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Investigations on nuclease activity of mixed ligand-copper(II) complexes with aromatic oximes and heterocyclic bases

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ABSTRACT

Mixed ligand complexes of copper(II) with salicyladoxime (SAO), 2-hydroxy acetophenone oxime (HAO) as primary ligands and pyridine(Py), imidazole (Im) as secondary ligands have been synthesized and characterized by molar conductivity, magnetic moments, electronic, IR and ESR spectral data. Cyclic voltammetric studies show quasi-reversible reduction attributable to Cu^{2+}/ Cu^{+}. The binding interactions between metal complexes and calf thymus DNA have been investigated by using UV-Visible titrations and cyclic voltammetry studies. The cleavage activity of complexes was carried out on double-stranded pBR322 circular plasmid DNA by using gel electrophoresis. All complexes show increased nuclease activity in the presence of oxidant (H_2O_2). The nuclease activity of mixed ligand complexes are compared with that of the parent copper(II) complexes. Controlled experiments suggest that the complexes cleave DNA predominantly *via* an oxidative mechanism.

KEYWORDS

Copper(II) complexes;
Mixed ligand complexes;
Aromatic oximes;
DNA binding;
Oxidative cleavage.

INTRODUCTION

Investigations on transition metal complexes to probe nucleic acids are becoming more prominent in the research area of bioinorganic chemistry[1-7]. Copper complexes are well established as chemical nucleases and known to cleave DNA by different mechanisms *viz*. hydrolytic[8] and oxidative[9,10]. Oxidative cleavage of DNA could take place by chemical or photochemical means. Chemists are in search of new transition metal complexes as chemical nuclease. Oximes are widely recognized ligands in different fields of chemistry[11-14]. Survey of literature revealed that metal complexes of such important ligands are not exploited much in DNA studies. Transition metal complexes of ortho substituted aromatic oximes have attracted much attention as they give *cis* and *trans* geometrical isomers. Copper complexes are known to assume *trans* structure while cobalt complexes have *cis*- structure. Investigations of DNA interactions and cleavage activity of metal complexes with oximes are very limited.

In the light of above and in continuation of our ongoing[15,16] research work on DNA interactions with metal complexes, herein we describe synthesis, characterization, DNA cleavage/binding studies on mixed ligand complexes with salicylaldoxime(SAO), 2-hydroxyacetophenone oxime (HAO) as primary ligands and pyridine (Py) and imidazole (Im) as secondary ligands.

EXPERIMENTAL

Materials and methods

All the reagents used in the preparation of ligands and their metal complexes were of reagent grade (Merck). The solvents used in the synthesis of ligands and metal complexes were distilled before use. All other chemicals were of AR grade and used without further purification. pBR322 DNA and Calf thymus DNA were purchased from Bangalore Genie(India). Agarose, Tris. HCl and ethidium bromide were procured from Sigma-Aldrich. All other chemicals were of Merck make.

The elemental analyses were performed using Perkin Elmer 2400 CHNS elemental analyzer. Magnetic moments were determined in the polycrystalline state on a PAR model 155 vibrating sample magnetometer operating at field strength of 2-8 kG. High purity Ni metal (Saturation moment 55e.m.u/g) was used as standard. The molar conductance of the complexes in DMF (10^{-3} M) solution was measured at 28 ± 2^0C with a Systronic model 303 direct-reading conductivity bridge. The electronic spectra were recorded in DMF with a Schimadzu UV-160A spectrophotometer. The FAB-mass spectra were recorded at Indian Institute of chemical Technology, Hyderabad on Joel SX 102/DA-6000 mass spectrometer using m-nitrobenzylalcohol as the matrix. FT-IR spectra were recorded in the range 4000-50 cm^{-1} with a Bruker IFS 66V in KBr and polyethylene medium. ESR spectra were recorded on Varian E-122 X-band spectrophotometers at liquid nitrogen temperature in DMF. The voltammetric measurements were performed on Bio-Analytical systems, (BAS) CV-27 assembly in conjunction with an X-Y recorder. Measurements were made on the degassed (N_2 bubbling for 5min) solution in DMF (10^{-3}M) containing 0.1M-tetraethylammonium perchlorate ($TEAClO_4$) as the supporting electrolyte. Three-electrode system consisted of a glassy carbon (working) platinum (auxiliary) and Ag/AgCl (reference) electrode.

Preparation of ligands

The ligands SAO and HAO were prepared by the reaction of hydroxylammoniumchloride ($NH_2OH.HCl$) with salicylaldehyde or 2-hydroxyactophenone. Hydroxylammoniumchloride (6.5g, 0.1mol) and salicylaldehyde (13ml, 0.1mol) or 2-hydroxyacetophenone (15ml, 0.1mol) were taken in 50% aqueous methanol. The resulting mixture was acidified with few drops of glacial acetic acid and refluxed for 2hrs. The precipitated compound was filtered, washed with cold water, dried under vacuum and recrystallized from aqueous ethanol. SAO: Yield: 87%, MS: (m/z) - 137(M^+), 119 (M^+-H_2O); 93 ($C_6H_5O^+$); 65($C_5H_5^+$); 39($C_3H_3^+$). ^{1}H-NMR δ : 9.91(s) 1H, Oxime-OH; 8.26(s) 1H, aldehydic proton;7.73(s) 1H phenolic-OH; 6.8-7.1 (m) 4H aromatic. HAO: Yield 92%, MS: (m/z)-151(M^+), 134(M^+-OH); 105 ($C_6H_5N^+CH_3$); 93($C_6H_5O^+$), 65($C_5H_5^+$); 39($C_3H_3^+$). ^{1}H-NMR δ: 11.5 (s) 1H Oxime-OH; 8.2 (s) phenolic-OH; 6.8-7.4 (m) 4H, aromatic; 2.4 (s) 3H, methyl protons.

Copper oximes complexes

Complexes were prepared by mixing copper(II) chloride (4.3g, 0.025mol) and oxime [SAO (6.9g, 0.05mol) or HAO (7.5 g, 0.05mol)] in 1:2 ratio in 50% aqueous ethanolic medium. The reaction mixture was stirred for 30 min. The precipitate formed was filtered, washed with hot water and then with cold methanol. The complexes were dried at 110^0C.

Mixed ligand complexes with pyridine.

Copper(II) complex (0.005 mol) of SAO or HAO was placed in a Schlenk tube and dissolved in pyridine (3ml). The solution was stirred magnetically for 30 min and n-hexane (25ml) was added. After standing for 3-4 days, the resulting dark green product formed was washed with water and n-hexane and then dried under reduced pressure over $CaCl_2$.

Mixed ligand complexes with imidazole

The copper(II) complex (1.8 gm, 0.01mol) was placed in a 250-ml round bottom flask. Imidazole (1.5gm, 0.05mol) dissolved in CH_2Cl_2 was added to the contents of the flask. The reaction mixture was refluxed on water bath for 2hrs. A dark green precipitate was formed. It was filtered, washed with cold hexane and dried under vacuo over $CaCl_2$.

DNA -binding studies

Solution of CT DNA in 0.5 mM NaCl/5 mM Tris-HCl (pH=7.0) gave a ratio of UV absorbance at 260 and 280nm, A_{260}/A_{280} of 1.8-1.9, indicating DNA was sufficiently free of protein. Concentrated stock solution

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TABLE 1 : Analytical data, molar conductivity and magnetic susceptibility of copper complexes and their adducts

S.no.	Complexes	Colour	Melting point ^{0}C	Elemental analysis			Molar conductance ($ohm^{-1}cm^2mol^{-1}$)	μ_{eff} (B.M)
				Carbon found (Cal.)	Hydrogen found (Cal.)	Nitrogen found (Cal.)		
I	$Cu(SAO)_2$	Green	208-210	50.32(50.27)	3.38(3.5)	8.19(8.32)	5.8	2.18
II	$Cu(SAO)_2(Py)_2$	Dark Green	180-184	57.75(58.32)	3.81(4.42)	11.44(11.39)	12 .0	2.48
III	$Cu(SAO)_2(Im)_2$	Bluish Green	185-188	47.35(47.44)	3.78(3.74)	19.54(19.58)	14.5	2.42
IV	$Cu(HAO)_2$	Green	234-237	51.67(52.59)	4.89(4.92)	7.62(7.66)	7.0	1.83
V	$Cu(HAO)_2(Py)_2$	Dark Green	225-227	56.56(56.60)	4.91(5.16)	9.41(9.44)	12.6	2.58
VI	$Cu(HAO)_2(Im)_2$	Dark Green	227-229	52.31(52.52)	4.72(4.94)	12.42(12.65)	12.9	2.42

of DNA was prepared in 5 mM Tris-HCl / 50mM NaCl in water, pH = 7.0 and the concentration of CT DNA (in nucleotide phosphate, NP) were determined by UV absorbance at 260nm after 1:100 dilutions. The molar absorption coefficient was taken as 6600 M^{-1}[17].

Solutions for titrations were prepared with the appropriate copper complexes (35μM of a 2.0 mM solution in DMF), calf-thymus DNA (225 μM in NP), NaCl (final concentration 50mM) and Tris-HCl buffer (pH =7.0, final concentration 50 mM) and diluted with H_2O to a total volume of 1 mL. After equilibration (ca.10 min), spectra were recorded against an analogous blank solution containing the same concentration of CT DNA and Tris-HCl / NaCl buffer.

The data were then fitted to eq (1) to obtain the intrinsic binding constant, K_b [18]

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

Where ε_a, ε_f and ε_b are apparent, free and bound metal complex extinction coefficients respectively. A plot of [DNA]/ $(\varepsilon_a - \varepsilon_f)$ Vs [DNA] gave a slope of 1/ $(\varepsilon_b - \varepsilon_f)$ and a Y-intercept equal to $1/K_b(\varepsilon_b - \varepsilon_f)$, K_b is the ratio of the slope to the Y-intercept.

Assay of DNA cleavage activity

The DMF solution containing metal complexes was taken in a clean Eppendroff tube and 1μl of plasmid DNA was added. The contents were incubated for 2hr at 37^{0}C and loaded on 0.8% agarose gel after mixing 5μl of loading buffer (0.25 % bromophenol blue + 25% xylene cyanol + 30% glycerol). Electrophoresis was performed at constant voltage (80 V) till the bromophenol blue reaches to ¾ of the gel. Further the gel is stained for 10 min by immersing it in ethidium bromide solution (5 μg/ml of H_2O). The gel was then de-stained for 10 min by keeping it in sterile distilled water and plasmid band were visualized by viewing the gel under transilluminator and photographed. The efficiency of the 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 by quantitatively estimating the intensities of the bands using the *Vilber Lourmat*(V 99.01) Gel Documentation System. The reactions were carried out in presence and absence of H_2O_2. Control experiments were done in presence of DMSO, glycerol and *tert.* butyl alcohol as free radical scavenger.

TABLE 2 : Electronic spectral data (cm^{-1}) of copper (II) complexes in DMF solvent

Complexes	Charge transfer (CT)	π- π*	d – d
I	35710	28570	14950
II	35710	28560	15150
III	35070	28560	15380
IV	36360	29762	15625
V	38410	29760	17180
VI	38460	28570	16660

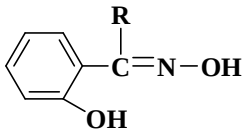


Figure 1 : Structure of ligands. Where R= H SAO, R= CH_3HAO

RESULTS AND DISCUSSIONS

The present ligands contained two functional groups viz., oxime and phenolic (-OH) groups (Figure 1). All complexes are stable at room temperature, non hygroscopic, insoluble in water, but partially soluble in methanol, ethanol and completely soluble in dimethylform amide (DMF). The colour, melting point, analytical data, molar conductance and magnetic moment data are summarized in TABLE 1. Analytical data support the formulae of complexes. The molar conductivity data suggest that the complexes are non-electrolytes. The magnetic moment data indicate that the complexes are

TABLE 3: Selected I.R. bonds (cm^{-1}) with tentative assignment

Complex /Ligand	νC=N	νC=N Py/im	νAr-H	νM-N	νM-N Py/Im	νM-O
SAO	1618	--	1500-1350	--	--	--
I	1585	--	1590-1340	470	--	570
II	1568	1556	1580-1350	474	263	592
III	1565	1540	1500-1340	475	244	580
HAO	1634	--	1600-1420	--	--	--
IV	1624	--	1600-1350	472	--	575
V	1620	1560	1500-1350	472	255	520
VI	1605	1528	1500-1400	465	232	570

monomers.

The electronic spectral data of metal complexes recorded in DMF are given in TABLE 2. A single d-d band is observed in the electronic spectra of complexes in 14900- 17200 cm^{-1} region. This band is assigned to $^2E_g \rightarrow {}^2T_{2g}$ transition, in favor of octahedral structure facilitated by coordination of DMF solvent molecules in axial position. Due to increase in the ligand field strength a blue shift is observed in the d-d band of mixed ligand complexes[19].

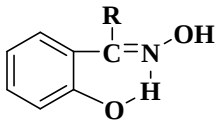
Mass spectra

The FAB mass spectra of parent complexes and mixed complexes are used to compare the stoichiometric composition. The molecular ion peak M+ for parent complexes were observed at m/z =334 and 362, suggesting the stoichiometry of parent complexes as ML_2. The molecular ion peak of mixed ligand complexes were observed at m/z =413 and 441 for pyridine adducts and 402 and 430 for imidazole adducts respectively. The stoichiometry of the mixed ligand complexes as $ML_2L'_2$.

Elemental analysis values are in close agreement with the values calculate from molecular formula assigned to these complexes, which is further supported by the FAB-mass studies of representative complexes.

I.R spectra

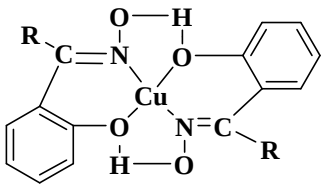
The important IR spectral bands of ligands / complexes and their assignments are given in TABLE 3. Strong bands observed at 3300, 3350 cm^{-1} in the IR spectra of SAO and HAO respectively are assigned to υOH vibration of phenolic group. The low νOH values are possibly due to intramolecular hydrogen bonding as shown below.



This strong band is absent in copper chelates suggesting the deprotonation of phenolic group and formation of covalent bond between phenolic oxygen and metal. Strong band observed at 1618, 1634cm^{-1} in the IR spectra of SAO and HAO respectively are assigned to >C=N- stretching vibration. This band is shifted to lower frequency in complexes revealing the participation of azomethine nitrogen in chelation. The non-ligand bands observed in the far IR spectra of metal complexes are assigned (TABLE 3) to νM-N, M-O stretching vibrations. Additional bands observed (1610-1600, 1540-1520, 260-220, 245-230cm^{-1}) in the IR spectra of mixed ligand complexes are presumably due to the binding of bases (pyridine/ imidazole) to copper via nitrogen donor atoms preferably in axial positions[20].

In the IR spectra of SAO and HAO bands observed respectively at 3260 and 3200 cm^{-1} are assigned to oxime OH stretching vibrations. These bands are respectively observed at 3200 and 3150 cm^{-1} in the chelates, indicating the involvement of oxime OH in strong hydrogen bonding leading to the formation of stable 5-membred ring structure. From above observation it is concluded that oxime –OH is neither deprotonated nor participated in chelation. Observance of three evenly distributed bands in 620-450 cm^{-1} region is the characteristic of *trans*-structure for the complexes[21]. IR spectral data together with electronic and magnetic moment data suggest the *trans* square planar structure for cuproxime and *trans* octahedral structure for mixed ligand copper(II) complexes (Figure 2a-b).

ESR spectra of all complexes were recorded in DMF at liquid nitrogen temperature. The ESR spectra



Where R= H, SAO; R=CH_3 HAO

Figure 2a: Structure of copper (II)-Oxime (parent) complexes

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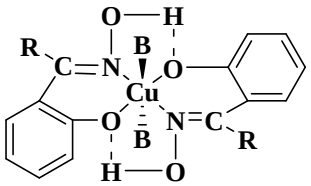

Where R= H, SAO; R=CH_3HAO;
B= Pyridine / Imidazole

Figure 2b: Structure of mixed ligand copper (II) complexes

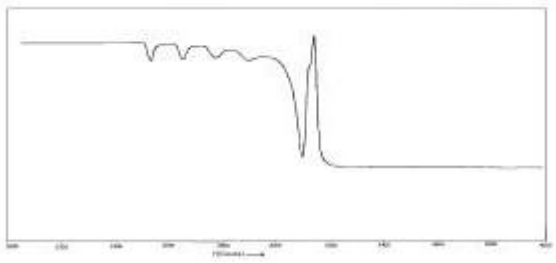

Figure 3: X-band ESR spectra of IV at liquid nitrogen temperature

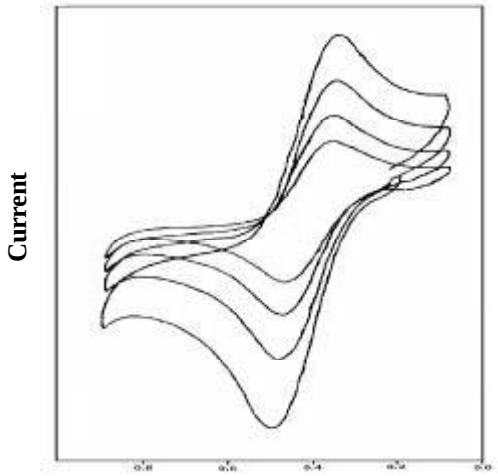

Figure 4: Cyclic voltammetry of [Cu(HAO)$_2$Im$_2$], at repeated scans at different scan rates 25, 50, 100 and 200 mVs^{-1}

of mixed ligand complexes exhibit a set of four well-resolved peaks in the low field region and single signal in high field region; corresponding to $g_{||}$ and $g_{\perp}$ respectively. A typical spectrum of [Cu(HAO)$_2$Im$_2$] shown in figure 3. The spin Hamiltonian, orbital reduction and bonding parameters of these complexes are given in TABLE 4. The trend $g_{||} > g_{\perp} > 2.0023$ observed for the present copper is typical of a copper(II) (d^9) ion in axial symmetry with unpaired electron present in the $d_{x^2-y^2}$

TABLE 4: E.S.R data of copper (II) complexes at liquid nitrogen temperature

Complex	$g_{||}$	$g_{\perp}$	g_{avg}	G	$K_{||}$	$K_{\perp}$
I	2.203	2.032	2.089	6.757	0.512	0.542
II	2.211	2.038	2.062	5.762	0.513	0.587
III	2.221	2.031	2.058	3.787	0.513	0.526
IV	2.374	2.044	2.087	4.118	0.639	0.645
V	2.301	2.057	2.172	7.279	0.681	0.727
VI	2.347	2.051	2.149	7.078	0.7124	0.885

TABLE 5 : Cyclic voltammetric data of copper (II) complexes*

Complex	Redox couple	Ep_c/V	Ep_a/V	ΔEp/mV	$E_{1/2}$	I_{Pa} / I_{Pc}
I	II / I	0.34	0.59	250	0.47	1.18
I + DNA		0.32	0.55	230	0.44	1.03
II	II / I	0.34	0.56	220	0.45	1.15
II+DNA		0.32	0.52	200	0.42	1.08
III	II / I	0.33	0.53	200	0.43	1.12
III +DNA		0.30	0.50	200	0.40	1.05
IV	II / I	0.35	0.59	240	0.47	1.19
IV+ DNA		0.33	0.57	240	0.46	0.92
V	II / I	0.42	0.60	180	0.51	1.13
V +DNA		0.38	0.59	210	0.48	1.02
VI	II / I	0.34	0.48	140	0.41	1.02
VI +DNA		0.28	0.44	160	0.36	0.95

*Recorded in DMF at room temperature with Et_4NClO_4 as supporting electrolyte; glassy carbon as working electrode; Pt wire as auxiliary electrode Ag/AgCl as reference electrode; Scan rate 50 mVs^{-1}. CT DNA – [5µl]

orbital. The observed $K_{||} < K_{\perp}$, relation indicates the presence of in-plane π - bonding[22]. The axial symmetry parameter (G) for these complexes indicates that there is no interaction between copper centers in DMF medium. Massacesi[23] reported $g_{||}$ values in the range 2.2 to 2.4 for complexes containing nitrogen and oxygen donor atoms. The present complexes have $g_{||}$ = 2.2 - 2.4 in conformity with both Cu-O and Cu-N bonds.

Electro chemical data of complexes obtained at glassy carbon electrode in DMF are given in TABLE 5. The cathodic peak current function values were found to be independent of the scan rate. Repeated scans as well as different scan rates showed that dissociation does not take place. Figure 4 shows the profile of Cu(HAO)$_2$Im$_2$ complexes at different scans rates.

All copper complexes are redox active and show cyclic voltammetric response in the potential range of 0.32- 0.60 V which is assignable for the reduction peak of Cu (II) / Cu (I). All these complexes exhibit a quasi-reversible behavior as indicated by the non-equivalent current intensity of cathodic and anodic peaks (i_c/i_a = 0.512-0.322 V). The difference $\Delta E_p = E_{Pc} - E_{Pa}$ exceeds the Nernstian requirement of 59/n mV (n = num-

ber of electrons involved in reduction reaction) which suggests the quasi-reversible character of complexes. All complexes have large separation between anodic and cathodic peaks (100-250 mV) indicating the quasi-reversible character. The ΔE_p values of parent complexes are greater than the mixed ligand complexes.

The $E_{1/2}$ values of present complexes are comparable with other copper complexes showing nuclease activity[24]. The $E_{1/2}$ values of mixed ligand complexes are less than that of parent complexes suggesting that former complexes undergo a more facile redox change which is considered as a requirement for DNA cleavage[25].

DNA binding studies

UV-visible titrations

The interaction of copper complexes with DNA was monitored by UV-Vis spectroscopy(Figure 5). The absorption spectra of copper complexes are compared with and without CT DNA. In presence of increasing amounts of CT DNA the spectra of all complexes showed hypochromicity and bathochromic shift (1- 4 nm). The change in absorbance values with increasing amount of CT DNA were used to evaluate the intrinsic binding constants (K_b) for all the complexes which are shown in TABLE 6.

The higher binding constant of $Cu(HAO)_2$ complex when compared with $Cu(SAO)_2$ is presumably due to the presence of electron donating methyl group present in the former complex. The higher binding constants of mixed ligand complexes with imidazole may be attributed to the presence of additional nitrogen donor atoms of imidazole that can interact more with DNA bases through hydrogen bonding. The higher binding constant of mixed ligands may be attributed to the π stacking or hydrophobic interactions of excess hetero aromatic ligand.

Redox titration

Binding nature of these complexes is further confirmed by redox investigation studies. Figure 6 show profile diagram of Cu $(HAO)_2Py_2$ in presence and absence of CT-DNA. On addition of CT-DNA, the complexes experience a shift in $E_{1/2}$ values as well as ΔEp values at the scan rate of $50mVs^{-1}$. The ratio of anodic to cathodic peak currents Ip_a/Ip_c in free copper complexes is decreased on addition of CT-DNA (TABLE 5), suggesting that CT-DNA moiety is bound to the complexes[26,27]. The change in formal potential of free

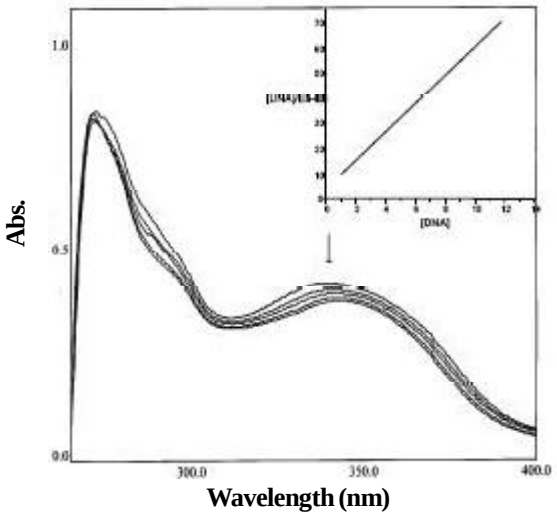

Figure 5: Absorption spectra of [Cu $SAO)_2Py_2$] in the absents and presences of increasing amount of DNA [0 - 100μM]. [Cu(L)] = 35μM. Arrow shows the decrease in absorbance upon increasing Conc. of DNA

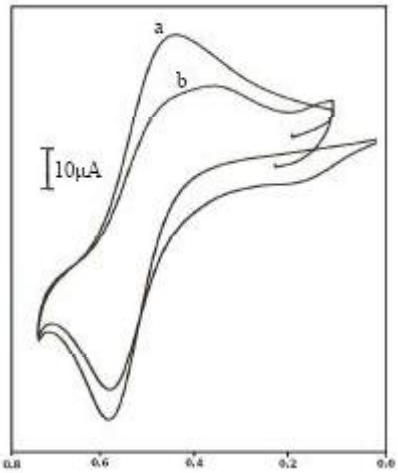

Figure 6: Cyclic voltammograms of 1mM $Cu(HAO)_2Py_2$] complex (a) in absence of CT DNA and (b) in presence of CT DNA (5 □μl) at scan rates

TABLE 6: Effect of CT DNA on the absorbance bands and binding constant of copper complexes and adducts

Complex	λ max/nm		Δλ/nm	H (%)	K_b/M^{-1}
	Free	Bound			
I	341.0	342.5	1.5	-10.6	2.5×10^4
II	339.5	343.5	4.0	-14.1	3.3×10^5
III	351.0	352.3	1.3	-16.4	6.1×10^5
IV	334.0	335.5	1.4	-11.2	5.7×10^4
V	335.0	337.2	2.2	-14.6	4.8×10^5
VI	335.5	336.6	1.1	-16.2	5.9×10^5

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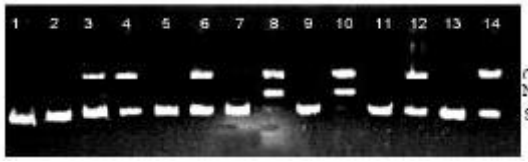

Figure 7: Agarose gel (1%) showing the results of electrophoresis of 2 µl of (0.10 µg/ml) pBR 322 plasmid DNA, 5µl of 0.1 M Tris-HCl/NaCl (pH 8.0) buffer: 1 µl (100 µM) complex in DMF; 6µl of sterilized water and 2 µl of 9.0 mM H_2O_2 were added, respectively, incubation at 37°C (120 min) : Lane 1: DNA; Lane 2: DNA + H_2O_2; Lane 3: DNA + $Cu(SAO)_2$; Lane 4: DNA + $Cu(SAO)_2$ + H_2O_2; Lane 5: DNA + $[Cu(SAO)_2]Py_2$; Lane 6: DNA + $[Cu(SAO)_2]Py_2$ + H_2O_2; Lane 7: DNA + $[Cu(SAO)_2]Im_2$; Lane 8: DNA + $[Cu(SAO)_2]Im_2$ + H_2O_2; Lane 9: DNA + $[Cu(HAO)_2]$; Lane 10: DNA + $[Cu(HAO)_2]$ + H_2O_2; Lane 11: DNA + $[Cu(HAO)_2]Py_2$; Lane 12: DNA + $[Cu(HAO)_2]Py_2$ + H_2O_2; Lane 13: DNA + $[Cu(HAO)_2]Im_2$ and Lane 14: DNA + $[Cu(HAO)_2]Im_2$ + H_2O_2

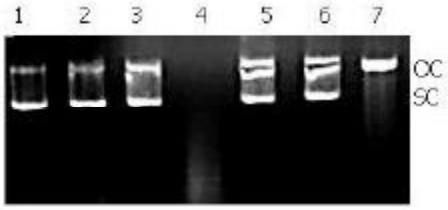

Figure 8 : Agarose gel electrophoresis of pBR322 plasmid DNA (3.3 µl) treated with mixed complex (1 µl of 2mM), 5µl of 0.1M Tris-HCl/NaCl buffer of pH 8.0, H_2O_2 (24 mM)and potential inhibitors. Incubation time: 2hr (37°C). DMSO (4 µL), *tert.* butyl alcohol (2 M) and glycerol (5 µl; Lane 1: DNA, Lane 2: DNA+ Cu(II) Lane 3: DNA + $[Cu(SAO)_2]Im_2$, Lane 4: DNA+ $[Cu(SAO)_2]Im_2$ +H_2O_2, Lane 5: DNA+ $[Cu(SAO)_2]Im_2$ + DMSO, Lane 6: DNA + $[Cu(SAO)_2]Im_2$ + *tert.* butyl alcohol and Lane 7: DNA + $[Cu(SAO)_2]Im_2$ + glycerol

TABLE 7: DNA (pBR322, 2 µl) cleavage data of copper complexes (For figure 7)

Reaction condition	% Form I	% Form II	% Form III
DNA control	92.5	7.5	--
DNA+ H_2O_2	92.0	8.0	--
DNA+ I	79.6	17.1	3.3
DNA+I + H_2O_2	59.9	31.9	6.5
DNA+ II	84.4	14.6	--
DNA+ II + H_2O_2	57.2	39.8	--
DNA+ III	78.5	15.2	--
DNA+ III + H_2O_2	28.5	50.5	21.0
DNA+ IV	75.5	--	20.1
DNA+ IV + H_2O_2	20.6	45.8	32.4
DNA+ V	73	27	--
DNA+ V + H_2O_2	51.4	48.6	--
DNA+ VI	84.1	25.8	--
DNA+ VI + H_2O_2	46.3	41.1	6.8

copper(II) complex in the presence of DNA reveal the binding of complex with DNA .The binding constant K_b and redox potential values suggest these complexes are week binders.

DNA cleavage activity

The nuclease activity of present copper complexes has been investigated on pBR322 supercoiled plasmid DNA by agarose gel electrophoresis in the presence and in the absence of oxidant (H_2O_2) at 120 min incubation period. The gel electrophoresis diagrams are shown in the Figure 7. The nuclease activity of all copper(II) complexes has fairly increased in presence of oxidant (H_2O_2) (Figure 7a; all even no. lanes). $Cu(SAO)_2$ complex cleaved super coiled form(SC) to nicked circular (NC) both in presence and absence of H_2O_2 which is evident from lanes 4 and 3 respectively. The higher cleavage activity in presence of oxidant (lane 4) is evident from TABLE 7. $Cu(HAO)_2$ complex show complete degrading of SC form to NC and OC(open circular or linear form) in presence of oxidant (lane 10). In lanes 6 & 12, $Cu(SAO)_2Py_2$ and $Cu(HAO)Py_2$ respectively converted SC form to NC in presence of H_2O_2. No such cleavage is observed in the absence of oxidant (lane 5 and 11). SC form has been degraded into NC and OC forms in lanes 8 and 14 containing imidazole complexes. In the absence of oxidant no such conversion was observed (lanes 7 and 13). From TABLE 7 the cleavage efficiency of mixed ligand complexes are arranged in order based on cleavage efficiency (TABLE 7). The order is as follows:

$Cu(HAO)_2Im_2 > Cu(SAO)_2Im_2 > Cu(HAO)_2Py_2 > Cu(SAO)_2Py_2$.

Control DNA cleavage experiment reveal the involvement of ·OH free radical as cleavage active species. In lanes 6, 7 & 8 containing DMSO, glycerol and *tert.* butyl alcohol are added to reaction mixture as hydroxyl radical scavenger agents. In the presence of hydroxyl radical scavengers, especially with DMSO and *tert*-butyl alcohol (lanes 5 and 6), cleavage activity is diminished significantly indicating the involvement of the hydroxyl radical as active species in the cleavage process.

CONCLUSIONS

While reports on DNA interactions of copper com-

plexes of polypyridyl and phenanthroline ligands are numerous in the literature, those of interactions of metal oximates and, more so of mixed ligand copper complexes with oximes and aromatic bases are not investigated so far. In this study, we have attempted to unravel the DNA interactions and nuclease activity of mixed ligand copper complexes. The reduction in peak intensity (absorption spectra) is in analogy with similar observation made earlier for other mixed ligand complexes[28,29]. Since the binding constants values are appreciable and less than 10^6 M^{-1} and since the complexes contain aromatic hydrophobic groups these complexes may be better regarded as partial intercalators of DNA. Nuclease activity of imidazole complexes is more than the corresponding pyridine complexes. The mixed ligand complexes of imidazole are found to be better DNA binding agents and efficient nucleases than the corresponding pyridine complexes possibly due to the additional nitrogen atom that may facilitate to strengthen DNA interaction *via* hydrogen bonding with DNA bases.

The present studies revealed that the copper complexes show insignificant nuclease activity in the absence of oxidant. However the activity is greatly enhanced in the presence of oxidant facilitated by the production of hydroxyl free radicals, which can damage DNA via oxidative path. Insignificant nuclease activity of complexes in the absence of oxidant is presumably due to the more stability of complexes attributed to their *trans* structure.

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REFERENCES

[1] Yan, L.Si-Dong, D.Shu-Yi, J.Liang-Nian, M.Zong-Wan; J.Inorg.Biochem., **100**, 1586 **(2006)**.
[2] Y.Z.Cheng, Z.Jing, B.W.Yan, X.Y.Cai, and Y.Pin; J.Inorg.Biochem., **101,** 10 **(2007)**.
[3] A.R.Chakravarty; J.Chem.Sci., **118**, 443 **(2006)**.
[4] S.Dhar, A.R.Chakravarty; Inorg.Chem., **44**, 2582 **(2005)**.
[5] A.Sreedhara, J.A.Cowan; Chem.Commun., 1737 **(1998)**.
[6] R.Cejudo, G.Alzuet, M.G.Alvarez, J.G.Gimenez, J. Borras, M.L.Gonzalez; J.Inorg.Biochem., **100,** 70 **(2006)**.
[7] V.Uma, M.Kanthimathi, T.Weyhermuller, U.N. Balachandran; J.Inorg.Biochem., **99**, 2299 **(2005)**.
[8] Y.An, M.L.Tong, L.N.Ji, Z.W.Mao; Dalton Trans., 2066 **(2006)**.
[9] K.Dhara, J.Ratha, M.Manassero, X.Wang, S.Gao, P.Banerjee; J.Inorg.Biochem., **101**, 95 **(2007)**.
[10] J.K.B.Leigh, M.Z.Jeffrey; Curr.Opin.Chem.Biol., **9**, 135 **(2005)**.
[11] M.A. Akbar, S.F.Livingstone; Coord.Chem.Rev., **13**, 101 **(1974)**.
[12] A.Chakravorthy; Cord.Chem.Rev., **13**, 1 **(1974)**.
[13] P.S.Reddy, K.H.Reddy; Polyhedron, **19**, 168 **(2000)**.
[14] D.Mandal, B.D.Guptha; Organometallics, **24**, 1501 **(2005)**.
[15] M.S.Surendra Babu, K.H.Reddy,P.G.Krishna; Polyhedron, **26**, 572 **(2007)**.
[16] K.H.Reddy, M.S.Surendra Babu, P.S.Babu, S. Dayananda; Ind.J.Chem.Sect.A., **43**, 1233 **(2004)**.
[17] M.E.Reichmann, S.A.Rice, C.A.Thomas, P.Doty; J.Am.Chem.Soc., **76**, 3047 **(1954)**.
[18] A.Wolfe, G.H.Shimer, T.Meehan; Biochemistry., **26**, 6392 **(1987)**.
[19] C.R.K.Rao, P.S.Zacharias; Polyhedron, **16**, 1201 **(1997)**.
[20] A.Jaggi, S.Chandra, K.K.Sharma; Polyhedron, **4**, 163 **(1985)**.
[21] K.Nakamoto; 'Infrared Spectra of Inorganic and Coordination Compounds', 2nd Ed., Wiley-Interscience, New York, **(1970)**.
[22]] B.J.Hathaway; Struct.Bond., **14,** 60 **(1973)**.
[23] M.Massacesi, D.G.Ponticelli, A.V.Budha, V. Krishnan; J.Mol.Struct., **48**, 55 **(1978)**.
[24] K.H.Reddy, P.S.Reddy, P.R.Babu; J.Inorg. Biochem., **7**, 169 **(1999)**.
[25] A.A.Kumbhar, S.B.Rendye, D.X.West, A.E.Libetra; Trans.Met.Chem., **16**, 276 **(1991)**.
[26] J.Annaraj, S.Srinivasan, K.M.Ponvel, P.Athappan; J.Inorg.Biochem., **99** 669, **(2005)**.
[27] S.Parveen, F.Arjmand; Ind.J.Chem.Sect., **44A**, 1151 **(2005)**.
[28] C.C.Cheng, S.E.Rokita, C.J.Burrows; Angew. Chem., **32**, 273 **(1993)**.
[29] Y.Wang, N.Okabe, M.Odoko; Chem.Pharm.Bull., **53**, 91 **(2005)**.