SYNTHESIS OF CUO NANOPARTICLES AND INVESTIGATION OF THEIR EFFECTS ON BACTERIAL STRAINS, BOTH GRAM-POSITIVE AND NEGATIVE

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Abstract
Chemical precipitation method was used to synthesis of copper oxide nanoparticles (CuO NPs). Afterwards, the size distribution, shape, and size average of the CuO NPs were ascertained by means of field emission scanning electron microscopy (FE-SEM), transition electron microscopy (TEM), and X-ray diffraction (XRD), respectively. CuO NPs then showed their antibacterial effectiveness against Gram-positive and negative bacteria pathogens, including Escherichia coli and Staphylococcus aureus. After being incubated for 12 hours at 25°C, the CuO NPs at a concentration of 20 and 50 ppm had an exceptional antibacterial activity, killing both Gram-positive and negative bacteria.

Keyword: CuO nanoparticles; Chemical precipitation method; Gram-positive and negative bacteria.

Introduction

Bacterial infections continue to be a leading cause of morbidity and mortality even with the abundance of powerful antibiotics and other antimicrobial treatments available today.[1] In order to replace the conventional organic agents, which have limited applications due to their low heat resistance, high decomposability, and short life, researchers are focusing on developing new bactericides based on inorganic materials in response to the emergence of microorganisms that are becoming resistant to a variety of antimicrobial agents [2]. Strong action against both broad-spectrum Gram-positive and Gram-negative bacterial species has been shown by inorganic nanoparticles [3]. Both the nature and content of the nanoparticles as well as the type of bacteria involved determine how hazardous they are to bacteria [4]. Copper oxide nanoparticles have proven to be good, efficient antibacterial and anticancer agents within the inorganic nanoparticle family [5]. Copper oxide nanoparticles can efficiently battle both Gram-positive and Gram-negative viruses and bacteria [6]. Iron oxide (Fe_3O_4), copper oxide (CuO), and zinc oxide (ZnO) are examples of metal oxide nanoparticles (NPs) that are gaining increasing attention these days due to their unique optical, physical, and biological properties.

[7-9] One of these metal oxide nanoparticles, CuO NPs, is becoming more well-known because of its significance in the environmental remediation and biopharmaceutical industries. [10] Numerous processes, including electrochemical synthesis, sonochemical synthesis, sol-gel procedures, microemulsion systems, precipitation synthesis, microwave irradiation, and environmentally friendly methods, can be used to create CuO nanoparticles.[11-17] Precipitation chemical technique is a preferred approach for creating CuO nanoparticles because it is simple, quick, and relies on using a suitable precipitation factor.[18] $CuCl_2.2H_2O$ was employed as a precursor in this work to synthesize CuO-NPs, and their antibacterial activities were investigated.

Experimental part

Synthesis of CuO NPs

The Cu^{+2} solution was made by dissolving $CuCl_2.H_2O$ (0.00588 mol) in 50 milliliters of deionized water. Next, with a stirrer, the (0.1 M) NaOH solution was gradually added to the solution at room temperature. Filtering , gathering the precipitate and calcining $Cu(OH)_2$ at 600 °C for six hours, and then washing it with ethanol and deionized water produced CuO NPs precipitate (see figure 1).

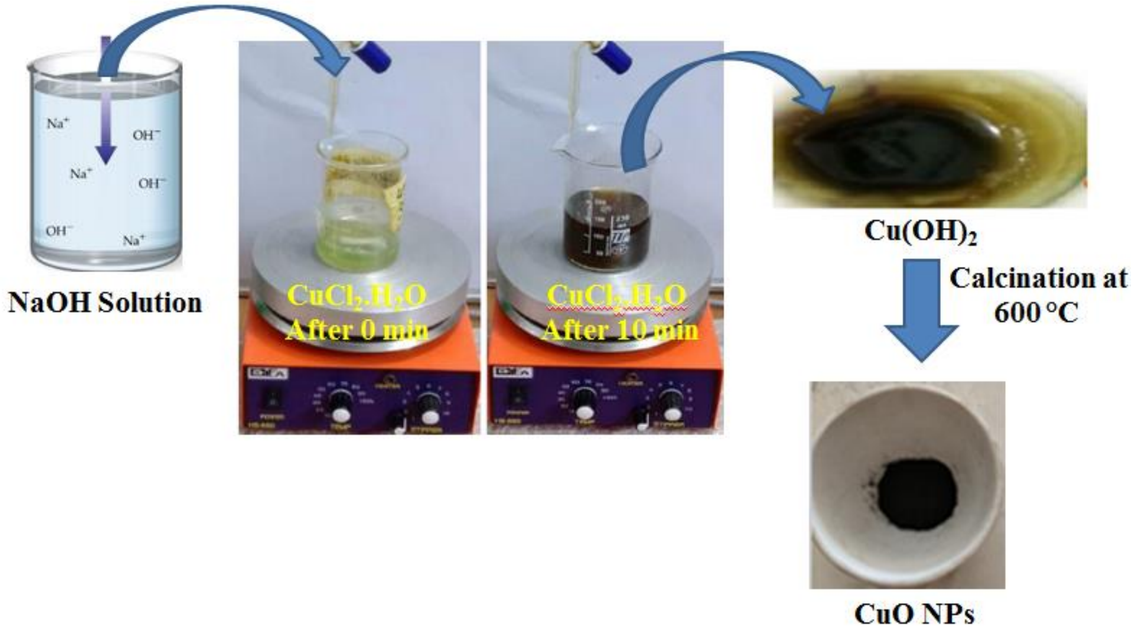

Fig.1. CuO nanoparticles synthesized by chemical precipitation method.

Synthesis of Culture Media

To make Muller Hinton agar, 38 grams were dissolved in 1.0 liter of distilled water, and the slurry was then poured onto sterile plates and refrigerated until needed. The procedure involved autoclaving the mixture for 15 minutes at 121 degrees Celsius and 15 pounds of pressure.

The Agar Well Diffusion Method for Evaluating Antimicrobial Activity

Many bacterial colonies have been moved by loop in order to prepare the suspended bacteria and insert them in tubes containing brain heart infusion broth to activate the bacteria. For 18 to 24 hours, the tubes were incubated at 37 °C. The suspended bacteria were contrasted with 1.5

x 10^8 cells/mL of the standard MacFarland solution. The germs were then spread out onto Muller-Hinton agar plates using a sterilized brush. After that, the plates were left to air-dry for a while. The culture media was punctured with a 5 mm diameter hole using a sterile cork borer. Using a micropipette, 100 µL of the CuO NPs (concentration 20 and 50 mg/mL) was added to each hole independently. The dishes should then be incubated at 37°C. Escherichia coli and Staphylococcus aureus were placed in the middle of petri dishes together with quantities of Penicillins (50 µg), Sulfonamides (50 µg), streptomycin (50 µg). According to the guidelines provided by the Clinical and Laboratory Standards Institute, these concentrations were selected. The effectiveness of each concentration and antibiotic was evaluated by measuring the diameter of the inhibitory zone encircling each hole.

Result and Discussion

Characterization of produced CuO nanoparticles: A chemical precipitation technique was used NaOH solution as a precipitation agent was to synthesis of copper oxide nanoparticles, which were then examined using XRD, FE-SEM, DLS, TEM and UV spectra of the resonance band at around 260–270 nm. Surface plasmon resonance, which has been utilized in optical spectroscopy to determine the size of these nanoparticles using the Haiss equation, is one of the unique properties of metal nanoparticles.

$$d= \ln (\lambda SPR - \lambda°)/L_1/L_2$$

The values L1 and L2 are equivalent to 6.53 and 0.0216, while d stands for the particle diameters of CuO NPs. λSPR and λ° indicate the wavelengths of maximum and minimum absorption, respectively. The UV-Vis spectra display the CuO NPs' distinctive absorption peak at 265 nm with diameter size of nanoparticles about 14 nm, which is depicted in figure 2.

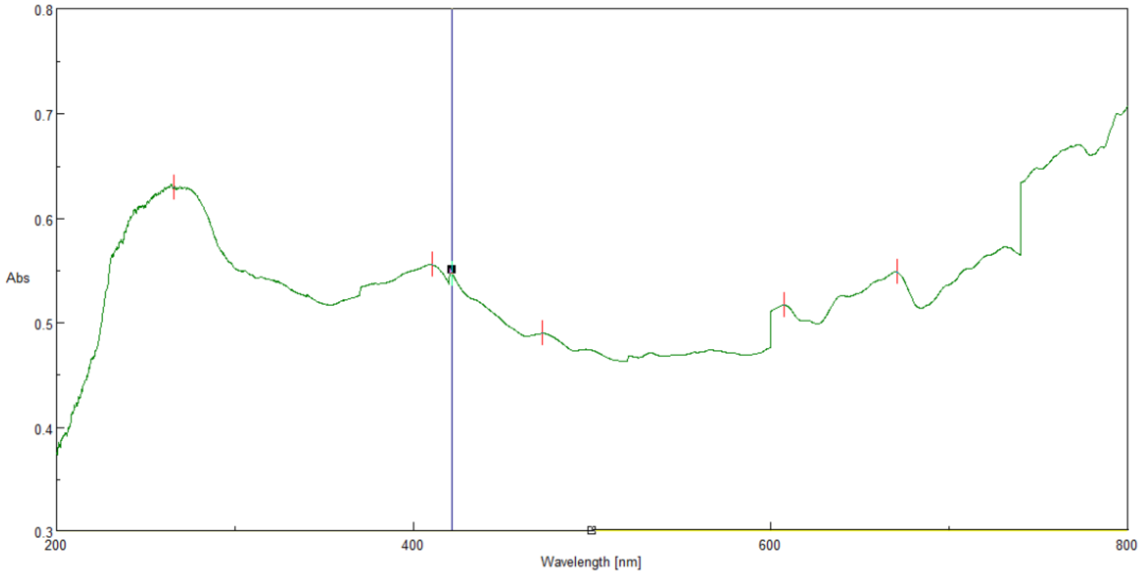

Fig. 2. UV-Vis spectra of CuO NPs synthesized

The XRD of CuO NPs synthesis using the chemical precipitation technique is shown in Figure 3. CuO NPs have been seen to exhibit a monoclinic structure, with cell parameters a=4.6530 Å, b=3.4100 Å, c=5.1080 Å, and β=99.480°, with an average crystalline size of 31.84 nm. The formation of the copper oxide nanocrystalline phase is indicated by the enormous peaks that are visible.

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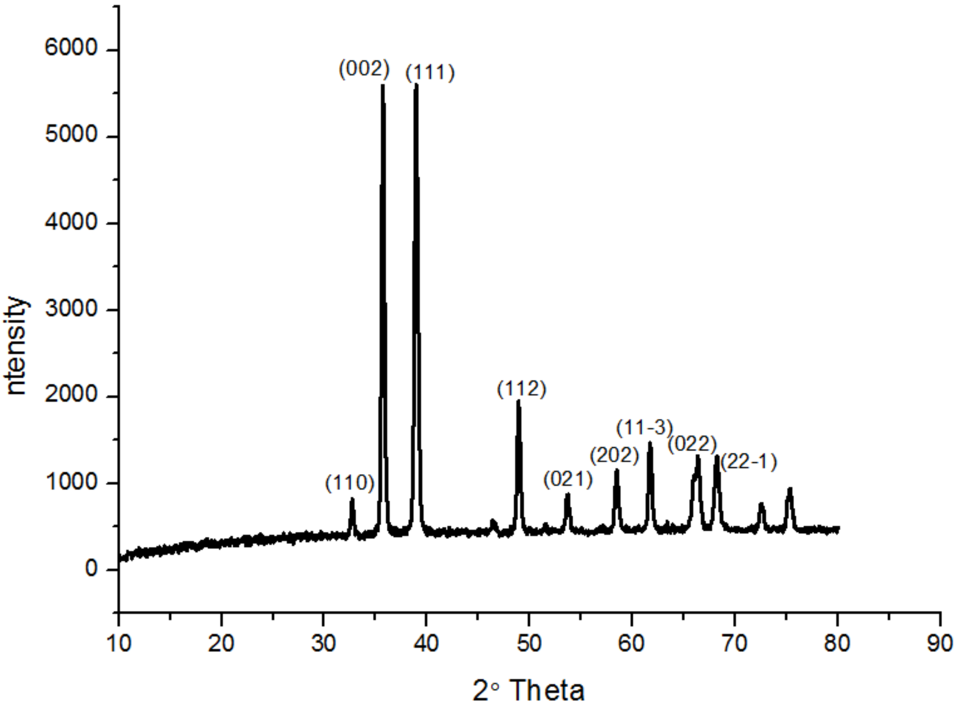

Fig.3. XRD pattern of CuONPs synthesized by chemical precipitation method

The morphology of copper oxide nanoparticles was examined using FE-SEM and TEM methods, which allowed the size and form of the particles to be seen. Figure 4 shows the copper oxide nanoparticle FE-SEM micrographs. The produced copper oxide nanoparticles, with a lamellar shape and an average size of around 74.4 nm.

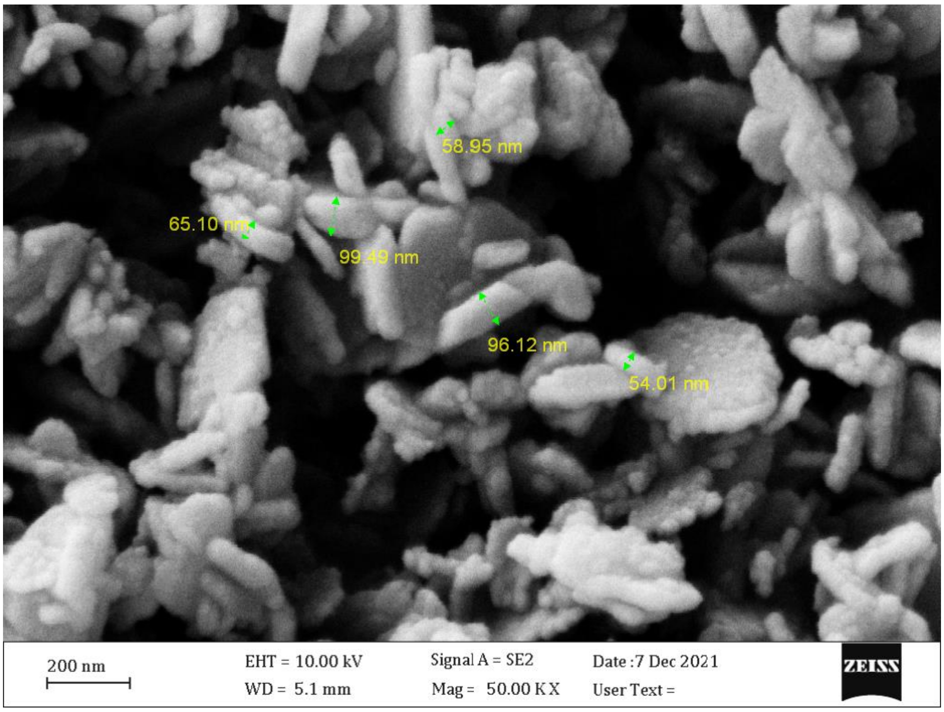

Fig.4. FE-SEM image of CuO nanoparticles prepared.

Figure 5 displays a photo of the CuO nanoparticles taken with a transmission electron microscope (TEM) at 100 and 200 nm. The irregular and cubic shapes of copper oxide nanoparticles ranged in size from 17 to 22 nm.

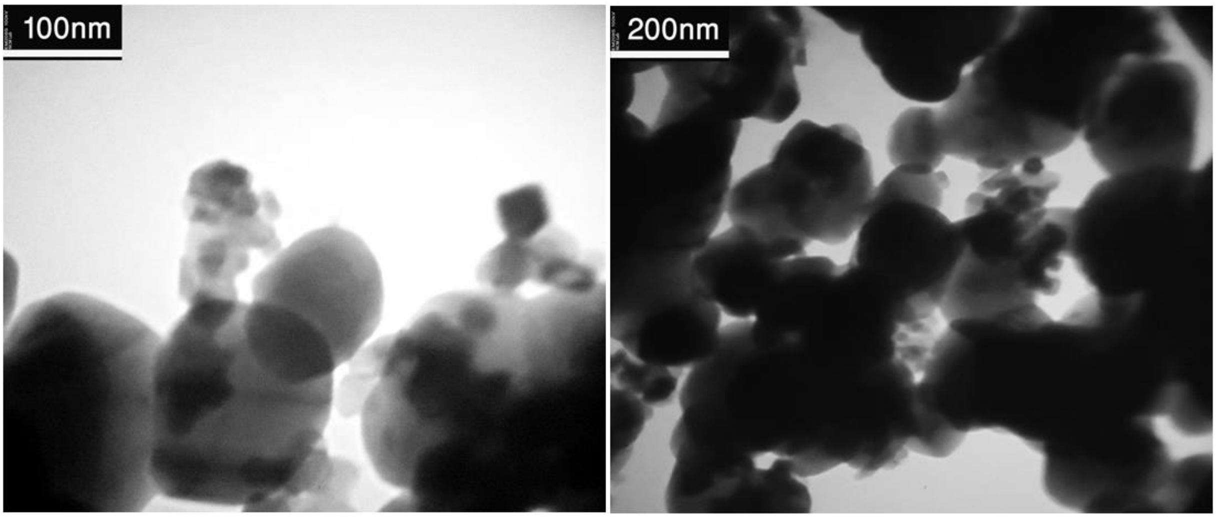

Fig.5. TEM image of CuO nanoparticles prepared via chemical precipitation method.

Bioactivities

The biological activity of artificially produced CuO nanoparticles was examined against two pathogenic bacterial isolates discovered in the general Al-Muqdadiya Hospital using the disk diffusion technique. Two gram-positive (Escherichia coli) and gram-negative (Staphylococcus aureus) bacteria were among the isolates. Hospitals frequently harbor the Escherichia coli and Staphylococcus aureus, which is why they were selected. CuO nanoparticles' efficacy against bacteria was evaluated by comparison with traditional antibiotics, including streptomycin, penicillins, and sulfonamides. In comparison to conventional antibiotics, the study's explanation of the produced CuO nanoparticles' antibacterial activity at two concentrations (20 and 50 mg/mL) is shown in Table 1, which confirmed the CuO nanoparticles have biological activity moderate to good.

Table 1**.** The result of CuO nanoparticles against Gr+ve **and** Gr-ve bacteria

Bacteria / Materials	*Staphylococcus aureus*		*Escherichia coli*	
	20 ppm	50 ppm	20 ppm	50 ppm
CuO NPs	13	27	24	38
Penicillins	R	R	9 mm	16 mm
Sulfonamides	R	R	18 mm	23 mm
streptomycin	R	11 mm	11 mm	19 mm

Conclusion

The fast and simple chemical precipitation approach was successfully used to create copper oxide nanoparticles (CuO NPs). The shape of CuO NPs' monoclinic structure has been verified by XRD, FE-SEM, and TEM analyses. In comparison to conventional antibiotics, the produced

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copper oxide nanoparticles had a moderate to good effect on the chosen bacteria when tested for antibacterial activity.

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