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DNA BINDING AND NUCLEASE ACTIVITY OF STRUCTURALLY CHARACTERIZED COPPER(II) COMPLEX

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Keywords:

Copper(II) complex, Spectral properties, DNA binding studies and nuclease activity

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ABSTRACT: A mononuclear copper(II) complex $[Cu(bpy)_2(NO_3)]$ NO_3 H_2O (bpy = 2,2'-bipyridine) has been synthesized and characterized based on physico-chemical spectral data. The complex is characterized based on electronic, IR and ESR spectroscopies. The structure of the complex is determined by single crystal X-Ray crystallography. The complex crystallizes in monoclinic space group P-1. Structural analysis reveals that the complex adopts square pyramidal geometry with CuN_4O chromophore. The interactions of these complexes with calf thymus DNA have been investigated using absorption spectrophotometry. The high binding constant 4.89 x 10^6 $M^{-}1$ may be due to the strong electrostatic attraction between the cationic complex and the negatively charged phosphodiester backbone of DNA. Nuclease activities of complexes are investigated on double stranded pBR322 plasmid DNA using gel electrophoresis experiments under different conditions. The complex cleaves DNA more effectively in the presence of oxidant.

INTRODUCTION: Copper plays several important roles in biology and medicine. It is constituent of many enzymes and proteins [1]. Penta coordinated metal plays significant role in several proteins and enzymes. For example, pent coordinate copper is present in Cu-Zn superoxide dismustase. Improper regulation of copper causes severe diseases such as Wilson's and Menke's diseases and some neurological disorders [2]. Classical polypyridyl ligands such as 2,2-bipyridine and 1,10-phenanthroline are strong bidentate ligands which form stable chelates with many transition metals.

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These ligands have a starring role in the field of chemistry and molecular recognition due to their usefulness in medicine and in the industry [3]. Various mixed ligand complexes with these ligands have been synthesized and their antibacterial and antifungal activities have been studied [4-6].

Interaction of metal complexes with nucleic acids is an exciting area of research due to their potential use as drugs, tools for biochemical and biomedical applications in gene regulation. Deoxyribonucleic acids (DNA) is the primary target for most anti-cancer drugs [7, 8]. Nuclease activity of copper- 1,10-phenanthroline complex was investigated in the year 1979. Since then the Copper complex are widely used as foot printing DNA molecules and cleaving agents [9-11] over the past two decades. DNA binding and cleavage activities of Copper complex with 1,10-phenanthroline are reported from our laboratories. Crystal structure and electronic properties of bis (2,2'-bipyridyl)-nitratocopper(II)

nitrate monohydrate complex are reported [12] in the year 1981. In continuation ongoing research work [13-15] from our laboratories we have revisited the structure determination of the complex with good resolution and considered worthwhile to investigate DNA binding and nuclease activity of structurally characterized pentacoordinate copper (II) complex, $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$

Experimental:

Chemicals:

Analytical grade 2, 2'- bipyridyl, and $Cu(NO_3)_2.\ 3H_2O$ were obtained from Merck. The solvents were distilled before use. Calf thymus DNA (CT-DNA) and plasmid pBR322 (cesium chloride purified) were purchased from Genie Bio labs, Bangalore, India. Agarose (molecular biology grade) and ethidium bromide (EB) were obtained from Sigma. Solutions of CT-DNA in 50 µM Tris-HCl (pH, 7.0) gave the ratio of UV absorbance at 260 and 289 nm of 1.8 indicating that the DNA was sufficiently free of protein concentration. The DNA concentration was determined by UV absorbance at 260 nm using molar absorption coefficient 6600 M^{-1}. Stock solutions were kept at 4^oC and used after not more than four days. DNA binding studies were performed in 50 mM NaCl/5mM Tris base, pH, 7.0 buffer.

Physical measurements:

The elemental analyses were performed using a Perkin Elmer 2400 CHNS elemental analyzer. The molar conductance of the complex in DMF (10^{-3} M) solution was measured at 28°C with a Systronic Model 303 direct reading conductivity bridge. The electronic spectra were recorded in DMF with a Perkin Elmer UV Lamda−50 spectrophotometer. FT–IR spectra in KBR disc were recorded in the range 4000–400 cm^{-1} with a Perkin Elmer spectrum 100spectrometer. The cyclic voltammetry was performed with a CH instruments 660C electrochemical analyzer and a conventional three electrode, Ag/AgCl reference electrode, glassy carbon working electrode and platinum counter electrode.

Nitrogen gas was purged and measurements were made on the degassed (N_2 bubbling for 5 min) complex solution in DMF (10^{-3} M) containing 0.1 M tetrabutylammonium hexaflourophosphate (TBAHEP) as the supporting electrolyte.

Preparation of complex:

To a stirring solution of $Cu(NO_3)_2.\ 3H_2O$ (1.21 g, 5 mmol) in MeOH (10 mL), a solution of 2, 2'-bipyridyl (4.95 g, 25 mmol) in MeOH (50 mL) was added slowly. The stirring was continued for 30 min. The dark blue coloured complex was formed. It was collected by filtration and washed with a small quantity of MeOH. Yield: 69%; M.P. 228-230 ^oC,

X-ray crystallography:

Crystal data were collected using the Enraf–Nonius CAD4- MV31 single crystal X-ray diffractometer, Indian Institute of Technology-Madras, Chennai. Enraf–Nonius CAD4- MV31 single crystal X-ray diffractometer is a fully automated four circle instrument controlled by a computer. It consists of an FR 590 generator, a goniometer, CAD4F interface and a microVAX3100 equipped with a printer and plotter. The detector is a scintillation counter. A single crystal is mounted on a thin glass fiber fixed on the goniometer head. The unit cell dimensions and orientation matrix are determined using 25 reflections and then the intensity data of a given set of reflections are collected automatically by the computer. An IBM compatible PC/AT 486 is attached to micro VAX facilitating the data transfer on to a DOS floppy of 5.25″ or 3.5″. Maximum X-ray power is 40 mA × 50 kV. The data collected were reduced using SAINT program. The trial structure was obtained by direct method using SHELXS-86, which revealed the position of all non-hydrogen atoms and refined by full-matrix least squares on F^2 (SHELXS-97) and graphic tool was DIAMOND for windows. All non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were treated with a mixture of independent and constrained refinements.

DNA Binding Study:

Interaction of Copper complex with calf thymus DNA was studied by UV–Vis spectroscopy. after no more than 4 days. The electronic spectra of metal complexes in aqueous solutions were monitored in the absence and presence of CT-DNA. Absorption titrations were performed by maintaining the metal complex concentration 20 ×

10^{-6} M and varying the nucleic acid concentration (0–7.36× 10^{-6} M). Absorption titration experiments were performed by maintaining the metal complex concentration constant while gradually increasing the concentration of CT-DNA with each addition of 10 µl DNA. The ratio of r = [complex]/[DNA] values vary from 23.41 to 2.60. Absorption spectra were recorded after each successive addition of DNA solution. The intrinsic binding constant (K_b) was calculated by using the equation,

$$[DNA]/(\varepsilon_a-\varepsilon_f) = [DNA]/(\varepsilon_b-\varepsilon_f) + 1/ K_b (\varepsilon_b-\varepsilon_f) \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where [DNA] is the molar concentration of DNA in base pairs, ε_a, ε_b and ε_f are apparent extinction coefficient (A_{obs}/[M]), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively.

Gel electrophoresis:

The extent of cleavage of DNA by the copper(II) complexes was monitored using agarose gel electrophoresis with pBR 322 DNA. After incubation for 30 min at 37°C, the samples were added to the loading buffer containing 0.25% bromophenol blue + 0.25% xylene cyanol + 30% glycerol, and solutions were loaded on 0.8% agarose gel containing 100 µg of ethidium bromide. Electrophoresis was performed at 75 V in TBE buffer until the bromophenol blue reached to 3/4 of the gel. Bands were visualized by UV transilluminator and photographed. The efficiency of DNA cleavage was measured by determining the ability of the complex to form open circular (OC) or nicked ircular (NC) DNA from its supercoiled (SC) form. The reactions were carried out under oxidative and/or hydrolytic conditions. Control experiments were done in the presence of hydroxyl radical scavenger DMSO (4 µL).

RESULTS AND DISCUSSION:

The complex is stable at room temperature, non-hygroscopic, partially soluble in water, methanol, ethanol and readily soluble in acetonitrile (CH_3CN), DMF and DMSO. Analytical data (**Table 1**) are consistent with the proposed molecular formulae of complex.

TABLE 1: ANALYTICAL DATA OF COMPLEX

Complex formula	Elemental analysis, Found (Calcd.)			
	C	H	N	M
$[Cu(bpy)_2(NO_3)]$ NO_3 H_2O	45.85 (46.33)	3.45 (3.47)	16.18 (16.21)	11.15 (11.19)

The complex is partially soluble in water, methanol, ethanol and readily soluble in acetonitrile (CH_3CN), DMF and DMSO. The complex was dissolved in DMF to perform conductivity measurements. Molar conductivity (90 $\Omega^{-1}cm^2$ mol^{-1}) suggest that the complex (**1**) is 1:1 electrolyte. The magnetic moment of complex **(1)** is found to be 1.68 BM. Magnetic moments of complex correspond to spin-only value (1.73 BM) of mononuclear copper(II) complexes.

The electronic spectrum (**Fig. 1)** of copper(II) complex in DMF solvent was recorded**.** The complex (**1**) exhibits two strong bands and one weak band at 36800, 33154 and 16512 cm^{-1} respectively assigned to π - π*, CT and d-d transitions. The position of d-d band is suggestive of square pyramidal geometry[16, 17]

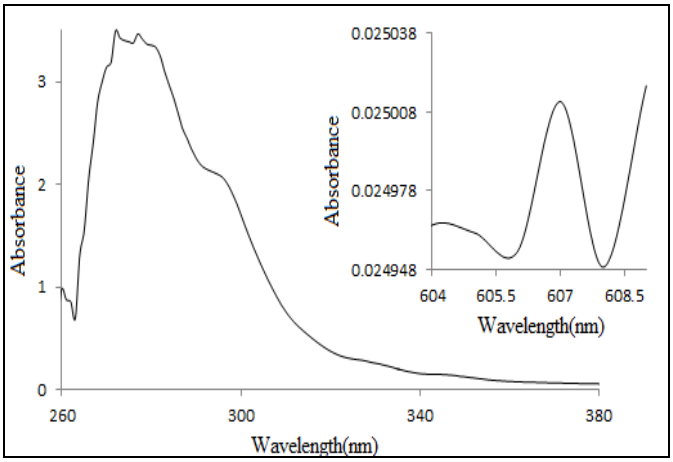

FIG 1: ELECTRONIC SPECTRUM OF $[Cu(bpy)_2(NO_3)]$ NO_3 H_2O

IR spectrum studies:

Infrared spectrum of the complex is shown in **Fig. 2**. Selected IR spectral bands are given **Table 2.** IR spectrum of complex 1 shows strong absorptions at 1473, 1255 and 1031 cm^{-1}, which are typical for ν (NO) of mono-coordinated nitrate based on C_{2v} symmetry.

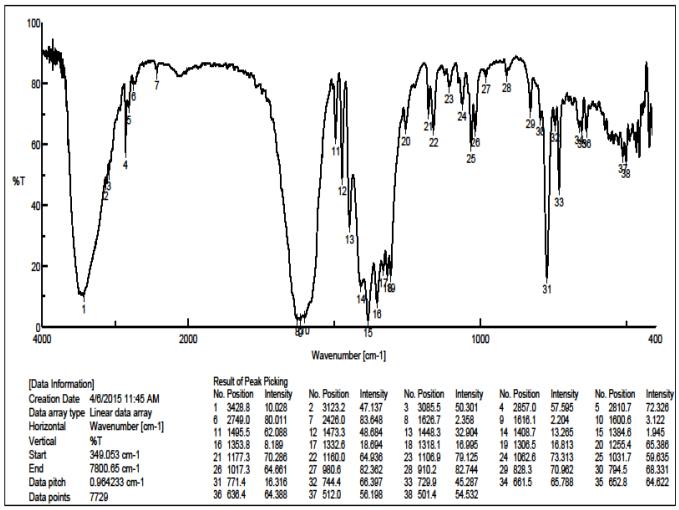

FIG. 2: IR SPECTRUM OF [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O.

TABLE 2: SELECTED IR SPECTRAL DATA OF [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O COMPLEX

Band frequency (cm^{-1})	Assignment
3428	O-H stretch of hydrated water molecules
3085	Aromatic C-H stretch
2857	---
1626	ν(C-N) bpy ring str.
1473	ν_{NO} of nitrato (B_2)
1384	Ionic nitrate
1332	--
1255	ν_{NO} of nitrato (A_1)
1031	ν_{NO} of nitrato (A_1)
771	--
501	

A strong peak is present at 1384 cm^{-1} in IR spectra of[Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O complex indicating the presence of ionic nitrate (D_{3h} symmetry), which is in agreement with the results of the conductivity experiments. Complex also exhibits a strong band at 3,432 cm^{-1} assigned to –OH stretching of a water ligand.

Description of crystal structure of complex, [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O (1):

Slow evaporation of methanolic solution of complex **1** gave a single crystal of size 0.300 x 0.250 x 0.250 mm^3 and was mounted on glass fibre. The cell parameters and the intensity data were obtained using Bruker Smart Apex CCD diffractometer equipped with a fine –focused sealed tube. The complex [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O crystallizes in monoclinic with space group of P21/c and with the unit cell a = 12.1125(3) A^0, α= 61.7570(10)°, b = 14.6202(4) A^0 , β= 85.531(3)°, c = 15.0912(4) A^0 , γ = 67.572 (2)° and V= 2158.87(10) Å^3 , Z= 4. Crystal data and structure refinements are shown in **Table 3**. The molecular structure of complex **1** is shown in **Fig. 3** together with the numbering scheme in the metal coordination sphere. Unit Cell structure of the complex is shown **Fig. 4**

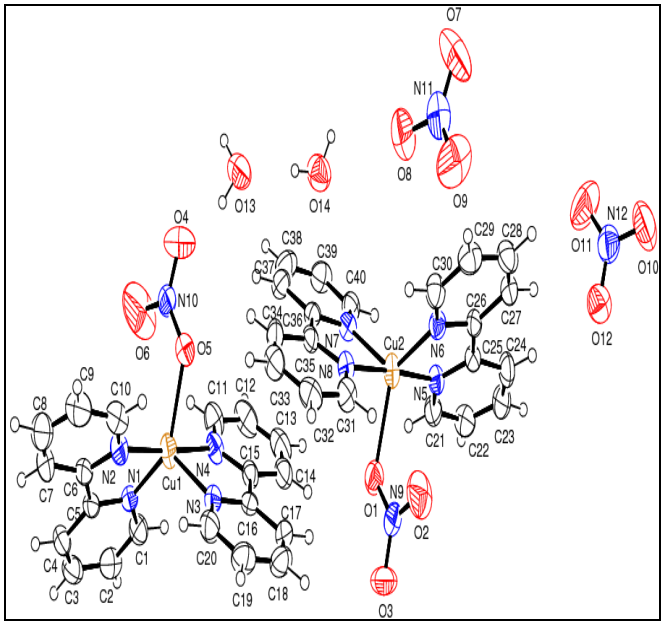

FIG. 3: ORTEP VIEW OF COMPLEX (1)

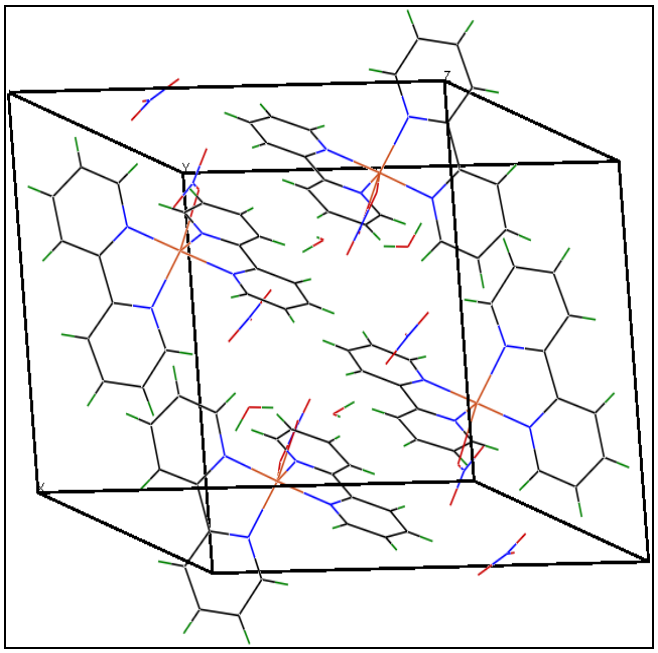

FIG. 4: UNIT CELL STRUCTURE OF

The complex: (1)

There are two independent molecules in the asymmetric unit amounting to FOUR molecules in the unit cell.

TABLE 3: CRYSTAL DATA AND STRUCTURE REFINEMENT FOR $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$

Parameter	Data
Empirical formula	$C_{20}\ H_{18}\ Cu\ N_6\ O_7$
Formula weight	517.94
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 12.1125(3) Å a= 61.7570(10)°. b = 14.6202(4) Å b= 85.531(3)°. c = 15.0912(4) Å g = 67.572(2)°.
Volume	2158.87(10) $Å^3$
Z	4
Density (calculated)	1.594 Mg/m^3
Absorption coefficient	1.068 mm^{-1}
F(000)	1060
Crystal size	0.300 x 0.250 x 0.250 mm^3
Theta range for data collection	2.094 to 24.999°.
Index ranges	-14<=h<=14, -17<=k<=17, -17<=l<=17
Reflections collected	40835
Independent reflections	40835 [R(int) = ?]
Completeness to theta = 24.999°	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7799 and 0.7401
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	40835 / 194 / 626
Goodness-of-fit on F^2	1.022
Final R indices [I>2sigma(I)]	R1 = 0.0649, wR2 = 0.1622
R indices (all data)	R1 = 0.1391, wR2 = 0.2051
Extinction coefficient	n/a
Largest diff. peak and hole	0.482 and -0.686 $e.Å^{-3}$

Selected bond lengths and bond angles are presented in **Table 4**. In the complex (**1**) the Cu ion is coordinated by four nitrogen atoms of two bipyridine ligands. Thus the four coordinate sites of copper are occupied by two bpy ligands. The fifth coordination site of copper is completed by oxygen atom of nitrate ligand (**Fig. 3**).

TABLE 4: BOND LENGTHS [Å] AND ANGLES [°] FOR $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$.

Bond	Bond lengths [Å]:-
N(1)-Cu(1)	2.011(6)
N(2)-Cu(1)	1.967(6)
N(3)-Cu(1)	2.079(6)
N(4)-Cu(1)	1.958(7)
N(5)-Cu(2)	1.963(6)
N(6)-Cu(2)	2.011(6)
N(7)-Cu(2)	2.075(6)
N(8)-Cu(2)	1.964(6)
O(1)-Cu(2)	2.199(5)
O(5)-Cu(1)	2.111(6)
Bonds	**Bond angles (^{0})**
N(4)-Cu(1)-N(2)	176.8(3)
N(4)-Cu(1)-N(1)	98.2(3)
N(2)-Cu(1)-N(1)	81.7(3)
N(4)-Cu(1)-N(3)	80.4(3)
N(2)-Cu(1)-N(3)	102.2(3)
N(1)-Cu(1)-N(3)	126.2(2)
N(4)-Cu(1)-O(5)	88.5(3)
N(2)-Cu(1)-O(5)	89.5(3)
N(1)-Cu(1)-O(5)	141.1(3)
N(3)-Cu(1)-O(5)	92.6(3)
N(5)-Cu(2)-N(8)	176.9(2)
N(5)-Cu(2)-N(6)	81.3(3)
N(8)-Cu(2)-N(6)	99.9(3)
N(5)-Cu(2)-N(7)	101.3(3)
N(8)-Cu(2)-N(7)	80.2(3)
N(6)-Cu(2)-N(7)	129.7(2)
N(5)-Cu(2)-O(1)	88.9(2)
N(8)-Cu(2)-O(1)	88.2(2)
N(6)-Cu(2)-O(1)	135.1(2)
N(7)-Cu(2)-O(1)	95.1(2)

Symmetry transformations used to generate equivalent atoms.

View of hydrogen bond net work and close packing are shown in **Figs. 5 and 6 (a, b c)** respectively. Hydrogen bonding data are given in **Table 5.**

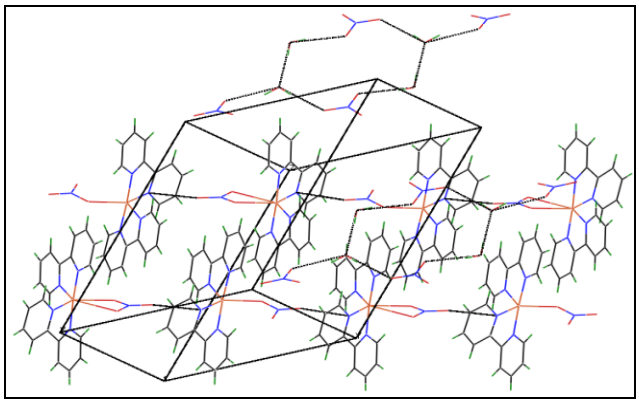

FIG. 5: VIEW OF HYDROGEN BOND NET WORK OF COMPLEX

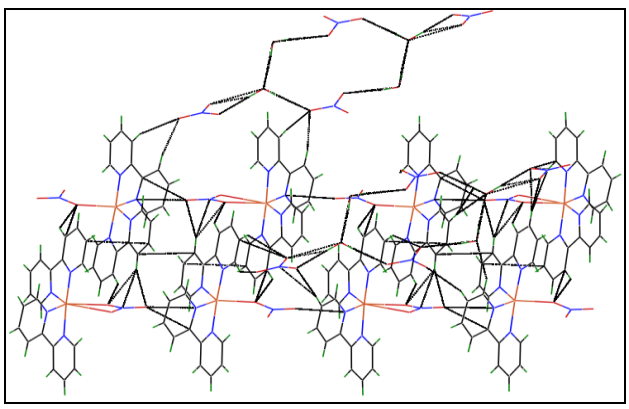

FIG. 6 (a): CLOSE PACKING VIEW

TABLE 5: HYDROGEN BONDING DATA FOR [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O [Å and °]

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
C(1)-H(1)...O(14)#1	0.93	2.50	3.336(11)	149.1
C(4)-H(4)...O(7)#2	0.93	2.42	3.338(12)	168.5
C(7)-H(7)...O(7)#2	0.93	2.56	3.425(12)	154.3
C(10)-H(10)...O(11)#3	0.93	2.52	3.241(12)	134.8
C(12)-H(12)...O(8)#4	0.93	2.64	3.188(13)	118.5
C(14)-H(14)...O(1)	0.93	2.43	3.264(11)	149.6
C(17)-H(17)...O(1)	0.93	2.63	3.468(10)	150.5
C(17)-H(17)...O(3)	0.93	2.59	3.433(10)	151.2
C(21)-H(21)...O(9)#5	0.93	2.38	3.198(11)	147.0
C(24)-H(24)...O(12)	0.93	2.47	3.399(11)	173.7
C(27)-H(27)...O(12)	0.93	2.47	3.390(11)	171.6
C(29)-H(29)...N(11)	0.93	2.67	3.477(12)	145.1
C(29)-H(29)...O(9)	0.93	2.51	3.429(12)	171.8
C(32)-H(32)...O(10)#6	0.93	2.48	3.247(12)	140.0
C(34)-H(34)...O(5)	0.93	2.32	3.084(10)	139.3
O(13)-H(13A)...O(12)#7	0.92(4)	2.02(5)	2.875(10)	154(9)
O(13)-H(13B)...O(8)#4	0.90(4)	2.03(5)	2.886(10)	157(8)
O(14)-H(14A)...O(13)	0.87(4)	2.10(5)	2.964(10)	170(10)
O(14)-H(14B)...O(10)#8	0.88(4)	2.06(5)	2.924(10)	164(9)

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y+1,-z+1 #2 x+1,y,z-1 #3 x,y,z-1
#4 -x,-y+1,-z+1 #5 x+1,y,z #6 -x,-y,-z+2
#7 x,y+1,z-1 #8 -x,-y+1,-z+2

The crystal data for complex (1) are different from previously reported [29] data of [(bpy)2 Cu(NO$_3$)]NO$_3$ CH$_3$OH and (bpy)$_2$Cu(NO$_3$)]NO$_3$.

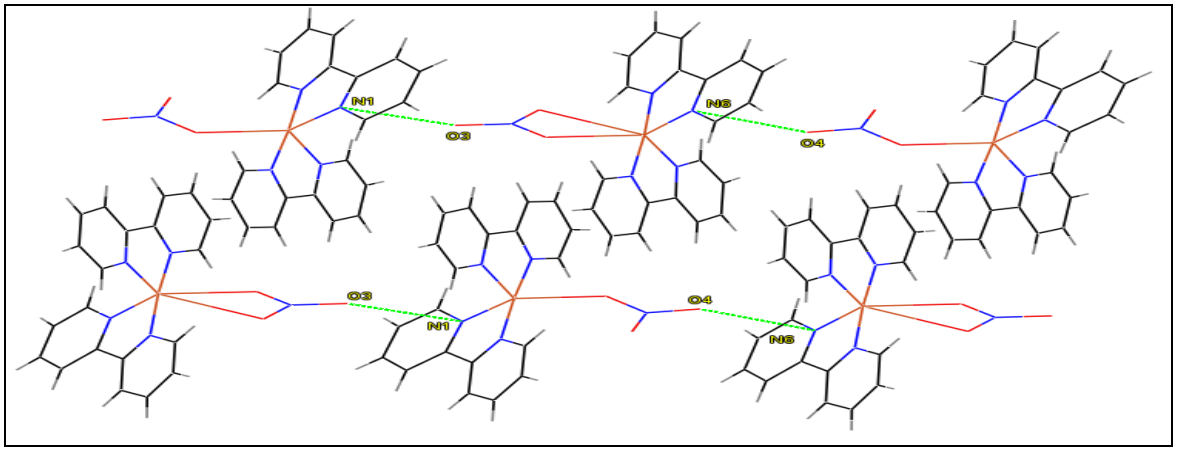

FIG. 6 (b): VIEW OF CLOSE PACKING SHOWING H- BONDING IN [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O.

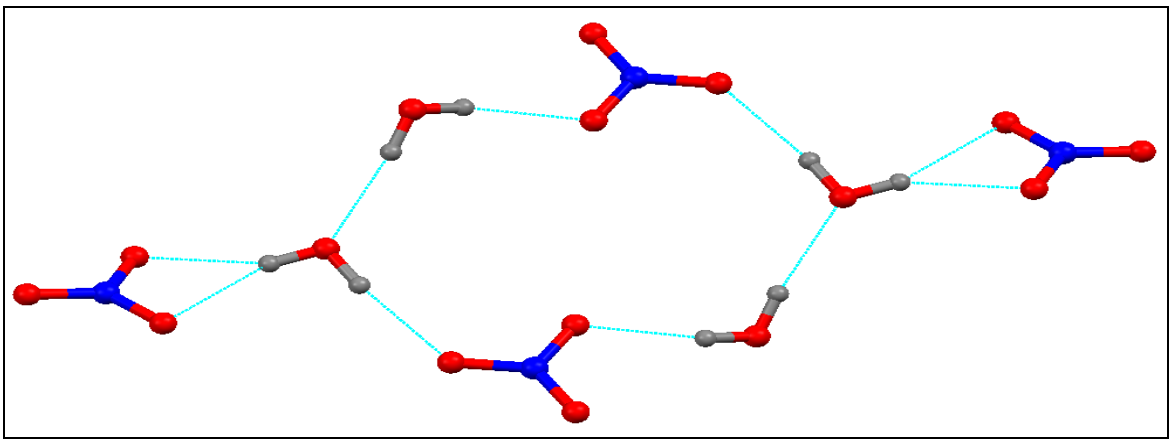

FIG. 6 (c): VIEW SHOWING H- BONDING BETWEEN H$_2$O AND NO$_3^-$ IN [Cu(bpy)$_2$(NO$_3$)] NO$_3$ H$_2$O

1. Non classical hydrogen bond of N···O type is present between the bipyridil nitrogen and the oxygen atom (not involved in bond formation with metal) of coordinate nitrate ion.
2. Ionic nitrates hydrogen bond with non coordinated water molecules.

ESR spectral studies:

ESR spectra of $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$ complex at room temperature and at liquid nitrogen temperature both in solid state and DMF medium are shown in **Fig. 7**. The $g_{\|}$ and $g_{\perp}$ were computed from the spectra using tetracyanoethylene (TCNE) as a 'g' marker. From the spectrum of the complex at 300 and 77 K in the solid state, it is clear that $g_{\|} > g_{\perp} > 2.00$ and the G values falling within the range 1.84- 3.12 are consistent with a d_{x2-y2} ground state in a square planar or square pyramidal geometry [18, 19]. According to Hathaway, if G > 4, the exchange interaction is negligible, whereas G < 4 indicates considerable exchange interaction between the metal centres in the solid complex. Thus, in the present case, the G values indicate some exchange interaction between the copper(II) atoms,.

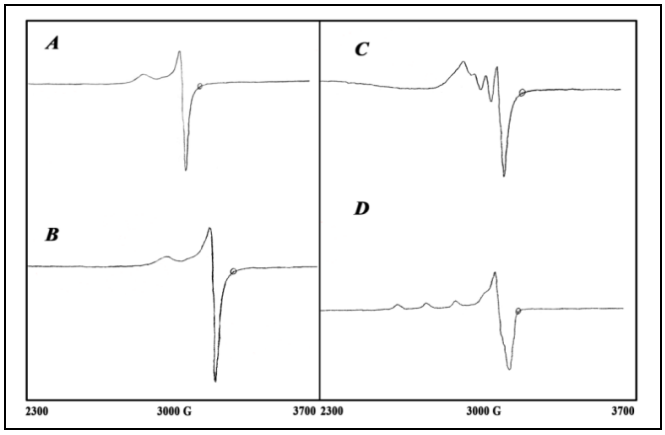

FIG. 7: X-BAND ESR SPECTRA OF $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$ AT (A) AT 300K, (B) AT LNT, (C) IN DMF SOLUTION AT 300K AND (D) AT LNT IN DMF SOLUTION

The ESR spectra of the complexes were also recorded in DMF at 300 K and at liquid nitrogen temperature and exhibit a set of four well-resolved peaks in the low-field region and one or two weaker signals at high field corresponding to the $g_{\|}$ and $g_{\perp}$ respectively. The spin Hamiltonian and orbital reduction parameters of DMF solution of complex(at LNT) are given: $g_{\|}$ (2.16); $g_{\perp}$ (2.06); g_{avg} (2.11); G (2.73); $A_{\|}X10^{-5}$ (0.0128); $A_{\perp}X10^{-5}$ (0.018); $K_{\|}$(1.65); $K_{\perp}$ (0.63); λ (351); α^2(0.23).

Electrochemical studies:

Redox behaviour of the complex has been investigated by cyclic voltammetry in DMF using 0.1M tetrabutylammonium hexafluorophosphate as supporting electrolyte. **Fig. 8** shows the profile of complex **(1)** at 25, 50, and 75 mVs^{-1} scan rates. Electrochemical data obtained at the glassy carbon electrode are given in **Table 6.**

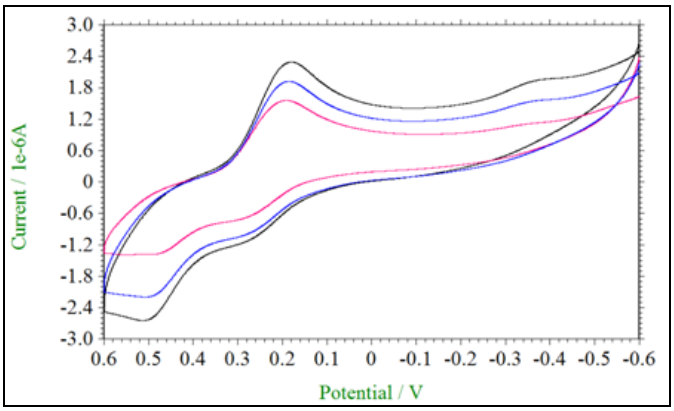

FIG. 8: CYCLIC VOLTAMMETRIC PROFILE OF $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$

The cathodic peak current function values were found to be independent of the scan rate. Repeated scans as well as various scan rates showed that dissociation does not take place in these complexes. The non-equivalent current intensity of the cathodic and anodic peaks [ic/ia = 0.945 (**1**) and 1.252 (**1**)] indicates quasi-reversible behaviour.

The potential difference (ΔEp = Epc – Epa) for complex exceeds the Nernstian requirement 59/n mV (n = number of electrons involved in the redox process) which suggests quasi-reversible character. The complexes have large separation (290mV) between the anodic and cathodic peaks, indicating the quasi-reversible character.

TABLE 6: CYCLIC VOLTAMMETRIC DATA OF COPPER(II) COMPLEX

Complex	Redox Couple	Cathodic peak Epc	Anodic peak Epa	Δ_{EP}(mV)	$E_{1/2}$ (V)	ic/ia
$[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$ **(1)**	II/I	0.212	0.502	290	0.357	1.252

Electronic absorption titrations:

The binding interactions of the complexes with CT-DNA were monitored by comparing their absorption spectra with and without CT-DNA. With increasing DNA amounts, the hypochromism of π - π* absorption band increased up to +15.47, together with red shift of 1 nm indicating the binding of the complexes to DNA. **Fig. 9** shows absorption spectra of complex **1** in the presence of increasing amounts of DNA.

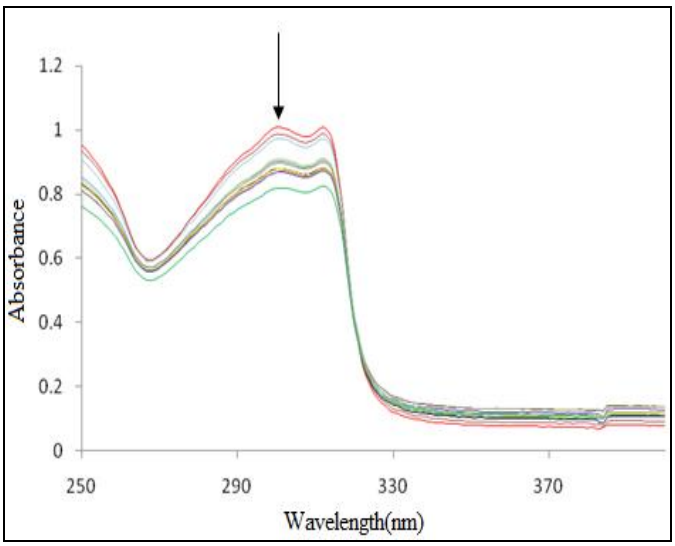

FIG. 9: ABSORPTION SPECTRA OF $[Cu(BPY)_2(NO_3)]$ NO_3 H_2O IN THE ABSENCE AND PRESENCE OF INCREASING AMOUNT OF DNA. ARROWS SHOWS THE DECREASE IN ABSORBANCE UPON INCREASING CONC. OF DNA (0 - 20µM)

The binding of an intercalative molecule to DNA is generally characterized by large hypochromism and significant red shift due to strong stacking interactions between the aromatic chromophore of the ligand and DNA base pairs, with the extent of hypochromism and red shift commonly consistent with the strength of intercalative interaction. However, in the present case, the magnitude of hypochromism and red shift observed for the Copper complex are lower than those observed for typical classical intercalators or partially intercalating complexes. To enable quantitative comparison of DNA binding affinities, the intrinsic binding constants Kb of the complexes for binding were obtained using Equation 1 given in experimental section. Electronic absorption spectral data upon addition of CT-DNA and binding constants of these complexes are given in the **Table 7.**

TABLE 7: ELECTRONIC ABSORPTION DATA UPON ADDITION OF CT-DNA TO THE COMPLEX

Complex formula	λ_{max}/nm		Δλ/nm	H%	K_b (M^{-1})
	Free	Bound			
$[Cu(bpy)_2(NO_3)]$ NO_3 H_2O	314	315	1	+15.47	4.89 x 10^6

The K_b value for the complex 1 is high, probably due to the strong electrostatic attraction between the cationic complex (1) and the negatively charged phosphodiester backbone of DNA [29, 30]. Since the complex is bulky, groove binding [20] of the complexes with DNA is suggested (rather than base pair intercalation).

Nuclease activity: Nuclease activity of complex **1** has been studied by agarose gel electrophoresis using pBR322 plasmid DNA in *Tris*-HCl/ NaCl (50mM/ 5mM) buffer (pH, 7) in the presence and absence of H_2O_2 after 30 minutes incubation period at 37°C. Nuclease activity of complex was also investigated in presence of free radical scavenger (DMSO) and reducing agent DTT. DNA cleavage activity of complex was studied at different concentrations. It was found that there is a nominal effect of concentration. Even at lower concentrations the complexes show much nuclease activity. From **Fig.10** and **Table 8,** it is evident that Copper complex cleave DNA more effectively in the presence of oxidant indicating that the Cu(II) complex may be reduced by the peroxide to produce hydroperoxo species. Lanes 5 & 7 of **Fig. 10** are almost invisible. It indicates that the DNA is completely degraded by the complex in presence of the oxidant. The hydroxyl free radical formed in the second step leads to DNA damage. This is consistent with the production of hydroxyl radicals by cuprous ions similar to the well known Fenton reaction [21, 22].

$$Cu(II) + H_2O_2 \rightleftharpoons Cu\,(I) + \dot{O}OH + H^+$$

$$Cu\,(I) + H_2O_2 \rightleftharpoons Cu(II) + \dot{O}H + {}^{-}OH$$

These hydroxyl radicals participate in the oxidation of the deoxyribose (sugar) moiety. In presence of free radical scavenger (DMSO) nuclease activity of Copper complex is diminished whereas the reducing agent DTT enhances the cleavage activity of Copper complex. This may be due to formation

of copper(I) complex by catalytic reduction which causes the production of more hydroxyl radicals which may support the oxidative cleavage.

The percentage of the three forms of DNA is presented in the **Table 8.** The decrease in percentage of supercoiled form of DNA may be considered to estimate the cleavage activity of complex**.** In the absence of H_2O_2 the complexes cleaved supercoiled DNA (Form 1) into nicked DNA (Form II) only.

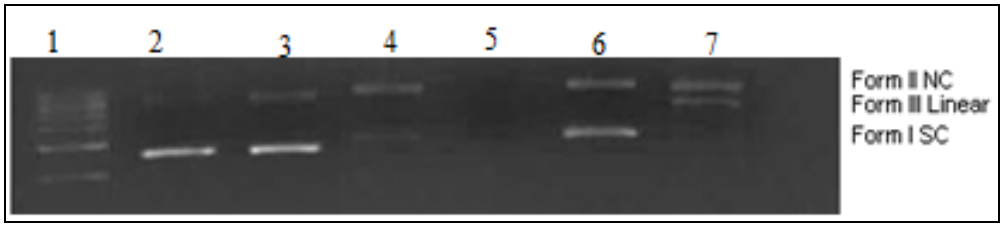

FIG. 10: **IMAGES OF DNA CLEAVAGE BY $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$**

Lane 1: 1kb DNA ladder; Lane 2: DNA control; Lane 3: DNA + H_2O_2; Lane 4: DNA + Nitrate complex (200µM); Lane 5: DNA + Nitrate complex + H_2O_2; Lane 6: DNA + Nitrate complex + DMSO; Lane 7: DNA + Nitrate complex + DTT

TABLE 8: SELECTED SC PBR322 DNA CLEAVAGE DATA OF $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$

Lane No.	Reaction Condition	Percentage of		
		Form – I	**Form –II**	**Form – III**
1	1kb ladder	-	-	-
2	DNA control	55	45	ND
3	DNA + H_2O_2	38	62	ND
4	DNA + Complex (200 µM)	29	44	27
5	DNA + Complex + H_2O_2(200 µM)	04	76	20
6	DNA + Complex + (DMSO)	37	43	20
7	DNA + Complex + (DTT)	08	32	60

CONCLUSION:

The complex $[Cu(bpy)_2(NO_3)]NO_3H_2O$. has been characterized based on molar conductivity, electronic and IR spectra. The structure of $[Cu(bpy)_2(NO_3)]\ NO_3\ H_2O$ complex is determined using single crystal X-Ray diffraction studies.. The complex is also investigated using ESR spectroscopy and cyclicvoltammetry. DNA binding constant of the complex is determined using absorption spectroscopy. Nuclease activity of the complex is investigated using gel electrophoresis experiments. Even at lower concentrations the complex shows much nuclease activity. The complex cleaves DNA more effectively in the presence of oxidant.

Supplementary Material: CCDC 1051153 contains the supplementary crystallographic data for Cu complex. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/ retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

Supplementary information is available at www.ias.ac.in/chemsci.

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REFERENCES:

1. Hussain Reddy K: Bioinorganic Chemistry, New Age International Pvt. Ltd, New Delhi 2003.
2. Wang T, Guo Z; Curr. Med. Chem, 2006; 13:525.
3. Agwara MO, Ndifon PT, Ndosiri NB, Paboudam AG, Yufanji DM Mohamadou A: Bull. Chem. Soc. Ethiop. 2010; 24:383.
4. Esther OO, Adeoye IO, Florence A OA: J. Inorg. Chem, 2013; 3:26.
5. Maurya RC, Chourasia J, Sharma P: Indian J Chem, 2007; 46A:1594.

6. Prashanthi Y, Kiranmai, K, Sathish, Kumar K, Vijay Kumar C, Shivaraj M: Bioinorganic Chemistry and Applications, 2012: Article ID 948534, 8pages.
7. Kelland L R: Eur J. Cancer, 2005: 41:971.
8. Jacques R, Antoine V, Philippe P, Jacques B: Crit. Rev. Oncol. Hemat. 2004: 51, 205.
9. Wang XL: J. Inorg. Biochem, 2004; 98: 423.
10. Dhar S, Nethaji M,. Chakravarthy AR: J. Inorg. Biochem, 2005; 99: 805.
11. Li LZ: J. Inorg. Biochem. 2005; 99: 1076.
12. Faraday RJ, Hodgson P, Suresh T, Hathaway BJ: JCS Dalton, 1981; 2070.
13. Hari Babu P, Patil YP, Hussain Reddy K, Nethaji M: Inorg. Chim Acta, 2012; 392: 478.
14. 14. Hari Babu P, Patil YP, Hussain Reddy K, Nethaji M: Trans Met Chem., 2014: 39:167.
15. Chandrasekahr S, Hussain Reddy K: J. Indian Chem. Soc, 2016: 93:309.
16. Chakravarthy AR, Reddy PAN, Santra BK and Thomas AM: Proc. Indian Acad. Sci (Chem. Sci.) 2002; 114: 391.
17. Chailueecha C, Youngme S, Pakawatchai, C Chaichit N, Van Albada GA Reedujk J: Inorg. Chim Acta, 2006; 359: 4168.
18. Zavalij PY, Burton BL, Jones Jr, WE: *Crystal Structure Commun*, 2002: C58: m330-333
19. Philip V, Sunil V, Kurup MRP, Nethaji M: Polyhedron, 2005; 24:1133.
20. Selvi PT, Palaniandavar M: *Inorg. Chim. Acta*, 2002; 337; 420.
21. Song YM, Xu JP, Ding I, Hou Q, Liu JW and Zhu ZL: J Inorg. Biochem, 2005; 103:396.
22. Raja K, Suseelamma A, Hussain Reddy K: J. Chem. Sci, 2016; 128:23.

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