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Studies on Nucleation and Growth Kinetics, Molecular Structure, Spectral Characterization and Antibacterial activity of DL-Methioninium Maleate Compound for Biological Applications

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ABSTRACT

Single crystals of DL-methioninium maleate (abbreviated as DLMM) were synthesized by slow evaporation technique from aqueous solution at room temperature. Before growing the crystal, the nucleation kinetics was analysed to estimate the critical supersaturation required for the growth of good quality crystal. The classical theory was employed to analyse the growth kinetics of the crystal. Using single crystal X-ray diffraction analysis, the lattice dimensions of the title compound were found to be a = 11.07 Å, b = 5.74 Å, and c = 19.69 Å. The crystallized compound belongs to monoclinic system (space group $P2_1/c$). From the powder X-ray diffraction study the different planes of the reflections have been distinguished and the results were confirmed. The molecular structure of the grown crystal DLMM was also analysed by using SHELXT-2014/7 program. The results indicate that the cationic form of the DL-methioninium molecule consists of a protonated amino group and an uncharged carboxylic acid group. The maleic acid molecule exists in its mono-ionized state. By observing the UV-Visible absorbance spectrum, the range and percentage of absorption and the material band gap were evaluated for the grown material. The absorption coefficient, complex refractive index and the optical transparency of the compound depend upon the optical band gap of the material. From the value of band gap (3.96eV) of the material, the absorption coefficient can be assessed. The absorption coefficient is an additional factor to elucidate the antibacterial behaviour of the material. Fourier transform infrared (FTIR) spectra have been used to determine the vibrational frequency of several DL-methioninium maleate functional groups. Using proton and carbon nuclear magnetic resonance (NMR) spectra, the presence of hydrogen and carbon in the formed crystal was verified. The presence of sulphur

component in the grown material is one of the prime factors of the bioactive material. By using the Agar disc diffusion method, the antibacterial properties of the grown compound were also examined. The anti-bacterial activities of DLMM compound suggest that it can be employed to treat a number of bacterial infections in the field of pharmaceutical science.

Keywords: Nucleation kinetics, Single crystal XRD, FTIR, NMR, Antibacterial activity

I. INTRODUCTION

Crystalline salts of amino acids open the door to anti-bacterial activities that have recently drawn researchers looking for practical uses. A vital amino acid, called methionine is also main supply of sulphur, which is necessary for the growth of normal metabolism. The most thorough searches for these crystals have been conducted [1-3], and the results are more interesting. Because of this, we are interested in exploring two sulphur components of DL-methionine and cystein [4]. According to a review of the literature, DL-methionine demonstrates the solid state change at 321 K in solutions [5], a dual oxidation process [6], and a greater bulk density [7]. It might function as an electrostatically activated genetic switch, according to Phillips et al. [8]. Many spectroscopic studies of the inorganic acid complexes of different amino acids as well as their derivatives have been conducted [9–11]. DL-methionine has many biological applications such as treatment of liver conditions and prevention of hypercholesterolemia and uses in animal feed, food, pharmacy, and medicine. The interaction of DL-methionine with inorganic acids of phosphonic, nitric, sulfuric, and perchloric has also been studied using vibrational spectroscopy [12, 13]. Similar studies were also conducted on DL-methionine using organic acids such as picric acid, orthophosphoric acid, trichloroacetic acid, etc. [14, 15]. In the current study, DL-methioninium maleate single crystals of good quality were synthesized from an aqueous solution using a slow evaporation approach. A brief description of nucleation kinetics [16, 17] was also included to evaluate the critical supersaturation required for growing of good quality of crystal. The grown crystals were characterized using several techniques such as XRD, UV-Vis analysis, FTIR and NMR. XRD studies were used to analyze the crystallographic data and structure of the grown material for ascertaining the single crystal nature of grown crystal. The spectral characterization studies have been carried out with a view to study the optical absorption characteristics, functional groups and molecular structure of the grown compound. The study of molecular structure is very important to confirm the sulphur component present in the material which exhibits bioactivity. At the end of the investigation antibacterial activity of the grown material has also been discussed to find applications in the field of pharmaceutical science. The spectral characterization correlates their results with antibacterial study of the grown material.

II. GROWTH OF DLMM CRYSTAL

(i) Nucleation Kinetics

According to classical nucleation theory [18], the critical free energy is given by

$$\Delta G^* = \frac{16\pi\sigma^3}{3\Delta G_v^2} \qquad (1)$$

where σ is the surface energy per unit area and ΔG_v is the bulk free energy change per unit volume.

$$\Delta G_v = -\frac{kT}{v}\ln s \quad (2)$$

Here's' is the supersaturation and 'v' is the specific volume. The nucleation rate is given by

$$J = A\exp\left(\frac{-\Delta G^*}{kT}\right) \quad (3)$$

When J=1, the nucleation rate is reasonable for achieving required supersaturation to confirm the good quality crystal during growth kinetics. This supersaturation is known as critical supersaturation (S_c). It is estimated as 1.12 at room temperature 300 K. The supersatuarion of the solution should be just below 1.12 to regulate the growth kinetics. If the supersaturation is exceeds 1.12 we will end up with the polycrystalline material.

(ii) Growth Kinetics

From the knowledge of critical supersaturation ascertained from nucleation theory, the solution comprising maleic acid (Loba chemie-99%) and DL-methionine (Loba chemie-99%) in a 1:1 molar ratio at ambient temperature was prepared with supersaturation just below 1.12. The mixture was then thoroughly agitated for about 5 hours to produce a saturated aqueous solution. To remove contaminants, whatman cellulose filter paper was used to filter the solution. A porous, perforated foil cover was placed over the solution, and it was left undisturbed. After 10 days, the translucent DLMM single crystals were formed due to supersaturation of the solution. DLMM crystals were purified through the procedure of repeated recrystallization [16]. The supernatant liquid was taken with burette and tested for supersaturation. It was found to be approximately 1.15 which is closer to the estimated value. Fig. 1 depicts the grown DLMM single crystal. The reaction formula is given below:

$$C_5H_{11}NO_2S + C_4H_4O_4 \rightarrow [C_5H_{12}NO_2S]^+.[C_4H_3O_4]^- \quad (4)$$

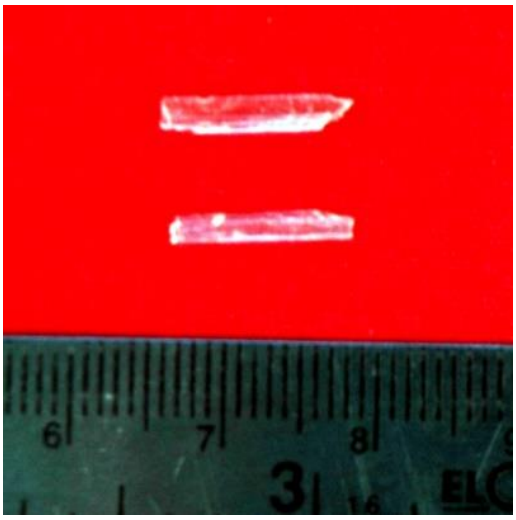

Figure 1: As-grown DLMM single crystals

(iii) Characterization Techniques

DLMM crystal was subjected to single crystal X-ray diffraction examination utilizing a BRUKER SMART APEX II single crystal X-ray diffractometer, and its lattice parameters were calculated. Powder X-ray diffraction investigation was also performed using a BRUKER D8 powder diffractometer with CuK_α (λ = 1.54056 Å) to determine the lattice parameters to confirm the crystallinity of the compound. The molecular structure of the grown crystal DLMM was analyzed by using SHELXT-2014/7 program. The optical properties of the crystals were studied by using a Perkin Elmer LAMDA 35 UV visible spectrophotometer in the wavelength range of 300 nm to 750 nm. To determine the various functional groups present in the DLMM compound, the middle infrared FTIR analysis of DLMM was performed between 400 cm^{-1} and 4000 cm^{-1} using a Burger IFS66 V FTIR spectrometer with KBr pellet technique. The sample was scanned over the range 10-60°C at the rate of 20°C/ min. Proton NMR and carbon NMR spectra of DLMM compound were recorded by using D_2O as a solvent on a BRUKER AV III 500 MHz FT NMR Spectrometer at 22°C to confirm the molecular structure of grown compound. The antibacterial properties of the grown DLMM crystal were evaluated using the Agar disc diffusion method.

III.RESULTS AND DISCUSSION

(i) Single crystal XRD

The grown DLMM single crystal was subjected to single crystal X-ray diffraction analysis to identify the crystallographic data of the material. It is confirmed that the grown crystal belongs to monoclinic system with space group $P2_1/c$. It is seen that the lattice parameters of DLMM are found to be in good agreement with the literature values [19]. Table 1 lists the current lattice parameter values as well as those reported previously for comparison.

(ii) Powder XRD

The X-ray powder diffraction technique is another tool to confirm the crystallographic data of the material. Fig. 2 displays the powder XRD pattern for the DLMM compound obtained in the temperature range of 10-60° at the rate of 20°C/min. The monoclinic system was indexed for the Bragg's peaks. Using the CELREF programme, the cell parameters were determined as a = 11.07 Å, b = 5.74 Å, c = 19.69 Å, α = γ = 90°, and β = 102.34°, with a cell volume V = 1229 3 Å^3. The computed lattice parameters agree with the values published earlier [19].

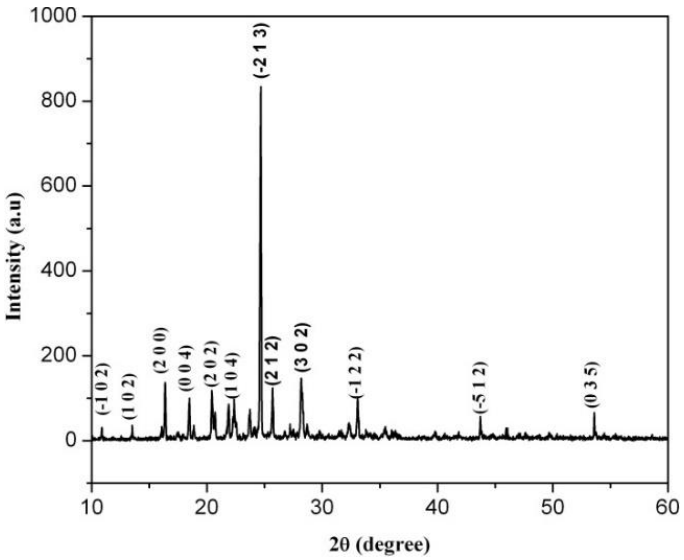

Figure 2: Powder XRD pattern of DLMM single crystal

Table 1

Lattice parameters of DLMM single crystal

Parameters	Reported values	Present work	
		Single XRD	Powder XRD
a	11.07 Å	11.07 Å	11.07 Å
b	5.74 Å	5.77 Å	5.74 Å
c	19.69 Å	19.69 Å	19.69 Å
α	90°	90°	90°
β	102.34°	102.61°	102.34°
γ	90°	90°	90°
Volume	1229 Å^3	1229 Å^3	1229 Å^3
Crystal system	Monoclinic	Monoclinic	Monoclinic

(iii) Computational method of solving molecular structure

The atom-numbering technique for the molecular structure of the DLMM molecule is shown in Fig. 3. The cationic form of the DL-methioninium molecule consists of a protonated amino group and an uncharged carboxylic acid group. The maleic acid molecule exists in its mono-ionized state. Similar to crystal structure of maleic acid, the semi-maleate ion has an intramolecular hydrogen bond between the atoms O_3 and O_5. Through hydrogen bonding, the semi-maleate ions do not directly interact. They produce a double layer that is parallel to the plane instead, acting as a mediator for interactions between methionine molecules.

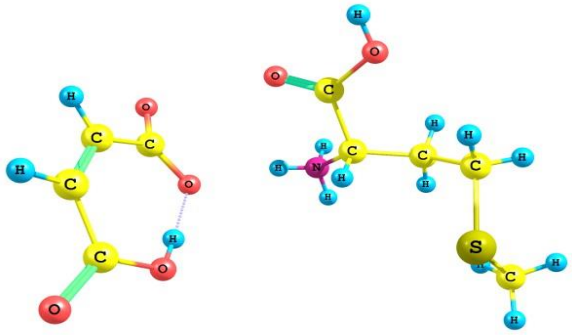

Figure 3: Molecular structure of DLMM compound

Layers that are alternately hydrophobic and hydrophilic are produced by the methioninium cations that border these layers on either side. There are no interactions involving hydrogen bonds between these double layers. It is interesting to note that six methioninium cations can be found close by a pair of maleate ions that are associated with an inversion. The presence of maleic acid and the sulphur component is mainly responsible for the bioactive nature of compound.

(iv) FTIR studies

The middle infrared FTIR analysis of DLMM was carried out between 400 and 4000 cm^{-1}. FTIR spectra (Fig. 4) as shown N-H asymmetric stretching vibration at 3432 cm^{-1}, CH_2-S asymmetric stretching at 2918 cm^{-1}, NH out of plane bending at 1618 cm^{-1}, $(NH_3)^+$asymmetric stretching vibration at (3186, 3083 cm^{-1}) and CH_3 asymmetric deformation at 1464 cm^{-1}[20].

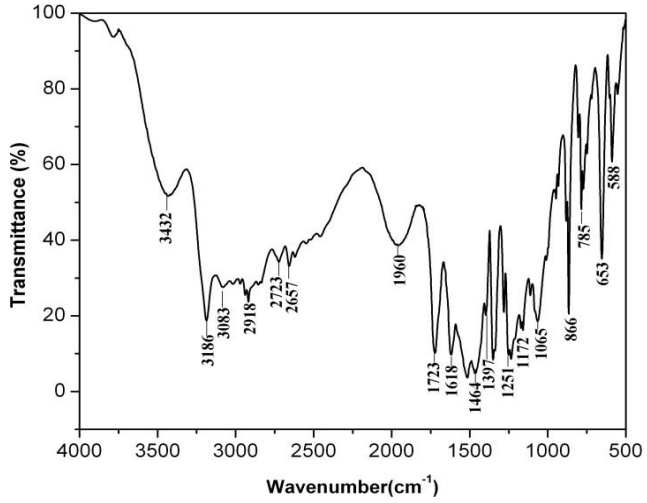

Figure 4: FTIR spectra of DLMM compound

The wave number region 1700-2900 cm^{-1} has been attributed to overtones and combination bands. The ionization of carboxyl group is evident from the absorption band at 1397 cm^{-1} which is due to symmetric stretching COO^- at 1172 cm^{-1} and 588 cm^{-1} that are due to rocking and wagging mode COO^-. The CH_2 asymmetric stretching (2938 cm^{-1}), wagging (1350 cm^{-1}), twist (1281, 1251, 1235 cm^{-1}) and rock (1009, 719, 653 cm^{-1}) vibration peaks are also seen. The rocking of NH_2 gives peak at 866 cm^{-1}. The tentative assignments for the powdered sample of DLMM are also listed in Table 2. We are thus able to predict the functional groups present in the grown compound.

Table 2

Band assignments of FTIR spectra of DLMM

Wavenumber (cm^{-1})	**Various stretching modes assigned**
3432	N-H asymmetric stretching
3186, 3083	$(NH_3)^+$ asymmetric stretching
2971	CH_3 asymmetric stretching
2938	CH_2 asymmetric stretching
2918	CH_2-S asymmetric stretching
2900-1760	Overtones and combination bands
1618	NH out of plane bending
1515	NH_3^+ symmetric deformation
1464	CH_3 asymmetric deformation
1397	COO^- symmetric stretching
1350	CH_2 wagging mode
1281,1251,1235	CH_2 twist
1172	COO^- rocking
1159	C-H stretching; (NH_3) rocking
1112	(NH_3^+) rocking
1009,719,653	CH_2 rocking
947,932,805,785,772	C-C stretching
882	CH_3 rocking; NO_2 rocking
866	NH_2 rocking
748	NO_2 wagging mode
588	COO^- wagging mode
552	NO_2 rocking; C-C deformation

(v) NMR studies

An effective method for identifying organic molecules is proton nuclear magnetic resonance (NMR) [21]. The signals and related chemical shifts of DLMM are displayed in the ^{1}H NMR spectrum (Fig. 5). The peak at 6.232 ppm, which is one of the doublet, is attributed to the maleic acids -CH=CH-. The triplets at 3.937 ppm, 3.949 ppm, and 3.962 ppm belong to the COO-C-H group of esters.

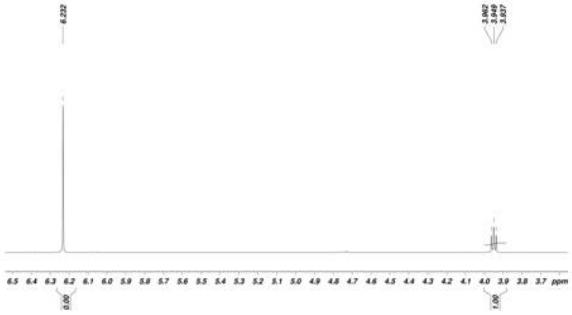

Figure 5: ^{1}H NMR spectrum of DLMM compound

Another crucial analytical method utilized to investigate the structure of DLMM single crystals is ^{13}C NMR spectrum analysis. According to the ^{13}C NMR spectrum (Fig. 6), the signal at 13.869 ppm is due to the existence of a CH_3 sulphur group that contains DL-methionine. Sulfur in DL-methionine causes protein to collect and become physiologically active in the grown material. Due to the influence of the nearby CH group, the signals at 28.623 and 29.137 ppm are grouped into a doublet. The aromatic groups of carbons are responsible for the signal at 133.03 ppm. A signal at 52.534 ppm is attributed to the CH_2 and CH carbon of the amino acid. Maleic acid has a carbonyl carbon of COOH group which is responsible for the doublet peaks at 170.223 and 172.615 ppm. The molecular structure of the title compound is thus confirmed from the FTIR and NMR studies. It is understood from the above studies in the present investigation that the maleate compound of DL-methioninium exhibits bioactivity.

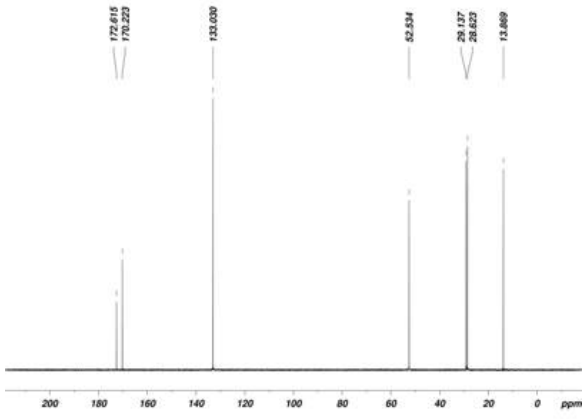

Figure 6: ^{13}C NMR spectrum of DLMM compound

(vi) UV-Vis absorption spectrum

Fig. 7 depicts UV-Vis absorption spectrum of the grown compound measured in the 300–750 nm regions. The insert of Fig. 7 displays the curve between hυ and $(\alpha h\upsilon)^2$ from which optical band gap has been calculated as 3.96eV.

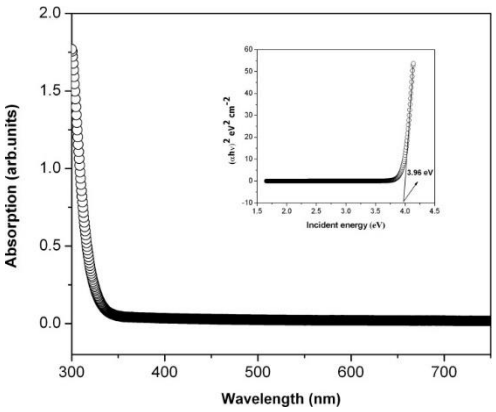

Figure 7: Absorption spectrum of DLMM compound

The value of optical band gap suggests that the material possess good optical properties with the moderate value of optical absorption coefficient. The lesser value of optical absorption coefficient is one of the additional requisite conditions for the material to exhibit antibacterial activity.

(vii) Antibacterial activity

Stock cultures were kept on a slant of nutritional agar at 4 °C. By transferring a loop of cells from the stock cultures into test tubes of nutrient broth for bacteria that were cultured for 24 hours at 37° C and active

cultures for tests were created. The agar well diffusion method was used to conduct the assay [22, 23].

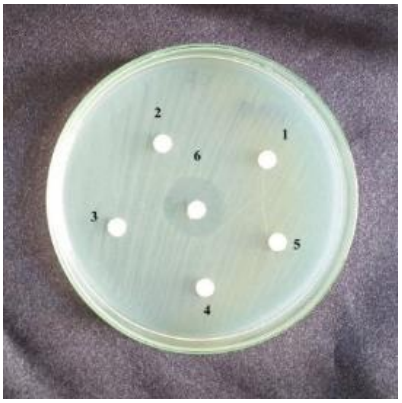

Figure 8 a: Zone of inhibition in Enterococcus faecalis

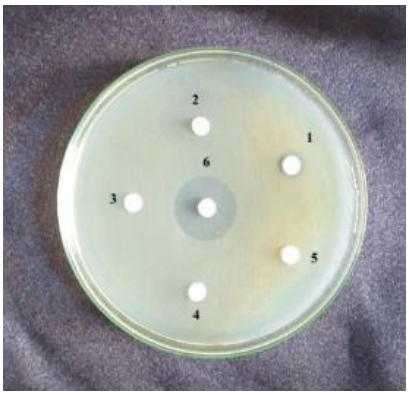

Figure 8 b: Zone of inhibition in Klebsiella pneumonia

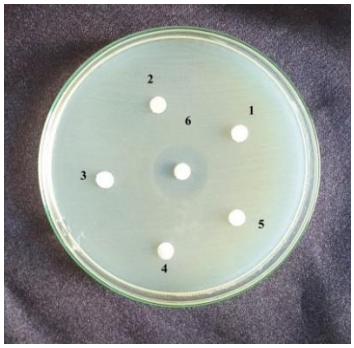

Figure 8 c: Zone of inhibition in Sraphylococcus aureus

In Muller Hinton agar (MHA) medium, the disc diffusion method was used to test the sample's antibacterial activity. 1 gm of agar was added after dissolving the Muller Hinton agar medium, which weighed 3.8 gm, in 100 ml of distilled water. The medium was then stored to undergo sterilization. The media was put into sterile petri plates after sterilization, and they were given one hour to harden. The inoculums were applied to the solid plates with sterile swabs wet with the bacterial suspension after the medium had hardened. 20 µl of the DLMM sample at the specified concentrations (1000 g, 500 g, 250 g, and 125 g) were used to create the discs. DMSO in 20 µl and a positive control Streptomycin (1 mg/ml) was added to 20 µl of MHA plates. At 37°C, these plates were incubated for 24 hours. The diameter of the zone of inhibition was then measured to assess the rate of microbial growth. From the study, it is observed that the growth of microorganism Sraphylococcus aureus and Enterococcus faecalis were very well inhibited by the positive control 20 µl of Sraphylococcus aureus (Table 3).

Table 3. The diameter of zone of inhibition in bacterial activity

Microorganisms	Zone of Inhibition in mm					
	Concentrations (µg)				DMSO (20 µl)	Streptomycin (20 µl)
	1000	500	250	125		
Klebsiella pneumonia	11	7	-	-	-	22
Sraphylococcus aureus	-	-	-	-	-	19
Enterococcus faecalis	-	-	-	-	-	21

IV.CONCLUSION

The slow evaporation method was used to grown DL-methioninium maleate (DLMM) single crystal at room temperature correlating the study of nucleation kinetics. The grown crystal is found to exist in monoclinic system, according to the single crystal and powder X-ray diffraction investigations. The results of molecular structure indicate that the cationic form of the DL-methioninium molecule consists of a

protonated amino group and an uncharged carboxylic acid group. The maleic acid molecule exists in its mono-ionized state. The DLMM optical absorption study has been used to evaluate the optical band gap of the grown crystal as 3.96 eV. Fourier transform infrared spectroscopy was used to identify the various vibrational functional groups of the DLMM. The molecular structure of DLMM was confirmed by FT-NMR spectroscopic investigation. The sulphur content was confirmed from the molecular structure of the crystal. The sulphur content is mainly responsible to exhibit the antibacterial activity. The material has positive control to successfully prevent the development of the microorganisms Staphylococcus aureus and Enterococcus faecalis, according to biological activities of DLMM crystal. Due to the presence of sulphur, it has been discovered that the material has antibacterial properties to prevent some bacterial growths in living things. The antibacterial activity of the grown material DL-methioninium maleate can find biological applications such as treatment of liver conditions and prevention of hypercholesterolemia.

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