

DNA BINDING AND NUCLEASE ACTIVITY OF MIXED LIGAND COPPER(II)
COMPLEXES

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ABSTRACT

The copper(II) complex, $[\text{Cu}(\text{bpy})(\text{NO}_3)_2]$ (**1**) obtained by the reaction of 2,2'-bipyridine (bpy) with $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in methanol (MeOH) solvent, reacts with sodium benzoate in MeOH solvent to form stable mononuclear complex, $\text{Cu}(\text{bpy})(\text{bz})_2 \cdot \text{H}_2\text{O}$ (**2**) (where, bz = benzoate) The complexes are characterized based on electronic, IR and ESR spectroscopies. The electrochemical behaviour of the complexes are investigated by cyclic voltammetry. The interactions of the complexes with calf thymus DNA have been investigated using absorption spectrophotometry. Nuclease activity of complexes is investigated on double stranded pBR322 plasmid DNA using gel electrophoresis experiments under different conditions.

KEYWORDS: Mixed ligand Copper(II) complexes, Bipyridine, Benzoate, DNA binding and DNA cleavage.

INTRODUCTION

DNA (deoxyribonucleic acid) is one of the most important biomacromolecules in the life process since it contains all the genetic information required for cellular function. The development of the novel small molecules that have the distinct affinity to DNA has attracted much attention in the field of nucleic acid chemistry. The study of interactions 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. Considerable efforts are being made to design sequence-specific DNA cleaving agents that bind DNA at any desired sequence and cleave DNA efficiently at the binding site.^[1]

Several artificial DNA binding metallo-nucleases developed for the cleavage of DNA have potential applications as therapeutic agents, and as versatile replacements for nucleases as laboratory tools. Investigation of transition metal complexes with catalytic activity and substrate specificity, mimicking natural enzyme, have important applications in molecular biology, and perhaps in the development of new therapeutics. DNA has become an interesting target for artificial enzymes, partly because of possible therapeutic applications.^[2] In past two decades, a variety of transition metal complexes have been used in developing artificial nucleases by either hydrolytic or oxidative pathway, with or without sequence specificity.^[3]

Currently, there is much interest to investigate catalytic activity of penta-coordinate copper complexes. Copper complexes have been extensively investigated in this

application since they possess biologically accessible reductive potentials and high affinity with nucleic acids. Copper complexes of heterocyclic bases show good DNA binding propensity. Copper(II) complexes with heterocyclic bases have continued to attract attention of coordination chemists due to its various interesting structural features. In the recent past we have reported^[4-6] nuclease activity of several copper(II) complexes. In continuation of our research activity, towards designing DNA cleaving agents, here in we report the Synthesis, spectral characterization, DNA binding and nuclease activity of mixed ligand copper(II) complexes.

EXPERIMENTAL

MATERIALS AND METHODS

Analytical grade 2, 2'- bipyridyl, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ and sodium acetate were obtained from Merck. The solvents used for synthesis of the metal complexes 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 280 nm of 1.8 indicating that the DNA was sufficiently free of protein. The DNA concentration was determined by UV absorbance at 260 nm using molar absorption coefficient 6600 M^{-1} . Stock solutions were kept at 4°C 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 complexes 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 Lambda-50 spectrophotometer. FT-IR spectra in KBr disc were recorded in the range 4000–400 cm^{-1} with a Perkin Elmer spectrum 100 Spectrometer. The cyclic voltammetry was performed with a CH instruments 660C electrochemical analyzer and a conventional three electrodes, 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 hexafluorophosphate (TBAHEP) as the supporting electrolyte.

PREPARATION OF COMPLEXES

Preparation of $[\text{Cu}(\text{bpy})(\text{H}_2\text{O})(\text{NO}_3)_2]$ (1): The complex was prepared by using literature procedure.^[7] To a stirring solution of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ (1.21 g, 5 mmol) in MeOH (10 mL), a solution of 2, 2'-bipyridyl (0.99 g, 5 mmol) in MeOH (10 mL) was added slowly. The stirring was continued for 30 min. The blue complex was filtered off and washed with a small quantity of MeOH. Yield: 1.56 g (81%). M.P. 256–258 °C, F.W. 361.77. Anal. Calc. for $\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_7\text{Cu}$; C: 33.20%; H: 2.79%; N: 15.49%; Found: C, 33.0; H, 2.6; N, 15.1 %.

Preparation of $\text{Cu}(\text{bpy})(\text{bz})_2 \cdot \text{H}_2\text{O}$ (2): To a stirring solution of complex (1) (0.77 g, 2 mmol) in MeOH (20 mL), excess sodium benzoate was added, and stirring was continued for 1 h. The dark blue solution was filtered and then evaporated slowly at room temperature. Dark blue crystals suitable for single-crystal XRD were obtained after three days. Yield: 69%, M.P. 218–220°C, F.W. 480. Anal. Calc. for $\text{C}_{24}\text{H}_{20}\text{CuN}_2\text{O}_5$; C: 60.05%; H: 4.16%; N: 5.83%; Found: C, 60.00%; H, 4.10; N, 12.08%.

DNA BINDING STUDY

The electronic spectra of metal complexes in aqueous solutions were monitored in the absence and in the presence of CT-DNA. Absorption titrations were

performed by maintaining the metal complex concentration at 20×10^{-6} M and varying the nucleic acid concentration ($0-7.36 \times 10^{-6}$ M). The titrations were carried out by gradually increasing the concentration of CT-DNA with each addition of 10 μL DNA. The ratio (r) of $[\text{complex}]/[\text{DNA}]$ value 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, $[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b (\epsilon_b - \epsilon_f)$ (1)

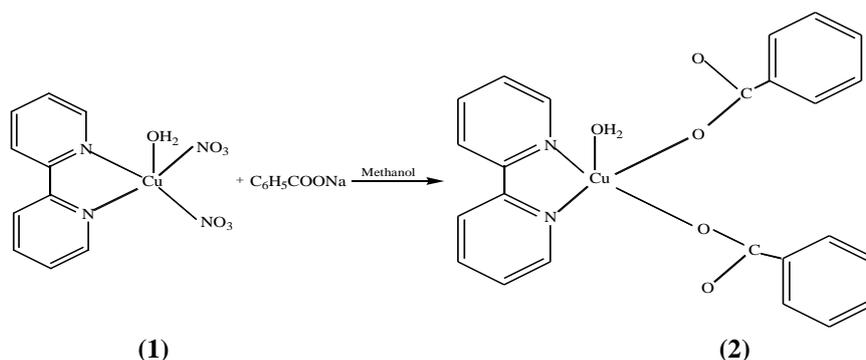
where $[\text{DNA}]$ is the molar concentration of DNA in base pairs, ϵ_a , ϵ_b and ϵ_f are apparent extinction coefficient ($A_{\text{abs}}/[\text{M}]$), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively.

GEL ELECTROPHORESIS

The extent of cleavage of DNA by the copper (II) complexes was visualized using 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 for about 1.5 hours 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 circular (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) and DTT (2 μL).

RESULTS AND DISCUSSION

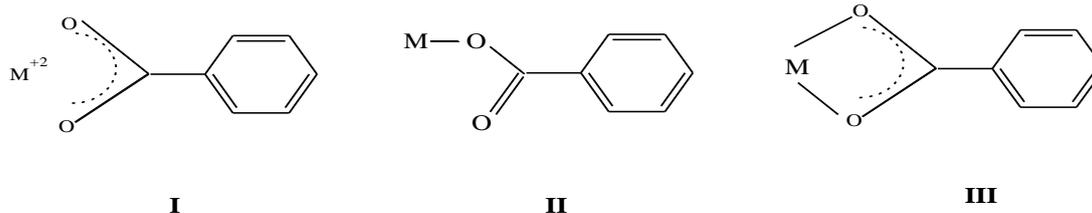
The reaction of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ with an equimolar amount of bpy in methanol results in the formation of a blue colored complex (1). It reacts with benzoate ligand in methanol to form the stable mononuclear complex (2) as shown in scheme 1. Molar conductivities of complexes 1 and 2 are found to be 6.0 and 9.6 $\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$ respectively. These data suggest that the complexes 1 and 2 are non-electrolytes.



Scheme 1 – Preparation of $\text{Cu}(\text{bpy})(\text{C}_6\text{H}_5\text{COO})_2 \cdot \text{H}_2\text{O}$ (2)

MAGNETIC AND ELECTRONIC SPECTRAL STUDIES

The electronic spectra of copper(II) complexes **1** and **2** exhibit two strong bands and one weak band at 37520, 30320 and 14,800; 37313, 30674 and 16924 cm^{-1} respectively. These bands are respectively assigned to π - π^* , CT and d-d transitions. The broad d-d bands of complexes are suggestive of square pyramidal geometry. The observed magnetic moment values for (1) and (2) complexes are 1.68 and 1.78 BM respectively, which are almost correspond to spin-only value (1.73 BM) of mononuclear copper(II) complexes.



In a series of metal salts having structure II, the anti symmetric COO stretching frequency will increase and symmetric COO stretching frequency will decrease, as the M-O bond becomes stronger IR spectral data for complex (2) suggest that the benzoate ligand binds copper as shown in structure II.

ESR SPECTRAL STUDIES

ESR spectra of $[\text{Cu}(\text{bpy})(\text{C}_6\text{H}_5\text{COO})_2\text{H}_2\text{O}]$ complex at room temperature and at liquid nitrogen temperature both in solid state and DMF medium are shown in **Figure 1**. The spin Hamiltonian and orbital reduction parameters of these complexes are given in **Table 1**. The g_{\parallel} and g_{\perp} were computed from the spectra using tetracyanoethylene (TCNE) as a 'g' marker. From the spectra of the complexes at 300 and 77 K in the solid state, it is clear that $g_{\parallel} > g_{\perp} > 2.00$ and the G values

INFRARED SPECTRAL STUDIES

The IR spectrum of complex **1** shows strong absorptions at 1,384 and 1,238 cm^{-1} , in a region typical for $\nu(\text{NO})$ of mono coordinated nitrates. Complex **1** also exhibits a strong band at 3,459 cm^{-1} assigned to -OH stretching of a coordinated water ligand. In the IR spectrum of complex **2**, a strong band is observed at 1555 cm^{-1} assigned to $\nu\text{C-C}$ (aromatic) of benzoate ligand. The benzoate anion (bz^-) coordinates the metal in one of the following ways:

falling within the range 3.39-3.86 are consistent with a $d_{x^2 - y^2}$ ground state in a square planar or square pyramidal geometry. According to Hathaway, if $G > 4$, the exchange interaction is negligible, whereas $G < 4$ indicates considerable exchange interaction between the metal centers in the solid complex. Thus, in the present case, the G values indicate considerable exchange interaction between the copper(II) atoms, which supports inter molecular interactions the complex.

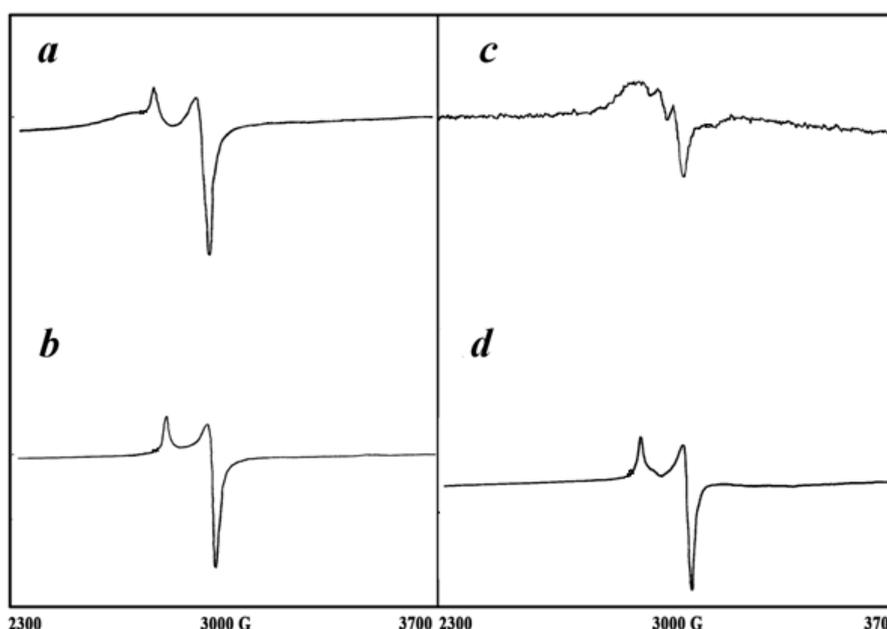


Fig: 1 X-band powder ESR spectra of $[\text{Cu}(\text{bpy})(\text{C}_6\text{H}_5\text{COO})_2\text{H}_2\text{O}]$. (a) at 300K, (b) at LNT (c) in DMF solution at 300K and (d) at LNT in DMF solution

Table 1. The Hamiltonian and orbital reduction parameters of copper(II) complex.

S.No.	Complex	In solid state				In DMF solution									
		g_{\parallel}	g_{\perp}	g_{avg}	G	g_{\parallel}	g_{\perp}	g_{avg}	G	$A_{\parallel} \times 10^{-5}$	$A_{\perp} \times 10^{-5}$	K_{\parallel}	K_{\perp}	λ	α^2
1.	[Cu(bipy)(H ₂ O)(NO ₃) ₂]	2.15	2.08	2.10	1.84	2.23	2.06	2.12	3.86	0.0124	0.0013	1.93	0.57	444	0.88
2.	[Cu (bipy)(C ₆ H ₅ COO) ₂ H ₂ O]	2.10	2.01	2.04	1.30	2.28	2.08	2.14	3.39	0.0128	0.0101	0.50	0.13	637	0.19

ELECTROCHEMICAL STUDIES

Redox behaviour of complexes has been investigated by cyclic voltammetry in DMF using 0.1M tetrabutylammonium hexafluorophosphate as supporting electrolyte. **Table 2** gives the electrochemical data obtained at the glassy carbon electrode in DMF. **Figure 2** shows the profile of complex (2) at 25–75 mVs⁻¹ scan rates. 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 of complex does not take place in solution state. The non-

equivalent current intensity of the cathodic and anodic peaks [$i_c/i_a = 0.945$ (1) and 1.642 (2)] indicates quasi-reversible behavior. The difference $\Delta E_p = E_{pc} - E_{pa}$ in all these complexes exceeds the Nernstian requirement $59/n$ mV ($n =$ number of electrons involved in the redox process) which suggests quasi-reversible character.^[8] The complexes have large separation (153–304mV) between the anodic and cathodic peaks, indicating the quasi-reversible character.

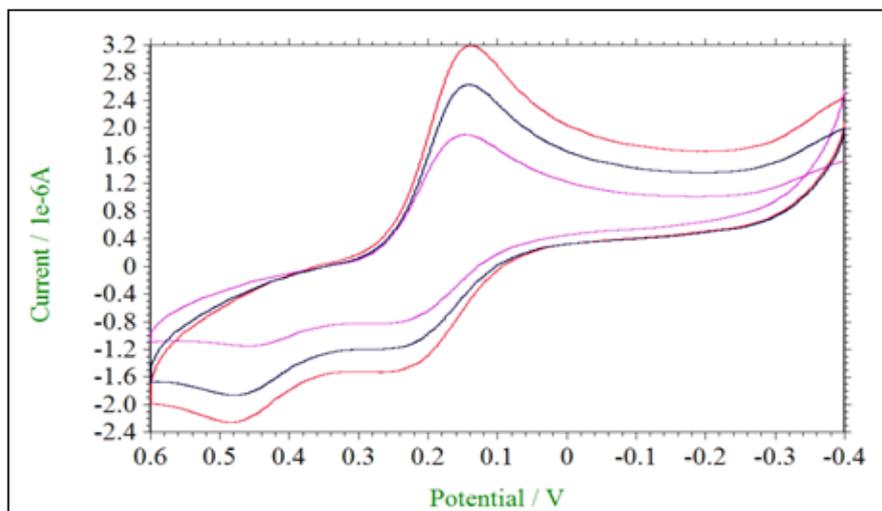


Fig: 2 Cyclic voltammetric profile of Cu(bpy) (C₆H₅COO)₂H₂O.

Table: 2 - Cyclic voltammetric data of copper(II) complexes

S.no	Complex	Redox couple	C _V		ΔE_p (mv)	E _{1/2}	i _c /i _a	Log K	-ΔG
			cathodic	anodic					
1.	[Cu(bipy)(H ₂ O)(NO ₃) ₂]	II/I	E _{pc}	E _{pa}	153	0.268	0.945	0.0823	473
			0.281	0.128					
2.	[Cu(bpy)(C ₆ H ₅ COO) ₂ (H ₂ O)]	II/I	0.147	0.451	304	0.299	1.642	0.1105	634

ELECTRONIC ABSORPTION TITRATIONS

The binding interactions of the complexes with CT-DNA were monitored by comparing their absorption spectra with and without CT-DNA. Addition of increasing amounts of CT-DNA, to the complex shows decrease in molar absorptivity (hypochromism, Δε. 2- 5%, Table 6.8) of $\pi - \pi^*$ absorption band, together with red shift of 1 nm indicating the binding of the complexes to DNA. **Figure 3** shows absorption spectra of complex 2 in the presence of increasing amounts of DNA. 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 complexes 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 (K_b) of the complexes are given in the **Table 3**. Since the complexes are bulky and lack planarity, groove binding^[9] of the complexes with DNA is suggested (rather than base pair intercalation). We predict groove binding of complex to DNA via hydrophobic interactions involving the phenyl groups of the complex.

Table: 3. Electronic absorption data upon addition of CT-DNA to the complex

S.No.	Complex	λ_{max} /nm		$\Delta\lambda$ /nm	H%	K _b (M ⁻¹) x 10 ⁶
		Free	Bound			
1.	[Cu (bipy)(H ₂ O)(NO ₃) ₂]	312	313	1	+1.96	3.92
2.	[Cu (bipy)(C ₆ H ₅ COO) ₂ H ₂ O]	312	313	1	+4.98	2.73

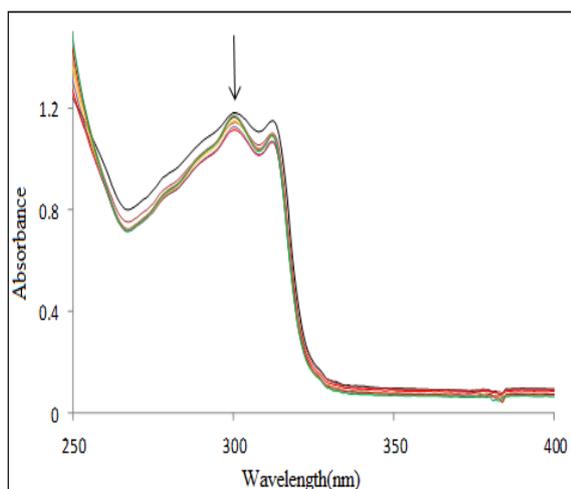
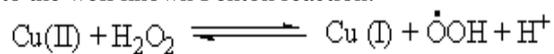


Fig: 3. Absorption spectra of of [Cu(bpy) (C₆H₅COO)₂ H₂O]. in the absence and in the presence of increasing concentration of CT-DNA; top most spectrum is recored in the absence of DNA.

NUCLEASE ACTIVITY

Nuclease activity of complexes **1** and **2** 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 H₂O₂ after 30 minutes incubation period at 37°C. Nuclease activity of complexes was also investigated in presence of free radical scavenger (DMSO) and reducing agent DTT. DNA cleavage activity of complexes 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. The percentage of the three forms of DNA is presented in the **Table 4 & 5**. The decrease in percentage of supercoiled form of DNA may be considered to estimate the cleavage activity of complex. In the absence of H₂O₂ the complexes cleaved supercoiled DNA (Form I) into nicked DNA (Form II) only. From Figures and Tables, it is evident that copper complexes cleave DNA more effectively in the presence of oxidant indicating that the Cu(II) complex may be reduced by the peroxide to produce hydro peroxy species. Lane 5 of **Figs 4** and **5** 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.^[10]



These hydroxyl radicals participate in the oxidation of the deoxyribose (sugar) moiety. In presence of free radical scavenger (DMSO) nuclease activity of copper complexes is diminished whereas the reducing agent DTT enhances the cleavage activity of copper complexes. 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 of DNA by the complex in the presence of H₂O₂.

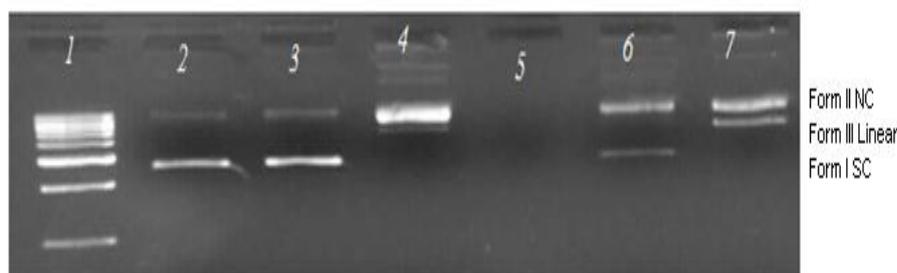


Fig. 4 - DNA Cleavage of Cu bipyridyl parent Complex:

Lane 1: DNA ladder; Lane 2: DNA Control; Lane 3: DNA + H₂O₂; Lane 4: DNA+ Complex (150μM) ; Lane 5: DNA + Complex + H₂O₂ ; Lane 6: DNA + Complex + DMSO; Lane 7: DNA + Complex + DTT.

Table 4: Selected SC pBR322 DNA Cleavage of Cu bipyridyl parent complex

Lane No	Reaction condition	Percentage of		
		FORM-I	FORM-II	FORM-III
2	DNA control	96	03	ND
3	DNA + H ₂ O ₂	73	27	ND
4	DNA + Complex (150μM)	36	45	20
5	DNA + Complex + H ₂ O ₂ (150 μM)	33	30	38
6	DNA + Complex + (DMSO)	24	51	25
7	DNA + Complex + (DTT)	12	07	81

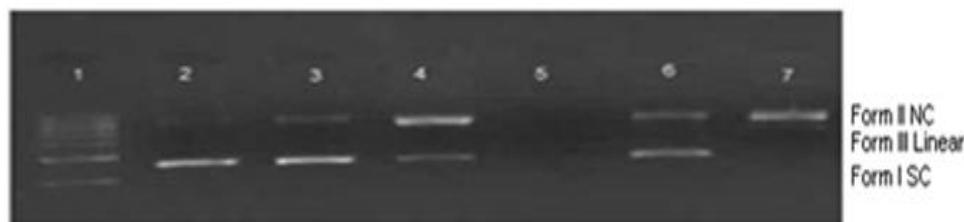


Fig: 5 DNA Cleavage of [Cu(bpy) (C₆H₅COO)₂H₂O].

Lane 1: 1kb DNA ladder ; Lane 2 : DNA control ; Lane 3 : DNA + H₂O₂ ; Lane 4: DNA + Benzoate complex (200μM) ; Lane 5: DNA + Benzoate complex + H₂O₂ ; Lane 6: DNA + Benzoate complex + DMSO ; Lane 7: DNA + Benzoate complex + DTT

Table: 5 Selected SC pBR322 DNA cleavage data of [Cu(bpy) (C₆H₅COO)₂H₂O].

Lane No	Reaction condition	Percentage of		
		FORM-I	FORM-II	FORM-III
1	1kb ladder	-	-	-
2	DNA control	55	45	ND
3	DNA + H ₂ O ₂	38	62	ND
4	DNA + Complex (200μM)	25	54	11
5	DNA + Complex + H ₂ O ₂ (200 μM)	04	78	18
6	DNA + Complex + (DMSO)	57	43	15
7	DNA + Complex + (DTT)	07	85	ND

CONCLUSIONS

The complexes, [Cu (bpy)(H₂O)(NO₃)₂] and [Cu(bpy) (C₆H₅COO)₂H₂O]. have been characterized based on molar conductivity, electronic and IR spectra. The complexes are also investigated using ESR spectroscopy. Electrochemical properties complexes are uncovered using cyclic voltammetry. DNA binding constants of the complexes are determined using absorption spectroscopy. Nuclease activities of complexes are investigated using gel electrophoresis experiments. Even at lower concentrations the complexes show much nuclease activity. The complexes cleave DNA more effectively in the presence of oxidant.

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