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DNA Interaction Studies of Cu (II) and Mn (II) Complexes containing Mixed Ligand of 1, 10-phenanthroline and 2-hydroxy-4-methyl-1, 8-naphthyridine

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ABSTRACT

The DNA binding ability of the novel complexes [Cu $(L_2)_2L_1]$ $(PF_6)_2$ [Complex **(1)**] and [Mn $(L_2)_2L_1]$ $(PF_6)_2$ [Complex **(2)**] containing bioactive mixed ligand of the type L_1=2-hydroxy-4-methyl-1, 8-naphthyridine and L_2 =1, 10-phenanthroline were synthesized and structurally characterized by elemental analysis, IR and ^{1}H NMR spectral studies. The intrinsic binding constant K_b has been estimated at room temperature. The binding constant of 2.7 x 10^4 M^{-1} for complex (**1**) and 4.3 x 10^4 M^{-1} for complex (**2**) in 5 mM Tris-HCl/50 mM NaCl buffer at pH 7.2, respectively, as obtained from absorption spectra indicate that the complexes intercalate between the base pairs of the CT-DNA tightly. The oxidative cleavage activity of the complexes **(1)** and **(2)** were studied by using gel electrophoresis and the results show that complexes have potent nuclease activity.

Keywords: Cu (II) and Mn (II) Complexes, DNA Binding, Cleavage studies.

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INTRODUCTION

Transition metal complexes that induce DNA strand scission on photolysis are of importance for their potential applications in photodynamic therapy [1-4]. Studies of small molecules, which bind at specific sites along a DNA strand as reactive models for protein–nucleic acid interaction, provide routes toward rational drug design as well as means to develop sensitive chemical probes for DNA. Thus, a number of metal chelates are of current interest for important applications in nucleic acid chemistry as probes of DNA structure in solution, reagents for mediation of strand scission of duplex DNA under physiological conditions and chemotherapeutic agents and in genomic research [5-9].

Copper(I) complexes containing heterocyclic bases have received considerable current interest in nucleic acid chemistry due to their diverse applications following the discovery of the "chemical nuclease" activity of the $[Cu\ (phen)_2]^+$ (phen = 1,10- phenanthroline) complex in the presence of hydrogen peroxide and a reducing agent by Sigman and coworkers [10]. It has been reported that some ternary copper (II) complexes of phenantroline have antitumor activity [11]. The fiber EPR spectra of mixed ligand copper (II) complexes of amino acids and diimines have thrown light on the modes of binding of the complexes to DNA [12]. Chakravarty and coworkers have used several ternary copper (II) complexes of diimines like dipyridoquinoxaline (dpq) to cleave DNA by double-strand scission under physiological condition [13–15]. Copper (II) complexes of tren-based *tris*-macrocycles have been also used as effective chemical nucleases [16].

Metalloporphyrins can experience reversible redox reactions in which the site of electron transfer may be localized at either the central metal or the porphyrin ring. Manganese porphyrins have several interesting aspects of physical, chemical and biological properties which distinguish them from other metalloporphyrins [17-19]. Manganese porphyrins continue to be of interest as models for the behavior of cytochrome P-450 [20, 21], photosystem II [22, 23] and superoxide dismutase [24], DNA binding and cleavaging reagents [25-27] and as catalysts for the epoxidation of olefins [28-32]. Axial ligation of manganese porphyrins coupled with redox chemistry is very important in diverse biological functions [33-38].

Herein, we report the DNA binding and cleavage studies of newly synthesized Cu (II) and Mn(II) complexes of mixed ligand containing 1,10-phenanthroline and 2-hydroxy-4-methyl-1,8-naphthyridine.

MATERIALS AND METHODS

The reagents and solvents used were of AR grade. Solvents were purified and used. Cupric acetate, manganese acetate, NH_4PF_6 (ammoniumhexafluorophosphate), DMSO (Dimethylsulphoxide) Tris-HCl buffer were purchased from qualigens (Mumbai, India). Ligand 1, 10-phenanthroline was purchased from Sigma Aldrich (Bangalore). CT-DNA (Calf thymus Deoxyribonucleic acid) and pUC 19 DNA (Plasmid University of California 19 Deoxyribonucleic

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acid) were purchased from Bangalore Gene, Bangalore, India.

Experimental

Synthesis of Ligand (2-hydroxy-4-methyl-1, 8-naphthyridine)

A mixture of 2-aminopyridine (1 g, 0.01 mol) and ethylacetoacetate (1.3 g, 0.013 mol) was irradiated in a microwave oven for 8 min. and 2 to 4 drops of H_2SO_4 was added and again irradiated for 3 min. A yellowish product precipitated out with a yield of 82%. It was washed with cold ethanol, dried under vacuum and recrystallised from ethylacetate. Analysis: $C_9H_8N_2O$. calcd. (%): C 67.50; H 5.00; N 8.75; O 10.00. Found (%): C 67.48; H 4.98; N 8.68; O 9.97. IR, KBr pellets (ν, cm^{-1}): 1462 ν(C=N); 3149 ν(C-H, Ar-H); 3258 ν(OH). ^{1}H NMR (δ, ppm): 2.60 (m, 3H, CH_3), 4.70(s, H, Ar-OH), 7.80(m, 4H, Ar-H), 8.8(m, 8H, ArN-H), 8.1(m, 2H, CH=N).

Synthesis of Metal Complexes

Synthesis of Metal Complex [Cu (phen) $_2$] (CH_3COO) $_{23H2O}$

Cuppric acetate (0.20g, 1 mmol) and 1, 10-phenanthroline (0.396g, 1 mmol) were dissolved in hot methanolic solution and refluxed on the water bath for 3h. The contents were cooled to obtain precipitate. The complex was filtered and dried under vacuum before being recrystallized in acetone. Yield of the complex 74%.

Synthesis of [Cu (L_2)$_2$$L_1$] ($PF_6$)$_2$ [Complex (1)]

50 ml of hot methanolic solution of [Cu (phen) $_2$] (CH_3COO) $_{23H2O}$ (0.56 g, 1 mmol) was added to a hot methanolic solution of 2-hydroxy-4-methyl-1, 8-naphthyridine (L_1) (0.161g, 1mmol). The mixture was refluxed on the water bath for 3h. The contents were cooled and precipitated by the addition of hot methanolic solution of NH_4PF_6 to the filtrate. The complex [Cu (L_2)$_2$$L_1$] ($PF_6$)$_2$ was filtered and dried under vacuum before being recrystallized in acetone. Yield of the complex 70%. Analysis: $C_{33}H_{24}N_6OP_2F_{12}Cu$: C, 43.23; H, 2.62; N, 9.17; O, 1.74; Cu, 6.98. Found: C, 43.18; H, 2.58; N, 9.14; O, 1.69; Cu, 6.92. IR, KBr pellets (cm^{-1}): 721(Ar. Stretch), 1681ν(C=N), 3040ν(C-H), 3344ν(O-H). ^{1}H NMR (δ, ppm): 2.44 (m, 3H, CH_3), 4.82(s, H, Ar-OH), 7.93(m, 4H, Ar-H), 7.63(m, 2H, CH=N), 9.01(m, 8H, ArN-H).

Synthesis of Metal Complex [Mn (phen) $_2$] (CH_3COO) $_{2.3H2O}$

Manganese acetate (0.249 g, 1 mmol) and 1, 10-phenanthroline (0.396g, 1 mmol) were dissolved in hot methanolic solution and refluxed on the water bath for 3h. The contents were cooled to obtain precipitate. The complex was filtered and dried under vacuum before being recrystallized in acetone. Yield of the complex 72%.

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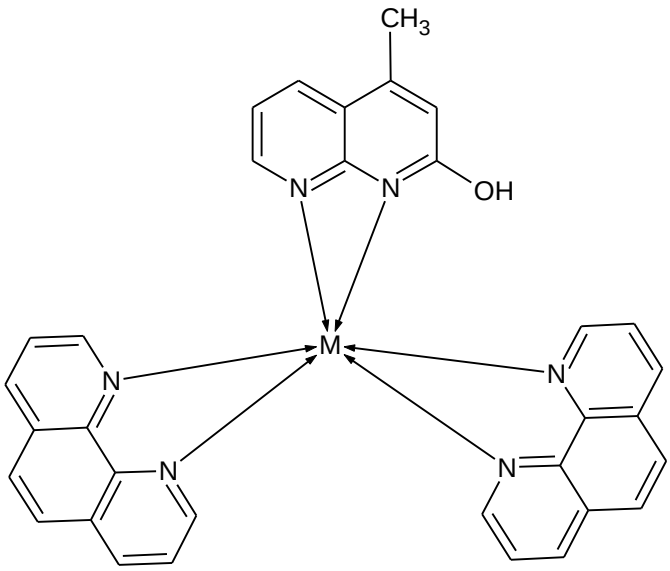
Synthesis of [Mn $(L_2)_2L_1$] $(PF_6)_2$ [Complex (2)]

To a hot methanolic solution of 2-hydroxy-4-methyl-1, 8-naphthyridine (L_1) (0.161g, 1mmol) 50ml of hot methanolic solution of [Mn (phen) $_2$] $(CH_3COO)_{2.3H2O}$ (0.58 g, 1 mmol) was added. The mixture was refluxed on the water bath for 3h. The contents were cooled and precipitated by the addition of hot methanolic solution of NH_4PF_6 to the filtrate. The complex [Mn $(L_2)_2L_1$] $(PF_6)_2$ **was** filtered and dried under vacuum before being recrystallized in acetone. Yield of the complex 67%. Analysis: C33H24N6OP2F12Mn: C, 43.66; H, 2.65; N, 9.26; O, 1.78; Mn, 6.08. Found: C, 43.61; H, 2.62; N, 9.22; O, 1.74; Mn, 6.04. IR, KBr pellets (cm^{-1}): 718(Ar. Stretch), 1694ν(C=N), 3060ν(C-H), 3320ν(O-H).

The structure of the investigated complexes is given in Fig.1.

Figure-1: The structure of the complexes where M is Cu^{2+} or Mn^{2+}



Spectral Measurements

Melting points were determined in open capillaries and are uncorrected. Microanalysis (C, H, and N) were performed in Carlo-Erba 1106 model 240 Perkin-Elmer analyzer at IISc Bangalore. The molar conductivity in Dimethylformamide(DMF) ($l0^{-3}$M) at room temperature was measured using Equiptronics digital conductivity meter. IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets at Acharya Pharmacy College, Bangalore. Bruker FT-NMR Spectrophotometer (400 MHz) was used for recording ^{1}H-NMR spectra at 25°C in MeOD (detoriated methanol) with TMS(tetra methyl silane) as the internal reference at IISc, Bangalore. UV-visible absorption spectra were recorded in Acharya Pharmacy College, Bangalore, using Shimadzu model UV-1650PC spectrophotometer at room temperature. Viscosity measurements were carried out on semi-micro dilution capillary

viscometer (Viscomatic Fica MgW) with a thermostated bath D40S at room temperature. Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 Spectrophotometer.

DNA Binding and Cleavage experiments

The concentration of CT-DNA per nucleotide [C(p)] was measured by using its known extinction coefficient at 260 nm (6600 M^{-1} cm^{-1}) [39] Tris HCl-buffer [5mM tris(hydroxymethyl) amino methane, pH 7.2, 50 mM NaCl] was used for the absorption, viscosity and thermal denaturation experiments.

Absorption titration experiments were carried out by varying the DNA concentration (0-140 μM) and maintaining the metal complex concentration constant. Absorption spectra were recorded after each successive addition of DNA and equilibration (approximately 10 minutes). The absorption data were analyzed for an evaluation of the intrinsic binding constant K_b using reported procedure [40].

Each viscosity experiment was performed three times and average flow time was calculated. Data were presented as (η/η_0) versus binding ratio where η is viscosity of DNA in the presence of complex and η_0 is the viscosity of DNA alone.

Thermal denaturation experiments were carried out with a Shimadzu Model UV-160A spectrophotometer coupled to a temperature controller (Model TCC-240A) by monitoring the absorption of CT-DNA (50 μM) at 260 nm at various temperatures, both in the presence (5-10 μM) and absence of each complex. The melting temperature (*Tm*, the temperature at which 50% of double stranded DNA becomes single stranded) and the curve width (σ_T, the temperature range between which 10% and 90% of the absorption increases occurred) were calculated as reported [41, 42].

The extent of cleavage of super coiled (SC) pUC 19 DNA (0.5μg) to the nicked circular (NC) form was determined by agarose gel electrophoresis in Tris HCl buffer (50mM, pH 7.2) containing NaCl (50 mM). The samples were then incubated for 1 hour at 37°C followed by addition to the loading buffer containing, 25% bromophenolblue, 0.25% Xylene Cyanol, 30% glycerol and finally loaded on 0.8 % agarose gel containing 1.0 μg/ml ethidium bromide(EB). Electrophoresis was carried out at 50V for 2 hours in Tris-borate ethylene diamine tetra acetate (TBE) buffer. Bands were visualized by UV light and photographed to determine the extent of DNA cleavage from the intensities of the bands using UVI tech Gel Documentation system. Due corrections were made for trace of NC DNA present in SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form.

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RESULTS AND DISCUSSION

Characterization of complexes

The elemental analysis, IR, ^{1}H NMR spectral data of the new complexes are summarized in experimental section. The elemental analysis data are agreed with the theoretical values within the limit of experimental error and confirmed the formula of the complexes. These complexes are soluble in DMF, DMSO and in buffer (pH 7.2) solution. The observed conductometric measurement values in DMF solutions fall in the region 45-75 Ω^{-1} cm^2 mole^{-1} indicate their electrolytic nature.

The IR spectra of ligand and complexes were recorded in the range of 4000-400 cm^{-1} on KBr pellets. The IR spectra of 2-hydroxy-4-methyl-1,8-naphthyridine show bands at 1462 cm^{-1} assigned to ν(C=N) aromatic hydrocarbon, 3181 cm^{-1} assigned to ν(C-H) group and 3396 cm^{-1} assigned to ν(O-H) group. In the spectra of complexes **(1)** and **(2)**, these bands were shifted to 1681 cm^{-1} and 1694 cm^{-1} for ν(C=N)group, 3040 cm^{-1} and 3060 cm^{-1} for ν(C-H) group and 3344 cm^{-1} and 3320 cm^{-1} for ν(O-H) group respectively. Besides, the complexes show new bands at 500-550 cm^{-1} are assigned to ν(M-N) bands [43]. In addition, the IR spectrum of the PF_6 salts of each complex showed a strong band at 756 cm^{-1} and 750 cm^{-1} ascribed to the counter anion and this band was absent for the corresponding salts [44].

In the ^{1}H NMR spectra of the complexes, the peaks due to various protons of mixed ligand of 1,10-phenanthroline and 2-hydroxy-4-methyl-1,8-naphthyridine are seen to be shifted on complexation with corresponding free ligands, suggesting complexation. The peaks due to CH_3 group at 2.60 ppm, (Ar-OH) group at 8.20 ppm and (Ar-H) group at 7.80 ppm in the spectra of the ligand are shifted to 2.50 ppm, 7.40 ppm and 7.70 ppm in the spectra of complex (**1**). The resonance due to (ArN-H) group at 8.80 ppm and (CH=N) group at 8.10 ppm in the spectra of the ligand are shifted to 9.00 ppm and 8.00 ppm in the spectra of complex (**1**) respectively. The coordination induced shifts indicates that the ligands are coordinated to the metal ion. Based on the above results, both the complexes are proposed to have octahedral geometry.

Absorption Spectral Studies

The binding of complex with DNA helix is characterized by electronic absorption spectroscopy. The magnitude of binding DNA with the complex through intercalation is determined by the change in absorbance (hypochromism) and red shift in wavelength, due to the intercalative mode involving a strong stacking interaction between the aromatic chromosphere and the DNA base pairs. The extent of hypochromism is commonly consistent with the strength of the intercalative interaction [45-47].

The Figures 2 and 3 represent the absorption spectra of the complexes (**1**) & (**2**) in the absence and presence of CT-DNA respectively. The well resolved bands were noticed at 294 nm for complex **(1)** and at 303 nm for complex **(2)** with increasing the DNA concentration (0-140

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μM). The result shows that the absorbance (hypochromism) decreased by the successive addition of CT- DNA to the complex solution. The hypochromism observed for the bands of complex **(1)** and **(2)** are accompanied by small bathochromic shift were 2 and 1 nm in Figures 2 and 3 respectively. The hypochromism and bathochromic shift are observed for the complexes suggest that binding is intercalative mode. In order to compare quantitatively, the DNA binding strengths of these complexes, the intrinsic DNA binding constants K_b are determined from the decay of the absorbance at 294 nm for complex **(1)** and 303 nm for complex **(2)** with increasing concentrations of DNA. The observed K_b values for complex **(1)** and **(2)** are equal to the classical intercalators bound to CT-DNA. The K_b values for complex **(1)** and **(2)** are 2.7×10^4 M^{-1} and 4.3×10^4 M^{-1} respectively. So, it is obvious that the present complexes are involved in intercalative interactions with CT-DNA.

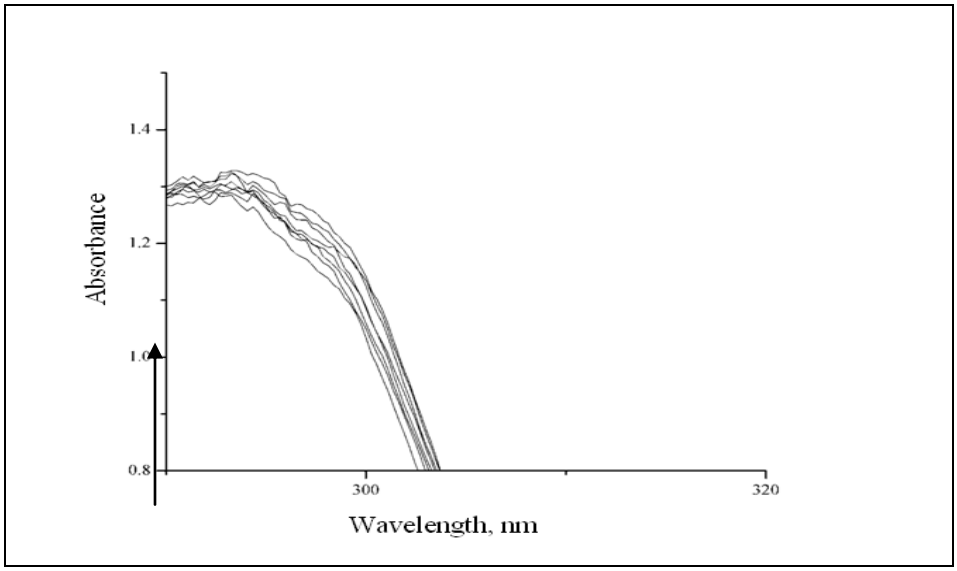

Figure-2: Absorption spectra of complex (1) in Tris-HCl buffer upon addition of DNA. [Cu] = 0.5 μM, [DNA] = 0-140 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

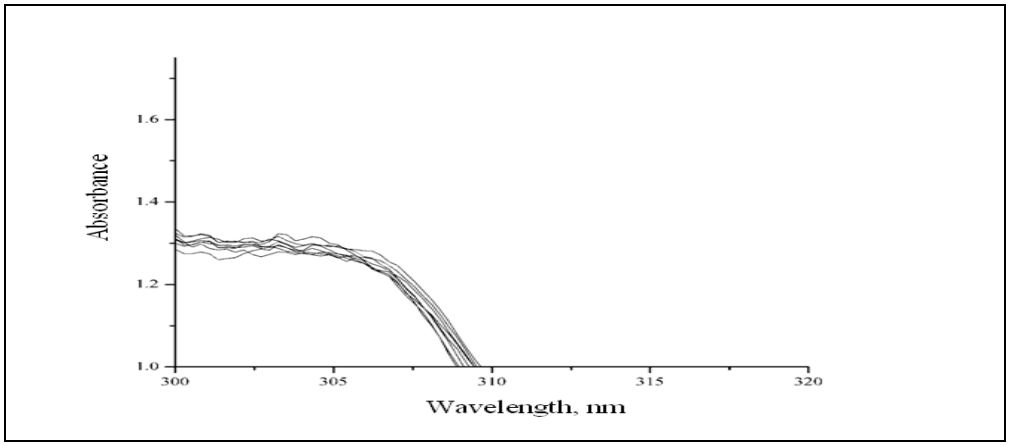

Figure-3: Absorption spectra of complex (2) in Tris-HCl buffer upon addition of DNA. [Mn] = 0.5 μM, [DNA] = 0-140 μM. Arrow shows the absorbance changing upon the increase of DNA concentration.

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Viscosity Measurements

The interactions between the complex and DNA were investigated by viscosity measurements. In fact, optical photophysical probes generally provide necessary but not sufficient clues to support the binding model. Viscosity measurements that are sensitive to length change of DNA are regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data [48]. A classical intercalation mode usually resulted in lengthening the DNA helix, as base pairs were separated to accommodate the binding complex, leading to an increase in DNA viscosity. In contrast, a partial and/or non classical interaction of ligand could bend (or kink) the DNA helix, reduce its effective length and concomitantly, its viscosity [49,50]. In order to further elucidate the binding mode of the present complex, viscosity measurements were carried out on CT-DNA by varying the concentration of the added complex. As seen in Figure 4, the viscosity of DNA increased as increasing the ratio of both complexes to DNA. This result further suggested an intercalative binding mode of the complexes with DNA and also parallel to the above spectroscopic results, such as hypochromism and bathochromism of complexes in the presence of DNA.

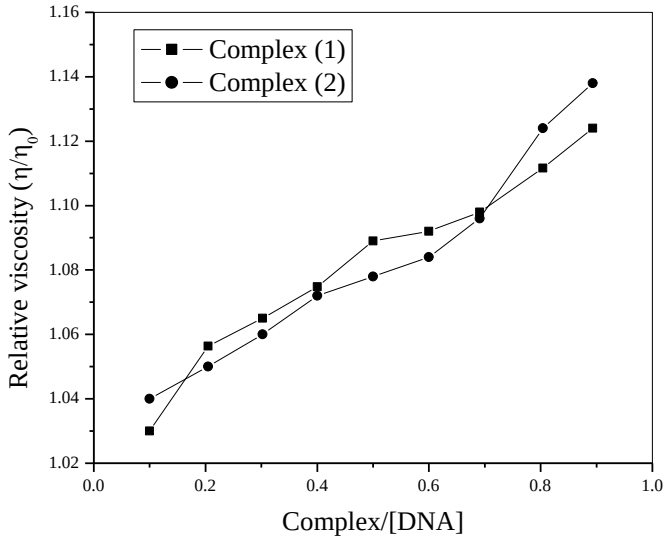

Figure-4: Plot of relative viscosity Vs [complex] /[DNA]. Effect of complex (1) and complex (2) on the viscosity of CT-DNA at 25 (±0.1)°C, [Complex] = 0-100 μM, DNA= 50 μM.

Thermal Denaturation studies

The thermal behavior of DNA is a measure of the stability of DNA double helix with temperature. An interaction between DNA and complexes were indicated by the increase in the thermal melting temperature (T_m). Thermal denaturation experiments also revealed that the intercalation of these metal complexes with DNA. In order to identify this transition process, the melting temperature T_m, which is defined as the temperature where half of the total base pairs is unbounded, is usually introduced. The increase in T_m and σ_T of DNA could be

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interpreted in terms of the stabilization that results from the intercalation of these metal complexes with DNA [51]. In the present thermal denaturation studies, thermal melting studies were carried out at DNA to complex concentration ratios of 25 and T_m and σ_T (the temperature range between 10% and 90% of absorption increase occurred) values were determined by monitoring the absorbance of DNA at 260 nm as a function of temperature. As shown in Figure 5, the T_m of DNA in the absence of any added drug was found to be 68 $\pm$ 1 ºC, under other experimental conditions. Under the same set of experimental conditions, the presence of complexes **(1)** and **(2)** increased the T_m by 2 ºC and 1 ºC respectively.

The observations made during the absorption titration, viscosity measurements and thermal denaturation experiments are reminiscent of those reported earlier for various metallointercalators, thus the results suggesting that the complexes **(1)** and **(2)** bound to DNA by intercalations [52-58].

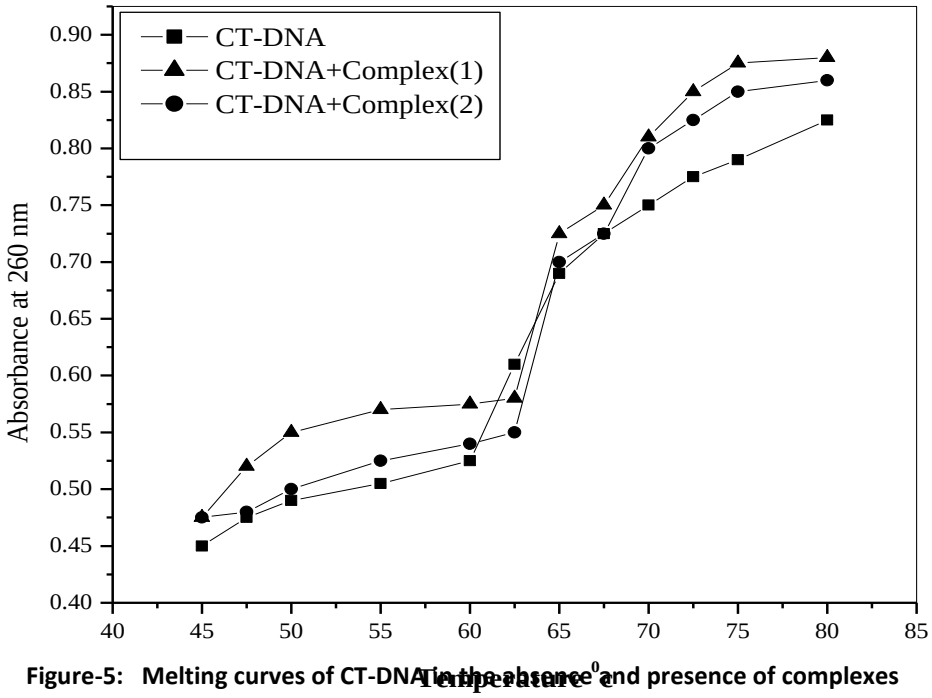

Figure-5: Melting curves of CT-DNA in the absence and presence of complexes

DNA Cleavage studies

The oxidative DNA cleavage activity of both the complexes were studied by gel electrophoresis [59-62] using super coiled(SC) pUC19 DNA (0.5 μg*)* in Tris-HCl buffer (pH, 7.2). Figure 6 shows the gel electrophoresis separations of pUC19 DNA after 1 hour incubation with varying concentrations of the complexes. Figure 6 summarizes the results of oxidative DNA cleavage experiments carried out with the complexes **(1)** and **(2)** (at the concentration of 40 μM, and 80 μM) as mentioned by the agarose gel electrophoresis method. Control experiments suggested that untreated DNA does not show any cleavage (lane 1; Figure 6). In

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the present study, pUC19 DNA gel electrophoresis experiment was conducted at 37 °C using the **(1)** and **(2)** in the presence of H_2O_2 as an oxidant. From the results of Figure 6 at higher concentration **(1)** and **(2)** shows better nuclease activity. Control experiments using H_2O_2 did not show any significant cleavage of pUC19 DNA (lane 1). At the concentration of 40 μM and 80 μM, complex **(1)** is able to convert 55 % and 65 % of the initial SC (Form I) to NC (Form II) (lane 3). The complex **(2)** is able to convert 60 % (40 μM) and 70 % (80 μM) of the initial SC (Form I) to NC (Form II) (lane 4 and 5).

Figure-6: Gel electrophoresis diagram showing the cleavage of SC pUC 19 DNA (0.5 μg), by the complexes (1) and (2) in a buffer containing 50 mM Tris-HCl and 50 mM NaCl 37 ºC in the presence of H_2O_2 : lane 1. DNA control; lane 2. DNA+40 μM (1); Lane 3. DNA+80 μM (1); Lane 4. DNA+40 μM (2); Lane 5. DNA+80 μM (2).

These qualitative findings could be quantified by densitometric analysis of the bands originating from SC and NC plasmids. Bands from the linear form, although clearly visible on the gels, were difficult to quantify. Large errors arise on weaker bands because the definition of the background is somewhat arbitrary in those cases. Therefore, parameters for quantification were chosen such that only the SC and NC bands were included in the procedure. The sum of intensity of both bands was standardized to 100% in all lanes.

CONCLUSION

In conclusion, we have synthesized and characterized two new complexes of the type $[Cu(L_2)_2L_1]$ $(PF_6)_2$ [Complex (**1**)] and $[Mn(L_2)_2L_1]$ $(PF_6)_2$ [Complex (**2**)]. Interactions of the new complexes with (double stranded) DNA were investigated by absorption spectra, viscosity and thermal denaturation studies. From the experimental results, it was confirmed that the complexes bound with the double stranded DNA with binding constant $K_b = 2.7 \times 10^4$ M^{-1} for complex (**1**) and $K_b = 4.3 \times 10^4$ M^{-1} for complex (**2**), respectively. The viscosity of solution of the DNA bound to the complexes increased with increase in concentration of the complexes. Thermal denaturation experiments revealed the intercalation of both complexes with DNA. Further, the cleavage studies show that the complex **(1)** and **(2)** have significant nuclease activity.

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