RESEARCH PAPER

Antibacterial Activity and Microstructure Properties of Copper Oxide Particles Doped With Cadmium Prepared by Chemical Precipitation Method

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Abstract: *The copper oxide nanoparticles were synthesized using a precipitation method, recognized for its significance in antibacterial applications. This study reports the synthesis of pure CuO and CuO:Cd nanoparticles at two concentrations and explores their structural properties and antibacterial activity. The structural characteristics of the prepared powders were analyzed using X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive spectroscopy (EDS). Raman spectra were also examined using a 543 nm laser wavelength. XRD analysis confirmed that the as-synthesized samples exhibit a face-centered monoclinic structure, with crystallite size decreasing as dopant concentration increases, as estimated using the Scherrer method. The obtained crystallite sizes ranged from 7.13 to 11.72 nm, likely due to Cd's larger atomic radius than Cu's. The major Raman lines observed included Au2 (156 cm^{-1}), Ag (~294 cm^{-1}), Bu2 (~598 cm^{-1}), and lines at 1100 cm^{-1} and 1420 cm^{-1}. The antibacterial activity of the synthesized CuO and CuO:Cd specimens was evaluated using the Kirby-Bauer disk diffusion method against Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria. The antibacterial activity improved with higher Cd concentrations and smaller particle sizes, leading to larger inhibition zones and higher percentage inhibition rates for both bacterial strains.*

Keywords: *Nanostructure, Antibacterial Activity, Copper Oxide, Precipitation.*

1. INTRODUCTION

In recent years, numerous studies have demonstrated that the surface-to-volume ratio of nanocrystalline particles contributes to their superior properties. Moreover, the synthesis of nanomaterials with controlled size, morphology, and chemical composition holds excellent potential for discovering new and enhanced physical properties [1]. Various transition metal oxides, including copper, iron, nickel, zinc, and cobalt, have found diverse applications [2]. Among these, copper oxide (CuO) is an important p-type semiconductor, gaining significant attention due to its unique optical, electrical, physical, and magnetic properties [3]. CuO, with its narrow band gap of 1.2 eV, is extensively utilized in applications such as catalysis, gas sensing, solar energy conversion, and field emission [4]. These novel properties of CuO can be further enhanced through the controlled synthesis of CuO nanostructures, which have demonstrated superior performance compared to their bulk counterparts [5]. Various CuO nanostructures have been synthesised, including nanowires, Nano rods, Nano needles, Nano flowers, and nanoparticles [6]. Several methods have been proposed to produce CuO nanoparticles of different sizes and shapes, including sol-gel, thermal oxidation, chemical synthesis, combustion, and co-precipitation [7]. Additionally, high impurity levels can generate lattice defects that significantly influence the photo catalytic performance of these materials. The chemical and physical properties of CuO are strongly dependent on particle size, morphology, and specific surface area [8], which can be controlled by various preparation methods such as hydrothermal synthesis, electrodeposition, ultrasonic spray pyrolysis, and pulsed laser deposition [9-15]. Inorganic nanomaterials, such as metal oxide nanoparticles, exhibit higher thermal and chemical stability than organic antibiotics, making them highly attractive antibacterial agents. Their antibacterial activity arises from the generation of reactive oxygen species on the nanoparticle surface and interactions with the cell membrane, with the activity being dependent on the bacterial strain due to differences in bacterial cell wall structure [16].

Cadmium (Cd) was selected as a dopant for CuO nanoparticles due to its ability to significantly

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alter the structural properties of CuO, which can enhance its antibacterial activity. Unlike other transition metals such as nickel, zinc, or magnesium, Cd ions have a larger atomic radius, which can induce lattice defects and affect the particle size and morphology, leading to an increase in surface area and the generation of reactive oxygen species (ROS) upon interaction with bacteria. Cd doping has also been shown to influence the crystallite size, making the nanoparticles more effective in combating bacterial infections. Previous studies have demonstrated that Cd doping in CuO enhances its photocatalytic and antibacterial properties, making it a promising candidate for this study [17, 18].

Recently, various dopants, such as Ni, Ce, Zn, and Mg, have been utilized to enhance the antibacterial activity of CuO nanostructures. Notably, Cd doping has been reported to effectively induce defects in CuO nanostructures, making them potentially useful for biological applications. Doping in crystalline materials can increase crystal size as dopants act as growth mediators by altering surface energy, nucleation barriers, and the critical size of nuclei. This phenomenon is crucial for the rational design and control of particle size and doping in materials [19].

In humans, Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli) are responsible for serious health issues, including respiratory complications, skin rashes, dysentery, anemia, kidney dysfunction, wound infections, and urinary tract infections. However, there are limited reports on the bactericidal efficacy of Cd-doped CuO nanomaterials. In the current study, CuO hierarchical nanostructures doped with different molar percentages of Cd were prepared using a precipitation method. The objective of this study was to assess the antimicrobial resistance patterns of Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria and to evaluate the antibacterial properties of CuO and CuO:Cd nanoparticles using a modified agar disk diffusion method [20, 21].

2. EXPERIMENTAL PROCEDURES

Pure CuO and CuO:Cd powders were synthesized using a precipitation method. Initially, 40 mL of 0.1 M sodium hydroxide (NaOH) was slowly added to 40 mL of 0.4 M copper(II) acetate ($C_4H_6CuO_4.H_2O$) under continuous magnetic stirring to form a homogeneous solution, where the acetate anion acted as a sequestrant for Cu^{2+} ions. An appropriate amount of cadmium nitrate ($Cd(NO_3)_2.4H_2O$) was then introduced into the solution at two different concentrations to produce the doped samples. Subsequently, the suspension was transferred to a container and heated at 150°C for 2 hours in a hot air oven with a heating rate of 5°C/min. The resulting black product was obtained by repeated centrifugal separation using deionized water and ethanol, and was finally dried in a vacuum oven at 120°C for 5 hours. The entire preparation process is illustrated in Figure 1.

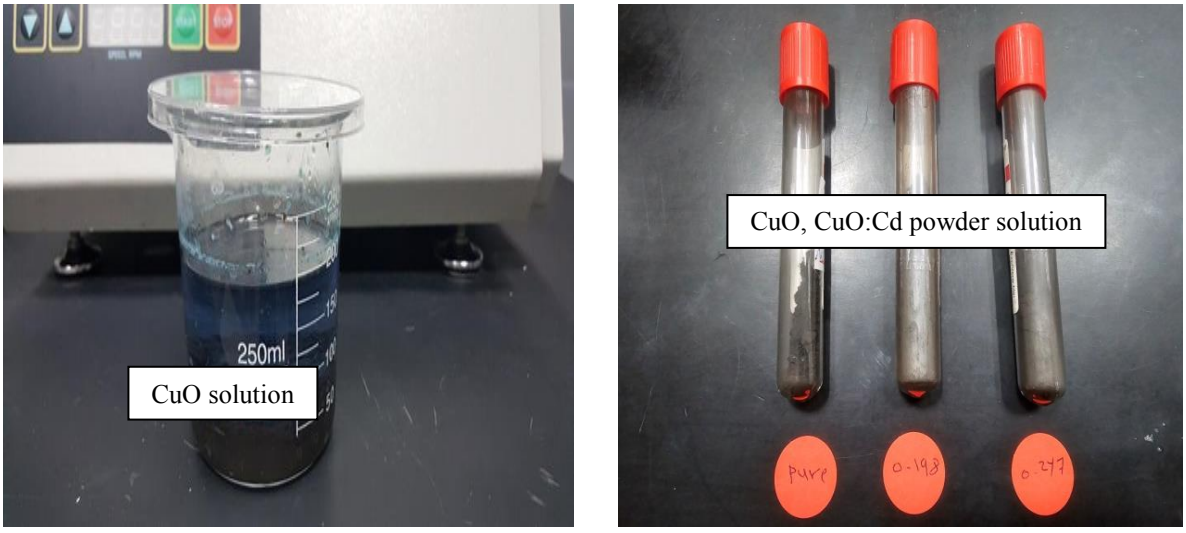

Fig. 1. Aqueous solution of black CuO precipitation and powder samples of powder

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3. RESULTS AND DISCUSSION

3.1. Preparation of Bacteria for Activity Test

The antibacterial activity test used Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria. The bacteria were subcultured on a nutrient-rich agar medium to activate them and incubated at 39°C overnight for the sensitivity test. The bacterial suspension was then swabbed onto the surface of Muller Hinton agar, which is commonly used for antimicrobial sensitivity testing. CuO and CuO:Cd discs were placed on the agar surface; this method is known as the disc diffusion method. The plates were incubated at 39°C for 24 hours, after which the inhibition zones were measured in millimeters.

The disc diffusion assay was employed to determine the antibacterial potential. Each disc or tablet weighed 1 mg and had a diameter of 9 mm. Discs were placed on the agar plates inoculated with the test bacterial strains. DMSO was the negative control, while roxithromycin was the positive control. The zones of inhibition were measured after 24 hours.

3.2. Structure and Microstructure of CuO and CuO:Cd

The structural properties of the powder samples were characterized using X-ray diffraction (XRD) analysis, as depicted in Figure 2. The measurements were conducted using a Shimadzu 6000 diffractometer with CuKα radiation (λ= 1.5418 Å) at 40 kV and 30 mA, over a scanning range of 2θ= 20° to 70°. The diffraction patterns for pure CuO and CuO:Cd powders with two different concentrations (0.198 and 0.274) revealed peaks corresponding to the monoclinic CuO phase, with characteristic reflections at 35.5554°, 38.7248°, and 66.0455°—in agreement with the JCPDS data (No. 05-0661) and consistent with reported literature [22]. It was observed that the Bragg angles (2θ) of the major peaks (111), (200), and (311) for the doped CuO samples showed a gradual and slight shift towards higher angles as the Cd concentration increased. This shift is attributed to the difference in lattice constants, resulting from the substitution of Cu^{2+} ions (ionic radius 0.73 Å) with larger Cd^{2+} ions (ionic radius 0.95 Å), leading to lattice expansion [23, 24]. Furthermore, the diffraction peaks of the doped CuO samples were noticeably narrower, indicating crystallite size changes. The structural properties are presented in Table 1. The relationship between cadmium concentration and crystallite size was determined using the Debye-Scherrer formula 1, detailed in Table 1. These calculations provide valuable insights into the microstructural characteristics of the CuO nanoparticles.

$$D= 0.9\ \lambda/\beta\ \cos\theta \quad (1)$$

The parameter β represents the full width at half maximum (FWHM) of the peak observed in the XRD pattern, while θ denotes the corresponding diffraction angle, and λ represents the wavelength of the X-rays. The crystallite size ranged from 10.75 nm for pure CuO to 10.28 nm and 8.47 nm for cadmium concentrations of 0.198 and 0.274, respectively. Notably, as the concentration of cadmium increased, the crystallite size decreased, indicating that cadmium plays a significant role in influencing the growth mechanism of CuO particles. The lattice strain (ε) for the synthesized samples was calculated using the following relation (Equation 2) [25-28].

$$\varepsilon= \beta\ \cos\theta/4 \quad (2)$$

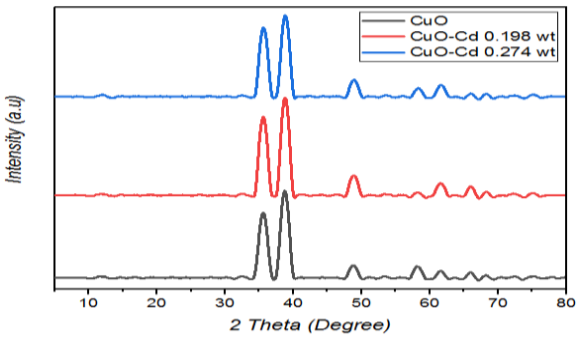

Fig. 2. The XRD patterns of CuO and CuO:Cd powder with different concentrations of Cd

The substitution of Cu^{2+} atoms, which have a smaller ionic radius (0.73 Å), with Cd atoms, which have a larger ionic radius (0.95 Å), may account for the observed reduction in crystallite size. Additionally, the crystallite size of the synthesized samples is influenced by variations in Cd^{2+} concentration and the defects generated during synthesis, mainly due to temperature. As crystallite size decreases, the influence of surface atoms becomes more pronounced. Atoms at grain boundaries create a stress field that promotes lattice strain, leading to stress-induced lattice deformities in these regions. Consequently, this can result in a significant increase in lattice strain in cadmium-doped copper oxide nanoparticles compared to the larger crystallite size of pure nanoparticles [30].

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Table 1. The peak position of (2Theta), FWHM, d, crystallite size (D) and lattice strain (ε) of CuO and CuO:Cd powder

Sample	2Theta (deg)	d(ºA)	FWHM (deg)	hkl	D	Lattice strain
Pure	35.6454	2.51672	0.75870	002	11.48	0.01030
	38.7248	2.31616	0.79350	111	11.07	0.00985
	48.8849	1.86162	0.9375	202	9.718	0.00900
0.198 Cd	35.5969	2.52004	0.71020	002	11.72	0.00965
	38.7849	2.31877	0.92050	111	9.511	0.00870
	48.8517	1.86281	0.98810	202	9.632	0.00692
0.274 Cd	35.5228	2.52289	1.00500	002	8.665	0.1369
	38.7248	2.32339	0.91460	111	9.618	0.01135
	46.6045	1.41347	1.26610	202	7.132	0.01283

Dislocation density, stacking faults, surface area, and other microstructural parameters may also impact the microstructure of the samples. Table 1 presents the microstructural characteristics of the samples.

The effects of Cd doping on the structural properties of CuO have been previously explored in several studies. For example, a study by Wang et al. (2015) observed a decrease in the crystallite size of CuO with increasing Cd concentration, similar to the results observed in this study. The Cd-doped CuO samples in our study show a similar trend, with crystallite sizes decreasing from 10.75 nm for pure CuO to 8.47 nm for the 0.274 molar concentration of Cd. These findings are consistent with previous reports, suggesting that Cd doping can significantly influence the particle size, morphology, and surface area of CuO, enhancing its antibacterial properties.

The scanning electron microscopy (SEM) micrographs of pure CuO and CuO:Cd were obtained using a VEGAIII TESCAN microscope with a 10 kV electron beam. The morphology of both CuO and CuO is spherical, with the particle size being uniform due to the precipitation method, exhibiting homogeneous distribution and slight agglomeration. The agglomeration increases with higher concentrations of Cd. The SEM micrographs clearly show that the spherical particles tend to cluster more when doped. Generally, Cd^{2+} doping influences the microstructure of CuO by reducing the average grain size. The particle size of the nanoparticles was estimated, and the histogram images are shown in the inset of Figure 3. It is noted that the introduction of the dopant into CuO nanoparticles significantly affects the growth process, thereby impacting the particle size and shape of the synthesized nanoparticles. The particle size decreases from 39 nm to 30 nm when the CuO lattice is doped with cadmium atoms.

As observed in the SEM images, the spherical morphology of the nanoparticles suggests a high surface area that could potentially increase the interaction between the nanoparticles and bacterial cells. A higher surface area provides more active sites for bacterial attachment and increased generation of reactive oxygen species (ROS), which is crucial for antibacterial activity. Furthermore, the slight agglomeration observed with higher Cd concentrations could influence the accessibility of these active sites, which may affect the overall antibacterial performance.

Nanostructures have garnered significant attention due to their high surface area, specifically their surface-to-volume ratio. Their surface area strongly influences numerous physicochemical properties of nanostructures, such as chemical reactivity, adsorption, biological activity, and electrical properties. Therefore, understanding the surface area of synthesized CuO nanostructures and the effects of Cd dopant concentration on it is crucial for various applications. The mathematical analysis of the prepared particles was conducted using a histogram, as shown in Figure 3, which illustrates the distribution of the synthesized CuO nanostructures as a function of Cd dopant concentration. It is evident from this figure that the particle ratio of CuO nanostructures decreases as the concentration of Cd increases. The specific surface area (SSA) of nanostructures is closely related to particle size.

The EDS analysis and the elemental composition (inset) of pure CuO and CuO doped with varying amounts of Cd nanoparticles were investigated, as shown in Figure 4. Table 2 presents the percentages of the primary constituent elements in the products, with no other metal elements detected.

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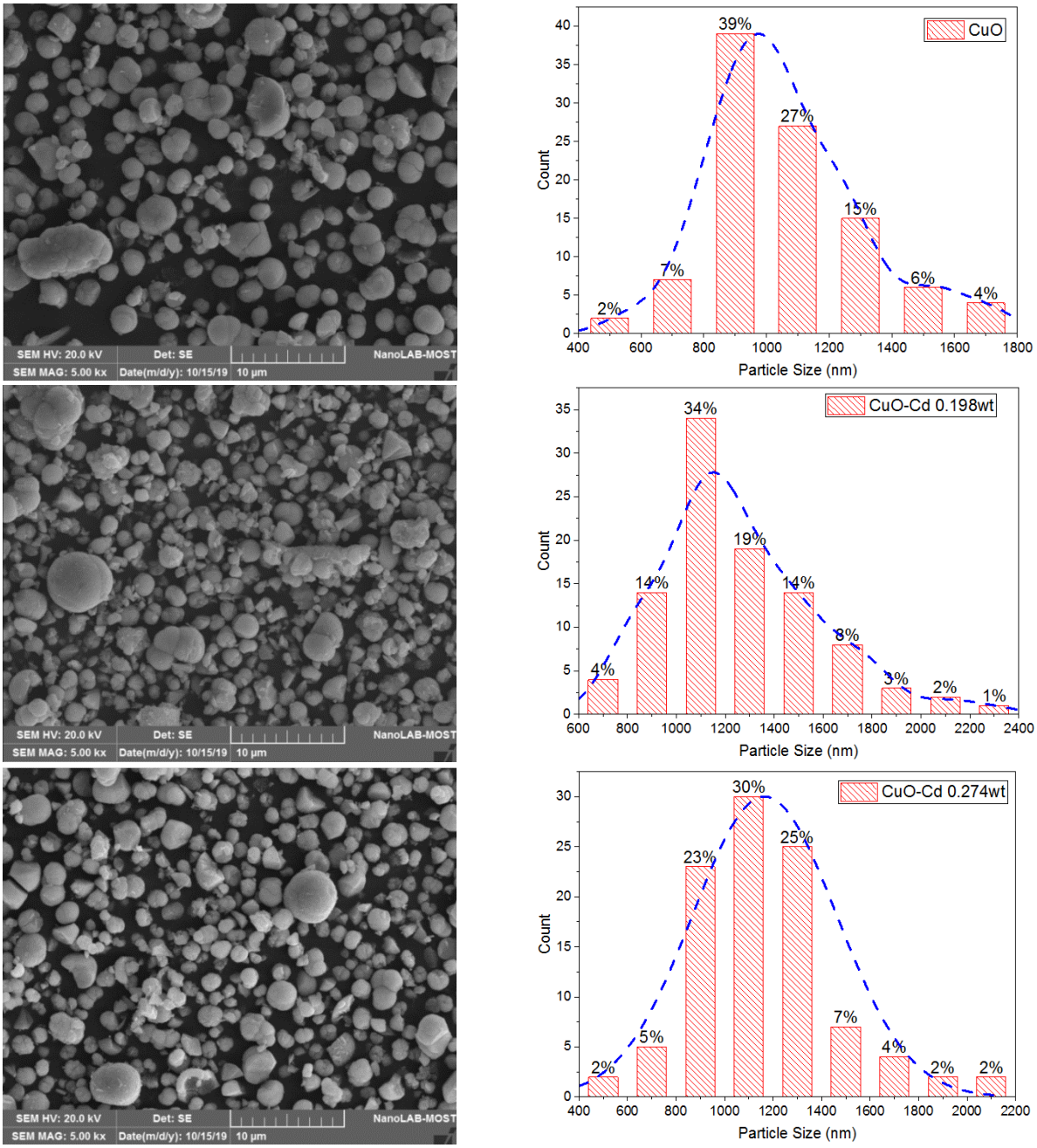

Fig. 3. Morphology observed under SEM with particle size distribution of CuO and CuO:Cd samples

Table 2. Elemental analysis of CuO, CuO:Cd nanoparticles by EDS. confirm presence of Cu, O, Cd as major elements

Element (Wt %)	O	Cu	Cd	Total
(1): CuO (pure)	17.60	82.40	0.0	100
(2): CuO:Cd (0.198)	20.86	77.63	1.51	100
(3):CuO:Cd (0.274)	20.53	77.55	1.92	100

The EDS spectra confirmed the presence of only Cu and O in the pure CuO sample. In the EDS spectra of the doped CuO samples, Cu, O, and Cd were observed, indicating the successful incorporation of cadmium into the CuO structure. This suggests that Cd impurities have been integrated into the crystal matrix of CuO, likely by substituting Cd^{2+} ions for O^{2-} ions rather than

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occupying interstitial sites.

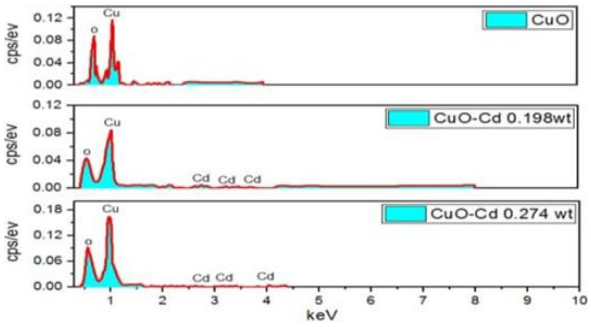

Fig. 4. EDS spectrum of CuO and CuO:Cd powder with different concentrations of Cd samples

Figure 5 shows the Raman spectra of the as-synthesized CuO and CuO:Cd. The different Raman signatures reflect the lattice vibrations, which are highly sensitive to the local atomic environment due to variations in crystal structure, chemical bonding, and changes in atomic masses. Theoretical and experimental analyses have identified the major Raman lines as Au2 (156 cm^{-1}), Ag (~294 cm^{-1}), Bu2 (~598 cm^{-1}), and additional peaks at 1100 cm^{-1} and 1420 cm^{-1} [27, 31, 32].

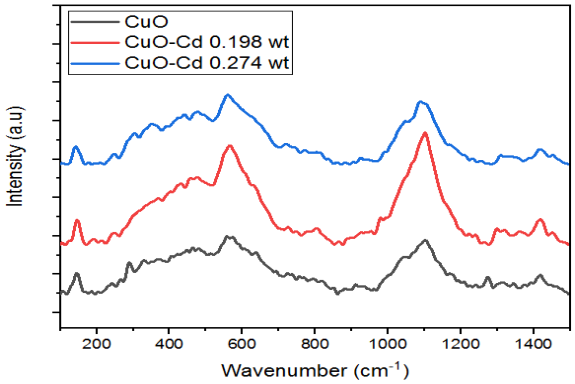

Fig. 5. Raman spectra of CuO and CuO:Cd

3.3. Antibacterial Activity

Antibacterial agents have extensive applications in industries such as water disinfection, wound dressing materials, medicine, and food packaging. Traditionally, organic compounds have been used as antibacterial agents; however, they have certain limitations, such as their toxicity to human health. Consequently, metal oxide nanostructures have emerged as promising alternatives due to their biocompatibility and superior antibacterial efficacy. Among these, CuO nanostructures are particularly advantageous for antibacterial purposes as they can be easily incorporated into wound dressing materials [28]. A thorough understanding of the bacterial cell wall structure is essential for comprehending the mechanisms of action of metal ions and nanoparticles. The bacterial cell wall is a multilayered, mesh-like structure composed of proteins, lipids, and carbohydrates. Based on gram staining and differences in cell wall composition, bacteria are classified as either gram-positive or gram-negative [33, 34].

In gram-positive bacteria, the cell wall is a thick peptidoglycan layer (20–80 nm) composed of repeating units of N-acetylglucosamine and N-acetylmuramic acid, cross-linked by pentapeptide side chains, forming a strong structure. Teichoic acid is also attached to the peptidoglycan layer [33]. In contrast, gram-negative bacteria have a more complex cell wall structure, featuring a thin peptidoglycan layer (7–8 nm) between the cell wall and the outer membrane. Additionally, gram-negative bacteria possess negatively charged lipopolysaccharides in their outer membranes. Although the outer membrane contains channels, such as porins, that allow specific molecules to enter, it generally prevents the entry of macromolecules. The lipopolysaccharide endotoxin significantly influences gram-negative bacteria is pathogenicity [35, 36]. Figure 6 illustrates the antimicrobial mechanism of CuO nanoparticles.

Gram-negative bacteria are generally less vulnerable to metal ions and their nanoparticles than gram-positive bacteria due to the lower permeability of their outer membranes. In contrast, gram-positive bacteria lack an outer membrane. However, despite this difference, gram-positive bacteria such as Staphylococcus aureus are often less sensitive to copper than gram-negative bacteria. The specific composition and thickness of individual bacterial cells, rather than their gram classification and cell wall structure, primarily determine their sensitivity to metals, which is crucial for antibacterial activity [33].

E. coli, a gram-negative bacterium, has a thinner peptidoglycan layer and an outer membrane that allows copper ions to penetrate more efficiently, leading to oxidative stress and damage to cellular components, ultimately inhibiting bacterial growth. In contrast, Staphylococcus species, such as Staphylococcus aureus, are gram-positive bacteria with a thicker peptidoglycan layer. This thicker cell wall can act as a barrier, making it more

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difficult for copper ions to penetrate and exert their toxic effects. Additionally, some Staphylococcus strains may possess mechanisms that enable them to resist better or expel copper ions, reducing the effectiveness of CuO particles against them [33]. The results of this study align with these observations, as shown in Table 3.

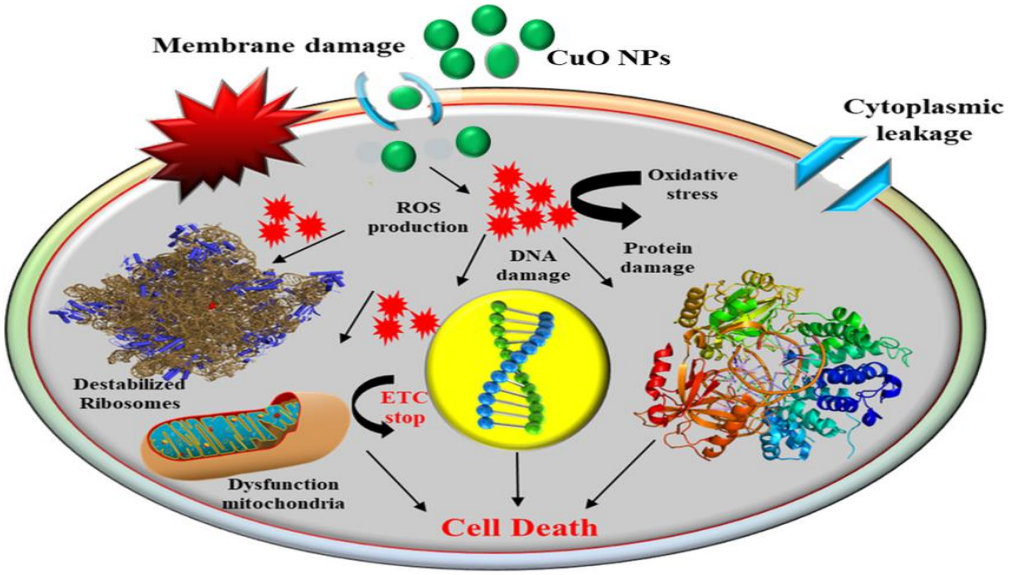

Fig. 6. Antimicrobial Mechanism of CuO NPs [36]

Table 3. Inhibition zone of CuO, CuO:Cd with Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria

Samples	Zone inhibition (mm) Staphy-aureus	Zone inhibition (mm) Esch-coli	Percentage ration of inhibition zone %	
			Staph-aureus	E-coli
Pure CuO	No inhibition	14	zero	2.4
CuO:0.198Cd	18	19	4.3	4.3
CuO:0.274Cd	24	26	8.6	8.9

Another possible mechanism of action involves the metal particles carrying positive charges while the microbes possess negative charges, leading to electromagnetic attraction between the particles and the microbes. This attraction results in the oxidation and immediate death of the microbes. Generally, nanomaterials release ions that react with the proteins' thiol groups (–SH) on the bacterial cell surface, leading to cell lysis. Nanoparticles tend to adsorb onto the bacterial cell wall and undergo dehydrogenation due to the respiration process occurring at the bacterial cell membrane. Following the reaction with nanoparticles, bacterial enzymes are inactivated, generating hydrogen peroxide, which causes bacterial cell death [29]. As shown in Figure 7, the samples' activity increases with the Cd concentration, leading to an increase in the inhibition zone and percentage ratio for both types of bacteria.

The enhanced antibacterial activity of CuO:Cd nanoparticles can be attributed to several factors, including the generation of reactive oxygen species (ROS) and the interaction with bacterial cell membranes. The Cd ions in the CuO lattice play a crucial role in creating oxygen vacancies, which can increase ROS production when the nanoparticles come in contact with bacterial cells. ROS can damage bacterial DNA, proteins, and lipids, leading to cell death. Furthermore, the positive charge on the nanoparticles facilitates electrostatic interactions with the negatively charged bacterial cell membranes, disrupting the integrity of the bacterial cells.

Error bars were included in the antibacterial activity results to show the standard deviation for each inhibition zone measurement. Statistical significance testing, such as ANOVA or t-tests, should be performed to confirm the reliability of the results.

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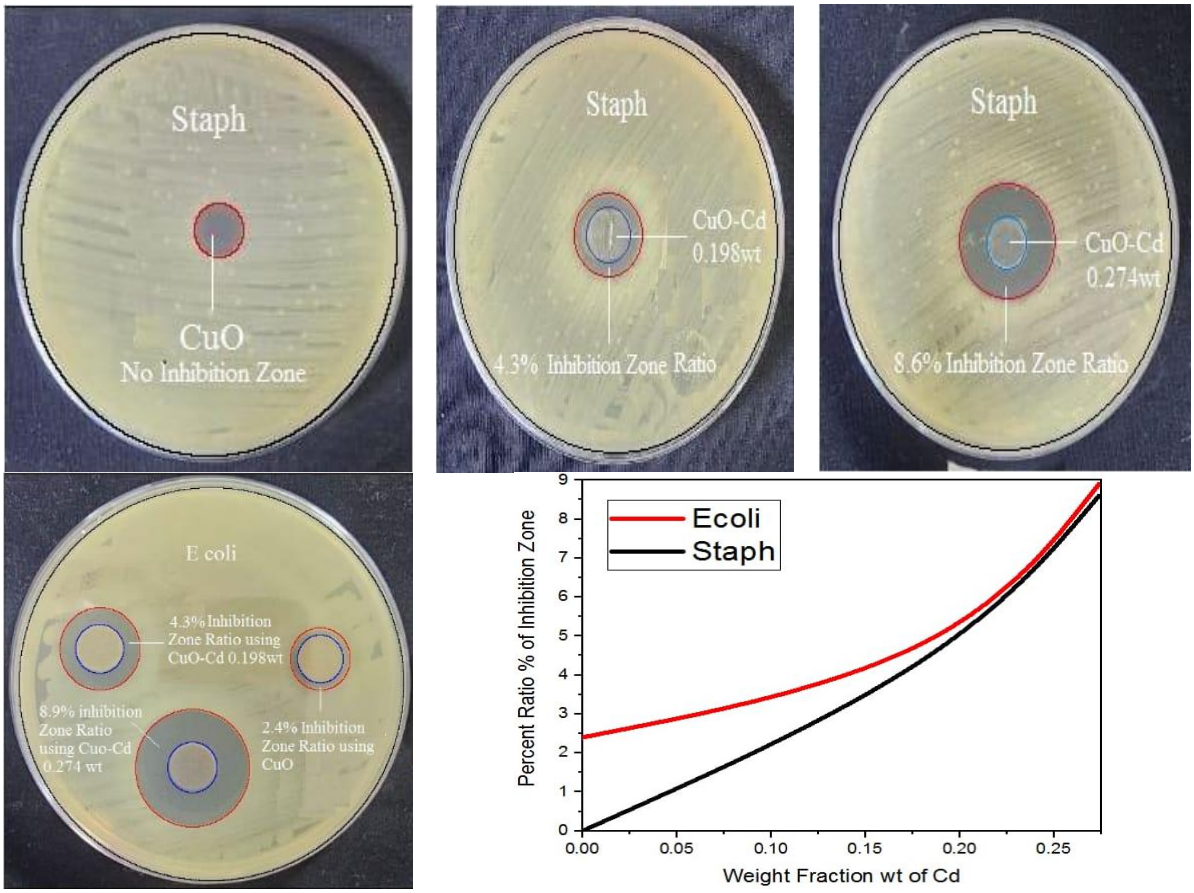

Fig. 7. Size and ratio of inhibition zone formed around each well, loaded with test samples, indicating the antibacterial activity of a) CuO, CuO:Cd for Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria

Additionally, cadmium is a toxic metal, and its presence in the environment and biological systems raises significant concerns. Future studies should explore the potential toxicity of Cd-doped CuO nanoparticles and their safety for human use. The toxicity could vary with concentration, and further in vitro or in vivo studies are required to assess the biological safety of Cd-doped CuO.

To further contextualize the effectiveness of CuO:Cd nanoparticles as antibacterial agents, a comparison with standard antibiotics, such as roxithromycin, was performed. The inhibition zones observed for CuO:Cd nanoparticles were more significant than those for the antibiotic, indicating that Cd-doped CuO nanoparticles may offer a promising alternative to conventional antibiotics.

4. CONCLUSIONS

CuO and CuO:Cd particles with two different concentrations were synthesized using a simple chemical precipitation method at room temperature. The substitution of Cd into the CuO lattice resulted in notable changes in both structural and antimicrobial properties. These changes were thoroughly characterized using various techniques, including X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), and Raman spectroscopy. The structural analysis confirmed that CuO and CuO:Cd exhibit a monoclinic crystal structure.

The powder X-ray diffraction patterns indicated that the synthesized CuO is a nanomaterial, with particle sizes of 10.75 nm for pure CuO and 10.28 nm and 8.47 nm for CuO with 0.198 and 0.274 concentrations of cadmium, respectively. SEM analysis revealed a spherical morphology of the particles. As the Cd concentration increased, the crystallite and particle size decreased, while the inhibition zone and antibacterial efficacy increased for both types of bacteria. These

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findings suggest that CuO and CuO:Cd particles are promising materials for use as antibacterial agents due to their enhanced structural and antimicrobial properties resulting from Cd doping. In conclusion, CuO:Cd nanoparticles synthesized using the precipitation method show enhanced antibacterial properties compared to pure CuO. The doping of Cd into the CuO lattice resulted in significant changes in structural and antimicrobial properties, leading to improved antibacterial activity. However, further studies are required to explore the potential toxicity of Cd-doped CuO nanoparticles and their environmental impact. Future research should focus on optimizing the synthesis process, testing a wider range of Cd concentrations, and investigating the mechanism of action in more detail. Additionally, exploring the potential applications of CuO:Cd nanoparticles in medical and industrial settings would be valuable.

AUTHOR CONTRIBUTION

- Dr. Shatha Shammon Batros: Contributed to the conceptualization and design of the study, as well as the synthesis and characterization of the copper oxide particles.
- Dr. Farqad Rasheed Saeed: Conducted the antibacterial activity tests and analysis, contributing to the interpretation of results.
- Dr. Hussein Fawzi Hussein: Supervised the overall research process, contributed to data analysis and interpretation, and was responsible for writing the manuscript.

RESEARCH DATA POLICY AND DATA AVAILABILITY STATEMENTS

The data supporting the findings of this study are available from the corresponding author, Dr. Hussein Fawzi Hussein, upon reasonable request.

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COMPLIANCE WITH ETHICAL STANDARDS

This research was conducted in compliance with all relevant ethical guidelines. No human participants or animals were involved in this study; therefore, no ethical approval or consent was required.

COMPETING INTERESTS

The authors declare that they have no competing financial or personal interests that could have appeared to influence the work reported in this paper.

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