient against bacteria that are resistant to antibiotics. *Escherichia* and *Staphylococcus* bring on enteric sickness and other illnesses. This study used co-precipitation to create Co_3O_4 nanoparticles. SEM, X-ray diffraction (XRD), and Fourier-transform infrared (FTIR) spectroscopy were used to analyze these oxide nanoparticles. The XRD pattern validated the Co_3O_4 crystalline match. SEM also showed the morphology of Co_3O_4 nanoparticles. As determined by X-rays, Co_3O_4 has an average diameter of around 37.08 nm. The Co_3O_4 nanoparticles were highly pure, showed by an energy-dispersive EDX pattern. Both on their own and in conjunction with cephalexin, these metal oxide nanoparticles demonstrated antibacterial efficacy against *S. aureus* and *E. coli*. These Co_3O_4 nanoparticles showed high growth inhibition compared with using cephalexin individually. These conclusions found that the presence of cephalexin with Co_3O_4 showed high growth inhibition. The antibacterial activity of Co_3O_4 nanoparticles should be subjected for further studies.

Keywords: Spinel nanoparticles, Co_3O_4, Co-precipitation, Antibacterial activity.

International Journal of Drug Delivery Technology (2023); DOI: 10.25258/ijddt.13.3.37

How to cite this article: Hammadi M, Sabah R, Hummadi EH. Synthesis and Characterization of Co_3O_4 Spinel Nanoparticles with Antibacterial Activity. International Journal of Drug Delivery Technology. 2023;13(3):995-999.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Nanoparticles (NPs) are particles with scale-dependent, nanoscopic characteristics that range in size from 1 to 100 nm. Their high surface-to-volume ratio significantly impacts their biological factors and applications.[1] Nanoparticles can be created using chemical or physical processes such as hydrothermal, precipitation, vapor deposition, and sol-gel processing with biological activity against bacteria and agricultural uses. Physical methods include laser ablation, mechanical milling, and vapor deposition.[2] The most widespread technique used to produce nanoparticles is co-precipitation. It is a rapid and eco-friendly method because it is used with inorganic or organic substances and could avoid most other disadvantages of extraction techniques.[3] NPs research has recently developed as a fascinating area of scientific study due to its wide range of practical uses in medicine, agriculture, manufacturing, and food packaging.[4,5] For instance, NPs are employed in medicine to transport medications, identify diseases, probe the DNA structure, and combat highly antibiotic-resistant pathogens.[6] Due to their safety and efficiency, copper and zinc NPs have gained more interest as antibiotics. NPs may be classified according to their scale, morphology, and physicochemical characteristics into different distinct categories. Several types of NPs fall under this category: carbon-based NPs, NPs based on metals like Cu, Ag, Au, Co, and Zn, NPs based on semiconductors, ceramics, NPs, NPs based on lipids, and polymeric NPs. Much research on metallic NPs such as ZnO, Co_2O_4, Fe_2O_3, and Ag_2O as potential antimicrobials has been conducted within these classes.[7,8] Combined with conventional antibiotics, These compounds exhibit a potent antibacterial action. For instance, conjugating ceftazidime and ciprofloxacin with ZnO-NPs will increase their effectiveness against multidrug-resistant bacteria.[9] When combined with silver nanoparticles, cephradine, vildagliptin, and ceftriaxone have increased biological activity against a wide range of clinical pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Klebsiella pneumonia*, *staphylococcus aureus*, and *Bacillus cereus*.[10,11] Co_3O_4NPs were created using the co-precipitation method in this investigation. The structural property of Co_3O_4NPs was tested using energy-dispersive X-ray diffraction spectroscopy-equipped SEM and XRD.

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A broth microdilution experiment investigated Co_3O_4NPs' antibacterial activity alone and combined with cephalexin against human pathogenic bacterial strains *E. coli* and *S. aureus*.

MATERIALS AND METHODS

Chemicals of analytical grade were utilized to create the solutions. Alfa Aesar (Germany) provided the ($CoCl_2.6H_2O$), (NaOH), and ethanol. Microplate made of polystyrene with 96 wells from Greiner Bio-One in Germany. We bought cephalexin from Ajanta Pharma in India. Media of a cultural nature from HiMedia Laboratories (India). *E. coli* and *S. aureus* cultures were obtained from the Al-Kindi Teaching Hospital's Microbiology Research Laboratory in Baghdad, Iraq. These strains were grown on nutrient agar and kept at 4°C.

Synthesis of Co_3O_4 Nanoparticles

The co-precipitation method was used to create the (Co_3O_4) nanoparticles. In 50 mL of deionized water, which was stirred, contained 0.9 g (CoCl2.6H2O). Gradually add 150 mL of (NaOH) in deionized water over the course of two hours with constant stirring at 80°C. The reaction mixture's pH was set to 8 and kept at 25°C for 12 hours. They were washed using EtOH and deionized water. The was dried for 24 hours at 25°C after being dehydrated for 30 minutes at 80°C and torched for two hours at 400°C.

Characterization Techniques

Co3O4NPs were characterized using a variety of methods, including scanning electron microscopy (SEM), fourier-transform infrared (FTIR) spectroscopy, and XRD. A Shimadzu (Kyoto, Japan) XRD with Cu-Kappa radiation was used to assess the nanoparticles' structure and crystallite size (γ-Kappa= 0.15406 nm). The KBr pellet for FTIR spectrophotometer from Shimadzu Tokyo, Japan. The German Zeiss (SEM) 200 kV was used for the SEM examination.

Antibacterial Activity

The substance's antibacterial activity was investigated using the broth microdilution technique. In 1-mg of Co_3O_4NPs was shaken into a stock solution of 1-mL of deionized water. For 30 minutes, the suspension was treated in an ultrasonic water bath. Before the bioassays, the vortex forcefully shook the rest while kept at 4°C. The bactericidal activity of the medicines and nanoparticles was calculated using the microdilution technique. Serial NPs or cephalexin dilutions (100–500 µg/mL) were prepared in sterile 96-well flat-bottom microplates. Using Mueller-Hinton Broth, the bacterial solution was changed to 0.5 McFarland. Each well-containing 100 µL of Co_3O_4NPs or cephalexin received an aliquot of 100 µL of bacterial inoculum. The only control was bacterial growth. It contained nothing but the blank culture medium. At 37°C, the microplate was incubated for 24 hours. Using a BioTek Synergy HTX, the suppression of bacterial growth was detected at 630 nm.

Determination of MIC

According to the recommendations of (CLSI, 2020), the MIC was measured. A 96-well plate was divided into aliquots containing 100 µl of each NP at a different concentration and 100 µl of the microbial inoculum. Plates were incubated at 37°C for 24 hours without shaking. The MIC read spectrophotometrically at 630 nm by a microplate reader.[12]

Combining of Co_3O_4NPs with Cephalexin

The activity of Co_3O_4NPs and cephalexin was also measured to evaluate the synergistic effect. At the required concentrations (75:25, 50:50, and 25:75 µg/mL). The 1:3, 1:1, and 3:1 (v/v) were chosen for the cephalexin: Co3O4NPs to final ratio. After 24 hours of incubation at 37°C, the optical density of the plate was calculated to be 630 nm.

Statistical Analysis

The data was analyzed using GraphPad Prism 9.0 (GraphPad Tech, La Jolla, CA, USA). Tukey's multiple comparison tests and one-way ANOVA were performed to distinguish between the studied classes. The results are shown as mean ± standard deviation (SD), and significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Investigation of the Morphology

Figure 1 displays the usual XRD patterns used to analyze Co_3O_4NPs spectra. The database acknowledged the typical cobalt oxide spectrum (JCPDS number 42–1467) consistent with the XRD cobalt oxide spectra. Using the Debye-Scherrer equation: $D = K\lambda/\beta\cos\theta$, it has been determined that the average particle size of crystalline Co3O4 is 37.08 nm.[13] Figure 2 illustrates the results of determining the product's chemical purity and elemental composition. The obtained sample does not contain any additional impurities, as shown by the EDX spectra of Co_3O_4NP, which only show the existence of Co and O peaks and no other distinctive peaks. A small (Au) peak appeared at 2.1 keV. The FTIR spectra of the Co_3O_4 NPs captured by scanning the samples over a wavelength range (400–4000 cm^{-1}) are shown in Figure 3. Significant absorption peaks were visible in the Co_3O_4 NPs at 671 and 578 cm^{-1}, corresponding to the Co-O bond.[14] The results from the XRD are supported by Figure 4, which shows that the Co_3O_4 NPs had an average size of about 59.09 nm in SEM. Investigative work was done on the NPs' morphology. The photos show that the Co_3O_4NPs are sphere-shaped and essentially uniform. Vander Waals for surface tension and electrostatic interactions between nanoparticles may be responsible for these weak forces.[15]

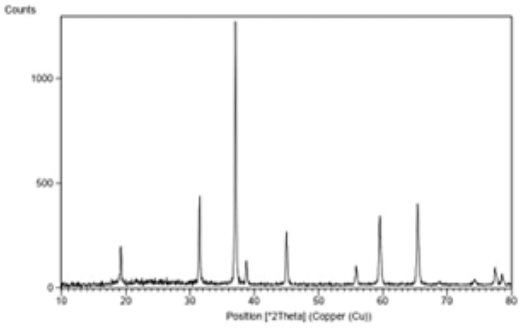

Figure 1: X-ray diffraction pattern: Co_2O_4NPs

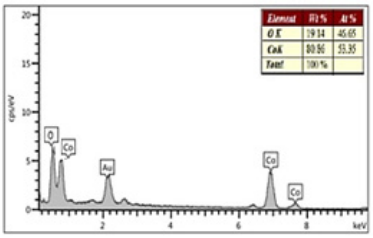

Figure 2: EDX spectra of Co_2O_4NPs

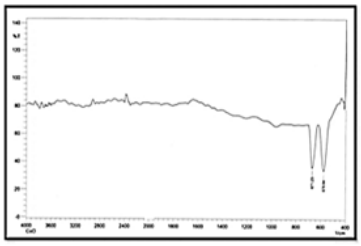

Figure 3: Fourier-transform infrared spectra Co_2O_4NPs

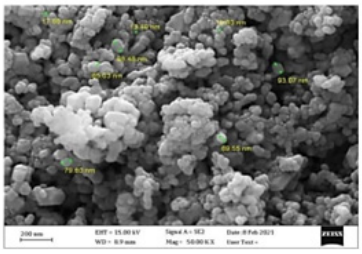

Figure 4: SEM images for Co_3O_4NPs

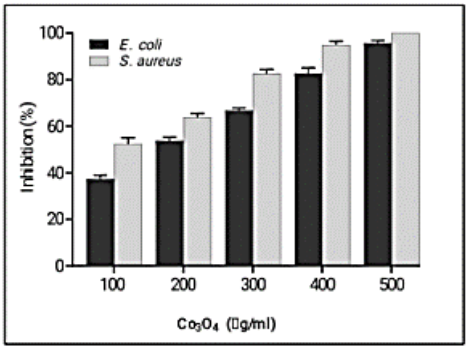

Figure 5: Effect of Co_3O_4NPs on *E. coli* and *S. aureus*

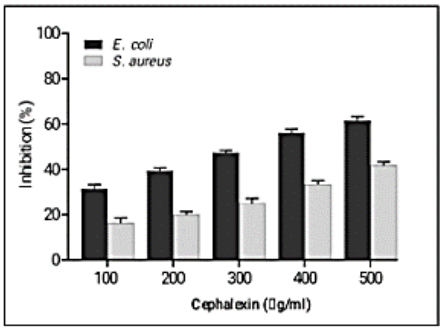

Figure 6: Effect of cephalexin on *E. coli* and *S. aureus*

Determination of MIC of Co_3O_4NPs

The Co_3O_4NPs' MIC values are 200 µg/mL for *E. coli* and 100 µg/mL for *S. aureus*. However, the electrostatic interaction between NPs and bacterial surfaces, which disrupts the exchange of the cell wall with the cell surfaces and changes the permeability of membranes by which NPs can enter the cell, maybe the cause of NPs' antibacterial effectiveness. This interaction causes the synthesis of reactive oxygen species (ROS) and cellular oxidative stress of the constituents.[16]

Antibacterial Activity Co_3O_4NPs

Investigations were made into the antibacterial effects of Co_3O_4NPs on the human pathogenic microorganisms *S. aureus* and *E. coli*. *S. aureus* has been utilized as a model for research into how antibiotics work against bacteria because of its crucial significance in various human and animal diseases. In a dose-dependent way, the amount of GI% reflects the degree of sensitivity of bacteria. The results showed significant antibacterial action against the Co_3O_4NPs tested. The antibacterial assay reveals that Co_3O_4NPs significantly outperform cephalexin (Figures 5 and 6). NPs were additionally more effective against *S. aureus* than *E. coli*. The MIC index of the tested Co_3O_4NPs is displayed in Table 1. *E. coli* 200 µg/mL and *S. aureus* 100 µg/mL are the Co_3O_4NPs' MIC values for both bacteria. However the electrostatic activity between NPs and bacterial surfaces, which disrupts the cell wall, gives NPs their antibacterial power. This will impact the membranes' permeability and the activation of the creation of ROS.[17] It has been suggested that the interaction between NPs and antibiotics was caused by antimicrobial inhibition that damaged the membrane.[18] The Co_3O_4NPs created in this work might help increase the antibacterial effectiveness against harmful microorganisms at lesser concentrations. In this instance, NPs may not be toxic and suitable for therapeutic usage at these doses. However, it is advised that *in-vivo* toxicity testing of NPs be considered because they can seriously harm humans or animals.[19] These results suggest that Co_3O_4NPs may be effective antibacterial agents for bacterial strains resistant to antibiotics. It can be seen that there is a direct correlation between the Co_3O_4NPs concentration and the rate of bacterial inhibition in Figure 5, which depicts the impact of various Co_3O_4NP concentrations on the growth of *S. aureus* and *E. coli*. The highest inhibition was reached at 500 µg/mL concentration towards *E. coli* (96%). While the highest inhibition rate was for *S. aureus* 100%, the lowest inhibition rate at 100 µg/mL against *E. coli* was 37%, while the lowest inhibition was for *S. aureus* (51%). The biological cobalt nanostructures increase the generation of ROS in tissues, which could cause cellular harm. Previous research on rodent and human cells employing Co_3O_4NPs demonstrated that Co_3O_4NPs can boost the induction of ROS. Due to the nanoscale form's tiny nanoparticle size, it can enter cells more readily than the ionic form.[20]

Biological Activity of Cephalexin

The effects of cephalexin on the development of *E. coli* and *S. aureus* demonstrated a direct correlation between cephalexin concentration and GI%, as illustrated in Figure 6. The highest inhibition rate against *E. coli* was discovered at a concentration

Table 1: The biological activity of Co_3O_4 NPs plus cephalexin

Chemicals	Concentration (µg/ml)	Volume (µl)	Inhibition (%)	
			E.coli	*S. aureus*
Co_3O_4 (control)	500	100	96	100
Cephalexin (control)	100	100	30	16
Cephalexin + Co_3O_4	100:500 (1:3)	75 + 25	100	100
Cephalexin + Co_3O_4	100:500 (1:1)	50 + 50	100	100
Cephalexin + Co_3O_4	100:500 (3:1)	25 + 75	100	100

of µg/ml 500, while the highest inhibition rate against *S. aureus* was 40%. The lowest inhibition rate against *E. coli* was 30%, and the most deficient inhibition against *S. aureus* was 16% compared to the 100 µg/mL concentration. The beta-lactam cephalosporin group, or first generation, includes cephalexin. It prevents the bacterial cell wall's synthesis and ultimately results in cell death. It manages several bacterial infections, including dermatitis, osteitis, otitis media, respiratory tract infections, pharyngitis, and urinary tract infections. Beta-lactamase enzymes are produced by *S. aureus* and *E. coli* as resistance mechanisms. The presence of beta-lactamase enzymes produced by these bacteria is the cause of cephalexin's low action since they ineffectively break down beta-lactam by hydrolyzing the C-N bond on the beta-lactam ring.[21]

Study the Effect of Mixing Co_3O_4 NPs with Cephalexin

As indicated in Table 1, the produced spinel nanoparticles, which had a concentration of 500 µg/mL, were combined with cephalexin, which had a concentration of 100 µg/mL, in three different ratios: 1:3, 1:1, and 3:1. Both of the medicines' presence had a significant impact on both microorganisms. Due to the presence of numerous active groups in antibiotic molecules, including the hydroxyl and amine groups, which readily interact with oxide nanoparticles, the combined impact of oxide nanoparticles and antibiotics can be achieved through the breakdown of antibiotics. Therefore, the interaction between the antibiotics and the oxide nanoparticles may cause a synergistic impact. In summary, adding spinel nanoparticles to antibiotics rather than pure antibiotics alone can increase the effectiveness of bacterial inhibition.[22]

CONCLUSION

Spinel Co_3O_4 nanoparticles were synthesized in this study utilizing a co-precipitation technique and were analyzed using FTIR, XRD, and SEM. The average particle size of Co3O4 NPs was 37.08 nanometers. Using the broth microdilution technique, the biological activity of these particles was assessed, and it demonstrated outstanding activity against *S. aureus* and *E. coli* when compared to cephalexin. It has been shown that NPs, both alone and in conjunction with cephalexin, are more effective against both.

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