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Brief Report

Crystallization and Characterization of Copper Sulphate Crystal and its Antimicrobial Activity

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ABSTRACT

Crystal growth plays a prominent role in the present era of rapid technical and scientific advancement, where the applications of crystals have unbounded limits. Developments in technology have stimulated the importance of discovering new materials and modifying the already known materials. Growth of crystals and their structural and physical characterization come under the scientific research on crystalline materials. Analytical reagent and AR grade chemicals and double distilled water were used in the growth of crystals from aqueous solutions by free evaporation method at room temperature. X-ray Diffraction Data (XRD) were collected from powder samples of crystals using an automated X-ray powder diffractometer. The reflections were indexed. The XRD confirms the crystalline nature of the sample crystal. Optical absorption (UV–Vis) spectra were recorded for the grown crystals to characterize optical activity. It is possible to observe the typical wide band associated to CS crystals in the range between 200 nm and 1100 nm with a maximum at 221 nm. The antimicrobial assays, which demonstrates that enhanced activity, obtaining a better antimicrobial effect against *Pseudomonas, Penicillium, Rhizobium* and *A. flavus*. These results showed that the CS Crystals can be particularly suitable as antimicrobial agent for specific application.

Keywords: Copper sulphate crystals; XRD; UV-Vis; Anti-microbial activity

INTRODUCTION

Crystallography is concerned with the nature of the regular atomic arrangements within the crystal. Crystallographers had made remarkable studies about the crystal before the discovery of X-ray by crystals [1]. However, only after that, it became possible to know about the internal arrangement of atoms in the crystals, in a more developed way [2]. As there was a remarkable achievement in the study of internal atomic arrangements it led to the study of more physical properties [3]. This interest is shifted from the study of natural crystals to the laboratory grown crystals. Copper sulphate is an odourless blue powder or transparent blue crystal. It is most commonly used for agricultural purposes as a fungicide to treat fruit and vegetable crops for mildew, blights and other fungus [4]. Copper sulphate is also used in water treatment plants as an algaecide and has applications as an herbicide. Copper is an inexpensive material and recently, it has found use as an antimicrobial agent and has seen application in the lining of food packaging to reduce spoilage [5]. Copper, a well-known antimicrobial agent, although a powerful and effective antimicrobial agent [6], copper is expensive and so can have high costs when required to coat high-contact surfaces that often have large areas. Antibiotic therapy has resulted in the persistence of multidrug-resistant microorganisms in the environment [7]. The development of multifunctional antibacterial drugs has been a long-term strategy for treating bacteria-caused infectious diseases. Antibacterial drugs with an antibacterial action against both gram-positive and gram-negative bacteria to overcome the aforementioned obstacles are preferred [8]. Hence the present investigation was aimed at synthesizing the single organic crystals. Identifying the crystal structure by powder X-ray diffraction analysis, characterizing the grown crystals by UV-visible absorption spectra and to study the anti–microbial properties of the grown crystal.

MATERIALS AND METHODS

Growth of sample crystal

In the present study, we attempted to grow reasonable sized crystals which were sufficient for the proposed characterization. So, it proposed to grow the single crystals by slow (free) evaporation method. Analytical Reagent (AR) grade sample of copper sulphate and double distilled water were used for the growth of crystals. The calculated amount of copper sulphate salt and double distilled water were mixed in aqueous solution with continuous stirring. The solution was allowed to dry at room temperature. Transparent good quality crystals were harvested with in a period or 15 days by

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the method adopted by Mahadevan in 2020.

X-Ray powder diffraction measurement

X-ray diffraction finds the geometry or shape of a molecule using X-ray. X-ray primarily interact with electrons in atoms. When X-ray photons collide with electrons, some photons from the incident beam will be deflected away from the incident beam will be deflected away from the direction were they originally travel. If the wavelength of those scattered X-rays did not change the process is called Thompson scattering (elastic scattering). The scattered X-rays carry information about the electron distribution in materials. These are the X-rays that we measure in diffraction experiment.

UV-Vis-NIR spectral analysis

UV-Vis- NIR transmittance spectra were recorded for the grown crystals using a lambda 35 spectrophotometer in the wavelength range 200 nm-1100 nm.

Antimicrobial activity of CS crystals

The antimicrobial activity of CS crystals were determined by well diffusion method. Four bacterial pathogens such as *Pseudomonas, klebsiella, S. aureus* and *streptococcus* and four fungal pathogens such as *A. niger, penicillium Sp, Rhizobium Sp.* and *Aspergillus flavus* were used for this investigation. Fresh bacterial culture of 0.1 ml having 108 CFU was spread on Muller Hinton agar plates using sterile cotton swabs. The fungal strains were spread on potato dextrose agar wells of 6 mm diameter were punched off into medium with sterile cork borer and filled with 50 ml of powdered crystal extracts using micro pipette in aseptic condition. The plates were kept in refrigerator to allow pre-diffusion of extract for 30 min and then incubated at 37°C for 24 h and 28°C-37°C for 3-4 days for bacterial and fungal cultures respectively. The antimicrobial activity was evaluated by measuring the zone of inhibition.

Solubility data of CS

Temperature (°C): 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C.

Amount of salts (grams): 6.45, 6.81, 7.45, 7.56, 7.78, 7.95, 8.15.

RESULTS AND DISCUSSION

With the emergence of antibiotic resistance and the hospital acquired infection, the interest for antimicrobial agents has recently increased again in public health. Copper sulphate is recommended as a supplementary method increasing biological safety in the hospital environment. In the present investigation the growth of pure crystal was grown by slow evaporation technique at room temperature. The grown crystals were subjected to PXRD. UV-Vis spectroscopic techniques are used to characterize the grown crystals. The antimicrobial activity of pure copper sulfate crystals was determined. The results obtained in the present study are reported and discussed (Table 1 and Figure 1).

Table 1: Optimized growth condition for CS crystals.

Sample	Solvent chosen	Growth temperature	Period of growth	Size of the crystal
CS	Distilled Water	40	10-15 days	2.4 × 0.9 × 2.39mm^3

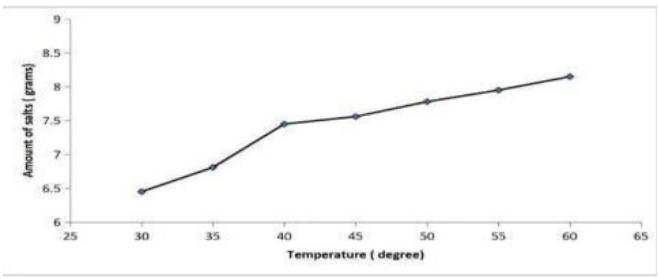

Figure 1: Solubility curve of CS crystal.

Crystals grown

The grown crystals can be represented as Copper Sulphate-CS:

Good quality crystals were harvested within a period of 15 days. The crystals grown in the present study are blue colour, stable and transparent. Figure 2, shows the photograph of the sample crystals grown. Crystal's upto a maximum of 2.4 × 0.9 × 2.39 mm^3 for CS were obtained. Both the crystal has orthorhombic system with Op24 space group. Table shows the optimized growth condition for CS crystals. The optimized growth condition for CS crystals are given in the below figure (Table 2).

Figure 2: Optimized growth condition for CS crystal.

Table 2: PXRD results of the synthesized pure $Cuso_4$ crystal.

Scan type	Coupled Two Theta/Theta
Scan mode	Continuous PSD fast
Scan status	Completed
Start	10.002°
End	80.010°
Step size	0.020°
Total time/step	40.00 s
Time/step	0.25 s
Axis offset	
Time started	0
Goniometer radius	200.5 mm
Goniometer radius primary	
Goniometer radius secondary	

X-Ray diffraction analysis

The grown pure copper sulfate crystals were subjected to powder X-ray diffraction analysis and the resultant spectrum confirmed the crystalline nature of the samples and the X-ray powder diffraction pattern of the grown crystals are shown in Figure 3.

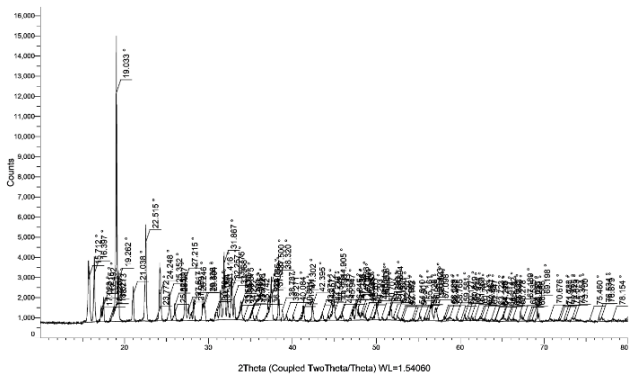

Figure 3: PXRD pattern of CuSo4 crystals.

Optical transmission spectrum

UV-Vis absorption spectrum gives information about the structure of the molecule [9]. The grown crystal with a typical absorption maximum at 221 nm. The UV-Vis transmission spectrum of pure crystal is shown in the Figure 4.

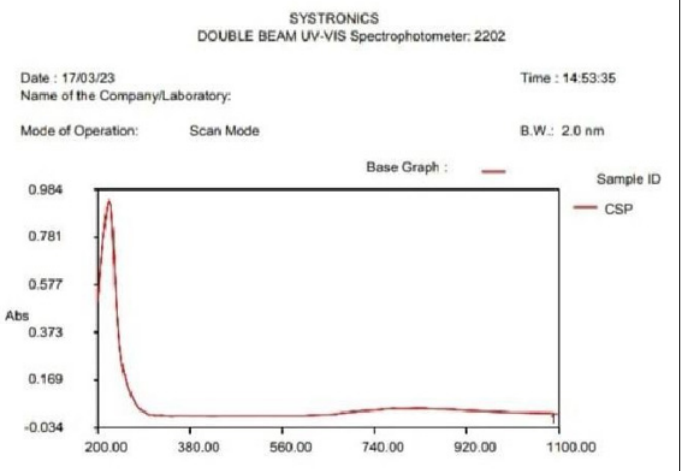

Figure 4: UV-Vis absorption spectrum of the $CuSo_4$ crystal.

Antimicrobial activity of CS crystal

Antimicrobial activities of CS crystals were determined by well diffusion method against various bacterial and fungal pathogens. The pure crystal showed inhibitory activity on *pseudomonas* (11 mm), *klebsiella* (8 mm), S. *aureus* (7 mm) and *streptococcus* (6 mm) respectively. In the antimicrobial activity assays Copper sulphate crystals can be particularly suitable for acting as an antibacterial agent, so they are excellent candidates for specific applications (Table 3 and 4, Figures 5 and 6) [10-27].

Table 3: Shows the Absorption peak of the UV-Vis spectrum of pure CuSo4 crystal.

Crystal	Absorption edge (nm)
CS	221

Table 4: Antimicrobial activity of CS crystals.

Sl. No	Test organisms	CS
1	*Pseudomonas*	11 mm
2	*Klebsiella*	8 mm
3	*S. aureus*	7 mm
4	*Streptococcus*	6 mm
5	*A. niger*	8 mm
6	*Penicillium Sp.*	11 mm
7	*Rhizobium Sp.*	3 mm
8	*A. flavus*	5 mm

Note: Zone of inhibition in mm.

Figure 5: Shows the antifungal activity of CS crystals.

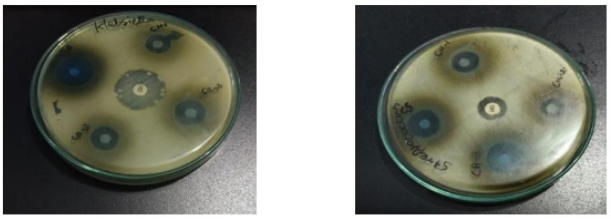

Figure 6: Shows the antibacterial activity of CS crystals.

CONCLUSION

Copper sulphate crystals were successfully grown by analytical reagent and AR grade chemicals by free evaporation method at room temperature. X-Ray Diffraction Data (XRD) were collected from powder samples of crystals using an automated X-ray powder diffractometer. The reflections were indexed. The XRD confirms the crystalline nature of the sample crystal. Optical absorption (UV-Vis) spectra were recorded for the grown crystals to characterize optical activity. It is possible to observe the typical wide band associated to CS crystals in the range between 200 nm and 1100 nm with a maximum at 221 nm. The antimicrobial assays, which demonstrates that enhanced activity, obtaining a better antimicrobial effect against *Pseudomonas*, *Penicillium*, *Rhizobium* and *A.flavus*. These results showed that the CS Crystals can be particularly suitable as antimicrobial agent candidate for specific application.

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