ZnO-CuO nanocomposites: synthesis, characterization and antibacterial activity

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Abstract. Nanotechnology deals with the study of manipulation of the materials on molecular or an atomic scale. Nanomaterials and nanocomposites possess unique characteristics such as miniature particle sizes with a close particle size distribution properties and pose as a highly dispersed and non-agglomerative mass. The typical blend and diverse characteristics of these particles prove to be of added advantage in typical applications such as medical and pharmaceutical treatments for bacterial infections. This paper describes the detailed process of synthesizing nanocomposite oxide mixtures of copper and zinc through the sol-gel formulation route technique. The particle size distribution is assessed using Brunauer-Emmett-Teller and Scanning Electron Microscopy analysis. The mineralogical analysis of the nano-composite is carried out using X-Ray Fluorescence and X-Ray Diffraction techniques. The synthesized ZnO-CuO particles possess antibacterial properties against both gram-(positive/negative) bacteria and hence, they exhibited good results on E.coli, Staphylococcus aureus and Pseudomonas aeruginosa.

1. Introduction
Nanotechnology is of growing significance in many research branches because of the opportunity for miniaturization as well as appealing properties linked with small particle size. Several basic properties of nanomaterials such as optical, mechanical, electrical and so on, can be expressed as a function of their composition, size and structural order. The nano composites are miniature sized, have close size distribution, are non-dispersive, non-aggregative and non-agglomerative in nature and these properties make their applications in many commercial systems inevitable [1]. Nanotechnology can detect, prevent and eliminate environmental contaminants in water, air and soil by being cost-effective and eco-friendly. Nanotechnology and nanoscience are likely to have thoughtful implications such as energy production and efficiency, agriculture and food, cosmetics, automotive industry, medical systems, drugs, computers, household appliances and weapons [2, 3].

CuO possess excellent electrical, magnetic and optical properties. Copper oxide nanoparticles find diverse applications and have been abundantly utilized in medical and biomedical applications, ceramics, gas and chemical sensing devices, solar energy transformer, magnetic storage devices, photo conductor, semiconductor and surface antigens [4]. The semiconducting metal oxide nanomaterials such as ZnO, titanium oxide (TiO2) and CuO have gained wide industrial applications due to their sole concert in optoelectronics, sensing and catalysis.
behaviour [5, 6, 7]. ZnO commonly available in powder form is one of the important gradient in calamine medication which finds application in treatment of human skin diseases. ZnO also finds applications in manufacture of ceramics, paints and food preservatives [4, 8].

Nanocomposites like ZnO-CuO possess several advantages owing to its size and surface area. This helps it to be used as an antimicrobial agent in microorganism behavioural studies. Large surface area increases the antimicrobial activity and hence, effectively kills the microorganism and bacterial growth is reduced significantly. This creates an urge to have proper synthesis mechanisms for nanocomposites like ZnO-CuO. Hence, this study serves as a guideline for the synthesis of ZnO-CuO nanocomposites. A systematic characterization procedure is followed to evaluate the synthesis process. This is followed by subjecting the generated nanocomposites to antimicrobial activity tests, which is a crucial marker for the analysis of the synthesized composites.

The rest of the paper is as follows. Section 2 briefs the methodology followed for the synthesis process by detailing the materials used and the inherent steps involved. The results of the tests for checking the goodness of the synthesis is presented in Section 3. The paper concludes in Section 4 with a future roadmap of the study.

2. Methodology
The techniques used to synthesize nanoparticles are a sol-gel method, colloidal method and chemical/physical vapour deposition methods. The sol-gel technique is the best method for medical and biomedical applications, provides flexibility in obtaining a diverse range of materials and the unique morphology as observed on nanoscale [9]. The advantages of the sol-gel method are that it can produce particles at an ultra-low temperature, can synthesize multiple particles simultaneously, can control the structure of the final product and also the physical/chemical/mechanical properties of the final product [10, 11]. Hence, in this study, we have exclusively made use of the sol-gel method.

2.1. Materials used
In this study, the chemicals used for synthesising ZnO-CuO nanocomposites are Zinc acetate dehydrate (ZnC$_4$H$_6$O$_4$ · 2H$_2$O), Copper sulfate pentahydrate (CuSO$_4$·5H$_2$O), ethanol (C$_2$H$_5$OH), Citric acid (C$_6$H$_8$O$_7$) and 1M sodium hydroxide (NaOH) solution. All other reagents are analytical grade and distilled water is used for the preparation of the solution.

![Figure 1. Sequence of steps in the synthesis of ZnO-CuO](image)

2.2. Preparation of ZnO-CuO nanocomposite particles
The sequence of steps involved in the preparation of ZnO-CuO is as shown in Figure.1. As discussed earlier, the ZnO and CuO nanocomposite particles are prepared by sol-gel method owing to the benefits it provides over the other methods. 25 ml of distilled water, 25 ml of ethylene glycol, 5.32 gm of zinc acetate dehydrate and 6.3 gm of citric acid are added and
stirred constantly with a magnetic stirrer for a duration of 60 minutes. At time intervals of one hour duration, 0.42, 0.67, 0.94 and 1.27 grams of copper sulfate pentahydrate are added to the suspension. Constant temperature is maintained at 600°C for 3 hours, then the mixture is stored for 48 hours in a dark space to attain gel formation. The gel is dried in an oven at a temperature of 120°C for 6 hours to remove residual moisture content and obtain ZnO-CuO nanocomposite particles in the powdered form.

The prepared nanocomposite particles are characterized using Scanning Electron Microscopy (SEM) analysis, X-Ray Diffraction (XRD) test, X-Ray Fluorescence (XRF) analysis and Brunauer-Emmett-Teller (BET) analysis.

3. Results and discussions

3.1. XRD analysis

The nanocomposite sample of ZnO-CuO is analyzed by XRD technique. The XRD analysis used scanning speed $2\theta$ in the range of 10 to 90° using a Bruker AXS D2 PHASER diffractometer. The interaction between X-rays and the crystalline material generated using a single wavelength results in diffraction pattern which shows $2\theta$ versus relative intensity of the diffracted beam. Elastic scatter of the incident photons contributes to the Bragg peaks. The sample is scanned at a rate of $1°\text{ min}^{-1}$ with CuK$_\alpha$ radiation having $\lambda = 1.540$ nm. The sturdy and pointed peaks in the patterns revealed the good crystalline life of the samples. No other fused phase was found, which indicates that the nanocomposite consists of two different phases of ZnO and CuO. Figure 2 (a) exhibits good pattern of ZnO-CuO nanocomposite materials. The diffraction peaks are well defined and are in good agreement with Joint Commission Powder Diffraction Standards (JCPDS) card numbers. The sharp peaks represent that the obtained nanocomposite has a high crystallinity nature. XRD analysis exhibits ZnO-CuO particles have highest peak $2\theta$ at 53.3, 83.9 according to JCPDS 46-1648, 76-0660, respectively.

![XRD and SEM images](image)

**Figure 2.** (a) XRD of ZnO-CuO nanocomposite sample (b) Surface morphology of ZnO-CuO nanocomposite particles

3.2. SEM analysis

The cleared ZnO-CuO powder is examined using SEM. The surface morphologies of the solid ZnO-CuO nanocomposite materials can be obtained by using SEM, format JEOL, version 1.1, SEM instrument JSM-6360 with platinum coating by a sputter coater (BAL-TEC/SCD 005). The clean morphology of the synthesized ZnO-CuO nanocomposite powder surface is examined by SEM and the results are given in Figure. 2 (b). From the figure, it can be observed that the powder has a large number of densely stacked nanomaterials, thereby causing the agglomerated structure. It is observed that CuO comprises of a large number of densely stacked nanoflakes at
terminal position of ZnO hexagonal rods. Usually the ZnO particles have hexagonal rod-type structure, while CuO is a nanoflakes type structure [11]. It is also evident from the SEM image that the obtained ZnO-CuO nanocomposite material has uniform shape and size with very high agglomeration. Hence, they did not form a massive cluster.

| Component | ZnO | CuO | SO3 | Al2O3 | SiO2 | SO2 | Fe2O3 | MgO | CuO | P2O5 | NaO | K2O |
|-----------|-----|-----|-----|-------|------|----|-------|-----|-----|------|-----|-----|
| Result    | 49.3| 29.3| 16.6| 4.02  | 0.34 | 0.08| 0.06  | 0.03| 0.02| 0.01 | 0.01| 0.01|

3.3. XRF analysis
It is observed from the Table 1, that oxides of zinc and copper nanocomposite material have the highest in weight percentages. It is observed from the results that ZnO and CuO has highest oxide in weight wt%. This analysis provides proper elemental analysis of ZnO-CuO nanocomposite materials.

3.4. BET analysis
For the purpose of the assessment of the surface area, the nitrogen adsorption-desorption isotherms of all nanocomposite materials are evaluated as per the works in [12]. Nitrogen gas is passed all the way through the holder containing sample material and based on the adsorption-desorption of nitrogen gases, the surface area is computed. The equation used in the BET analysis is,

\[
\frac{1}{v}[(P_0/P) - 1] = (C - 1)(P/P_0)(V_mC) + 1/(V_mC)
\]

where, \(P_0\) and \(P\) are the adsorbate’s saturation and equilibrium pressures, \(V\) adsorbed gas volume and \(C\) is the BET constant. The computed surface area using the above equation is 1.148 m\(^2\)/gm and the pore volume is 0.0036 cc/gm. Pore volume is basically the total volume of very minute openings in a bed of adsorbent particles. The larger pore volume indicates the availability of space for the adsorption in the adsorbate molecules on the surface of adsorbent samples. Large surface area of ZnO-CuO increases the antimicrobial properties due to ultraviolet light absorption. The ZnO-CuO nanocomposites are hence a putative markers to kill the bacterial cells owing to the increased production of Zn\(^{2+}\) ions and reactive oxygen species [13].

3.5. Antibacterial activity test
In recent years, there is a rise in interest in the field of research and development of antibacterial agents for bacterial resistance. Several methods such as the well diffusion, agar diffusion, agar or broth dilution and zone of inhibition are adapted for the antibacterial tests. The zone of inhibition is a commonly used test method. It is also called as Kirby-Bauer, a qualitative approach that is used to measure antibacterial resistance. All the tools required for the antibacterial tests are sterilized properly by washing them and covering them in a paper in an autoclave for 25 minutes. Agar medium is prepared by dissolving 9.8 gm of nutrient agar to each 350 ml of distilled water in a conical flask. The liquid broth is prepared by dissolving 1.3 gm of nutrient broth to each 100 ml of distilled water in a conical flask. Agar medium and liquid broth are sterilized in an autoclave at 121°C for 40 minutes. The agar medium is poured to the petri dish near the flame in a laminar airflow chamber and then kept for 24 hours for the gel formation.

Each microorganism is immersed in liquid broth by using a wire loop and the flasks are kept in the incubator for a period of 48 hours to enhance the growth of microorganisms. Concentrations
of nanocomposite particles are prepared in small disposable tubes and small disks are immersed in the tubes. The liquid broth containing microorganisms is poured to Petri dish containing agar medium near the flame and is spread uniformly using a spreader. It is allowed to settle for a few minutes. The disks are then placed on the petri dish and then kept in an incubator for 48 hours. After 48 hours, the apparent zone around each disk, i.e. ”Zone of Inhibition” is observed which means that the growth of microorganisms has stopped. The zone of inhibition is then measured to find the efficacy of different concentrations of prepared nanocomposite particles.

Figure 3 (a) through (c) shows the media culture of bacteria E.coli, Pseudomonas aeruginosa and S.aureus, respectively. From the antibacterial test results, as shown in Figure. 4, we come to know that the zone of inhibition is found which means that ZnO-CuO nanocomposite particles are effective in killing the microorganisms. The ZnO-CuO nanocomposite materials shows the best antibacterial activity with an apparent zone of 1.9 mm for E.coli, 1.7 mm for S.aureus and 1.4 mm for Pseudomonas aeruginosa.

Figure 4. Antibacterial activity test

4. Conclusions and future roadmap

In a nutshell, the study focuses on the synthesis of ZnO-CuO nanocomposites by using the sol-gel technique. The morphology of nanoparticles display consistent shape and size. ZnO nanoparticles possess nanoflake structure and that of CuO nanoparticles are hexagonal and rod-shaped structure. ZnO-CuO nanocomposite materials exhibit low level of agglomeration. XRF analysis of nanocomposite reveal CuO as 29.3% and ZnO is 49.3% respectively. The surface area of nanocomposite ZnO-CuO from BET analysis is found to be 1.148 m$^2$/gm and pore volume is 0.0036 cc/gm. As a future work, the authors would like to create guidelines for other nanocomposites which are as significant as that of ZnO-CuO.


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