

Antibacterial Studies of Copper Sulphide Nanoparticles Prepared by Chemical Precipitation Method

Renuka Pawar¹ and Sandip Patil²

Shri. Vijaysinha Yadav College, Peth Vadgaon^{1,2}

renukapawar71@gmail.com

Abstract: Copper sulphide nanoparticles (CuS NPs) are synthesized by chemical precipitation method using precursor copper chloride and thiourea. The morphology and crystalline nature of CuS NPs were characterized by XRD, UV-Vis., Raman and SEM analysis. XRD pattern indicating formation of the hexagonal covellite CuS phase. The optical band gap energy is 2.35eV. The raman spectra confirms the successful formation of covellite-structured CuS nanoparticles with good crystallinity and phase purity. The morphological study by scanning electron microscopy shows spherical nature of CuS nanoparticles. CuS NPs tested against gram positive (*S. aureus*, *E. faecalis*, *B. cereus*) and gram negative (*E. coli*, *P. aeruginosa*) bacteria by agar disk diffusion method. Copper sulphide nanoparticles are more sensitivity towards gram positive and gram negative bacteria. In vitro mechanistic study revealed that CuS NPs kills the bacteria by damaging their cell membrane and producing reactive oxygen species.

Keywords: Copper sulphide, Chemical precipitation, X-ray diffraction, Band gap, Antibacterial activity.

I. INTRODUCTION

Nanotechnology has emerged as one of the most promising fields of modern science due to its wide-ranging applications in medicine, electronics, catalysis, environmental remediation and biotechnology [1, 2]. Nanoparticles exhibit unique physical, chemical and biological properties compared to their bulk counterparts because of their high surface-to-volume ratio and quantum confinement effects [3]. The semiconductors of chalcogenide nanostructures, such as ZnS, CdS, NiS, CoS and CuS have a potential applications in photovoltaic cells, gas sensors, LEDs, photocatalytic activity and etc. [4-8]. Among various metal and metal sulphide nanoparticles, copper sulphide (CuS) nanoparticles have attracted significant attention owing to their excellent optical, electrical, catalytic and antimicrobial properties.

Copper sulfide is a p-type semiconductor with a narrow band gap and exhibits remarkable optical absorption in the visible and near-infrared regions. These properties make CuS nanoparticles suitable for applications in solar cells, sensors, photocatalysis, energy storage devices and biomedical fields [5, 6]. CuS nanoparticles alongwith semiconductor applications shows emerging and promising platform for photothermal cancer therapy, biomolecular imaging and biomolecular sensing [9-11]. The synthesis of CuS nanoparticles can be achieved through various methods such as chemical precipitation [12], hydrothermal [13], solvothermal [14], microwave-assisted [15] and green synthesis techniques [16]. Among these, the chemical precipitation method is widely preferred due to its simplicity, low cost and ability to produce nanoparticles with controlled size and morphology.

Characterization of synthesized CuS nanoparticles is essential to understand their structural, morphological and optical properties. Techniques such as X-ray diffraction (XRD), UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM) are commonly employed to investigate crystal

structure, particle size, morphology, functional groups and optical band gap. These analyses provide valuable information regarding the quality and properties of the synthesized nanoparticles.

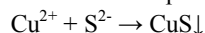
In recent years, the increasing prevalence of antibiotic-resistant microorganisms has created an urgent need for the development of alternative antimicrobial agents. Metal sulphide nanoparticles have shown considerable potential in combating pathogenic microorganisms due to their ability to generate reactive oxygen species, disrupt cell membranes and interfere with essential cellular processes [17]. CuS nanoparticles, in particular, have demonstrated effective antibacterial and antifungal activities against a broad spectrum of microorganisms. Their enhanced antimicrobial performance is attributed to their nanoscale dimensions, high surface reactivity and controlled release of copper ions.

The present study focuses on the synthesis of CuS nanoparticles by a suitable chemical method, followed by detailed characterization using various analytical techniques. Furthermore, the antimicrobial activity of the synthesized CuS nanoparticles is evaluated against selected gram positive and gram negative microbial strains to assess their potential as effective antimicrobial agents for biomedical and environmental applications.

II. EXPERIMENTAL DETAILS

2.1 Materials and Methods:

Copper sulphide (CuS) nanoparticles were synthesized by a simple chemical precipitation method using copper sulfate (Sigma-Aldrich Chemie, India) as the copper precursor and thiourea (Merck) as the sulfur source. 0.4M 40ml copper sulfate and 0.4M 40ml thiourea solution was prepared in deionized water and stirred separately for 30 min. The thiourea solution was added slowly to the copper sulfate solution under continuous magnetic stirring at room temperature. The reaction mixture was stirred continuously to ensure homogeneous mixing of the reactants. During the reaction, thiourea provided the sulfide ions required for the formation of copper sulphide, resulting in the nucleation and growth of CuS nanoparticles. The formation of CuS can be represented by the following simplified reaction:



The resulting precipitate was separated from the reaction mixture by filtration and washed several times with deionized water to remove unreacted precursor ions and soluble by-products. The obtained precipitate was subsequently washed with ethanol and dried at an appropriate temperature until a constant mass was obtained. The dried material was then finely ground to obtain CuS nanopowder. The synthesized CuS nanoparticles were subjected to further characterization using techniques such as X-ray diffraction (XRD), UV-Visible spectroscopy, scanning electron microscopy (SEM) and other suitable analytical techniques to investigate their structural, optical, and morphological properties.

2.2 Characterization techniques:

The CuS nanoparticles were characterized by using different techniques. The structural analysis of CuS nanoparticles was obtained by X-ray Diffractometer (Ultima IV of Rigaku Corporation, Japan) with Cu ($K\alpha$) ($\lambda=1.54056 \text{ \AA}$). UV-Vis. spectra of CuS nanoparticles were obtained using (UV-Vis (DRS)-NIR V-770, JASCO, Japan spectrophotometer) in the wavelength range from 200 to 800 nm. The structural analysis was done by Raman spectroscopy in the range 0 to 800 cm^{-1} . Fourier transform infrared (FT-IR) spectrum was taken using (FT-IR JASCO-4600) scanning from 4000 to 400 cm^{-1} in the % transmittance mode. The morphological study was stemmed from Scanning Electron Microscopy instrument SEM JEOL JSM- 6360.

III. RESULT AND DISCUSSION:

X-ray Diffraction (XRD) Analysis of CuS Nanoparticles:

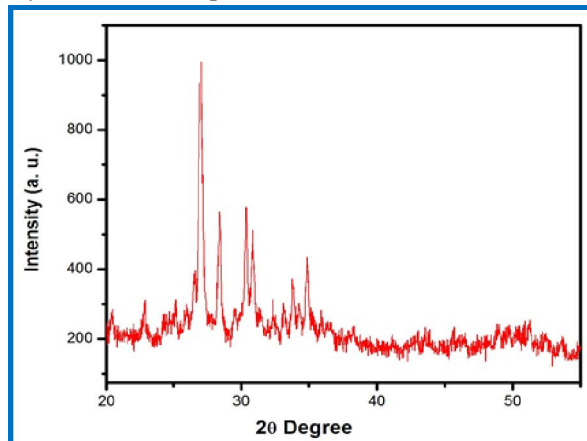


Fig. 1 XRD pattern of CuS nanoparticles

The X-ray diffraction (XRD) pattern of the synthesized CuS nanoparticles is shown in Fig. 1. The XRD pattern of the synthesized CuS nanoparticles exhibits distinct diffraction peaks at approximately $2\theta = 27.5^\circ$, 29.3° , 31.0° , 32.8° , and 34.6° , which are indexed to the hexagonal covellite CuS phase. The diffraction peaks agree well with the standard JCPDS data (Card No. 06-0464), confirming the successful formation of crystalline CuS [18]. The absence of impurity peaks indicates high phase purity. Peak broadening observed in the diffraction pattern is attributed to the nanoscale crystallite size and lattice strain effects. The sharp and intense diffraction peaks demonstrate the good crystallinity of the synthesized CuS nanoparticles.

3.2 UV-Visible Absorption Spectrum of CuS Nanoparticles:

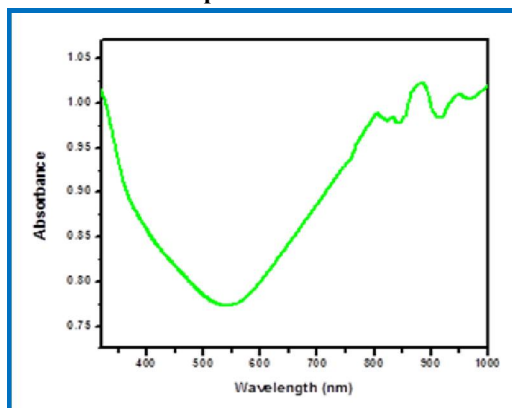


Fig. 3 UV-Vis. absorption spectrum of CuS nanoparticles

The UV-Vis absorption spectrum of CuS nanoparticles exhibits broad absorption in the visible and near-infrared regions (350–1000 nm). A minimum absorbance is observed around 550 nm, followed by a gradual increase toward longer wavelengths. The extended NIR absorption is attributed to localized surface plasmon resonance arising from copper-deficient CuS, while the broad spectral profile confirms the nanocrystalline nature of the material. These optical characteristics demonstrate the potential of CuS nanocrystals for photocatalytic, photothermal, and optoelectronic applications.

3.3 Optical Band Gap Analysis of CuS Nanoparticles:

The optical band gap energy of the synthesized CuS nanoparticles was determined from the Tauc plot of $(\alpha h\nu)^2$ versus photon energy ($h\nu$), assuming a direct allowed transition as displayed in Fig. 4. The linear region of the plot was extrapolated to intersect the photon energy axis, yielding a band gap value of **2.35 eV**. The enlarged band gap compared to bulk CuS is attributed to quantum confinement effects associated with the nanoscale particle size. The obtained band gap confirms the semiconducting nature of the CuS nanoparticles and demonstrates their suitability for photo catalytic and optoelectronic applications.

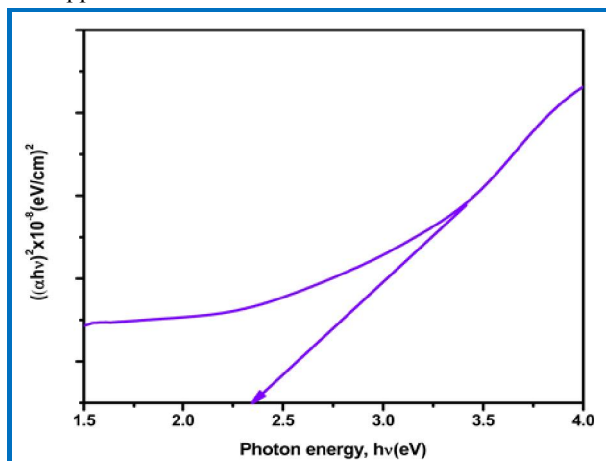


Fig. 4 Band gap energy of CuS NPs prepared by Precipitation method

3.4 Raman Spectral Analysis of CuS Nanoparticles:

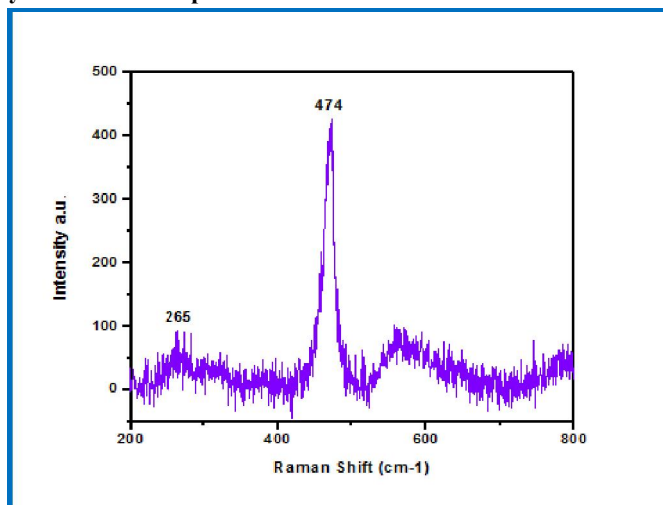


Fig. 5 Raman spectrum of CuS NPs prepared by Precipitation method

The Raman spectrum of the synthesized CuS nanoparticles recorded in the range of 200–800 cm^{-1} is presented in Fig. 5. The spectrum exhibits two characteristic vibrational bands centered at approximately 265 cm^{-1} and 474 cm^{-1} , confirming the formation of crystalline copper sulphide. The weak Raman band observed at 265 cm^{-1} can be attributed to the Cu–S lattice [19, 20] vibrational mode, corresponding to the stretching and bending vibrations involving copper and sulfur atoms in the CuS crystal lattice. This peak is characteristic of covellite-phase CuS and indicates the presence of metal–sulfur bonding within the nanostructure. A prominent and intense Raman peak is observed at 474 cm^{-1} , which

is assigned to the S–S stretching vibration (A_g mode) of covellite CuS. This mode originates from the vibration of sulfur pairs present in the hexagonal CuS structure and is considered the fingerprint Raman band of covellite-phase copper sulfide [20]. The high intensity and sharp nature of this peak suggest good crystallinity and phase purity of the synthesized nanoparticles.

3.5 SEM Analysis of CuS Nanoparticles:

The surface morphology and microstructural characteristics of the synthesized CuS nanoparticles were investigated using Scanning Electron Microscopy (SEM) at different magnifications as shown in Fig. 6. The SEM micrographs reveal that the CuS sample consists of a large number of fine nanoparticles distributed uniformly over the surface. At lower magnification, the particles appear densely packed and homogeneously dispersed, indicating successful nucleation and growth of CuS nanocrystals throughout the sample. No large cracks or significant structural defects are observed, suggesting good sample integrity. With increasing magnification, the individual nanocrystals become more distinguishable. The particles exhibit predominantly quasi-spherical to irregular morphologies and tend to form clusters due to their high surface energy. Such agglomeration is commonly observed in sulfide nanomaterials and arises from strong interparticle interactions during the synthesis and drying processes.

At the highest magnification, the micrograph clearly shows that the CuS nanocrystals are composed of nanosized grains with relatively uniform morphology. The particles are closely packed, forming a porous network structure with interconnected grains. This porous arrangement can provide a large specific surface area and abundant active sites, which are advantageous for photocatalytic, sensing, and electrochemical applications.

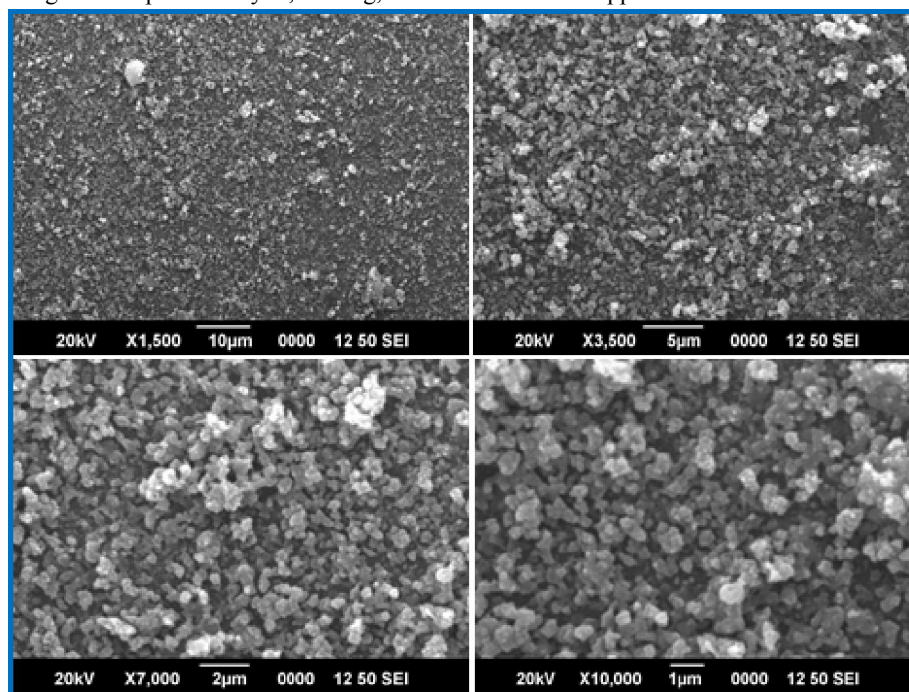


Fig. 5 SEM images of CuS NPs prepared by Precipitation method

3.6 Antimicrobial Activity of CuS Nanoparticles:

The antimicrobial activity of the synthesized copper sulphide (CuS) nanoparticles was evaluated against both Gram-negative and Gram-positive bacterial strains by the agar diffusion method. The well diffusion method was used to check the antimicrobial activity of the sample against the respective bacterial pathogen for this sterile Muller and

Hinton agar plate was used. Muller and Hinton agar plate was prepared having 0.7 cm diameter and then 100 ml sample was added into the well-kept for diffusion in the refrigerator for 5 min. The plates were incubated at 37 °C for 24 hours. Copper sulphide nanoparticles were tested against microorganisms included *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Bacillus cereus*.

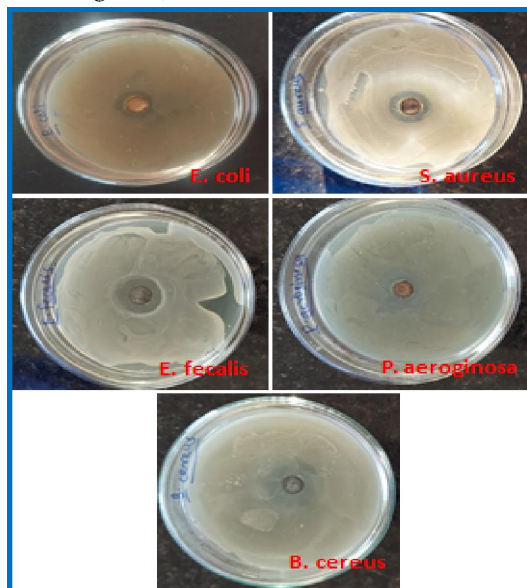


Fig. 6 Antibacterial activity of synthesized CuS nanoparticles

As shown in Fig. 6, visible zones of inhibition were observed around the CuS nanoparticle-treated discs/wells, confirming the antibacterial potential of the synthesized nanoparticles. Among the tested organisms, distinct inhibition zones were observed against *E. coli*, *S. aureus*, *E. faecalis*, and *B. cereus*, indicating that CuS nanoparticles can effectively inhibit the growth of these bacterial strains. The inhibition observed against *P. aeruginosa* was comparatively less pronounced, suggesting that this organism may exhibit relatively greater resistance or reduced susceptibility to the nanoparticles under the experimental conditions.

The antibacterial activity of CuS nanoparticles may be attributed to the release of copper ions (Cu^{2+}), generation of reactive oxygen species (ROS), and direct interaction of nanoparticles with the bacterial cell membrane. These mechanisms can cause membrane disruption, increased membrane permeability, oxidative stress, leakage of intracellular components, and damage to essential cellular proteins and nucleic acids, ultimately resulting in inhibition of bacterial growth or cell death. The small size and large surface area of nanoparticles may further enhance their interaction with bacterial cells.

IV. CONCLUSION

The present study successfully demonstrated the preparation, characterization, and antimicrobial evaluation of CuS nanoparticles synthesized by the precipitation method. The XRD analysis confirmed the formation of crystalline CuS with a covellite crystal structure, indicating the successful synthesis of the desired phase. The optical study revealed band gap energy of approximately 2.35 eV, confirming the semiconducting nature of the synthesized CuS nanoparticles. Raman spectroscopy provided further evidence for the formation of CuS through characteristic vibrational modes associated with the CuS lattice, while FT-IR analysis confirmed the presence of characteristic Cu-S bonding and the functional groups associated with the synthesized material. SEM analysis revealed the morphology and nanoscale features of the prepared CuS particles, supporting the formation of CuS in nanoparticulate form. The

antimicrobial studies demonstrated that the synthesized CuS nanoparticles exhibited inhibitory activity against the tested bacterial strains, although the extent of inhibition varied among the microorganisms.

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