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Mixed ligand Co(II) Complexes: Synthesis, Characterization, DNA binding and Photonuclease Studies

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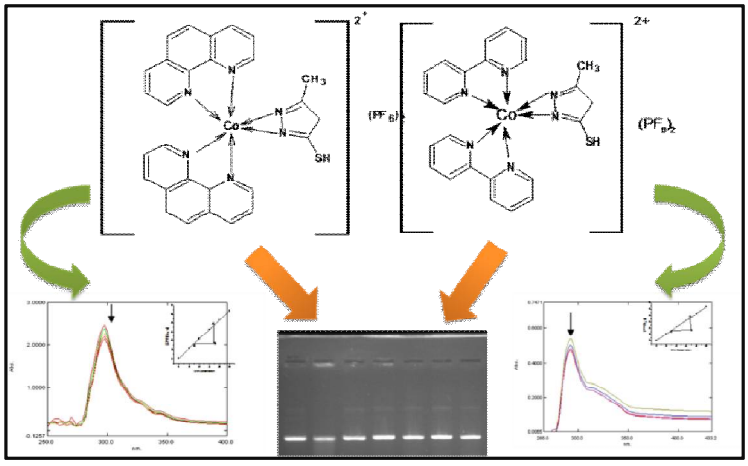
ABSTRACT

A new cobalt complex of the $[M(L1)_2(L2)]^{n+}$ where M is a Cobalt metal ion and L1= phenanthroline/ bipyridine, L2= 5-methyl-1,3,4-thiadiazole-thiole, have been synthesized and characterized by elemental analysis(CHN), FT-IR and UV-visible(UV-Vis) spectroscopic techniques. The DNA-binding property of the complexes has been investigated employing absorption spectroscopy, viscosity measurements and thermal denaturation study. The DNA cleavage experiments were carried out by gel electrophoresis method using pUC19 DNA. The experimental results show that both complexes can bind to DNA in an intercalation mode.

Graphical Abstract



Keywords: Thiadiazole, Elemental analysis, Electrophoresis, Intercalation

INTRODUCTION

There has been significant interest in the synthesis of transition metal complexes that are suitable for binding and cleaving nucleic acids due to their various applications in nucleic acid chemistry, like foot-printing studies and sequence specific binding agents an also exists as anticancer drugs. Many transition metal complexes, copper, ruthenium, palladium, platinum, etc., are used now-a-days extensively to study metal complex-DNA interaction.

In recent studies reveals that, the interaction of DNA with cobalt complex has attracted much attention because cobalt was accepted as an essential metal element widely distributed in the biological systems such as cells and body [1-2]. Cobalt involves itself in the regulation of DNA synthesis indirectly and in cobalt-dependent proteins which make it an essential biological element [3]. Cobalt(III) complexes have been widely investigated in coordination chemistry and biochemistry [4] owing to their therapeutic activities [5].

Deoxyribonucleic acid (DNA) plays a significant role in the life process because it carries the inheritance information and leads the biological synthesis of proteins and enzymes through the replication and transcription of genetic information in living cells. DNA is especially a good target for metal complexes as it gives a wide variety of potential metal binding sites [6-8]. For example, the rich DNA electron bases and phosphate groups are suitable for direct covalent coordination at the metal centre. There are noncovalent binding behaviors such as hydrogen bonding and electrostatic binding in the grooved regions of the DNA, along with the intercalation of planar aromatic ligands in the stacked base pairs [9–15]. The interaction of DNA with transition metal complexes has gotten intensive attention in the last few years in order to develop new novel nonradioactive probes of DNA structure [16, 17], new therapeutic agents that cleave DNA [18-20] and DNA-mediated electron transfer reactions [21].

Metal complexes of S-, N-, and O chelating ligands have attracted considerable attention because of their interesting physicochemical properties, pronounced biological activities and their use as models for metalloenzymes active sites [22]. Thiadiazole is an important class of S, N-containing heterocycles and has been reported to be biologically active compound [23]

The increasing clinical importance of drug-resistant microbial pathogens has lent additional urgency in microbiological and antifungal research [24-26]. In this regard, thiadiazoles have occupied an important place in drug industry; in particular 1,3,4-thiadiazoles derivatives have been used widely in various fields. Earliest application was in the pharmaceutical area where sulfonamides antibacterial were used as drugs. Some of the later uses are as antitumor and anti-inflammatory agents, pesticides, dyes, lubricants and analytical reagents [27]. Interest in 1,3,4-thiadiazole and its derivatives also arises from the fact that they possess also cover a wide spectrum of therapeutic action liking used as anticonvulsant herbicidal, pesticidal, amoebicidal, CNS depressant, antibacterial, and antiviral drugs [28].

Recently, binding of metal complexes with 1,10-phenanthroline or modified phenanthroline moieties to DNA has attracted much attention [29-31]. Synthesis of new cobalt(II) complexes enabled chemists to extensively study the ability of these complexes to act as probes in investigating the structure of DNA, when these metal complexes are incorporated with either 1,10-phenanthroline/2’2 bipyridine or a modified phenanthroline/bipyridine moiety as one of the ligands.

During our studies, it occurred to us that complexes of the type $[M(L1)_2(L2)]^{n+}$ where M is a transition metal ion and L1=phenanthroline/bipyridine, L2=5-methyl-1,3,4-thiadiazole-2-thiole, ligands containing N/S/O donor atoms are well-suited for this purpose. Although DNA interactions of a number of $[M(L1)_2(L2)]^{n+}$ type complexes have previously appeared in the literature, relatively less attention seems to have been paid to systematic investigations inquiring into the effects brought about

by changing M ion in such complexes. We have been interested to know the effect of variation of the metal ion on the ability to bind and photocleave DNA in mixed ligand complexes containing the phenanthroline/ bipyridine family of ligands. In this paper, we reported the synthesis, characterization, DNA binding and photocleavage studies of Cobalt complexes containing the same ligand (L2= 5-methyl-1,3,4-thiadiazole-2-thiole) as in our previous article [32].Various physico-chemical and biochemical techniques including UV/Visible, viscometric titration, thermal denaturation, and gel electrophoresis have been utilized to probe the nature of interaction of these complexes with the duplex. Also reported in this paper is a detailed mechanistic investigation on the DNA photocleavage by $[Co(phen/bpy)_2(L2)]^{2+}$.

MATERIALS AND METHODS

All common chemicals, solvents as well as 2,2-bipyridine, 1,10-phenanthroline $CoCl_2.6H_2O$, ammonium hexaflurophosphate (NH_4PF_6) were purchased from Merck (India). All the solvents were purified before use as per the standard procedures. Deionised, triply distilled water was used for preparing various buffers. Highly polymerized calf thymus DNA (CT-DNA) and super coiled (SC) pUC19 DNA were purchased from Bangalore Genie (India).Tris-HCl buffer, Agarose (molecular biology grade) and ethidium bromide were purchased from Himedia. Solution of DNA in 5mM Tris-HCl, 50 mM NaCl buffer (pH 7.2) gave a ratio of UV absorbance at 260 and 280 nm of 1.8-1.9:1, indicating that the DNA was sufficiently free of protein. The concentration of DNA was determined spectrophotometrically using molar absorptivity 6600^{-1} cm^{-1} at 260nm. The stock solutions were stored at 4°C and used over no more than 4 days.

Physical measurements: Micro analyses (C, H, and N) were performed in Carlo-Erba 1106-model 240 Perkin-Elmer analyzer. Melting points were determined in open capillaries and are uncorrected IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. ^{1}H-NMR spectra were recorded on a Bruker AC-P500 spectrometer (300 MHz) at 25°C in $CDCl_3$ with TMS as the internal reference. UV visible absorption spectra were recorded using Shimadzu 1650 PC model UV spectrophotometer at room temperature. Viscosity measurements were carried out on Brookfield viscometer at room temperature. Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier teyrrcontrolling programmer.

DNA binding and cleavage experiments

Absorption titration experiments: These experiments were performed by maintaining a constant concentration of the complex while varying the nucleic acid concentration. This was achieved by dissolving an appropriate amount of the metal complex in the DNA stock solution and by mixing various proportions of the metal complex and DNA stock solutions while maintaining the total volume constant (1 ml). This resulted in a series of solutions with varying concentrations of DNA but with a constant concentration of the complex. The absorbance (A) of the most red-shifted band of each investigated complex was recorded after successive additions of CT DNA. The intrinsic binding constant, K_b, was determined from the plot of [DNA]/(e_a– e_f) vs [DNA], where [DNA] is the concentration of DNA in base pairs, e_a, the apparent extinction coefficient is obtained by calculating A_{obsd}/[complex] and e_f corresponds to the extinction coefficient of the complex in its free form. The data were fitted to (1) where e_b refers to the extinction coefficient of the complex in the fully bound form.

$$[DNA]/(e_a – e_f) = [DNA]/(e_b – e_f) + 1/K_b(e_b – e_f). \quad (1)$$

Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(e_b – e_f)$ and a y-intercept of $1/K_b(e_b – e_f)$. K_b was determined from the ratio of the slope to intercept. An in-house nonlinear least square analysis program or the MicroCal Origin software package run on an IBM-compatible Pentium 166 computer was used for curve-fitting the data.

Viscometric studies: Viscosity measurements were carried out at 25±1°C by using semimicro dilution capillary viscometer at room temperature. The DNA concentration was fixed at $5x10^{-5}$ M and flow time was measured with a digital stopwatch. The mean values of three replicated measurements were used to evaluate specific viscosity η of the samples. The values for relative specific viscosity $(\eta/\eta_o)^{1/3}$, where η_o and η are the specific viscosity contributions of DNA in the absence (η_o) and in the presence of the Co(II) complex (η), were plotted against 1/R.

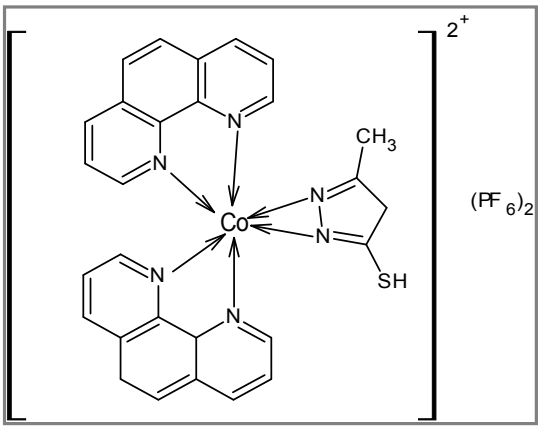
Thermal denaturation studies: DNA melting experiments were carried out by monitoring the absorption (260 nm) of CT DNA (160 mM) at various temperatures, in both the absence and the presence (0–10 mM) of each investigated complex. The melting temperature (T_m) and the curve width s_T (= temperature range in between 10% to 90% of the absorption increase occurred) were calculated as described [30]. The shape of the melting curves, T_m and s_T values for CT-DNA and for CT-DNA in the presence of $[Ru(phen)_3]^{2+}$ were consistent with the literature data [33]. Some of the metal complexes were seen to absorb at 260 nm, but control experiments suggested that this absorption is independent of temperature.

Gel Electrophoresis: Electrophoresis through Agarose is the standard method used to separate, identify or purify DNA fragments. This technique is useful for identifying bands containing as little as 1-10 ng of DNA can be detected by direct examination of the Agarose gel (stained with ethidium bromide) in the UV light. When an electric field is applied across the gel, DNA, which is negatively charged at neutral pH, migrates toward the anode. The intact supercoiled (Form I) DNA migrates faster than the single nicked (Form II) in the gel. This technique has been employed to identify the product/s of the DNA photocleavage, which was carried out in this work.

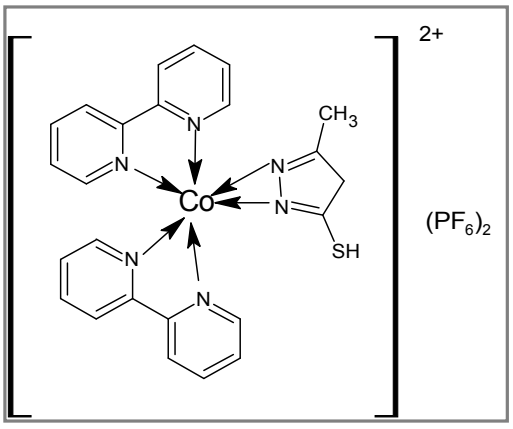
Preparation

Synthesis of ligand: Ligand 1,10-phenanthroline(L1), bipyridine (L1) and 5-methyl-1,3,4-thiadiazole-2-thiole (L2) were purchased from Sigma Aldrich (Bangalore).

Synthesis of Complexes: The complexes $[Co(phen)_2Cl_2]$/$[Co(bpy)2Cl_2]$ were prepared by literature method [34]. Solution containing $[Co(phen)_2Cl_2]$/$[Co(bpy)2Cl_2]$ (0.49 g, 1 mmol) and 5-methyl-1,3,4-thiadiazole-2-thiole (L2) (0.1322g, 1 mmol) (50 mL) in ethanol was refluxed for 1h with stirring and further stirred for 4-5 h under nitrogen. Then it was filtered and the crude complex was precipitated upon addition of saturated ethanolic solution of ammonium hexaflurophosphate. The complex was filtered and recrystallized (acetone-ether). The elemental analysis data of the ligand and its complexes are summarized in the table1.



Scheme 1. Structure of $[Co(phen)_2(L2)](PF_6)_2 .2H_2O$ [1].



Scheme 2. Structure of $[Co(bpy)_2(L2)](PF_6)_2 .2H_2O$ [2].

Table 1. Analytical and physical properties of mixed ligand Co(III) complexes

Compound	Yield %	Mol.wt	Found (Cal.)%			
			C	H	N	Co(III)
$[Co(phen)_2(L2)](PF_6)_3$	79	822	39.91 (40.87)	2.42 (2.55)	10.32 (10.21)	7.05 (7.17)
$[Co(bpy)_2(L2)](PF_6)_3$	82	774	37.45 (37.20)	2.68 (2.71)	10.89 (10.85)	7.56 (7.62)

RESULTS AND DISCUSSION

Characterization of complexes

IR-Spectra: The ligand 5-methyl-1,3,4-thiadiazole-2-thiol shows absorption bands at 2868 and 1590 cm^{-1} due to ν(SH) and ν(C=N), respectively. The ν(C=N) band shifted to 1580 cm^{-1} for complexes (**1**) and (**2**), respectively. This indicate that the nitrogen atom is involved in coordination to the Co(II) ion. Besides, the complexes show low frequency in the region 410–415 cm^{-1} are assigned to (Co-N) bands [35-39]. In addition the IR spectrum of the PF_6 salt of each complex showed a strong band in the region 843–847 cm^{-1} ascribable to the counter anion and this band was absent for the corresponding chloride salts [40].

UV-visible spectra: The absorption spectra of the ligand L_2 and these complexes with ligand L_2 were recorded in DMSO solvent in the range of 200-800 nm. The UV-visible spectra of this ligand 5-methyl-1,3,4-thiadiazole-2-thiole (L_2) was characterized by prominent bands at 300 nm due to intra ligand transition π- π* transition. The electronic spectrum of the Co(III) complexes shows 2 bands around 229-345 nm, which may be assigned to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ and $^4T^{1g}(F) \rightarrow {}^4A_{2g}(F)$ transition. There are absorptions around 230-255 nm, which are ascribed to metal-to-ligand charge transfer [41]. The UV-visible peaks corresponding to the $\pi \rightarrow \pi*$ and $n \rightarrow \pi*$ transitions in these ligands, and these were observed around 270-305 nm. The peaks belonging to the $\pi \rightarrow \pi*$ transitions are shifted to a longer wavelength as a consequence of coordination and this confirms the formation of Co(III) complexes [42]. Magnetic measurements were recorded at room temperature. Magnetic susceptibility measurements provide sufficient information to characterize the structures as shown in (Table 2). The Co(III) complexes are paramagnetic, and their magnetic susceptibility values are around 4.40-4.80 B.M. The values indicated are in good agreement with the literature [43]. These results indicate an octahedral geometry for the Co(III) complexes. The suggested structures of the complexes are shown in the Scheme.

Table 2. Electronic spectra, Molar conductance and magnetic moment data of ligands and Co(III) complexes

Compound	λ_{max}, nm (log ε)	Ω_M mohs cm^2 mol^{-1}	Magnetic moment μ_{eff} BM
L1=phen	226 (4. 54), 264 (4. 23)	--	--
L1=bpy	246 (4. 48), 278 (4. 39)	--	--
L2=5-methyl-1,3,4-thiadiazole-2-thiol	222 (4.45), 246(4.72), 297(4.35), 315(3.63)	--	--
$[Co(phen)_2(L2)](PF_6)_3$	229 (5.25), 279(5.29), 364 (4.47), 342 (4. 42)	24.2	4.46
$[Co(bpy)_2(L2)](PF_6)_3$	227(5. 08), 263 (5. 18), 287 (4. 62), 305 (4. 24)	34.5	4.80

Error limits: $\lambda_{max,}$ ± 1 nm; log ε, ± 10%

DNA binding Studies

Absorption spectra: Addition of increasing amounts of CT-DNA resulted in a decrease of absorbance for each investigated complex and also that of $[Co(phen)_2(L2)](PF_6)_3$ and $[Co(bpy)_2(L2)]$

$(PF_6)_3$ Representative spectra illustrating this hypochromicity and the presence of isosbestic points observed for the interaction of $[Co(phen)_2(L2)](PF_6)_3$ with CT-DNA are given in figure 1a and 1b. Change in absorbance at the peak maximum of the most red-shifted band of each complex with increasing concentration of DNA has been monitored for an evaluation of the intrinsic binding constant using (1) (see figure 1a and 1b inset, for the plot using(1)); the binding constants thus obtained are given in table 2. The observed K_b values also support the intercalative binding. In the present study as observed K_b values (given values) for Co(III) and Ni(II) complexes are equal to the K_b values observed for classical intercalators such as EthBr, K_b, $1.8x10^6$ M^{-1} in 25 mM Tris-HCl/40 mM NaCl buffer, pH 7.9) and partial intercalating metal complex $[Ru(phen)_2(dppz)]^{2+}$, dppz = dipyrido-[3,2-d: 2′,3′-f]-phenazine, [K_b>10^6 M^{-1}] bound to CT-DNA. So, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

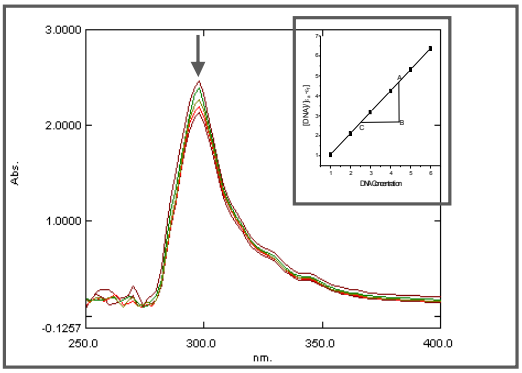


Figure 1(a). Absorption spectra of complex **(1)** in Tris-HCl buffer upon addition of DNA. [Co] = 0.5 μM, [DNA] = 0.1 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
The Inner plot of [DNA]/ $(\varepsilon_a-\varepsilon_f)$ vs[DNA] for the titration of DNA with Co(III) complex **(1)**.

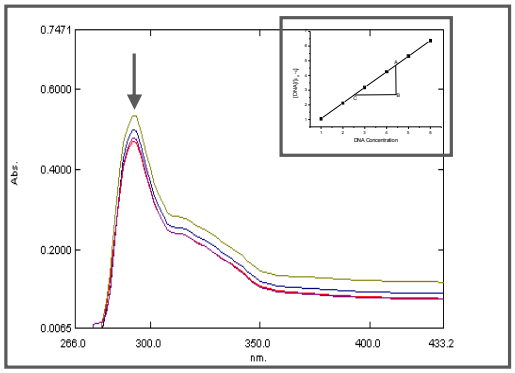


Figure 1(b). Absorption spectra of complex **(2)** in Tris-HCl buffer upon addition of DNA.[Co] = 0.5μM, [DNA] = 0.1μM. Arrow shows the absorbance changing upon the increase of DNA concentration.
The inner plot of [DNA]/ $(\varepsilon_a-\varepsilon_f)$ vs[DNA] for the titration of DNA with Co(III) complex **(2)**.

Viscosity measurements: Intercalation of a ligand to DNA is known to cause a significant increase in the viscosity of a DNA solution due to an increase in the separation of the base pairs at the intercalation site and, hence, an increase in the overall DNA molecular length. In contrast, a ligand that binds in the DNA grooves causes either a less pronounced change (positive or negative) or no change in the viscosity of a DNA solution [44]. The effect of each investigated complex on the viscosity of CT-DNA solution was studied in order to assess the binding mode and strength of these complexes with DNA. Representative plots of η/η_o vs [drug]/[DNA] are shown in figure 2 for the cobalt(III) complexes. As seen in this figure, positive and negative changes of viscosity with increasing addition of the complex are seen for $[Co(phen)_2(L2)](PF_6)_3$ and $[Co(bpy)_2(L2)]$ $(PF_6)_3$ suggesting an intercalative mode of binding by the Co(III) mixed ligand complex [45].

Thermal denaturation studies: Thermal denaturation curves for DNA in the presence and absence of a representative complex are given in figure 3 and the relevant data for all the complexes investigated in this study are summarized in table 3. In the present study the T_m DNA was found to be 60±1°C under experimental conditions.

Addition of complex $[Co(phen)_2(L2)](PF_6)_3$ and $[Co(bpy)_2(L2)](PF_6)_2$ increased T_m (±1°C) by 6°C and 4°C, respectively, which indicates that these complexes stabilize the double helix of DNA As shown in figure 3. The increased T_m value of the latter is comparable to that of the T_m value of classical intercalators [46]. So, from the above data it is concluded that the new Co(III) mixed ligand complex act as a new class of DNA intercalators.

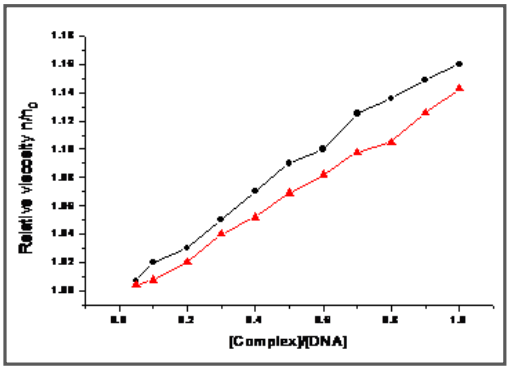


Figure. 2. Effect of increasing amounts of the complex $[Co(Phen)_2(L2)](PF_6)_3$ [------●-----] and $[Co(bpy)_2(L2)](PF_6)_2$[----◀----] on the relative viscosities of CT-DNA at 25 (±0.1)°C.

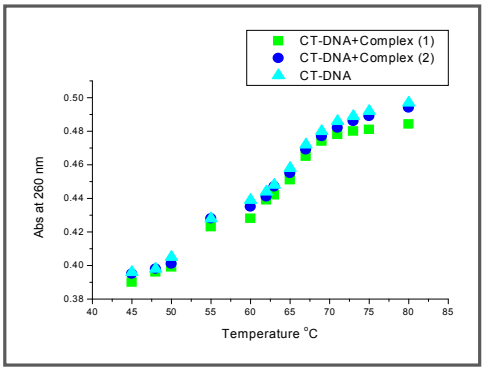


Figure 3. Melting curves of CT-DNA in the absence and presence of complexes $[Co(phen)_2(L2)]^{3+}$ and $[Co(bpy)_2(L2)]^{2+}$.

Table 3. Results of Absorption titration and Thermal melting experiments

Compound	K_b (M^{-1})	T_m°C (σ_T°)
DNA	--	60 (20)
$[Co(phen)_2(L2)](PF_6)_3$	$2.4x10^3$	66 (21)
$[Co(bpy)_2(L2)](PF_6)_3$	$1.3x10^3$	64 (25)

DNA photocleavage studies: The photocleavage of super coiled (SC) pUC19 DNA (0.1 µL (0.2 µg)) to its nicked circular (NC) form was determined by Agarose gel electrophoresis in Tris-HCl buffer (50 mM, pH 7.2) containing NaCl (50 mM). The cleavage reactions mixture containing, 0.53 µM, 1.06µM and 2.12 µM complex (1) and (2) in 20µL buffer were photo irradiated using monochromatic UV or visible light. The samples were then incubated for 1 h at 37°C followed by addition to the loading buffer containing 25%bromophenolblue, 0.25% xylene cyanol, 30% glycerol (3 µL) and finally loaded on 0.8% Agarose gel containing 1.0 µg mL^{-1} ethidium bromide. Electrophoresis was carried out at 50 V for 2 h in Tris-borate EDTA (TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using syngene Gel Documentation System.

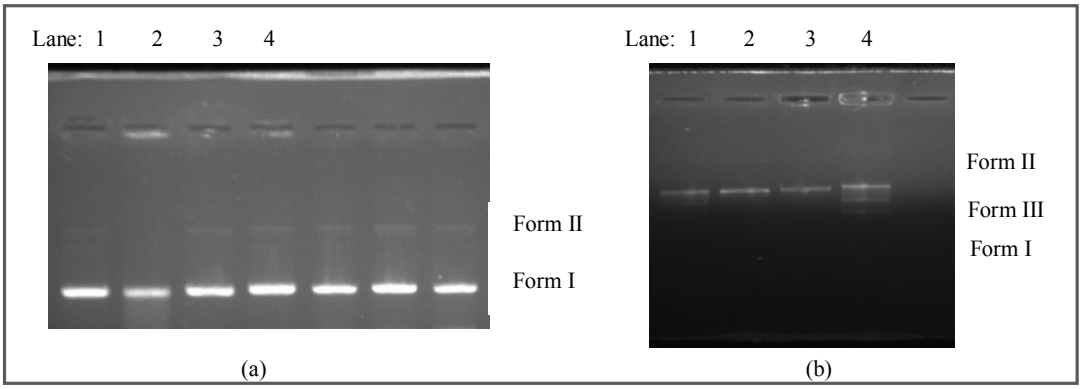


Figure 4. (a) DNA cleavage activity of the **complex 1. Lane 1**: Control, **Lane 2-4**: complex (1) with increasing concentration $0.17*10^{-3}$, $0.34*10^{-3}$ and $0.51*10^{-3}$ µg, **(b)** DNA cleavage activity of the **complex 2 Lane 1**: Control, **Lane 2-4**: Complex (2) with increasing concentration $0.17*10^{-3}$, $0.34*10^{-3}$ and $0.51*10^{-3}$ µg.

Figure 4 (a) shows that the complex **(1)** at lower concentration of (0.53 µM, 1.06 µM) show lower cleavage activity (Lane 2 and 3) compared to higher concentration of (2.12 µM), in which super

coiled (Form-I) DNA cleaved. This supercoiled relaxed and produced a slower moving nicked circular form (Form-II) (Lane 4).

Figure 4(b) in the case of complex **(2),** at lower concentration (0.53 μM, 1.06μM) the DNA cleaved from supercoiled (Form-I) to nicked circular form (Form-II) (Lane 2 and 3), but at higher concentration (2.12 μM) the super coiled plasmid DNA would cleaved to linear form (Form III) which is in between Form I and Form II.**)** From these results we infer that cobalt(III) complex **(1)** and **(2)** act as a potent cleaving agent. The wavelength used for the photo-induced DNA cleavage experiments were 365 nm.

APPLICATION

this study demonstrate that substitution by different metal ions in metallo–intercalators of the type $[M(phen/bpy)_2L2]^{n+}$ can bring about subtle modulation in the properties of this class of mixed-ligand complexes and, consequently, in their interactions with DNA.

CONCLUSIONS

A novel Co(III) complexes $[Co(phen)2(L2)](PF_6)_3$(1) and $[Co(bpy)_2(L2)]$ $(PF_6)_3$(2) have been synthesized and characterized. The experimental results indicate that the complexes bind to DNA via an intercalative mode of binding. By comparison with previous studies, probably the most striking result emerging from this study is a quantitative evaluation of the contribution of Cobalt metal ion to the DNA binding affinity. In summary, the results described in this study demonstrate that substitution by different metal ions in metallo–intercalators of the type $[M(phen/bpy)_2L2]^{n+}$ can bring about subtle modulation in the properties of this class of mixed-ligand complexes and, consequently, in their interactions with DNA.

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