Research Article

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Antibacterial Activity of Ag-Cu-O Nanocomposite - Thermal Reduction Method

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ABSTRACT

The development of nanocomposite with antimicrobial property and stability has gained greater interest nowadays. The incorporation of silver, whose microbial activity is well known in the copper oxide matrix, had resulted in an amazing property. This new nanocomposite is a potential material in medical, food packaging, water treatment and pharmaceutical fields. Copper and silver are abundant in nature and cost-effective too. To synthesis high purity Ag-Cu-O nanocomposite, novel thermal degradation method was used in the present work. Structure, surface and optical characteristics were studied in detail. The microbial activity of the synthesized nanocomposite in gram-positive and gram-negative bacteria were studied by disc method and compared with the existing reports.

Keywords: Antimicrobial activity; Nanocomposites; Silver copper oxide; Structural analysis; Surface morphology.

1. INTRODUCTION

Nanotechnology is playing a crucial role in many fields, including agriculture, medicine, environmental science, textile and food industries. Particularly in the field of medicine, metal nanoparticles like Ag, Au, Cu, Ti and Pt nanoparticles are showing good antibacterial and antifungal properties (Morones *et al.* 2005; Pinto *et al.* 2012). In recent times the interest is increasing in the field of nanocomposites where the combination of two are more nanoparticles will lead to superior properties. The physical and chemical properties of nanocomposites are different from their parent nanoparticles. Improved stability and hardness are the main characteristics of metal nanocomposites. In this context, the development of metal nanocomposite with antimicrobial activity is interesting because of its size and shape-dependent properties. Among all the metal nanoparticles, Ag and Cu have shown appreciable antibacterial activity in many bacterial strains. These nanoparticles are being used in various fields such as biomedical equipment, water treatment and food processing (Ruparelia *et al.* 2008; Llorens *et al.* 2012; Hajipour *et al.* 2012). The cationic release is the main advantage of silver nanoparticle's strong antimicrobial and fungal activity (AshaRani *et al.* 2009). The scientific community is always attentive of exploring alternative nanoparticles, which gives comparable or better results. Many researchers have reported that the copper nanoparticles are resulting analogous effects in many pathogen microorganisms (Turnlund *et al.* 1998; Yoon *et al.* 2007; Cady *et al.* 2011). In several instances, the metal oxide nanocomposites demonstrated better performance over the mono-metal oxides (Jiang *et al.* 2012; Tan *et al.* 2014; Wu *et al.* 2015; Darabdhara *et al.* 2017).

Based on this, the present work focuses on the synthesis of silver-copper oxide nanocomposite and the study of its biological activity by *in situ* method using gram-positive and gram-negative microorganisms. The purity of the material plays a vital role in biological properties; hence, thermal degradation method was used to synthesis silver-copper oxide (Ag-Cu-O) nanocomposite.

2. EXPERIMENTAL TECHNIQUES

The thermal degradation method is known for its controlled synthesis of metal nanoparticles. This is a unique method where the purity of the synthesized nanoparticles is more than 99.9 % as this method won't require any organic solvents to clean the resultant product. Further, the morphology and size of the samples can be easily controlled by varying the temperature without any capping agent. In the present work, Cu:Ag ratio was 8:2. All substances used were of analytical grade and of 99.99 % purity. The solvents used were distilled before use. 3-hydroxy 2-napthoic acid and aminoguanidine were purchased from Aldrich, and all other chemicals used were of commercially available grades. The synthesis of Ag-Cu-O nanocomposite is as follows:

2.1 Preparation of Copper-Silver oxide complex

A known weight of 3-hydroxy 2-napthoic acid (0.1881 g, 0.001 mol) was dissolved in 20 ml of double-distilled water. Aminoguanidine (0.544 g, 0.004 mol) was added to the above solution while stirring. The solution became clear, and the pH (7) was measured. Copper nitrate solution (0.1876 g) and silver nitrate solution (0.1688 g) were dissolved in 20 ml of water. The solution was poured into the metal solution, and the resulting solution was concentrated for five hours on a water bath. A polycrystalline substance obtained after two days was washed with distilled alcohol and dried in desiccators.

2.2 Synthesis of Metal Nanocomposite and Nano Metal oxides

Ag-Cu-O nanocomposite was synthesized by the thermal decomposition method. The complexes were weighed (12 g) and placed in a muffle furnace at a temperature of 38 ºC and raised steadily up to 800 ºC at a heating rate of 10 ºC per minute. When the temperature has reached 800 ºC, it was maintained for 6 hours. The metal oxides and composites were prepared in a similar manner using the same procedure.

2.3 Physico-chemical experiment techniques

The synthesized metal nanocomposites were inferred through physicochemical techniques like UV-DRS, FTIR, XRD and SEM with EDAX studies. The infrared spectra of the pure nano metal oxides and their composites were recorded on the IR Affinity – 1CE Schimadzu model spectrometer in the range 4000 – 400 cm. UV-Vis. spectra of pure metal oxides and their composites were recorded on Lab India UV 3000+ UV-Vis. spectrometer. XRD of the pure nano metal oxides and their composite were recorded on Brucker Advanced D8 X-ray diffractometer. SEM with EDAX was observed using Joel JSM-6390 LV SEM at 3-12 kV at different magnifications. Antibacterial studies were carried out in *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* bacterial stains by *in situ* method.

3. RESULTS & DISCUSSION

3.1 Powder X-ray diffraction

The diffractogram was recorded using a synthesized recorder at room temperature. The diffraction pattern was recorded from 10- 90º at the scan rate of 0.2 º/min. Fig. 1 shows the XRD pattern of Ag-Cu-O nanocomposite. The sharp peaks in the XRD pattern revealed that the formed materials are crystalline in nature. The synthesized metal oxide XRD peaks are in accordance with JCPDS card number 89-3722 (Ag_2O) and 65–2309 (CuO) (Darabdhara *et al.* 2017). The nanocomposite peaks were well-matched with the above results and indexed according to that. This confirms the formation of silver-copper oxide composite. The absence of impurity peaks in XRD pattern revealed the high purity of the synthesized nanoparticles. The structure of the nanocomposite was face centered cubic. Using the Scherrer formula, the average crystallite size was calculated and it was about 32 nm.

3.2 Fourier Transform Infrared spectroscopy (FTIR)

Fig. 2 shows the FT-IR spectrum of the nanocomposite. ATR mode was used to record the spectrum at room temperature. The spectrum displays two characteristics broad band centered at 3517 and 1521 cm^{-1} which are referring to the stretching and bending mode vibrations. The band at 739 cm^{-1} corresponds to the vibration of Metal-oxide stretching mode (Devi *et al.* 2010; Ahmed *et al.* 2012). The strong band at 425 cm^{-1} corresponds to the vibration of Ag-Cu bond. It could be seen from Fig. 3.1 that the broad absorption band centered at 3450 cm^{-1} is attributed to the band OH stretching vibrations due to the fact that the calcinated powders tend to physically absorb water. The small bands at 1219 cm^{-1} can be attributed to copper oxide.

3.2 Scanning Electron Microscopy (SEM)

SEM is a very effective tool to study the surface morphology of nanomaterials. A micrograph of the Ag-Cu-O nanocomposite is shown in Fig. 3. Well dispersed plate-like structure, and the tubes are visible on the surface of the synthesized material. Copper forms a plate-like structure, whereas silver prefers to have a tube-like structure. This was also confirmed with the EDAX analysis (Fig. 3). Elemental mapping was carried out individually at the plate and the tube and found that the wt. % of Cu and Ag is high, respectively (Fig. 4).

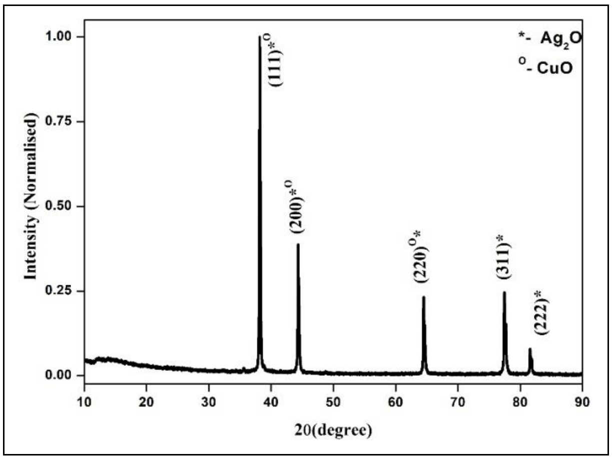


Fig. 1: XRD spectrum of Ag-Cu-O.

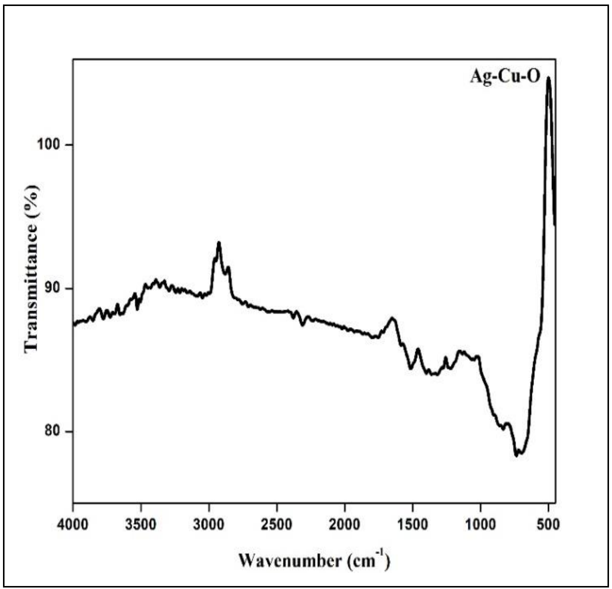


Fig. 2: FTIR spectrum of Ag-Cu-O.

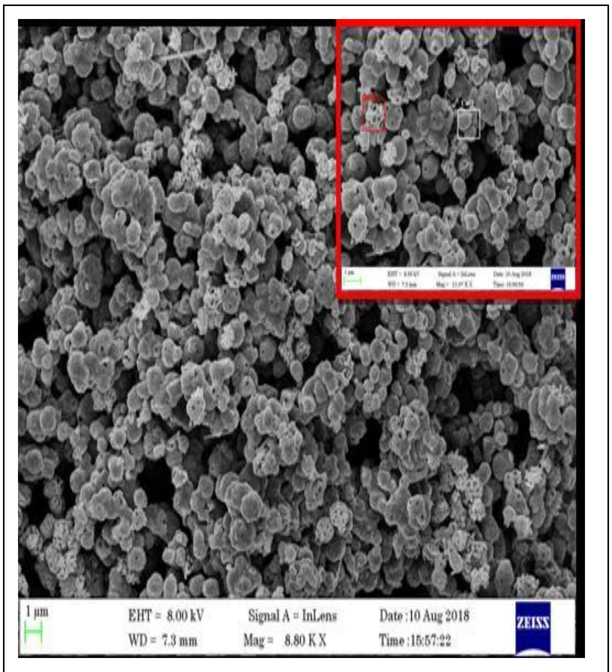
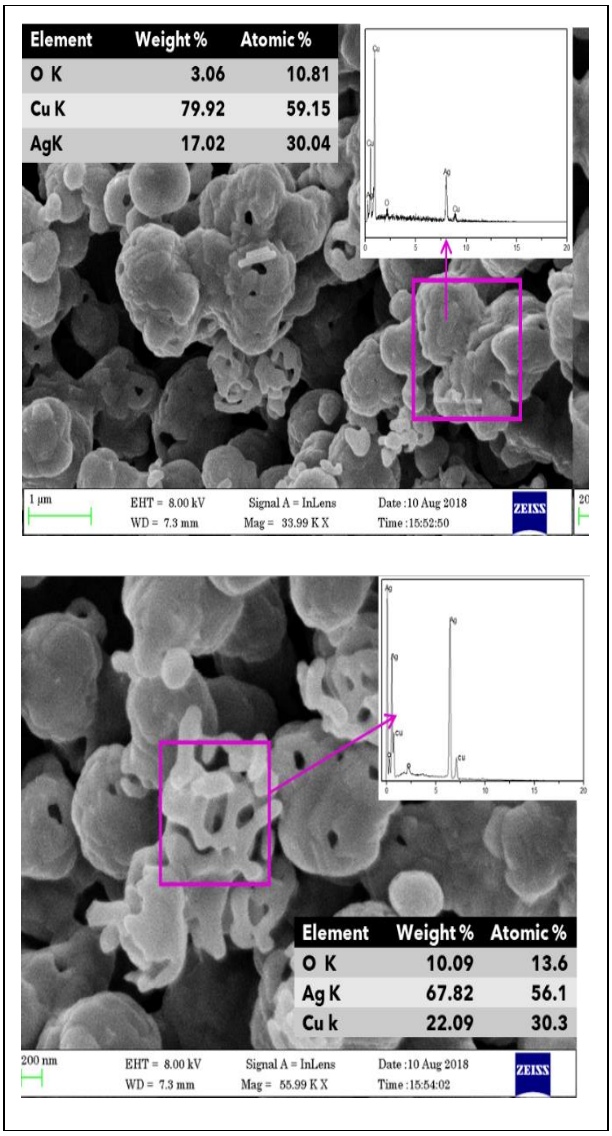


Fig. 3: SEM of Ag-Cu-O (Inset: Zoomed view).

3.4 UV - Visible Diffuse Reflectance Spectroscopy

UV-Vis. DRS is a unique tool to measure the reflectance of nanomaterials. Ag-Cu-O absorption spectrum is shown in Fig. 5. The broad absorption at 320 nm is a significant plasmon band of Ag and Cu metals. $\pi \rightarrow \pi^*$ energy transfer mechanism was attested from this absorption. Other than this absorption, the synthesized nanocomposite was transparent in the visible region.



Element	Weight %	Atomic %
O K	3.06	10.81
Cu K	79.92	59.15
AgK	17.02	30.04

Element	Weight %	Atomic %
O K	10.09	13.6
Ag K	67.82	56.1
Cu k	22.09	30.3

Fig. 4: Elemental mapping of the plate and the tube-like structures.

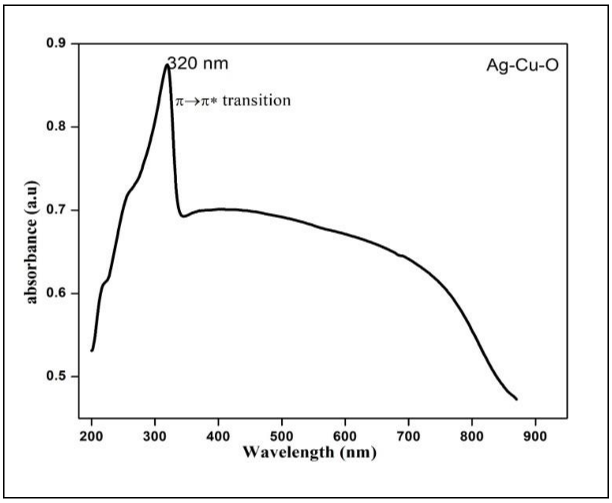


Fig. 5: UV-DRS spectra of Ag-Cu-O.

3.5 Antibacterial activity

Antibacterial activity of *in situ*-synthesized Ag-Cu-O nanocomposite was tested by standard disc method using *Staphylococcus aureus, Escherichia coli and Bacillus subtilis*.

3.5.1 Preparation of Inoculum

The inoculums for the experiment were prepared in fresh nutrient broths from preserved slant culture. The inoculums were standardized by adjusting the turbidity of the culture to that of McFarland standards. The turbidity of the culture was adjusted by the addition of sterile saline or broth or by further incubation to get the required turbidity. All the materials used, like cotton swabs and forceps, were sterilized using alcohol.

3.5.2 Disc Method - measurement of the zone of inhibition

The standardized inoculums are inoculated in the plates prepared earlier (aseptically) by dipping a sterile in the inoculums removing the excess of inoculums by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid, and finally streaking the swab all over the surface of the medium, 3 times rotating the plate through an angle of 60 ºC, after each application. Finally, the swab was passed around the edge of the agar surface. The inoculums were left to dry at room temperature with the lid closed. Each Petri dish is divided into 2 parts; in one part prepared Ag-Cu-O (100 µg) discs (discs were soaked overnight in sample solution) and in the other part standard Ciprofloxacin (10 µg), were placed in the plate with the help of sterile forceps. Then Petri dishes were placed in the refrigerator at 4 ºC or at room temperature for 1 hour for diffusion and incubated at 37 ºC for 24 hours. Finally, the zone of inhibition produced by different samples was observed and measured using a scale and the average of two diameters of each zone of inhibition was recorded. The results were incorporated in Fig. 6 and given in Table 1. From the results, it was obvious that the synthesized composite has antibacterial activity even without the presence of any antibodies. The better activity was observed in gram-positive bacteria.

Table 1. Antimicrobial activity of synthesized Ag-Cu-O nanocomposites.

S. No.	Organisms	Zone of Inhibition (mm)	
		Std.	Ag-Cu-O
1	*Staphylococcus aureus*	45	18
2	*Bacillus subtilis*	42	18
3	*Escherichia coli*	40	15

4. CONCLUSION

A unique thermal degradation method was employed to synthesis Ag-Cu-O nanocomposite. This method resulted in high purity, FCC structured, Ag-Cu-O and this was confirmed from the XRD analysis. The presence of Ag and Cu was attested by the strong absorption band in FTIR spectrum. Plasmon transfer mechanism was evidenced by the deep absorbance in UV spectrum. The prepared nanoparticles exhibited good antibacterial property in gram-positive and gram-negative bacteria which has shown that the synthesized particles were suitable for wound dressing materials and for food packaging materials.

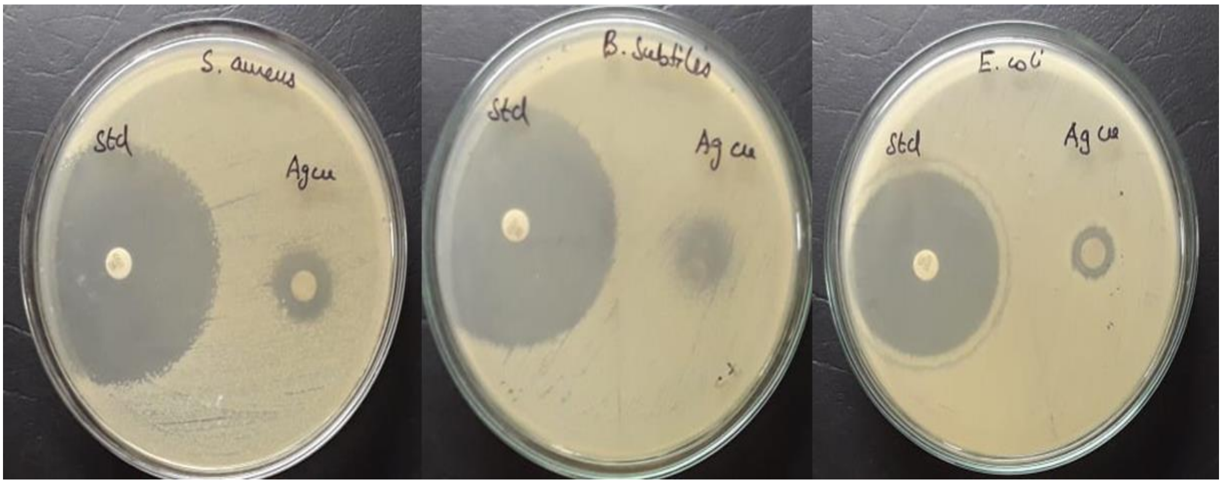


Fig. 6: Antimicrobial activity of Ag-Cu-O.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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