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Research Article

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Synthesis of ZnO-CuO Nanocomposite for Antibacterial Application

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Abstract

In this study, a 0.95ZnO-0.5CuO nanocomposite was successfully synthesized using the solution combustion method at a temperature of 500°C for 6 hours. The structural and optical properties of the material were analyzed using X-ray diffraction (XRD), and ultraviolet-visible (UV-Vis) spectroscopy. The antibacterial properties were tested using the agar well diffusion method against Escherichia coli (*E. coli*). The XRD analysis revealed sharp Bragg peaks, indicating high crystallinity of the nanocomposite. The material exhibited a mixture of hexagonal (ZnO) and monoclinic (CuO) phases. The crystallite size was calculated to be 20.18 nm, confirming the nanoscale structure of the composite. UV-Vis spectroscopy demonstrated optical activity under UV light, with a measured optical band gap of 3.11 eV. The antibacterial tests showed promising results, with the composite material achieving inhibition zone diameters of 15.12 mm at 15.6 mg/mL concentration against *E. coli*.

Keywords: *E. coli*; Composite; Structure; Optical Property; Antibacterial Activity

Abbreviations

XRD: X-ray diffraction; UV-Vis: Ultraviolet-Visible; CuO: Cupric Oxide; ZnO: Zinc Oxide; ROS: Reactive Oxygen Species; FWHM: Full Width at Half Maximum.

Introduction

Antibacterial activity refers to the ability of a substance or material to inhibit the growth and reproduction of bacteria or to kill bacterial cells. Bacteria, as microorganisms, can cause a wide range of infections and diseases in humans, animals, and plants. The rise of bacterial infections, particularly with the emergence of antibiotic-resistant strains, has led to increased interest in materials and substances that exhibit effective antibacterial properties [1]. Understanding the theoretical basis of antibacterial activity is crucial for developing new materials, drugs, and technologies aimed at combating bacterial infections.

Bacteria are classified into two main groups: gram-positive and gram-negative based on their cell wall structure, which can influence the susceptibility of bacteria to antibacterial agents [1,2]. Both types of bacteria differ primarily in the structure of their cell walls, which is a critical factor in how they respond to antibiotics and other antibacterial agents. Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, have a thick peptidoglycan layer that makes up the majority of their cell wall [1,2]. This layer is rigid and provides structural support, but it lacks an outer membrane. In contrast, gram-negative bacteria, like *Escherichia coli* and *Pseudomonas aeruginosa*, have a thinner peptidoglycan layer, but they possess an additional outer membrane composed of lipopolysaccharides [3]. This outer membrane serves as a protective barrier, making gram-negative bacteria more resistant to certain antibiotics and harmful substances. While both types of bacteria have cytoplasmic membranes and ribosomes, the outer membrane in gram-negative bacteria is a key distinguishing feature that affects their permeability and susceptibility to treatments. Despite these structural differences, both gram-positive and gram-negative bacteria can cause a wide range of infections in humans and are targeted in antibacterial research for effective treatments.

The development of resistance to antibacterial agents is a significant concern. Bacteria can evolve mechanisms to counteract the effects of antibacterial substances, such as by producing enzymes that degrade antibiotics or by altering their cell wall structure to prevent the entry of harmful substances. As a result, there is an ongoing need for new antibacterial materials and strategies that can effectively combat bacterial resistance. Multimodal antibacterial materials, which combine several mechanisms of action, are emerging as a promising solution to this challenge. For example, metal oxide nanocomposites that generate ROS, release metal ions, and disrupt cell membranes simultaneously can exert a more comprehensive antibacterial effect and reduce the likelihood of resistance development [4].

Zinc oxide (ZnO) and copper oxide nanoparticles (CuO) nanoparticles have garnered considerable attention due to their unique physicochemical properties, particularly in the field of antimicrobial materials. Both nanoparticles exhibit significant antibacterial activity against gram-positive and gram-negative bacteria. Recent reports confirmed that the combined ZnO and CuO nanoparticles to form ZnO-CuO nanocomposite exhibits enhanced antibacterial activity compared to their individual components. The interaction between ZnO and CuO in nanocomposites arises from the complementary mechanisms by which each nanoparticle acts against bacteria. The antibacterial activity of ZnO-CuO nanocomposite has been widely studied, with promising results in terms of inhibiting the growth of harmful microorganisms [5,6]. One of the key advantages of ZnO-CuO composites is their ability to disrupt bacterial metabolism more effectively than either material alone [5,6]. The nanocomposites leverage the complementary release of copper ions and ROS production, leading to heightened oxidative stress within bacterial cells. This results in significant damage to the cell membrane, leading to increased permeability and eventual cell death.

Experimental Details

Sample Preparation: To synthesize the ZnO-CuO nanocomposite, precise quantities of $Zn(NO_3)2.6H_2O$, $Zn(NO_3)2.6H_2O$ and $C_6H_8O_7$. H_2O were individually dissolved in distilled water for five minutes using a magnetic stirrer. Afterward, the solutions were mixed and stirred for 20 minutes at room temperature. During this process, ammonium hydroxide was gradually added drop by drop until the solution reached a pH of 7. The mixture was then heated on a hot plate at 90°C while continuously stirred with a magnetic stirrer for 1.5 hours. As the gel formed, the temperature was raised to 100°C, causing combustion and resulting in black powder formation. This black ash was ground for 3 hours using an agate mortar and pestle, and then calcined at 500°C for 6 hours in a furnace using a platinum crucible, producing the ZnO-CuO nanocomposite.

Antibacterial activity: The antibacterial activity of the synthesized nanoparticles was evaluated using the agar well diffusion method with *E. coli* (ATCC 25922) as test organisms. Penicillin was the positive control, and dimethyl sulfoxide was the negative control. Muller-Hinton agar was prepared, and bacteria were inoculated into the agar and incubated in an autoclave at 121°C for 20 minutes at 15 psi. The media was poured into sterile petri dishes, solidified, and swabbed with bacteria from overnight cultures. Wells were made in the agar, filled with 62 mg/ml of the nanomaterial, and allowed to diffuse for 30 minutes. After incubating at 37°C for 24 hours, the inhibition zones were measured in millimeters. The test was repeated three times for accuracy.

Characterization: The crystal structure and phase of the ZnO-CuO nanocomposite were analyzed using an X-ray Diffractometer (Philips X'Pert Pro) with Cu Kα radiation, scanning from 20° to 80° (2θ). Optical properties were studied using a UV-Vis NIRCCB Spectrophotometer (Model natV-770) in the 200-800 nm wavelength range.

Results and Discussion

XRD study

X-ray diffraction analysis is a technique used to determine the crystal structure, phase composition, and crystallite size of materials. When a material is subjected to X-rays, the rays diffract at specific angles based on the atomic arrangement. By measuring the intensity and angles of the diffracted beams, XRD produces a diffraction pattern that helps identify the material's crystalline phases and lattice structure. The technique is widely used for analyzing nanoparticles, thin films, and bulk materials.

Figure 1 shows the XRD patterns of the ZnO-CuO nanocomposite synthesized via the solution combustion method at 500°C for 6 hours. Sharp and well-defined Bragg peaks are formed, indicating the high crystallinity of the material. The diffraction peaks marked with an asterisk (*) at 2θ values of 31.89o, 34.60°, 36.42°, 47.61°, 56.71°, 63.03°, 66.52°, 68.07°, 69.23°, 72.59°, and 77.22° corresponding to the Miller indices (100), (002), (101), (102), (110), (103), (100), (112), (201), (004), and (202), confirm the formation of a single-phase hexagonal wurtzite ZnO crystal structure (JCPDS card no. 00-036-1451) with space group P63mc [7]. Additionally, the peak marked with an asterisk (#) at 2θ values of 39.02 corresponding to the Miller indices $(11\bar{1})$, planes, confirm the single-phase monoclinic CuO structure (JCPDS card no. 00-048-1548) with space group C_2/c. These results confirm the formation of ZnO-CuO material, verifying the crystalline phases of both ZnO and CuO in the sample through distinct diffraction peaks.

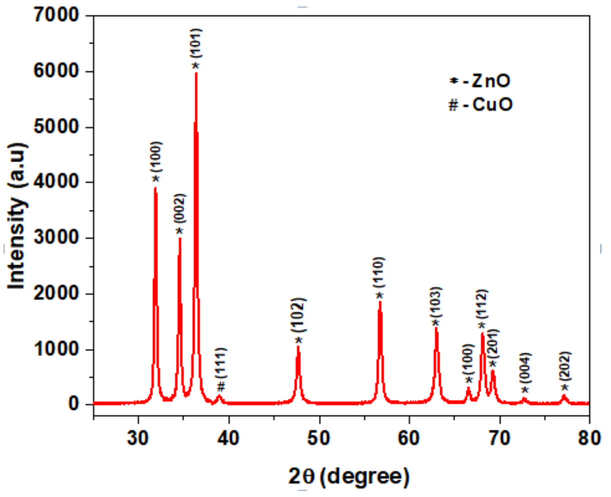

Figure 1: XRD pattern of ZnO-CuO nanocomposite.

The crystallite size (D) of ZnO-CuO nanocomposite was calculated using the Scherrer equation;

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where K is the shape factor (usually around 0.9), λ is the wavelength of the X-ray radiation (e.g., for Cu Kα, λ = 1.5406 Å), β is the full width at half maximum (FWHM) of the diffraction peak in radians, and θ is the Bragg angle (half of the diffraction angle, 2θ). The crystallite size of is found to be 20.48 nm. This confirms the formation of a nanocomposite material, as sizes in the nanometer range typically exhibit unique physical and chemical properties.

Optical Properties Study

UV-Vis spectroscopy is a technique that measures how much ultraviolet and visible light is absorbed by a substance. This information can help us understand the electronic structure of the substance. The method works by shining light through a sample and measuring how much light is absorbed at different wavelengths. Absorption occurs when electrons in the sample move between energy levels and the resulting spectrum can tell us a lot about the material's optical properties, band gap, and electronic transitions. This makes UV-Vis spectroscopy very important in different fields of science.

In this study, UV-Vis spectroscopy was employed to investigate the optical characteristics of the ZnO-CuO nanocomposite within the 200–800 nm wavelength range. The obtained spectrum is displayed in Figure 2. The UV-Vis spectra reveal significant absorbance peaks at 309 nm and 365 nm, confirming that the ZnO-CuO nanocomposite exhibits strong optical activity in the ultraviolet region. However, the sample shows minimal absorbance in the visible region, indicating its potential use for detecting or blocking ultraviolet radiation, making it suitable for o applications such as UV sensors or UV shielding materials. However, the ZnO-CuO nanocomposite exhibits better optical absorption compared to pure ZnO nanoparticles. This enhanced absorption suggests that the mixing of CuO into the ZnO matrix improves the material's optical properties, making it more effective in applications that require strong light absorption, such as photocatalytic activity.

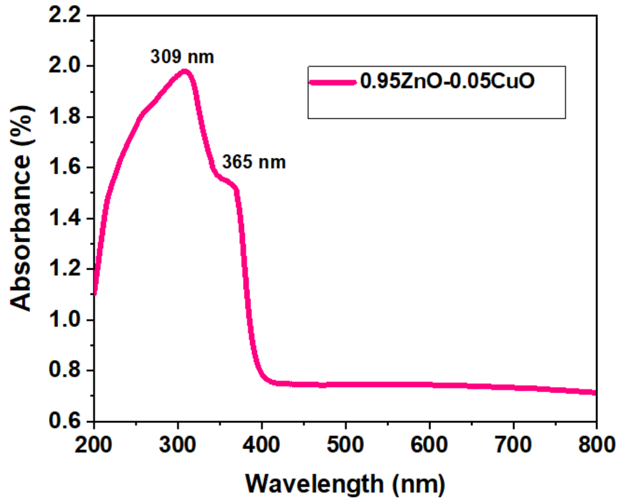

Figure 2: UV-V is absorption spectra of ZnO-CuO nanocomposite.

The direct optical band gap of the ZnO-CuO nanocomposite was calculated using Tauc's relation [8]:

$$\alpha h\nu = A\left(h\nu - E_g\right)^n$$

Where, hv represents the photon energy, α is the absorption coefficient, A is a constant, and n is either 1/2 or 2, corresponding to direct or indirect band gaps, respectively. The graphical plot of $(\alpha h\nu)^2$ versus hυ are depicted in Figure 3. From this figure, the direct optical band gap energy of the ZnO-CuO nanocomposite is determined to be 3.14 eV. This value is lower than the band gap of pure ZnO nanoparticles but higher than that of CuO nanoparticles, indicating that the ZnO-CuO nanocomposite exhibits an intermediate band gap between the two individual materials [9].

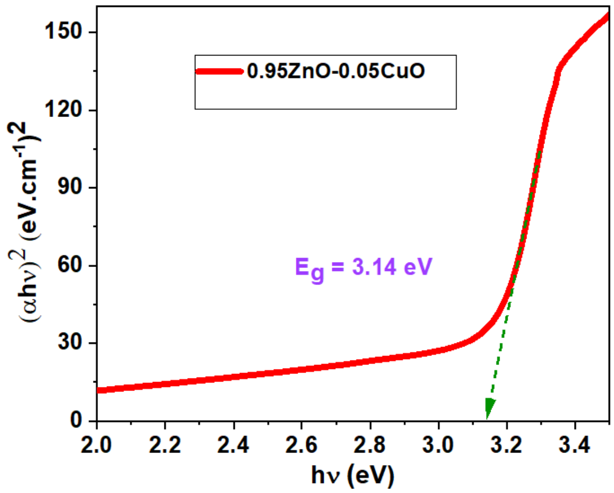

Figure 3: Direct energy band gap plot of ZnO-CuO nanocomposite.

Antibacterial Activity

The antibacterial properties of the ZnO-CuO nanocomposite synthesized via the solution combustion method at 500°C for 6 hours against *E. coli* are shown in Figure 4. DMSO was used as a negative control, while penicillin served as a positive control. The figure presents petri dishes containing the nanocomposite at varying concentrations (15.6, 31.25, 62.5, and 125 mg/mL) alongside the controls.

As seen in the figure, the inhibition zones against *E. coli* were 15.12, 14.10, 14.08, and 13.14 mm for concentrations of 15.6, 31.25, 62.5, and 125 mg/mL, respectively. This suggests that the antibacterial efficacy of the ZnO-CuO nanocomposite is dose-dependent. Interestingly, the lowest concentration (15.6 mg/mL) exhibits the highest antimicrobial activity with an inhibition zone of 15.12 mm (Figure 4). As the concentration increases, the inhibition zone progressively decrease, indicating reduced efficacy. The negative control (-Ve) shows no inhibition as expected, while the positive control (+Ve), penicillin, shows a 15.62 mm inhibition zone, slightly larger than the result for the 15.6 mg/mL concentration. The results obtained at lower doses in this study are higher than those reported by Lozhkomoev for 0.95ZnO-0.05CuO (13.76 mm) synthesized via the electrical explosion method, Aloucheh RM, et al. [10] for 0.75ZnO-0.25CuO (2.3 mm) using the sol-gel method, and Widiarti N, et al. [11] for ZnO-CuO (9.14 mm) via green synthesis. This confirms that the ZnO-CuO nanocomposite is a promising candidate for antibacterial activity against *E. coli* [12].

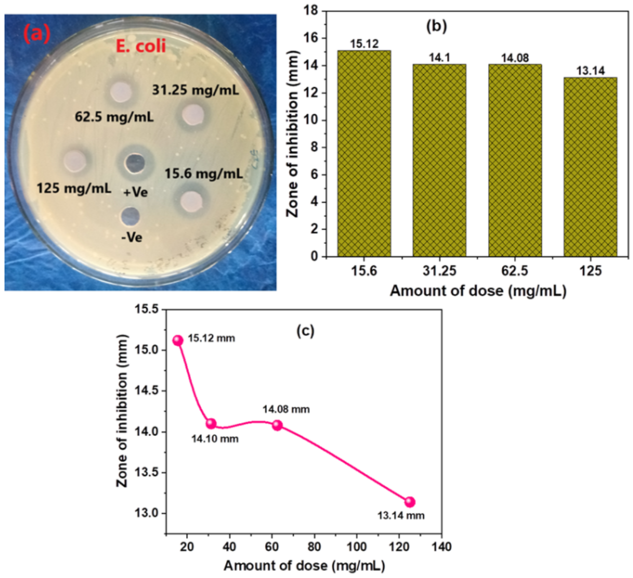

Figure 4: (a) antibacterial activities, (b) bar graph representation of the ZOI produced, and (c) ZOI values comparison against *E. coli* bacteria.

Conclusion

In this study, a ZnO-CuO nanocomposite was successfully synthesized using the solution combustion method with $Zn(NO_3)_2{\cdot}6H_2O$, $Cu(NO_3)_2{\cdot}6H_2O$, and $C_6H_8O_7{\cdot}H_2O$ precursors at 500°C for 6 hours $C_6H_8O_7{\cdot}H_2O$ used as both a fuel and a chelating agent. XRD analysis confirmed the formation of a nanocomposite with a high degree of crystallinity, consisting of hexagonal ZnO ($P6_3mc$ space group) and monoclinic CuO (C2/c space group) phases. The crystallite size of the composite was found to be 20.48 nm, indicating its nanoscale nature. UV-Vis spectroscopy showed strong optical absorption in the ultraviolet region, with a direct optical band gap of 3.14 eV. This suggests that the ZnO-CuO nanocomposite holds potential for UV-related applications, particularly in the range of 200 to 402 nm, such as blocking harmful UV radiation to protect human skin. The antibacterial activity of the ZnO-CuO nanocomposite was tested against *E. coli* using the agar well diffusion method, revealing a dose-dependent antibacterial effect, with the highest inhibition zone observed at the lowest concentration of 15.6 mg/mL. The synergistic interaction between ZnO and

CuO nanoparticles likely enhances the generation of reactive oxygen species (ROS), which disrupt bacterial cell walls and membranes, leading to cell death. Additionally, the ability of Cu^{2+} ions to interact with bacterial proteins and DNA might further inhibit bacterial growth. This study suggests that the ZnO-CuO nanocomposite could be an effective antibacterial agent, particularly for applications targeting *E. coli*, making it a promising material for use in healthcare, food packaging, and water treatment.

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