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Synthesis, Structure Determination, and Biological Activity Study of Copper Oxide/Hydroxide Nanoparticles Using UV-irradiation Method

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Abstract

This study involved the synthesis of copper oxide/hydroxide (CuO and $Cu(OH)_2$) nanoparticles via the UV-Vis method. The resulting nanoparticles were characterized using different analytical techniques such as X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), and transmission electron microscopy (TEM). The morphological and structural characteristics of the synthesized nanoparticles were analyzed, revealing that the particle sizes of the samples ranged from 21.66 to 15.95 nm. Their antibacterial properties were determined against two different types of bacterial strains, namely Gram-positive *Staphylococcus aureus* (+ve) and Gram-negative *Pseudomonas aeruginosa* (-ve), in addition to their activity against *Candida albicans fungi*. The results indicated that all the prepared nanoparticles exhibited a strong ability to inhibit the growth of bacteria and fungi. The $Cu(OH)_2$ nanoparticles provided a maximum inhibition zone for each bacterial and fungal strain at the highest concentrations of each type.

Keywords: Biological activity. Copper oxide/hydroxide. Nanoparticles. UV-irradiation. X-ray diffraction.

تخليق , تحديد البنية ودراسة الفعالية البيولوجية لدقائق أكسيد/هيدروكسيد النحاس النانوية باستخدام طريقة التشعيع بالأشعة فوق البنفسجية

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الخلاصة

تشتمل هذه الدراسة على تخليق جسيمات نانوية من أكسيد/هيدروكسيد النحاس (CuO و $Cu(OH)_2$) عن طريق استخدام الأشعة فوق البنفسجية – المرئية. تم تشخيص الجسيمات النانوية الناتجة باستخدام تقنيات تحليلية مختلفة مثل حيود الأشعة السينية (XRD)، ومجهر مسح الانبعاث الإلكتروني الميداني (FE-SEM)، ومجهر الإلكترون النافذ (TEM). وقد تم دراسة الخصائص المورفولوجية والبنيوية للجسيمات النانوية

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المُصنّعة، وكشفت أن أحجام جسيمات العينات تراوحت بين 21.66 و15.95 نانومتر. وقد حُددت خصائصها المضادة للبكتيريا ضد نوعين مختلفين من سلالات البكتيريا، وهما المكورات العنقودية الذهبية موجبة الجرام (ve+) والزائفة الزنجارية سالبة الجرام (ve−)، بالإضافة إلى نشاطها ضد فطريات المبيضات البيضاء. وأشارت النتائج إلى أن جميع الجسيمات النانوية المُحضرة أظهرت قدرة قوية على تثبيط نمو البكتيريا والفطريات. وقد وفرت جسيمات النانو $Cu(OH)_2$ منطقة تثبيط قصوى لكل من السلالة البكتيرية والفطرية عند أعلى تركيز من كل نوع.

1. Introduction

Nanotechnology has garnered significant interest due to the unique properties of nanoparticles, making them valuable for various applications across industrial, agricultural, and medical fields [1-3]. Certain nanoparticles are utilized in advanced diagnostic tools, imaging, targeted medical methods and products, pharmaceuticals, biomedical implants, and tissue engineering. Nanotechnology allows for the safer administration of high-risk treatments, such as cancer chemotherapy drugs. Furthermore, wearable devices can identify changes in biomarkers, cancer cell states, and inflammation that have already occurred in the body [4-6]. Among the diverse types of nanoparticles, those containing copper, especially $Cu(OH)_2$ and CuO, have attracted significant attention due to their catalytic and antimicrobial activities [7-9]. In addition, copper oxide and hydroxide nanoparticles have many applications, including electrochemical sensors [10], anti-cancer [11, 12], antioxidants [13], food preservation [14], electrode material [15], wastewater treatment [16], and nano-insecticides [17]. Thus, the implementation and synthesis of nanoparticles have evolved as a mainstay for study in the domain of materials and nanotechnology.

The UV irradiation method has been employed to produce nanoparticles with a uniform size and a smaller diameter, in addition to being a simple and low-cost method [18]. In recent years, the synthesis of nanomaterials by UV irradiation has become very important. It has attracted the attention of many researchers because of its ability to easily synthesize different types of nanomaterials, such as Metal nanoparticles (MNPs) and metal oxide nanoparticles (MONPs), where processes like photothermal and photochemical can be used in this field [19-22]. This technique delivers an adaptable way to create nanoparticles with customized properties, making them an attractive option for researchers working in the nanotechnology field.

The physicochemical properties of copper oxide/hydroxide nanoparticles depend on size, shape, surface charge/area, and agglomeration of nanoparticles. However, the physicochemical properties have a strong connection with synthesis methods in addition to capping, reducing, and stabilizing agents [23]. Several literature reviews evaluate the antimicrobial properties of copper oxide/hydroxide nanoparticles synthesized by different methods. Copper oxide nanoparticles synthesized by the hydrothermal method offer noteworthy antimicrobial properties [24]. The green synthesis method has shown promising results regarding antioxidant and antimicrobial efficacy. Additionally, numerous researchers have documented the use of various synthesis methods [25], biosynthesis using plant extracts [26], supercritical hydrothermal synthesis method [27], thermal decomposition method [28], electrochemistry [29], microwave irradiation [30], sonochemical method [31], and reduction [32].This study aims to utilize the UV-irradiation method to synthesize CuO and $Cu(OH)_2$ nanoparticles and analyze their structural, morphological, and antimicrobial properties. Diagnosing nanoparticles is essential to comprehend their behavior and their possible applications. To study the structural properties and morphology of the prepared nanoparticles, specific analytical techniques were used in this study.

The evaluation of antimicrobial properties for synthesized nanoparticles was achieved by using various bacterial and fungal strains. The *Pseudomonas* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Candida albicans* were selected as model organisms to evaluate the efficacy of $Cu(OH)_2$ and CuO nanoparticles in inhibiting the growth of bacteria and fungi. The antimicrobial properties of nanoparticle species are crucial in various fields, including food packaging [33], medicine [34], and environmental remediation [35]. Nanoparticles' capability to inhibit microbial growth can lead to the development of new antimicrobial agents and coatings with improved efficacy and decreased environmental impact compared to conventional antimicrobial agents.

This study could pave the way for the development of new antimicrobial agents and coatings with improved efficacy and less environmental impact compared to conventional antimicrobial agents, which is valuable for researchers for future research projects.

2. Experimental

2.1. Materials and methods

The nanoparticles were made with copper (II) nitrate Cu $(NO_3)_2$, oxalic acid $(COOH)_2$, sodium hydroxide (NaOH), and deionized water. All chemicals were acquired from BDH and were not purified in any way.

2.2. Synthesis of $Cu(OH)_2$ and CuO nanoparticles

The synthesis was based on the method described in reference with modifications [36], where photoirradiation of cells utilizing an ultraviolet light produced the nanoparticles $Cu(OH)_2$ and CuO. The photocell consists of a UV source immersed inside a quartz tube, and the latter is placed inside the reactor (Pyrex flask) containing the copper nitrate brine as the essential raw ingredient for the preparation. As a UV source, a 125 W medium-pressure mercury lamp with a maximum light intensity of 365 nm was used. To avoid a rise in temperature due to the UV source, the reactor was placed in an Ice bath to cool it down. As a result, a solution of (25 mL, 0.01 mol) $Cu(NO_3)_2$/DI water was added and stirred for 30 minutes with a magnetic stirrer, and then a solution of (25 mL, 0.01 mol) oxalic acid/DI water was gradually added, one drop at a time. The combination was exposed to photocell radiation for 25 minutes while being chilled to 4 °C, resulting in powder precipitation. It is separated and repeatedly washed with deionized water. The Cu $(OH)_2$ was precipitated and dried at ambient temperature for 24 hours to produce a blue powder. The black CuO nanoparticles were precipitated when 4 g of the product $Cu(OH)_2$ was calcined for 4 hours at 400 °C [37].

2.3. Biological activity test

The biological activity of two different pathogenic bacterial strains, including *Staphylococcus aureus* (+ve), *Pseudomonas* (-ve), and the fungus *Candida albicans*, (isolates were taken from a urinary catheter isolated from women) were assessed at four different concentrations (1024, 512, 256, and 128 µg/mL) of copper hydroxide and copper oxide nanoparticles. The culture medium was prepared according to the manufacturer's recommended protocol. In a 500 mL volumetric flask, about 7.6 g of Muller-Hinton agar (M-H) was added to 200 mL of distilled water for the bacterial activation test. At the same time, Sabouraud Dextrose Agar (SDA) was prepared separately, and about 13 g of Sabouraud agar was added to 200 mL of distilled water for the fungal activity test. The prepared solutions were mixed by using a magnetic heating stirrer for 10 min and then covered and autoclaved at 124 °C for 20 min. After autoclaving the solutions, they were poured into petri dishes. A full loop of the bacterial culture was streaked onto M-H agar, and a piece of fungal culture was cut and placed onto SDA agar. The DMSO was used as a control solution (solvent). Each plate contains 5 holes with a diameter of 5 mm. DMSO solution is placed in the middle, and the rest of the holes are filled with DMSO solution for nanoparticles at the following concentrations (1024, 512, 256, and 128 µg/mL). At 37 °C, the plates were incubated in an incubator (SD-310 RL, Dasol, Korea) for 24 h for bacterial culture and 48 h for fungal culture. The resulting inhibitory zones' sizes were measured and recorded [38-40].

3. Results and discussion

3.1. X-ray diffraction (XRD)

XRD patterns were obtained with a SHIMADZU XRD-6000 instrument employing Cu Kα radiation (λ = 1.54 Å) over a 2θ range of 10°–80°. Figure 1 displays the XRD patterns of the as-prepared $Cu(OH)_2$ and CuO nanoparticles, comparing their structures before and after calcination at 400 °C. The crystallite size can be estimated using the Debye-Scherrer equation $D = k\ \lambda / \beta COS\ \theta$ [41, 42]. Five characteristic peaks are observed in the 2θ range (12.22°, 19.73°, 35.97°, 39.11° and 58.49°). in the XRD pattern, which corresponds to the (020), (021), (002), (130), and (150) respectively, the crystal planes of $Cu(OH)_2$ is (JCPDS No. 13-0420) [36, 43] before calcination, its crystallite size (C.S) was 15.6 nm. After calcination at 400˚C the XRD pattern of CuO nanoparticles synthesized by photolysis method with additives oxalic acid, shows characteristic peaks at 32.56 ˚,35.65 ˚,38.84 ˚,48.8 ˚,58.46 ˚,61.78 ˚,66.51 ˚,68.31 ˚,72.56 ˚ and 75.19˚ corresponding reflecting planes are (110), (11-1), (111), (20-2), (202), (11-3), (022), (220), (221) and (22-2) respectively, pattern diffraction perfectly obvious peaks within the pattern suggested a good monoclinic nano product. From the results, it is possible to conclude that broader peaks signify smaller particle sizes, which reflect good experimental conditions for the crystal's growth. It was a clear improvement in the average crystallite size; in general, all peaks of the XRD reflections of CuO match that of (JCPDS no. 48-1548) corresponding to a monoclinic structure, its crystallite size (C.S) was 13.36 nm
[44, 45].

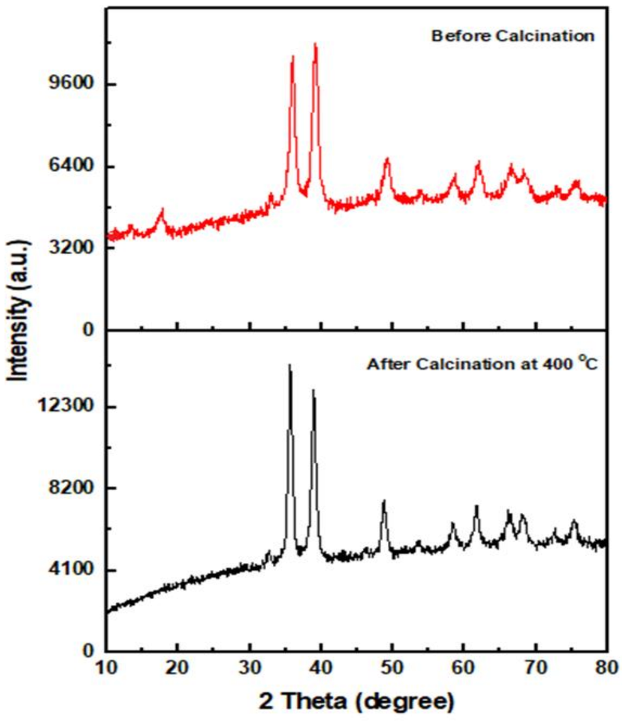
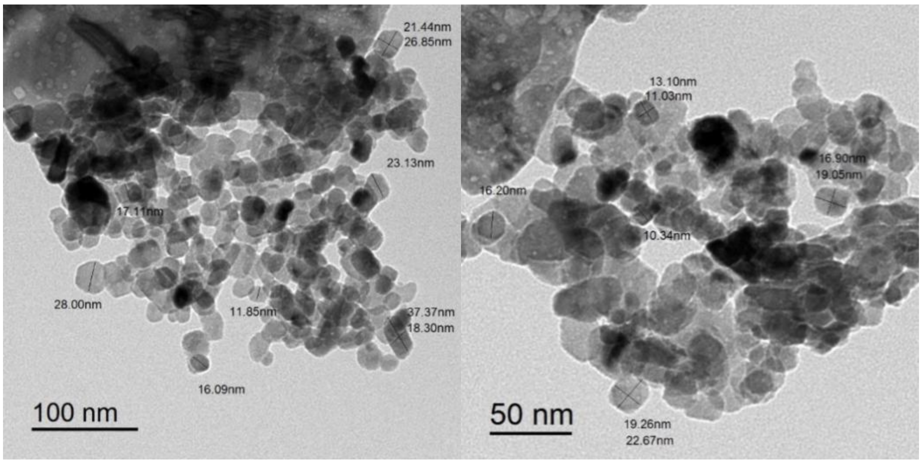


Figure 1: The XRD of $Cu(OH)_2$ and CuO NPs.

3.2. Transmission Electron Microscopy (TEM)

The morphology and particle size of nanoparticles were determined by Transmission Electron Microscopy (JEOL JEM -2100), as shown in Figure 2. The TEM images confirmed that the nanoparticles were synthesized with a spherical shape and exhibited no aggregations.

The use of oxalic acid as a surfactant, combined with UV irradiation, was crucial in producing well-defined nanoparticles. The synthesis followed a bottom-up approach, which included forming the nano nucleus when the surfactant was added with UV irradiation. Then, growth began until the required particle was formed. The large surface area is favorable, which benefits the enhancement of NP's performance for biological activity. The average particle sizes calculated randomly from the figures were 22.23 nm for $Cu(OH)_2$ and 16.06 nm for CuO [36].



a b

Figure 2: TEM image of (a) $Cu(OH)_2$ and (b) CuO NPs.

3.3. Field Emission Scanning Electron Microscopy (FE-SEM)

Field emission scanning electron microscopy (JEOL JSM-6510 LV) was employed to examine the morphology and size of nanoparticles. The analysis revealed a smooth surface with well-defined crystallization and spherical aggregates, indicating that a nearly uniform distribution of samples can be concluded, as shown in Figure 3. It can be noted that the samples contain a high porosity through the measurement images. The spherical shape is the dominant one in the copper oxide, and the spherical shape and agglomeration of peels are the dominant ones in the copper hydroxide sample. The average particle size of nano-samples is determined randomly on the FE-SEM images. The copper hydroxide and copper oxide were determined, with an average diameter of 21.66 and 15.95 nm, respectively. There are high proportions of homogeneous diameters in the measurement images and small sizes [36].

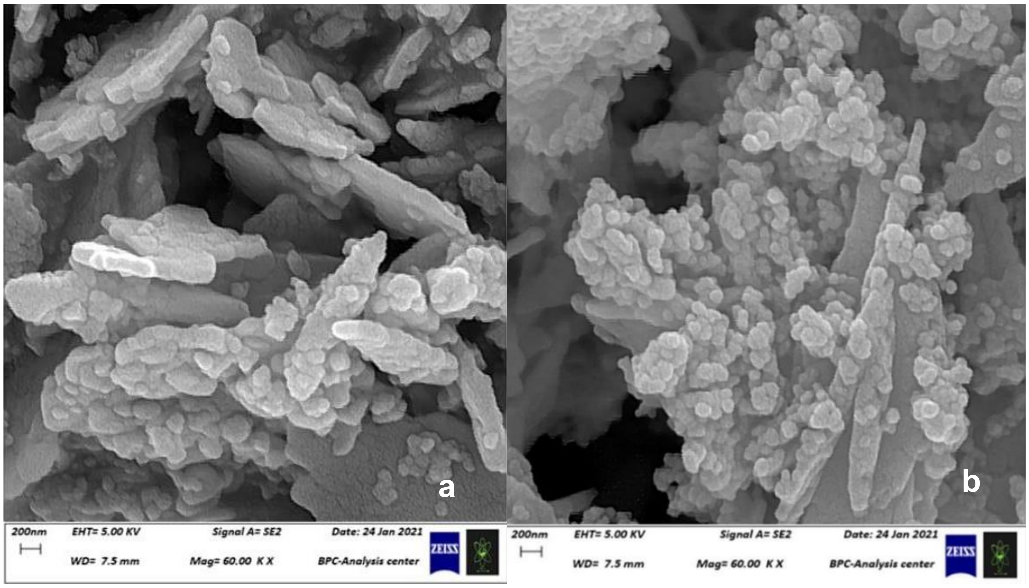


Figure 3: FE-SEM image of (a) $Cu(OH)_2$, (b) CuO NPs.

3.4. Evaluation of biological activity

Two different strains of pathogenic bacteria (*Staphylococcus aureus, Pseudomonas*) and fungus (*Candida albicans*) were employed to examine the nanoparticles' biological activity. These bacteria are recognized to be the primary cause of numerous illnesses and infections, and their resistance to the majority of available antibiotics, in addition to *Candida albicans* fungus.

McFarland was used as a reference point to measure biological activity. The recently prepared compounds were dissolved in DMSO, which served as a solvent. The DMSO solution was tested for different concentrations of nanoparticles after placing them in the agar medium at a temperature of 37 °C for 24 hours. Microbial growth was inhibited during the diffusion period of the test solution. On the plate, the inhibition zone of the bacteria strain (*Staphylococcus*) and the fungi (*Candida albicans*) at the $Cu(OH)_2$NPs. A concentration of 1024 µg/mL, showed the greatest zone inhibition compared to the other concentrations. The Pseudomonas strain exhibited no zone of inhibition in any of the $Cu(OH)_2$ and CuO NPs solutions at the concentration of 256 and 128 µg/mL, as presented in Table 1 and Figure 4. The findings indicate that $Cu(OH)_2$ NPs have roughly higher activity than CuO NPs toward the bacterial and fungal species [47-49]. The prepared NPs have great activity and an exceptional ability to kill bacteria and fungi, as the prepared NPs are distinguished by their ability to rupture bacterial cell membranes and to prevent growth as a result of the distribution of the external electrical potential of their membrane [50, 51]. The following justifications, in part or in all, can do that: The importance of the hydroxyl group in combating bacteria as a potent and successful group. The reduced polarization of the metal ion due to the fractional sharing of the positive charge is thought to be the second cause of the activity. As a result, the lipophilic nature of the bacterial membrane is enhanced, encouraging penetration through the lipid layers [52].

The prepared $Cu(OH)_2$ and CuO NPs were almost equally as efficient against fungi as they were against bacteria. This shows the strong interaction between these nanomaterials and the composition of the fungus. This interaction can occur in one of two ways: as antimetabolites, the nanomaterials interfere with the formation of key biomolecules within the cell. Or, as spindle inhibitors, such agents prevent correct cell division by interfering with the cytoskeletal components and the inability of one cell to divide into two parts [53].

Table 1: The effect of nanomaterials on two types of bacteria and fungi, represented by the inhibition zones (mm).

NPs.	*Staphylococcus*				*Pseudomonas*				*Candida albicans*			
	Inhibition zones (mm)											
Concentration (µg/mL)	**1024**	**512**	**256**	**128**	**1024**	**512**	**256**	**128**	**1024**	**512**	**256**	**128**
$Cu(OH)_2$NPs.	30	25	20	18	25	10	0	0	30	25	20	15
CuO NPs.	25	20	20	18	20	5	0	0	25	20	15	15

Conclusion

This study successfully synthesized nanoparticles free from aggregation. SEM analysis showed that the sizes of $Cu(OH)_2$ and CuO nanoparticles were approximately 33.40 nm and 25.50 nm, respectively. These nanoparticles were found to be pure and well-suited for biological applications. TEM measurements indicated sizes of 22.23 nm for $Cu(OH)_2$ and 16.06 nm for CuO. XRD analysis showed crystal sizes of 15.6 nm for $Cu(OH)_2$ before calcination and 13.36 nm for copper oxide after calcination. Testing against *Staphylococcus aureus* and *Pseudomonas* revealed vigorous antibacterial activity, with inhibition zones of 90–100%, while fungal cells (*Candida albicans*) were affected at all concentrations. Notably, copper hydroxide achieved a complete 100% inhibition zone for fungal cells. This positions these nanoparticles as highly promising agents for future biological applications.

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Declarations

Conflict of interest: The authors have no competing interests to declare that are relevant to the content of this article.

Author Contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Kamal Rashid Al-Jorani, Amal F. Kamil, Atheer Abdulsahib Ali, and Ahmed M. Rheima. Amal Fadil Kamil: Resources, Formal analysis. Kamal R. Al-Jorani: Writing – review & editing, Supervision, Resources, Data curation. Atheer Abdulsahib Ali: Resources, Investigation, Formal analysis. Ahmed Mahdi Rheima: Writing – original draft, Investigation, Formal analysis. All authors read and approved the final manuscript.

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