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DNA Binding and Cleavage Activity of Binuclear Metal Complexes with Benzil-α-Monoxime Thiosemicarbzone

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Abstract: Transition metal **c**omplexes of copper(II), nickel(II), cobalt(II) and iron(II) with benzil-α-monoxime thiosemicarbazone **(**BMOT) have been synthesized and characterized by molar conductance, magnetic moments, IR, electronic and ESR spectroscopy. Electrochemical behaviors of these complexes were investigated by cyclic voltammetric studies. The nuclease activity of these complexes has been investigated on double-stranded pBR322 circular plasmid DNA by using the gel electrophoresis experiments in presence and absence of oxidant (H_2O_2). In the absence of oxidant DNA cleavage by hydrolytically was observed a less discernable, whereas in presence of oxidant (H_2O_2) all complexes showed increased nuclease activity.

Keywords: Benzil-α-monoxime thiosemicarbazone, Transition metal complexes, Cyclic voltammetry, DNA cleavage, Oxidative cleavage

Introduction

Thiosemicarbazones and oximes are widely studied in organic synthesis, metal ion complexation[1-4]. These ligands have good complexing ability and their activity increases on complexation with transition metal ions. Thiosemicarbazones are broadly employed ligands containing sulphur-nitrogen for therapeutic[5], anti malarial, anti viral and anti microbial activity[6-8]. The toxicological importance of the -N-C=S moiety has been well established in antifungal, antibacterial and pesticides[9,10]. Coordination of thiosemicarbazone and oxime ligands with transition metal often enhances their biological activity[11-13]. Interaction of transition metal complexes with nucleic acids are of paramount importance for the designing

of chemotherapeutic drugs, regulating gene expression and designing tools for molecular biology[14-16]. Plenty of studies came up with that DNA is the major intracellular target of antitumor drugs because of the interaction between small molecules and these compounds can cause DNA damage in cancer cells, preventing the division of cancer cells and ending in cell death[17,18]. Although transition metal complexes of oximes[19] and thiosemicarbazones[20] have been extensively studied, much remains to be investigated on the ligational behavior of compounds containing both oxime and thiosemicarbazone functional groups. Survey of literature revealed that among ligands containing both oxime and thiosemicarbazone are less targeted for DNA interaction and cleavage activity. In the light of the above we report synthesis and characterization of benzil-α-monoxime thiosemicarbazone and its Cu(II), Ni(II), Co(II) and Fe(II) complexes and their DNA binding and cleavage activities against plasmid pBR322 DNA.

Experimental

All chemicals were of analytical grade obtained from Merck and were used without further purification. Agarose used in gel electrophoresis was purchased from Sigma, CT DNA and plasmid pBR322 were purchased from Genie Biolabs (Bangalore, India).

The elemental analyses were performed using a Perkin-Elmer 2400CHNS elemental analyzer. Molecular weights of the complexes were determined by cryoscopic method using camphor as solvent. Magnetic moments were determined in polycrystalline state on a PAR model-155 vibrating sample magnetometer operating at field strength of 2-8 kG. High purity Ni metal (Saturation moment 55 emu/g) was used as standard. The molar conductance of the complexes in dimethylformamide (DMF) (1073 M) solution was measured at (28+2) ºC with a systronic model 303 direct-reading conductivity bridge. The electronic spectra were recorded in DMF with a Schimadzu UV-160A spectrophotometer. FTIR spectra were recorded in the range 4000-50 cm^{-1} with a Brucker IFS 66V in KBr and polyethylene medium. ESR spectra were recorded on Varian E-122 X-band spectrophotometers at liquid nitrogen temperature (LNT) in DMF. The voltammetric measurements were performed on Bio-Analytical Instrument, BAS-CV-27 assembly in conjunction with an X-Y recorder. Measurements were made on the degassed (N2 bubbling for 5 min) complex solution in DMF (1073 M) containing 0.1 M tetraethyl-ammoniumperchlorate as the supporting electrolyte. A three-electrode system consisting of glassy carbon (working) platinum (auxiliary) and Ag/AgCl (reference) electrodes was used.

Assay of nuclease activity

The DMF solution containing metal complexes was taken in a clean Eppendorf tube and 3.3 ml of plasmid DNA was added. The contents were incubated for 2 h at 37 ºC and loaded on 0.8% agarose gel after mixing 5 μL of loading buffer (0.25% bromophenol blue + 25% xylene cyanol + 30% glycerol). Electrophoresis was performed at constant voltage (80 V) till bromophenol blue reached $3/4^{th}$ of the gel. Further the gel was stained for 10 min by immersing it in ethidium bromide solution (5 mg/mL of H_2O). The gel was then de-stained for 10 min by keeping it in sterile distilled water and plasmid bands were visualized by viewing the gel under transilluminator and then photographed.

DNA Binding studies

All measurements with CT DNA were performed in buffer Tris–HCl 5 mM (pH 7.2), 50 mM NaCl solution. The UV absorbance ratio $\lambda_{260}/\lambda_{280}$ was 1.8-1.9, indicating the DNA was sufficiently

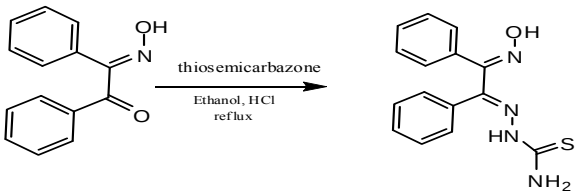
free of protein. The concentration of CT DNA per nucleotide was determined from the absorption intensity at 260 nm with the known ε value of 6600 M^{-1} cm^{-1}. The absorption titrations were performed by adding increasing amounts of CT DNA to a solution of the complex at a fixed concentration contained in a quartz cell and recording the UV–Vis spectrum after each addition. The absorption of CT DNA was subtracted by adding the same amounts of DNA to a blank. The data were then fitted to Eq. (1) to obtain the intrinsic binding constant, K_b[21].

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

Where ε_a, ε_f and ε_b are the 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 $1/K_b(\varepsilon_b - \varepsilon_f)$. Thus, K_b is the ratio of the slope to the y-intercept.

Synthesis of benzil-α-monoxime thiosemi-carbazone (BMOT)

Benzil-α-monoxime was prepared by following the literature procedure[21]. Benzil-*α*-monoxime (0.025 mol) and thiosemicarbazide (0.025 mol) are taken in 1:1 ration, add 500 mL of 1% HCl-ethanol in clean round bottom flask and refluxed for 10 h. The reaction mixture is cooled and left over night, yellow coloured product was formed, collected by filtration, washed several times with hot water, small quantities of cold methanol and hexane and dried in vacuo. Yield 86%, m.p. 158-160 °C; I.R.:- 3372(m), ν(OH) of oxime group, 3338(m), 3283(m), ν(NH), symmetric stretch of NH_2 ; 1631(s), ν(C=N) Schiff base/oxime; 1262(w), ν(C=S); 1118(m), ν(C-N). NMR :- DMSO-d_6, 7.36 - 7.93 (multiplet, 10H, phenyl protons); 8.45 - 8.82 (multiplet, 2H, amino protons), 11.79 (singlet, 1H, hydrazino proton) and 12.49 (singlet, 1H, =N-OH proton). MS *m/z* (%): 299 (M + 1); 298 (M^+) peaks.



Scheme 1. Synthesis of BMOT ligand

Synthesis of metal complexes

The metal complexes were prepared by mixing hot aqueous ethanolic solution of metal chlorides (expect iron(II) complexes, an aqueous solution of ferrous ammonium sulphate was used) and BMOT in the molar ratio of 1:1. The reaction mixture is heated under reflux for 3 h. The reaction mixture was then cooled and left overnight in refrigerator. Crystalline complexes which separated out were collected by filtration. The solid complexes are washed with hot distilled water and small quantity of cold methanol.

Results and Discussion

BMOT ligand containing two functional groups *viz.,* oxime and thiosemicarbazone, their metal complexes are stable at room temperature and non-hygroscopic in nature. All complexes are freely soluble in dimethylformamide (DMF), dimethyl sulphoxide (DMSO), partially soluble in alcohol and insoluble in water. Physical properties and molecular weight calculated by cryoscopic method are given in Table 1. The molar conductance values of these complexes measured in DMF solution (Table 2) suggest an electrolytic nature for these metal complexes. The magnetic moment values are found to be between 1.3 to 3.5 B.M ranges, which indicate the presence of unpaired electrons. Data reveal that all complexes

have low magnetic moment values, than the expected values which may be attributed to the presence of magnetically coupled metal centers in dimeric complexes. This is further supported by molecular weight calculated by cryoscopic method suggesting the presence of complex to metal ratio in 1:1.

Table 1. Physical, molar and magnetic properties of BMOT complexes

Name of the complex	Colour	Yield, %	Melting point, °C	Molecular weight Obs (Cal)	Molar conductance	Magnetic moment	Magnetic values
$[Cu(BMOT)]_2Cl_2$	Dark green	60	235-237	720 ±10 (729)	73.0	1.30	1.7 H.S/L.S
$[Ni(BMOT)]_2Cl_2$	Green	45	241-243	692 ±10 (704)	59.6	1.54	2.8 H.S/L.S
$[Co(BMOT)(H_2O)_2]_2Cl_2$	Black	70	232-240*	770 ±10 (782)	59.6	1.83	3.9 H.S / 1.7 L.S
$[Fe(BMOT)(H_2O)_2]_2SO_4$	Reddish brown	60	220-225	794 ±10 (810)	23.00	2.02	4.9 H.S / L.S

**Decomposed in the range indicated*

Table 2. Electronic spectral data, cm^{-1}

Complexes	d-d	Charge transfer
$[Cu(BMOT)]_2Cl_2$	24691	38461
$[Ni(BMOT)]_2Cl_2$	17543	37878
$[Co(BMOT)(H_2O)_2]_2Cl_2$	16666, 14705	38461, 33898
$[Fe(BMOT)(H_2O)_2]_2SO_4$	19890	37735

Electronic spectra

The electronic spectra were recorded in chloroform and DMF solvents. The electronic spectral data of metal complexes are given in Table 3. The spectral data of copper complexes are dominated by intense intra-ligand and charge transfer (CT) bands. The presence of a single d-d band may be attributed to the symmetric nature of ligand field. Charge transfer band in the spectrum of copper complex of BMOT is observed at 38461. The electronic spectra of nickel complex exhibit medium intensity bands at 17543 suggesting a square planar geometry for the complex. A charge transfer band at 37878 is also observed.

Table 3. Selective I.R. bands, cm^{-1} with tentative assignments

Complex	υ(N-H)	υ(Ar-H)	υ(C=N)	υ(C=S)	υ(M-N)	υ(M-S)	υ(M-O)
BMOT	3176	3065	1645	1190	-	-	-
$[Cu(BMOT)]_2Cl_2$	3128	3020	1625	1179	480	365	534
$[Ni(BMOT)]_2Cl_2$	3125	3040	1632	1180	479	360	546
$[Co(BMOT)(H_2O)_2]_2Cl_2$	3098	3007	1617	1175	488	366	548
$[Fe(BMOT)(H_2O)_2]_2SO_4$	3104	3042	1623	1115	465	362	538

The electronic spectra of cobalt complexes recorded in DMF show two distinct bands at 16666, 14705 cm^{-1}, attributed to the $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)(\nu_2)$ and $^4T_{1g}(F) \rightarrow {}^4A_{1g}(F)(\nu_3)$ transitions respectively in an octahedral field. Important ligand field parameters are respectively summarized for cobalt complexes of DAMOT, PPDOT and BMOT. B (Racah parameter)

720; 10 Dq, 7848; and β_{35}; 0.72. The calculated values lie in the same range as expected for an octahedral Co(II) complex. The ratio of ν_2 observed to ν_1 calculated is found to be 2.73 as required for octahedral cobalt(II) complex.

Iron(II) complexes exhibit an intense charge transfer band at 37735 cm^{-1} assigned to $T_{2g}(Fe) \rightarrow \pi^*(L)$ transition. The band observed at 19890 cm^{-1} (BMOT) in the electronic spectrum of iron(II) complexes is assigned to $^5T_{2g} \rightarrow {}^5B_{1g}$; which is generally observed in 6-coordinte iron(II) complexes. In electronic spectra of metal complexes recorded in either chloroform (or) DMF solvent, there is no appreciable shift in d-d transition suggesting that there is no coordination of DMF solvent.

IR spectral data

The important vibrational bands of metal complexes are given in Table 4. In principle, the ligand can exhibit thione-thiol tautomerism, since it contains a thioamide -NH-C=S functional group[22]. The absence of SH band at 2570 cm^{-1} and presence of NH band at 3200 cm^{-1} region in the IR spectrum of BMOT suggest that the ligand remains in thione forms at least in solid state.

A strong band appearing at 1185 cm^{-1} in the spectrum of BMOT is shifted to lower frequency 1180-1115 cm^{-1} in metal complexes indicating the involvement of thio-keto sulphur in coordination. A strong band observed around 3400 cm^{-1} range in the IR spectrum of BMOT is disappeared in the spectra of all complexes suggesting deprotonation of oxime OH in complex formation. The >C=N- (imine band) is observed at 1603 cm^{-1} in the IR spectrum of BMOT is shifted to lower wave numbers in the spectra of complexes suggesting the participation of imine nitrogen atom in coordination. Broad and strong bands in the IR spectra of Co(II) and Fe(II) complexes in 3400 - 3500 cm^{-1} region suggest the presence of coordinated water molecules. The appearance of bands 800-825 cm^{-1} region are assigned to wagging modes of water molecules in iron(II) and cobalt(II) complexes. Additional bands are observed in Far IR spectra of metal complexes in 500 - 430 and 360 - 300 cm^{-1} regions due to ν(M-N) and ν(M-S) modes respectively.

Table 4. Spin Hamiltonian and orbital reduction parameters of Cu(II) complexes

Parameters	$g_{\parallel}$	$g_{\perp}$	g_{av}	G	$A_{\parallel}^*$	$A_{\perp}^*$	A_{av}^*	$K_{\parallel}$
Cu(BMOT)	2.3322	2.0589	2.1956	5.8286	0.01581	0.00139	0.06619	0.9110

Units in cm^{-1}

ESR spectra

ESR spectra of copper complexes of BMOT are recorded in DMF at liquid nitrogen temperature. ESR spectra of copper complex are given in Figure 1. The spectra exhibit a set of four well-resolved peaks in the low field region and weakly resolved signals in high field region corresponding to $g_{\parallel}$ and $g_{\perp}$ respectively. The spin-Hamiltonian, orbital reduction and bonding parameters of these complexes are given in Table 5. The $g_{\parallel}$ and $g_{\perp}$ values are computed from the spectra using tetracyanoethylene (TCNE) free radical as g marker.

The 'g' tensor values of a Cu(II) complexes can be used to derive the ground state. In square-planar complexes the unpaired electron lies in the dx^2-y^2 orbital giving $^2B_{1g}$ as the ground state with $g_{\parallel} > g_{\perp} > 2.00$ while the unpaired electron lies in the dz^2 orbital giving $^2A_{1g}$ as the ground state with $g_{\perp} > g_{\parallel} > 2.00$. From observed values it is clear that $g_{\parallel} > g_{\perp} > 2.00$ which suggest the fact that the unpaired electrons lies predominately in the dx^2-y^2 orbital[23]. The g_{av} value for these complexes are greater than 2 indicating covalent property[24].

For in-plane π-bonding $K_{\parallel} < K_{\perp}$, while for out-of plane π-bonding $K_{\parallel} > K_{\perp}$. From Table 5, it is observed $K_{\parallel} > K_{\perp}$, relation indicates the presence of out-of-plane π-bonding. The axial symmetry parameter G (5.82) for these complexes indicates that there is no interaction between copper centers in DMF medium. The EPR X-band spectrum for the binuclear copper complex is also recorded in solid state at RT and liquid nitrogen temperature. At both temperatures the complex gives a broad signal in the low field region indication g spin-exchange interactions between two copper(II) ions. The two expected spin allowed EPR transition occur at 3140 and 3180 G yielding g_{iso} value 2.087 copper complexes. The absence of hyperfine structure indicates that the interaction would be mainly dipolar in nature. Based on magnetic, electronic, IR and ESR spectra a binuclear *trans* octahedral structure is assigned (Figure 2)

Table 5. Cyclic volta metric data of BMOT metal complexes

Complex	Gain	Redox couple	CV cathodic E_{pC}	CV anodic E_{pa}	ΔE_P	$E_{1/2}$ (V)	$-i_c/i_a$
$[Cu(BMOT)]_2Cl_2$	0.1	III/II	0.44	0.55	110	0.495	0.611
$[Ni(BMOT)]_2Cl_2$	0.1	III/II	-1.25	-0.92	330	-1.085	-
		II/I	-1.44	-1.30	140	-1.37	-
$[Co(BMOT)(H_2O)_2]_2Cl_2$	0.1	IV/III	-0.53	-0.34	190	-0.435	-
		III/II	-1.27	-0.85	420	-1.06	-
$[Fe(BMOT)(H_2O)_2]_2SO_4$	0.1	III/II	0.28	0.41	130	0.345	0.653

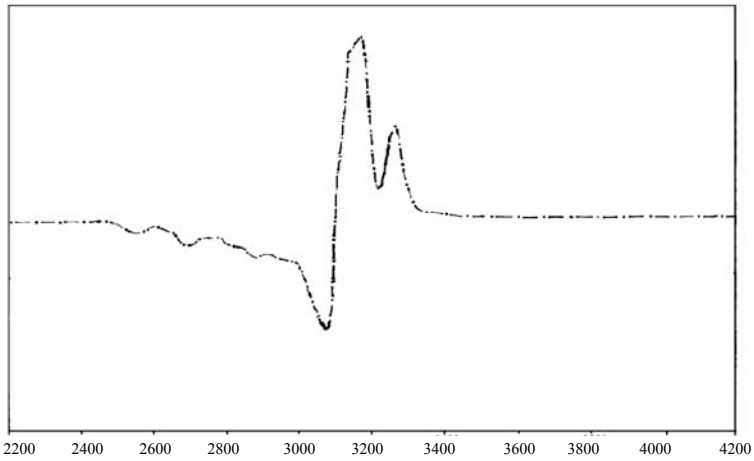


Figure 1. X-band ESR spectrum of $[Cu(BMOT)]_2Cl_2$ complex at liquid nitrogen temperature in DMF

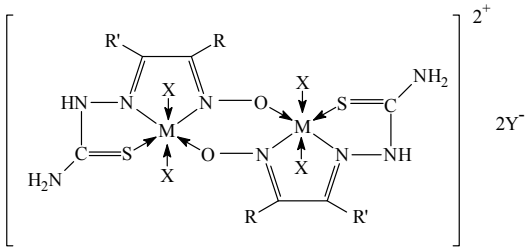


Figure 2. Structure of metal complexes where M = Cu(II), Ni(II), Co(II), and Fe(III), R = C_6H_5, X = Water molecule present in Co(II) and Fe(II) complexes, Y = 1/2 SO_4 in Fe(II); Cl in Cu(II), Ni(II) and Co(II) complexes.

Cyclic voltammetric data

Cyclic voltammograms (Figure 3) of the metal complexes are recorded in dimethyl-formamide in $TBAClO_4$ supporting electrolyte Ag/AgCl reference electrode. The redox behaviour of complexes has been investigated by cyclic voltammetry at a glassy carbon electrode. Table 5 gives the electrochemical data obtained at glassy carbon electrode in DMF. 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 in three complexes.

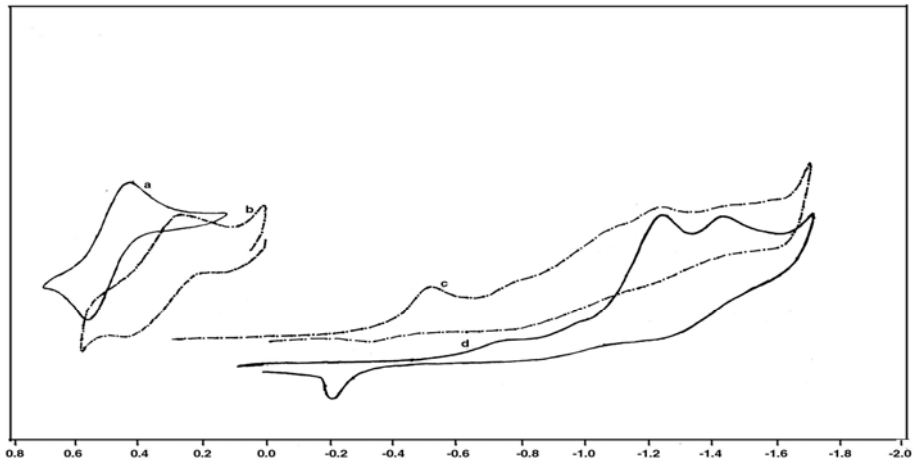


Figure 3. Cyclic voltammetric profile of (a) $[Cu(BMOT)]_2Cl_2$ (b) $[Fe(BMOT)(H_2O)_2]_2SO_4$ (c) $[Co(BMOT)(H_2O)_2]_2Cl_2$ (d) $[Ni(BMOT)(H_2O)_2]_2Cl_2$

The reduction peak of the Fe(III)/Fe(II) and Cu(III)/Cu(II) couple for these complexes is observed at the potential 0.345 and 0.495 Vs Ag/AgCl for BMOT respectively[25-27]. The non-equivalent current intensity of cathodic and anodic peaks (i_c/i_a = 0.611; 0.653 V at 100 mV S^{-1}) for copper and iron. Complexes of BMOT indicate a quasi-reversible behavior. The difference $\Delta E_P = E_{Pc} - E_{pa}$ in all these complexes exceeds the Nernstion requirement 59n/mV (n = number of electrons involved in oxidation reaction) which suggests the quasi-reversible character of these complexes. All these complexes have large separation (100 - 500 mV) between anodic and cathodic peaks indicating the quasi reversible character. The $E_{1/2}$ values of these complexes are comparable with other complexes of nitrogen and sulphur donor ligands[28].

Nuclease activity of present metal complexes

The nuclease activity on plasmid DNA can be monitored by gel agarose electrophoresis. By using the pBR322 plasmid DNA, nuclease activity was studied with BMOT and their copper(II), nickel(II), cobalt(II) and iron(II) complexes in presence and absence of hydrogen peroxide (oxidant). At micro molar concentration, the ligand exhibits no significant activity in absence and in the presence of the oxidant as shown in Figure 4. In absence of oxidant copper(II) and iron(II) complexes causes discernible DNA cleavage as shown by increase in intensity in form I (nicked) and form III (linear) with decrease in intensity in form I (super coiled) which is attributed to stepwise conversion of form I (super coiled) to form II(nicked) and to form III (linear). In presence of oxidant (H_2O_2) all the forms of DNA is degraded by

copper and Fe(BMOT) complex only form I (super coiled) is degraded, with increase in intensity in form II and form III respectively. Nickel complexes in the absence of oxidant show no significant nuclease activity, but in the presence of oxidant the activity is increased. The cobalt complexes of PPDOT, degraded all the three forms of DNA completely both in the presence and absence of oxidant (H_2O_2). While in Co(BMOT) complex, an increase in activity is shown in presence of oxidant compared to absence of oxidant.

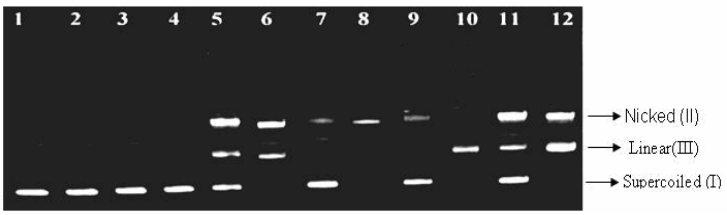


Figure 4. Agarose gel (1%) showing the results of electrophoresis of 1 μL of (0.10 μg/mL) pBR 322 plasmid DNA, 2 μL of 0.1 M Tris-HCl (pH 8.0) buffer: 250 μM complex in DMF; 10 μL of sterilized water and 2 μL of 9.0 mM H_2O_2 were added, respectively, incubation at 37 °C (120 min) Lane 1: DNA (control); Lane 2: DNA + H_2O_2 (control); Lane 3 : DNA + BMOT; Lane 4: DNA + BMOT + H_2O_2; Lane 5: DNA + $[Cu(BMOT)_2]$; Lane 6: DNA + $[Cu(BMOT)_2]$+ H_2O_2; Lane 7: DNA + $[Co(BMOT)_2]$; Lane 8: DNA + $[Co(BMOT)_2]$ + H_2O_2; Lane 9: DNA + $[Ni(BMOT)_2]$; Lane 10: DNA + $[Ni(BMOT)_2]$ + H_2O_2, Lane 11: DNA + $[Fe(BMOT)_2]$; Lane 12: DNA + $[Fe(BMOT)_2]$ + H_2O_2

In overall view all the complexes showed increased nuclease activity in the presence of oxidant. The increased activity of the complexes in the presence of oxidant may be due to the formation of hydroxyl (OH) free radical[29], which involves oxidation of the deoxyribose moiety followed by breakage of the sugar phosphate backbone. The nuclease activity of the complexes in the absence of oxidant may be due to the binding of metal ion to DNA and these metal ions can be reduced and then oxidized by dioxygen, leading to hydroxyl radical production close to the metal binding site which can damage DNA in site specific reactions or due to the reactive oxygen species (possibly singlet oxygen).

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