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DNA Binding Studies through Electronic Absorption and Electrochemical Method: Synthesis, Structural Elucidation, Thermal and Hardness Characterization of 2-Methyl Imidazolinium-3-Nitropthalate

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Abstract

A hydrogen bonded organic salt, 2-Methyl Imidazolinium-3-Nitropthalate ($2M_{I3}NP$) was synthesized and single crystals grown employing solution growth technique at ambient temperature. The ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded to establish the molecular structure. The crystal structure has been determined by single crystal X-ray Powder Diffraction (XRD) analysis and the title crystal belongs to monoclinic system with the space group, $P2_1/c$. Fourier Transform Infrared (FTIR) spectrum has been carried out to identify the various functional groups present in the synthesized compound. The thermal stability of the crystal was investigated by TG/DTA analyses. The mechanical strength was determined by Vicker's micro hardness test. The interactions of the synthesized compound with calf thymus DNA were studied using absorption spectroscopy and cyclic voltammetry which revealed that the compounds could interact with calf thymus DNA through intercalation having binding constant (K_b) value of 3.2×10^4 M^{-1}.

Keywords: Biomaterials; Crystal growth; Crystal structure; Spectroscopy; Thermal analysis

Abbreviations:

$2M_{I3}NP$: 2-Methyl Imidazolinium-3-Nitropthalate; NMR: Nuclear Magnetic Resonance; XRD: X-ray Powder Diffraction; FTIR: Fourier Transform Infrared.

Introduction

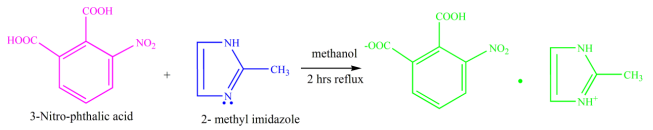
Crystal engineering plays a crucial role to obtain multi-component crystalline materials with enhanced physicochemical properties [1,2]. Much attention has been paid to design and synthesize materials based on hydrogen bonding interactions in the field of supra-molecular chemistry [3,4]. Hydrogen bonding is one of the important types of non-covalent bond interactions which play an important role in chemical and crystal engineering, as well as in supra-molecular chemistry [5-7]. The hydrogen bonding between carboxylic acids and heterocyclic nitrogen atoms is utilized to generate supra-molecular assemblies of organic molecules [8]. The proton transfer interaction of compound between organic acid (acceptor) and base (donor) leads to the enhancement of the antibacterial and antifungal activities, DNA binding and photocatalysts [9-11]. The supra-molecular synthon approach involving carboxylic acid and nitrogen containing functionalities is an important tool in the development of new crystal structures [12,13]. Hydrogen bond interactions are of attractive for the existence and great importance in life body, such as the double helix of DNA and the stabilization of secondary structure of protein and also useful in chemical, catalytic, and biochemical processes, chemical and crystal engineering, as well as in supra-molecular chemistry [14,15]. Many important biological molecules such as histidine, vitamin B12, DNA, purines, histamine and biotin contain imidazole ring system [16]. The protonated and deprotonated nitrogen present in the imidazole and its derivatives are involved to coordinate with organic and inorganic compounds which help to make the three dimensional supra-molecular assembly [17-19].

In this background, we report the formation of the proton transfer molecular salt obtained from the reaction of 2-methyl imidazole with 3-nitrophthalic acid. Crystal structure, supra-molecular assembly through hydrogen bonding interactions, thermal, mechanical and biological properties of the title crystal are presented in this paper.

Experimental Details

Synthesis and growth of $2M_{I3}NP$ single crystal

AR grade of 2-methyl imidazole and 3-nitrophthalic acid were purchased and used as such without further purification. 1:1 molar solution of reactants was prepared separately in methanol and henceforth mixed together. Then the solution was stirred well for about 3 hrs to dissolve completely and the resulting solution was filtered through a quantitative Whatman 41 grade filter paper to eliminate the suspended impurities. The filtrate was then collected in a 250 mL beaker and kept aside unperturbed in an atmosphere most suitable for the growth of single crystals. Good optical quality single crystals of the title compound were harvested in about 12^{th} day. The collected crystals were recrystallized using dry methanol to get good quality crystals (Scheme 1).



Scheme 1: The reaction between 2-methyl imidazole and 3-nitrophthalic acid.

Instrumentation techniques

The chemical compounds (Merck and Hi-Media) were of analar grade. The electronic absorption spectrum was recorded in DMSO employing a Systronics 2202 Double beam spectrophotometer in the wavelength range 200-600 nm. In order to confirm the presence of various functional groups, the title crystal was subjected to FTIR spectral analysis on a Perkin Elmer FTIR 8000 spectrophotometer in the frequency range 4000-400 cm^{-1} using the KBr pellet method. ^{1}H and ^{13}C NMR spectra were recorded employing a Bruker AV III 500 MHz spectrometer equipped with a 5 mm triple resonance broadband probe using TMS as an internal standard. The thermal stability of the title crystal was established by carrying out TG and DTA thermal analyses simultaneously on a NETZSCH STA 409 C/CD TG/DTA thermal analyzer in between 0 and 800°C in nitrogen atmosphere at a heating rate of 10°C min^{-1}. Vickers microhardness studies have been carried out on as-grown single crystals using HMV SHIMADZU tester.

Single crystal XRD analysis

Single crystal X-ray diffraction data of OPDP crystal was collected at 20°C on an Oxford Xcalibur Gemini EOS CCD diffractometer using Cu-Kα radiation equipped with a fine focused sealed tube. The unit cell parameters were determined and the data collections of $2M_{I3}NP$ crystal were performed using a graphite monochromated Mo-Kα radiation (0.71073 Å) by φ and ω scans. The structure of the crystal was solved by direct method [20] using SHELXS-97 and refined by full matrix least squares on F^2 (SHELXL-97) [21]. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were placed in calculated positions and refined as riding atoms.

DNA binding experiments

Absorption spectroscopic studies: All experiments involving the binding of compounds with CT-DNA were carried out in a doubly distilled water buffer with tris(hydroxy-methyl) aminomethane (Tris; 5 mM) and sodium chloride (50 mM) and adjusted to pH=7.2 with hydrochloric acid. A solution of CT- DNA in the buffer gave rise to UV absorbance of about 1.8-1.9 at 260 and 280 nm, indicating that the DNA was sufficiently free of protein. The CT-DNA concentration per nucleotide was determined spectrophotometrically by employing a molar absorptivity extinction coefficient of 6600 $dm^3mol^{-1}cm^{-1}$ at 260 nm. The compounds were dissolved in a mixed solvent of 5% DMSO and 95% Tris-HCl buffer. Stock solutions were stored at 4°C and used within 4 days. Absorption titration experiments were performed with fixed concentrations of the complexes (25 μM) with varying concentrations of DNA (0-50 μM). While measuring the absorption spectra, an equal amount of DNA was added to both the test solution and the reference solution to eliminate the absorption of DNA itself.

Cyclic voltammetry studies: Cyclic voltammetry studies were performed using a CHI 604A electrochemical analyzer. Experiments were carried out in a 15 mL three-electrode electrolytic cell. The working electrode was glassy carbon; a platinum wire was used as the counter electrode and an Ag/AgCl electrode saturated with KCl was used as the reference electrode. The cyclic voltammograms of the compound were recorded in (1:2) DMSO-buffer solutions at a rate of 100 mVs^{-1} where Tris-HCl buffer and 0.1 M Bu_4NClO_4 solution were used as the supporting electrolytes. Oxygen was removed by purging the solutions with pure nitrogen that had been previously saturated with solvent vapours. All electrochemical measurements were performed at 25°C.

Results and Discussion

The crystal data and the structure refinement are given in Table 1.

Empirical formula	$C_{12}H_{11}N_3O_6$
Formula weight	293.24
Temperature	296(2) K
Wave length	0.71073 Å
Crystal system	Monoclinic
Space group	P21/c
Unit cell dimension	a=12.4680(4) α=90°,
	b=12.0790(4) Å β=98.4540(10)°,
	c=8.6799(4) Å γ=90°
Volume	1293.00(8) $Å^3$
Z	4
Density (calculated)	1.506 Mg/m^3
Absorption coefficient	0.123 mm^{-1}
Crystal size	0.25 × 0.20 × 0.20 mm
Theta range for data collection	1.65 to 28.33°
Index ranges	-16<=h<=16, -15<=k<=16, -7<=l<=11
Reflections collected	32106
Independent reflections	3203 [R(int)=0.0368]
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9758 and 0.9698
Refinement method	Full-matrix least-squares on F2
Data/ restraints/ parameters	3203/0/194
Goodness-of-fit on F2	1.109
Final R indices [I>2sigma(I)]	R1=0.0601, wR2=0.1580
R indices (all data)	R1=0.0904, wR2=0.2039

Table 1: Crystal data and structure refinement for the $2M_{I3}NP$ crystal.

The crystal belongs to the monoclinic system with the space group, P21/c and the lattice parameters are a=12.4680 (4) Å, b=12.0790 (4) Å,

c=8.6799 (4) Å, α=90°, β=98.4540 (10)°, γ=90°. Figure 1 shows the ORTEP view of the molecule drawn at 50% probability thermal displacement ellipsoids with the atom numbering scheme. The asymmetric unit composes one 2-Methyl imidazolinium ion and one 3-Nitropthalate ion. In addition to N-H…O hydrogen bond, the O-H…O hydrogen bond also participates to stabilize $2M_{I3}NP$ in the crystal lattice. From the data, it is noted that the strength and directionality of the N-H...O, O-H...N and C-H....O hydrogen bonds between acidic part and basic part are sufficient enough for the formation of the salt crystal. This kind of interactions is very useful to construct the three-dimensional supra-molecular assembly and in the biological activity of the compound.

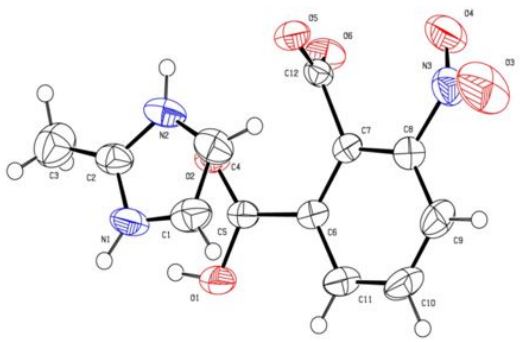


Figure 1: ORTEP diagram of $2M_{I3}NP$ crystal.

In FTIR spectrum, the carboxylic OH stretching vibration is observed as a broad band at 3680 cm^{-1}. The N-H symmetric stretching vibration is appeared at 3108 cm^{-1}. The C-H stretching of the methyl group and aromatic C-H stretching observed at 2900 and 2703 cm^{-1} respectively. The C=O and C=C stretching vibrations are observed at 1928 and 1680 cm^{-1} respectively. The bands at 1578 and 1350 cm^{-1} are assigned asymmetric and symmetric NO_2 stretching vibration [22]. The C-H bending vibration is appeared at 1455 cm^{-1}. The C-N stretching vibration is observed at 1314 cm^{-1}.

In ^1H NMR spectrum (Figure 2a), the downfield signal at δ 12.62 acid is assigned to COOH proton of 3-nitrophthalate moiety. The singlet signal observed at δ 1.26 owes to methyl protons of 2-Methyl imidazole moiety. The NH proton signal appears at δ 7.94 and two overlapping doublet signals centered at δ 7.59 is due to C-H Protons of 2-Methyl imidazole moiety. A triplet centered at δ 7.62 is attributed to C_4 aromatic proton present in Phthalic acid moiety. The C_3 and C_5 aromatic C-H protons are observed as two doublet signals at δ 8.11 and δ 7.99 respectively. From ^{13}C NMR spectrum (Figure 2b), the appearance of twelve distinct carbon signals explicitly confirms the molecular structure of the crystal. The appearance of two overlapping carbon signals at 166.65 and 166.61 owes to the presence of two carboxyl carbons of the Nitro Phthalic acid moiety. The singlet peak observed δ 148.43 is due to C_8 carbon of the same moiety. The signal appeared at δ 148.31 is assigned to C_2 carbon of 2-methyl imidazole moiety. The peaks observed at δ 134.25 and 134.14 are due to C_{11} and C_{10} carbons of Nitrophthalic acid moiety respectively. The carbon signals of C_9 and C_6 in Nitrophthalic acid moiety appear at δ 127.14 and 125.72 respectively. The signal observed at δ 119.34 ppm owes to C_7 carbons of Nitrophthalic acid moiety. The C_4 and C_5 carbon of same kind in aromatic ring of 2-methyl imidazole moiety are observed at δ 114.9. In up field, the signal observed at δ 11.3 is due to methyl carbon of imidazole moiety.

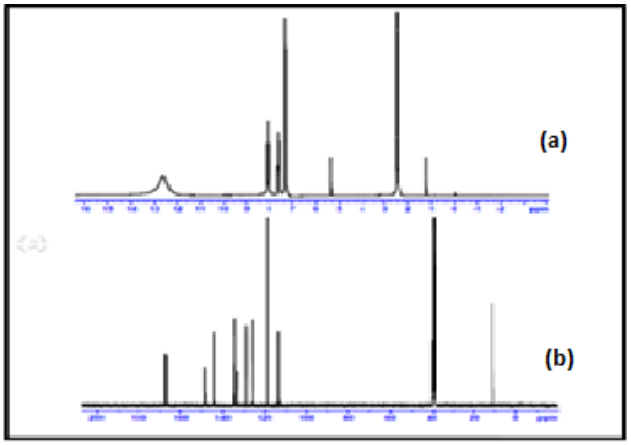


Figure 2: ^1H and ^{13}C NMR spectrum of $2M_{I3}NP$ crystal.

In the thermogram, the TG curve reveals that the compound is stable up to 200°C and decomposes into single stage immediately after the melting with the weight loss of 91.73%. The DTA reveals the same changes shown by the TG. The sharp endothermic peak in DTA at 200°C indicates the melting point of the compound. The sharpness of the endothermic peak shows good degree of crystallinity and purity of the compound. From the DTA curve it is observed that the material is stable up to 200°C the melting point of the substance then it undergoes irreversible exothermic changes at 293.47°C.

The hardness of the crystals was calculated using the relation, Hv=1.8544P/d2 MPa, where Hv is Vicker's microhardness number, P is the indentor load and d is the diagonal length of the impression. When the load was increased from 25 to 100 g, cracks were developed on the smooth surface of the crystal. It is observed that the grown crystal exhibits reverse Indentation Size Effect (ISE). By plotting log p verses log d, the value of the work hardening coefficient n was found to be 3.149. According to Onitsch, $1.0 \leq n \leq 1.6$ for hard materials and $n>1.6$ for soft materials [23]. Hence, it is concluded that crystal belongs to the soft material category.

DNA binding spectra of the compound in the absence and presence of CT-DNA is given in Figure 3a. These results suggest strong interactions between DNA and compounds, and it is also likely that these compounds bind to the DNA helix via intercalation. After the compounds intercalate to the base pairs of DNA, the π^* orbital of the intercalated compounds could couple with π orbitals of the base pairs, thus decreasing the $\pi \rightarrow \pi^*$ transition energies, hence resulting in hypochromism [24,25]. In the intrinsic binding constant (Kb) can be determined from the following equation [26],

$$DNA/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+1/Kb\ (\varepsilon_b-\varepsilon_f)$$

From the plot of $DNA/(\varepsilon_a-\varepsilon_f)$ versus [DNA], Kb is calculated by the ratio of slope to the intercept. The magnitude of intrinsic binding constant (Kb) value for compound is 3.2×10^4 M^{-1}. Further, the observed binding constant values are comparable to that of the classical intercalator ethidium bromide [27]. From the above DNA binding results, it is obvious that the ruthenium complexes are bound to DNA via intercalative mode.

Further, DNA binding mode of the compound was confirmed by using CV technique. The decrease in the peak current of the compound in the presence of CT-DNA may be the result of the slow diffusion of the compound into the DNA molecules, forming an equilibrium mixture of the free and DNA-bound $2M_{I3}NP$ compound to the electrode surface. The cyclic voltammograms of the title compound in the absence and presence of CT-DNA are shown in Figure 3b. The decrease of the voltammetric currents in the presence of DNA may be attributed to slow diffusion of the compound bound to CT-DNA. This in turn indicates the extent of binding affinity of the ruthenium complexes to DNA by intercalative binding mode [28,29]. The electronic spectroscopic and cyclic voltammetric studies confirm that the complexes bind to DNA by intercalation binding mode.

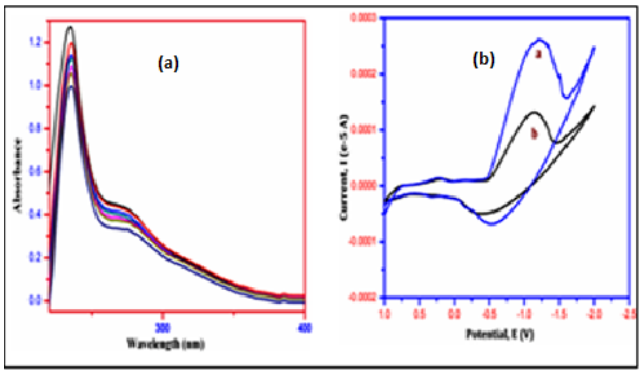


Figure 3: The study of DNA binding interaction with $2M_{I3}NP$ through Electronic absorption spectroscopy and CV technique.

Conclusion

The charge transfer compound was synthesized, and single crystals grown by the slow solvent evaporation solution growth method. The molecular structure of the crystal was established by NMR technique. Single crystal X-ray diffraction study revealed that the crystal belongs to monoclinic system with space group P21/c. The presence of various functional groups was confirmed by FTIR spectroscopic study. The thermal analyses were studied to investigate the thermal stability of the compound and it is found that the crystal is stable up to 200°C. The Vicker's micro hardness study reveals the soft nature of the crystal. Further, The DNA binding properties of the synthesized compound were examined using the absorption spectroscopic and cyclic voltammetry methods, which revealed that the compounds could interact with CT-DNA through intercalation. The results obtained from our presented work would be useful for understanding the mechanism of interactions of the compounds binding to DNA and helpful in the development of their potential biological, pharmaceutical and physiological implications in the future.

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