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Binding and photocleavage of DNA by mixed ligand Co(III) and Ni(II) complexes of thiophene[2, 3-b] quinoline and phenanthrolie/bipyridine

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Abstract In order to systematically perform an experimental and theoretical study on DNA binding and photocleavage properties of transition metal complexes of the type $[M(L)_2(L_1)](PF_6)_n \cdot xH_2O$ (where M = Co(III) or Ni(II), L = 1,10-phenanthroline or 2.2′ bipryidine, L_1 = Thiophene [2,3-*b*] quinoline (qt), n = 3 or 2 and x = 5 or 2) have been synthesized and characterized by elemental analysis, IR, ^{1}H NMR, UV and magnetic susceptibility data. The DNA-binding properties of these complexes have been investigated with UV-Vis, viscosity measurements, thermal denaturation and cyclic voltametric studies. It is experimentally found that all the complexes are bound to DNA via intercalation in the order $[Co(bpy)_2(qt)](PF_6)_3 > [Co(phen)_2(qt)](PF_6)_3 > [Ni(phen)_2(qt)](PF_6)_2 > [Ni(bpy)_2(qt)](PF_6)_2$. The photocleavage studies with pUC19 DNA shows that all these complexes promoted the conversion of SC form to NC form in absence of 'inhibitors'.

Keywords Mixed ligand complexes · Phenanthroline · Bipyridine · DNA binding · Photocleavage

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Abbrevations

SC	Super coiled
NC	Nicked circular
DNA	Deoxyribose nucleic acid
pUC	plasmid University of California

Introduction

Transition metal complexes often useful as chemical nucleases due to their versatile structure, redox behaviour and physicochemical properties (Sigman et al. 1996; Barton 1986; Pyle et al. 1990; Friedman et al. 1990). The binding and cleavage of transition metal complexes with DNA has received a great deal of attention during the past decade (Yang et al. 1998). The interaction of transition metal complexes with DNA has been extensively studied in the development of new tools for nanotechnology (Banerjee et al. 1993; Sardesai et al. 1994). Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids, in purifying nucleic acids (Barton and Lippard 1980), in probing the structure and biochemistry of nucleic acids.

Among the various metal complexes employed so for the mixed ligand Ru(II)/(III), Co(III) and Ni(II) complexes that contain both bpy or phen and modified bpy or phen moiety as ligands are prominent (Kuroda et al. 1992; Vaidyanathan and Nair 2003; Uma et al. 2005). In these mixed ligand complexes, the ligands or

metal ion may be varied in an easily controlled way to facilitate the individual application (Hiort et al. 1993). The transition metal complexes containing planar or fused ring systems can bind to DNA by non-covalent interactions such as electrostatic, intercalative and groove binding (Hartshorn et al. 1992; Pebg et al. 2007). All the studies reveal that modification of the metal or ligands would lead to subtle or substantial changes in the DNA binding modes, location and affinity. This gives valuable information to explore the various site-specific DNA probes and potential chemotherapeutical agents (Zhang et al. 2001; Frodl et al. 2002).

The DNA cleavage reactions are generally targeted towards its basic constituents, viz. heterocyclic base, sugar and phosphate. While the reactions targeted to the phosphodiester linkage proceed via hydrolytic cleavage pathways leading to the formation of fragments that could be relegated through enzymatic processes, the DNA cleavage by nucleobase oxidation and/or degradation of sugar by abstraction of sugar hydrogen atom(s) follows oxidative reaction pathway (Erkkila et al. 1999; Norden et al. 1996). Among different methodologies adopted for oxidative cleavage of DNA, the one based on irradiation with UV or visible light of longer or shorter wavelength has gained importance for their potential use in photodynamic therapy (PDT) of cancer (Sessler et al. 1994; Gupta et al. 2005).

Cobalt complexes have gained importance because of their application as potential hypoxia-activated prodrug (Blower et al. 2001) and the recent studies have shown that nickel complexes modify the antioxidant system and produces more active oxygen species that may be associated with the induction of chromosomal aberrations and mutation(Sugiyama 1994; Burrows and Rikita 1994). Thus, we chose to concentrate our studies on complexes of biometals, cobalt and nickel, which have the same interesting characteristics and DNA cleaving properties as ruthenium complexes, but have not received as much attention as the ruthenium(II) or (III) system (Arounaguiri and Maiya 1996).

Substituted quinolines are prominent building blocks in both organic and inorganic molecular chemistry with their π-stacking ability and coordination properties. The coordination chemistry of quinoline and its derivatives has been intensively explored due to the array of interesting electronic, photonic, magnetic, reactive and structural properties shown by the transition metal complexes of this family of ligands (Staniewicz and Hendricker 1977; Pyle et al. 1989). In light of our research, we have concluded that the precise nature (planar, aromatic and fused rings) of the ligand and metal are of paramount importance in the interaction of the complexes with the DNA molecule, which would help in the design of newer drugs and develop new selective and efficient DNA recognition and cleaving agents (Prabhakara et al. 2007; Ravikumar Naik et al. 2006). We aim at exploring the design of new metal complexes of quinolines containing sulfur donar atom, which possess more potent binding and DNA cleaving ability.

In the present paper, we report the synthesis and characterization of new cobalt and nickel complexes derived form phenanthroline/bypyridine and thiophene [2,3-*b*] quinoline ligands. The DNA binding on CT-DNA and photonuclease activity against pUC19 DNA were also reported.

Methods

Chemicals

All reagents and solvents were of AR grade, purchased commercially. All the solvents were purified before used. $CoCl_2 \cdot 6H_2O$, $NiCl_2 \cdot 6H_2O$, 1,10-phenanthroline, 2.2′ bipyridine and ammonium hexaflurophosphate (NH_4PF_6) were purchased from Qualigens Fine Chemicals (India). Tris–HCl buffer (5 mM Tris–HCl, 50 mM NaCl, pH-7.2, Tris = Tris(hydroxymethyl) amino methane solution was prepared using deionized double distilled water. Calf thymus DNA (Ct-DNA) and pUC19 DNA were purchased from Bangalore Genie (India).

Physical measurements

Melting points were determined in open capillaries and are uncorrected. Micro analyses (C, H, N and S) were performed in Carlo-Erba 1106-model 240 Perkin-Elmer analyzer. IR spectra were recorded with Shimadzu model FT-IR spectrophotometer by using KBr pellets. ^{1}H-NMR spectra were recorded on a Bruker FT NMR spectrometer (300 MHz) at 25°C in DMSO with TMS as the internal reference. UV Visible absorption spectra were recorded using Shimadzu model UV spectrophotometer at room temperature. Viscosity measurements were carried out using a semimicro

dilution capillary viscometer at room temperature. Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer.

Synthesis of Co(III) and Ni(II) complexes

The complexes $[Co(phen/bpy)_2Cl_2]Cl \cdot 3H_2O$ and $[Ni(phen/bpy)_2Cl_2]$ were prepared as reported previously (Vlcek 1967; Harris and McKenzie 1967).

Synthesis of $[Co(phen/bpy)_2(qt)](PF_6)_3 \cdot 5H_2O$ (**1**) & (**3**)

To a 50 ml ethanolic solution of $[Co(phen/bpy)_2Cl_2]Cl$ (1 mM) was added to a ethanolic solution of qt (1 mM). The mixture was refluxed for 4 hr with constant stirring under nitrogen. It was then filtered, and the complex was precipitated upon addition of a saturated ethanolic solution of ammonium hexafluorophosphate. The complex was filtered and further dried under vacuum before being recrystallized (acetone–ether) (Fig. 1).

Synthesis of $[Ni(phen/bpy)_2(qt)](PF_6)_2 \cdot 2H_2O$ (**2**) & (**4**)

A solution containing $[Ni(phen/bpy)_2Cl_2]$ (1 mM) and qt (1 mM) in ethanol was refluxed for 5 hr 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, recrystallized (acetone–ether) and further dried under vacuum (Fig. 1).

DNA-binding experiments

UV Visible absorption spectra of the complexes were recorded on a Shimadzu model UV spectrophotometer

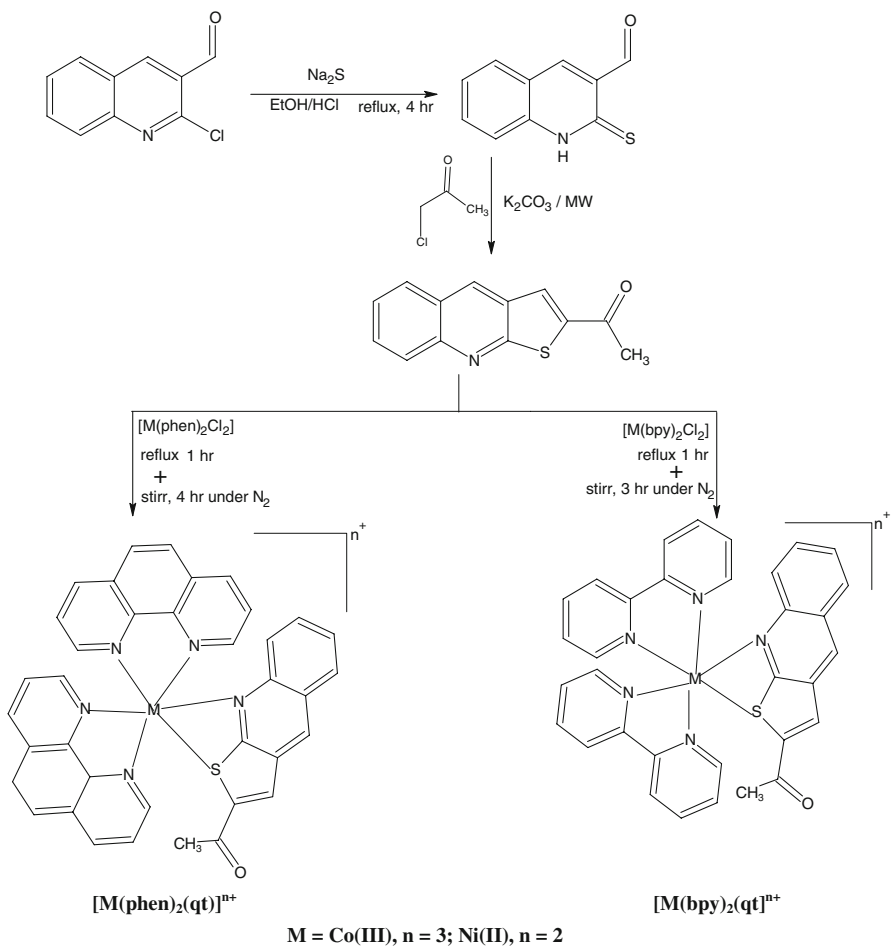

Fig. 1 Synthesis of $[Co(phen)_2(qt)]3+$, $[Ni(phen)_2(qt)]2+$, $[Co(bpy)_2(qt)]3+$ and $[Ni(bpy)_2(qt)]2+$

at room temperature. A solution of calf thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm about 1.8–1.9:1, indicating that the DNA was sufficiently free from protein (Ramakrishnan and Palaniandavar 2005). The concentration of CT-DNA was determined spectrophotometrically using the molar extinction coefficient at 6,000 M^{-1} cm^{-1} at 260 nm (Sasmal et al. 2007). Stock solutions were stored at 4°C and used within 48 h of preparation. The complex and DNA solutions were allowed to incubate for 10 min before the absorption spectra were recorded. The electronic absorption spectra of complexes in buffer (5 mM Tris–HCl, 50 mM NaCl pH 7.2) were performed by using a fixed complex concentration to which increasing amounts of DNA stock solution were added. To enable quantitative comparison of the DNA binding affinities the intrinsic binding constant K_b of the complexes for binding with CT-DNA were obtained by using Eq. (1) (Wolf et al. 1987)

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

where [DNA] is the concentration of DNA in base pairs, ε_a corresponds to the apparent absorption coefficient A_{abs}/[M], ε_f corresponds to the extinction coefficient for the free metal [M] complex and ε_b corresponds to the extinction coefficient for the metal [M] complex in the fully bound form. In plots of $[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_f)$ vs. [DNA]. K_b is given by the ratio of slope to the intercept.

Viscosity measurements were carried out using a semimicro dilution capillary viscometer (Viscomatic Fica MgW) with a thermostated bath D40S maintained at a constant temperature 25°C ± 0.1°C. Each sample was measured three times and an average flow time was calculated. Data were presented as (η/η_o) vs. binding ratio. Where η is the viscosity of DNA in the presence of complex and η_o is the viscosity of DNA alone.

Thermal denaturation studies were carried out with a Perkin-Elmer Lambda 35 spectrophotometer equipped with a Peltier temperature-controlling programmer (±0.1°C). The absorbance at 260 nm was continuously monitored for solutions of CT-DNA (0.1 μM) in the absence and presence of the complexes (0.5 μM). The temperature of the solution was increased by 1°C min^{-1}.

DNA photo cleavage studies

The extent of cleavage of super coiled (SC) pUC19 DNA (0.5 μl, 0.5 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). In the cleavage reactions, the 30 μM and 20 μM complexes in 18 μl buffer were photo-irradiated at 365 nm using monochromatic filter source. 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 containing1.0 μg/ml 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 UVITEC Gel Documentation System. Due corrections were made for the trace of NC DNA present in the SC DNA sample and for the low affinity of EB binding to SC DNA in comparison to the NC form. The wavelength used for the photo-induced DNA cleavage experiments was 365 nm.

Results and discussion

The hexafluorophosphate salts of the complexes employed in this work have been characterized by elemental analysis, UV, IR and ^{1}H NMR spectroscopic (for diamagnetic complexes) and magnetic susceptibility (for paramagnetic complexes) measurements. These data are summarized in Tables 1–3.

The IR spectra of the ligand shows strong band at 1,623 and 1,326 cm^{-1} were assigned to ν(C=N) of azomethine and ν(C–S–C) of thiophene group. These bands were shifted nearly 20−30 nm to lower frequencies in case of complexes indicates that the azomethine and thiophene groups are involved in the complexation. The band at 1,521 cm^{-1} can be attributed to the ring stretching frequencies of ν(C=C) of 1,10-phen/bpy. Very strong bands observed in the region 835–839 cm^{-1} have been assigned to PF_6 salt of each complex ascribable to the counter anion.

In the ^{1}H NMR spectra of the Co(III) complex, the peaks due to various protons of phen and qt are seen to be shifted in comparison with the corresponding

Table 1 Analytical and physical properties of the ligand and mixed ligand Co(III) and Ni(II) complexes

Compound	Yield (%)	Mol. weight	Found (Calc. %)				
			C	H	N	S	M
Ligand	69	227.28	68.70	3.99	6.16	14.11	–
$[Co(phen)_2(qt)](PF_6)_3$	78	1138.01	43.29 (43.80)	3.08 (3.25)	5.31 (5.85)	6.01(6.25)	5.08 (5.15)
$[Ni(phen)_2(qt)](PF_6)_2$	70	939.82	49.29 (49.57)	3.02 (3.12)	6.10 (6.30)	6.92 (6.38)	6.28 (6.00)
$[Co(bpy)_2(qt)](PF_6)_3$	76	1090.9	40.15 (40.40)	3.11 (3.00)	6.89 (6.59)	7.10(7.01)	5.16 (5.34)
$[Ni(bpy)_2(qt)](PF_6)_2$	79	891.78	46.28 (46.19)	3.93 (3.98)	6.82 (6.96)	7.34 (7.85)	6.23 (6.00)

Table 2 Some important IR stretching frequencies (cm^{-1}) and UV/Visible data of Co(III) and Ni(II) complexes

Complex	UV/Visible λ_{max} (nm) (log ε)[a]	IR (cm^{-1})[b]
$[Co(phen)_2(qt)](PF_6)_3$	232 (5.46), 294(5.49), 344 (4.75), 368 (4.21)	1586, 1291, 1431, 837
$[Ni(phen)_2(qt)](PF_6)_2$	238 (4.28), 281 (4.38), 356 (4.13), 359 (4.58)	1582, 1289, 1420, 839
$[Co(bpy)_2(qt)](PF_6)_3$	233(5.61), 246 (5.43), 295 (4.24), 332 (4.44)	1601, 1309, 1398, 836
$[Ni(bpy)_2(qt)](PF_6)_2$	240 (5.35), 254 (5.06), 274 (4.15), 329 (4.19)	1608, 1312, 1445, 839

[a] Spectra were measured in Tris HCl buffer: λ_{max}, ± 2 nm; logε, ± 10%

[b] Spectra were measured as KBr pellets

Table 3 Some important ^1H NMR and magnetic susceptability data of Co(III) and Ni(II) complexes

Complex	δ ppm[a]	μ_{eff} (BM)[b]
$[Co(phen)_2(qt)](PF_6)_3$	9.92 (d, 2H), 8.00 (m, 4H), 8.92 (d, 2H), 8.60 (d, 6H m), 8.29 (d, 2H), 8.59 (m, 8H), 7.70 (d, 4H)	Diamagnetic
$[Ni(phen)_2(qt)](PF_6)_2$	–	3.11
$[Co(bpy)_2(qt)](PF_6)_3$	9.42 (d, 2H), 8.28(m, 4H), 8.46 (d, 2H), 7.80 (d, 6H m), 8.18 (d, 2H), 8.39(m, 8H), 7.68 (d, 4H)	Diamagnetic
$[Ni(bpy)_2(qt)](PF_6)_2$	–	3.16

free ligands suggesting complexation (Table 3). Unlike the Co(III) complex was diamagnetic, Ni(II) complex was found to be paramagnetic with a μ_{eff} value of 3.11 and 3.16 ± 0.20 BM as expected for typical d^8 systems.

Absorption titration

The electronic absorption spectra of complexes in presence of increasing amounts of DNA in 5 mM Tris, 50 mM NaCl, pH 7.2 buffer are as shown in Fig. 2. In the UV region, the intense absorption bands with maxima of 296 nm for $[Co(phen)_2(qt)](PF_6)_3$, 288 nm for $[Ni(phen)_2(qt)](PF_6)_2$, 283 and 375 nm for $[Co(bpy)_2(qt)](PF_6)_3$ and 292 nm for $[Ni(bpy)_2(qt)](PF_6)_2$ were attributed to interligand $\pi - \pi^*$ transition of the complexes. Addition of increasing amount of CT-DNA resulted in the hypochromism and red shift in the UV-spectra of the complex. These spectral characteristics suggested that the complex might bind to DNA by an intercalative mode. After intercalating the base pairs of DNA, the π^* orbit of the intercalated ligand could couple with the π orbital of base pairs, thus decreasing the $\pi - \pi^*$ transition energy and further resulting in the red-shift. On the other hand, the coupling π orbit was partially filled by electrons, thus decreasing the transition probabilities and concomitantly, resulting in the hypochromism (Pyle et al. 1989).

The magnitude of hypochromism and red shift observed for all the Co(III) and Ni(II) complexes (Table 4.) are comparable to those observed to typical classical intercalators and partially intercalating metal complexes [EthBr, K_b, 1.8×10^6 M^{-1}, [VO(saltrp)(B)], saltrp = N-salicylidene-L-methionate, B = N,N-donor heterocyclic base, K_b, 7.2×10^5 M^{-1}, [Co(bpy)2(dpta)]3 + , dpta = dipyrido-[3,2-a: 2′,3′-

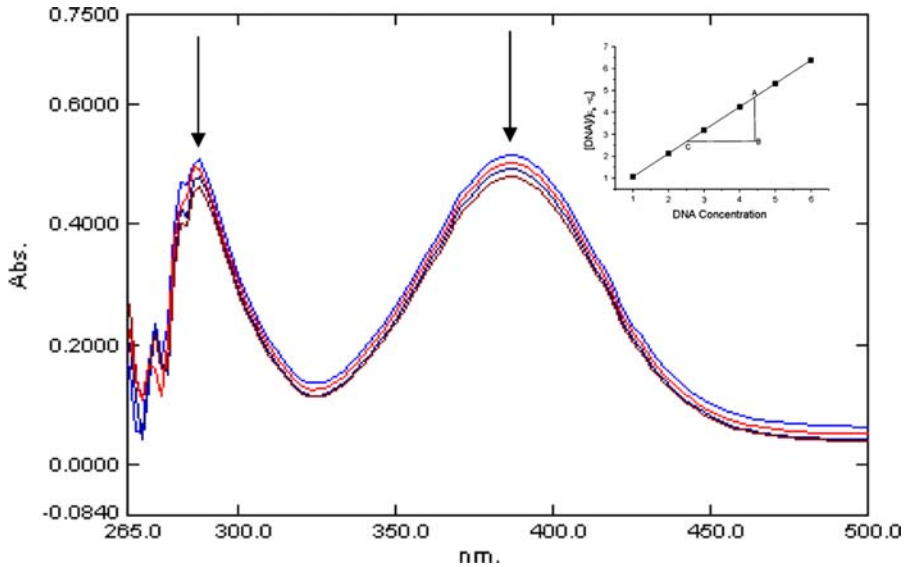

Fig. 2 Absorption spectra of complex $[Co(bpy)_2(qt)](PF_6)_3$ in Tris–HCL buffer upon addition of CT DNA. [Ni] = 0.5 μM, [DNA] = 0.1 μM. Arrow shows the absorbance changing upon the increase of DNA concentration. The inner plot of [DNA]/$(\varepsilon_a - \varepsilon_f)$ vs. [DNA] for the titration of DNA with Co(III) complex

Table 4 Absorption spectral properties of Co(III) and Ni(II) complexes bound to CT-DNA

Compound	Ligand –based				
	λ_{max} (nm)	Change in Abs.	Δε (%)	Red-shift (nm)	$K_b \times 10^5$ (M^{-1})
$[Co(phen)_2(qt)](PF_6)_3$	296	Hypochromism	32	4	2.4
$[Ni(phen)_2(qt](PF_6)_2$	288	Hypochromism	23	2	1.9
$[Co(bpy)_2(qt)](PF_6)_3$	375	Hypochromism	38	6	2.9
$[Ni(bpy)_2(qt)](PF_6)_2$	292	Hypochromism	29	2	1.6

c]-thien-[3,4-c]azine, in 25 mM Tris–HCl/40 mM NaCl buffer, pH 7.9) and partial intercalating metal complexes $[Ru(phen)_2(dppz)]^{2+}$, dppz = dipyrido-[3,2-d: 2′,3′-f]-phenazine, $K_b > 10^6$ M^{-1}] bound to CT-DNA (A. R. Chakravarty et al. 2007, Liang-Nian Ji et al. 2008). The intrinsic binding constant K_b obtained for Co(III) and Ni(II) complexes follow the order $[Co(bpy)_2(qt)](PF_6)_3 > [Co(phen)_2(qt)](PF_6)_3 > [Ni(phen)_2(qt)](PF_6)_2 > [Ni(bpy)_2(qt)](PF_6)_2$ suggesting that the Co(III) complexes shows more binding property than Ni(II) complexes.

Viscosity measurements

Furhtermore, the interactions between the complexes and DNA were investigated by viscosity measurements. Optical photophysical probes provided necessary, but not sufficient clues to support a binding model. Hydrodynamic measurements that were sensitive to length change (i.e., viscosity and sedimentation) were regarded as the least ambiguous and the most critical tests of binding mode in solution in the absence of crystallographic structural data (Sathyanarayana et al. 1993; Sathyanarayana et al. 1992). A classical interacalation model usually resulted in lengthening the DNA helix, as base pairs were separated to accommodate the binding ligand leading to the increase of DNA viscosity. As seen in Fig. 3 the viscosity of DNA increased as increases the ratio of complexes to DNA. This result further suggested an intercalative binding mode of the complex with DNA and also paralleled to the above spectroscopic results, such as hypochromism, bathochromism (red-shift) of complexes in the presence of DNA.

Thermal denaturation study

Thermal behaviours of DNA in the presence of complexes can give insight in to their conformational changes when temperature is raised, and offer information about the interaction strength of complexes with DNA. It is well known that when the

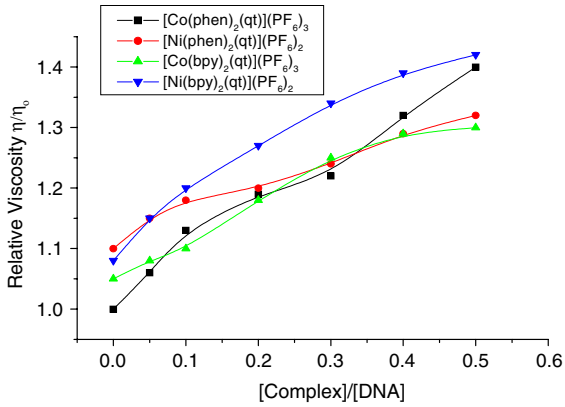

Fig. 3 Effect of increasing amounts of the complex $[Co(phen)_2(qt)]^{3+}$ [■], $[Ni(phen)_2(qt)]^{2+}$ [●], $[Co(bpy)_2(qt)]^{3+}$ [▲] and $[Ni(bpy)_2(qt)]^{2+}$ [▼] on the relative viscosities of CT-DNA at 25 (± 0.1)°C

temperature in the solution increases, the double stranded DNA gradually dissociates to single strands and generates a hyperchromic effect on the absorption spectra of DNA bases ($\lambda_{max} = 260$ nm). 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 are unbounded, is usually introduced. According to the literature the interaction of natural or synthesized organics and metallointercalators generally results in a considerable increase in melting temperature (T_m). Here DNA (100 μM) melting experiments revealed that T_m of CT-DNA is 75.3 ± 1°C in the absence of the complex as seen in Fig. 4. However, with addition of complex $[Co(phen)_2(qt)](PF_6)_3$ (30 μM), the T_m of

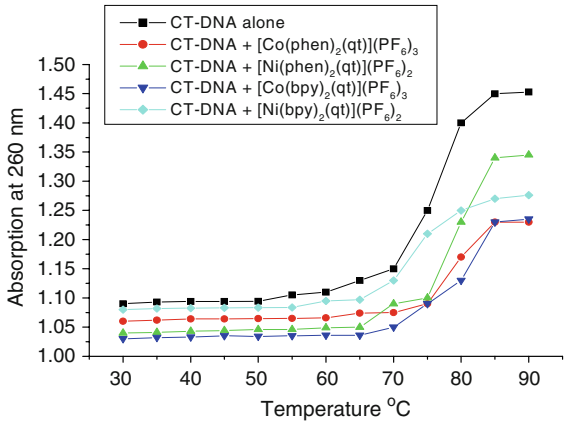

Fig. 4 Melting curves of CT-DNA in the absence and presence of complexes $[Co(phen)_2(qt)]^{3+}$, $[Ni(phen)_2(qt)]^{2+}$, $[Co(bpy)_2(qt)]^{3+}$ and $[Ni(phen)_2(qt)]^{2+}$

the DNA increases dramatically to 80.0 ± 1°C. Similarly, for $[Ni(phen)_2(qt)](PF_6)_2$, $[Co(bpy)_2(qt)](PF_6)_3$ and $[Ni(bpy)_2(qt)](PF_6)_2$ complexes the T_m increased to 79.2 ± 1°C, 83.0 ± 1°C and 78.3 ± 1°C, respectively. The increased T_m (~3–7.5°C) value of the DNA after addition of the complexes are comparable to that observed for classical intercalators (Arounguiri et al. 1996).

Cyclic voltammetric studies

The application of cyclic voltammetry to study of the interaction between complexes and DNA provides a useful compliment to the previously utilized methods of investigation such as UV-Vis and viscosity experiments. The typical cyclic voltammogram of 0.02 mM solution of $[Co(phen)_2(tq)]^{3+}$complexe without and with DNA at carbon paste electrode in Tris–HCl buffer were carried out (Fig. 5).

In the forward scan, a single cathodic peak was observed, which corresponds to the reduction of complex. In the reverse scan, no anodic peak was observed, which indicates that the process is irreversible. When CT-DNA is added to a solution of complex, marked decrease in the peak current height and shifts of peak potential to more −ve values were observed. The cyclic voltammetric behavior were not affected by the addition of very large excess of DNA, indicating that the decrease of peak current of complex after the addition of DNA due to the binding of $[Co(phen)_2(tq)]^{3+}$ complex to the DNA. When the concentration of DNA increased the changes in peak current and potential become slow. This reveals that the complexes were interact with CT-DNA.

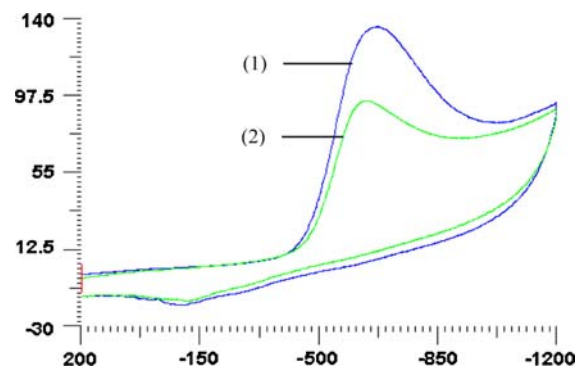

Fig. 5 Cyclic voltammograms of $[Co(phen)_2(qt)]^{3+}$ complex in Tris–HCl buffer in absence (**1**) and presence (**2**) of DNA

DNA cleavage studies using Gel electrophoresis

It is known that DNA cleavage is controlled by relaxation of supercoiled circular conformation of pUC19 DNA to nicked circular and/or linear conformations. When electrophoresis is applied to circular plasmid DNA, fastest migration will be observed for DNA of closed circular conformations (Form I). if one strand is cleaved, the supercoiled will relax to produce a slower-moving nicked conformation (Form II). If both strands are cleaved, a linear conformation (Form III) will be generated that migrates in between (Sitlani et.al. 1992; Liu et al. 2002).

Oxidative DNA cleavage

Figure 6 summarizes the results of oxidative DNA cleavage experiments carried out with the complexes of Co(III) and Ni(II) (at the concentration of 20 μM and 40 μM) as mentioned by the agarose gel electrophoresis method. Control experiments suggested that untreated DNA does not show any cleavage (Lane 1; Fig. 6a, b). It is shown that the complexes (**1**) and (**2**) at higher concentration (40 μM) show more cleavage activity in which supercoiled (Form-I) DNA cleaved and supercolied will relaxed to produce a slower moving nicked circular form (Form-II) (Lane 3 and 5; Fig. 6a) compared to lower concentration (20 μM) (Lane 2 and 4; Fig 6a). In the case of complexes (**3**) and (**4**) also at higher concentration (40 μM) the DNA cleaved from supercoiled (Form-I) to nicked circular form (Form-II) (Lane 3 and 5; Fig. 6b) than at lower concentration (20 μM) (Lane 2 and 4; Fig. 6b). In conclusion at higher concentration of 40 μM the new complexes shows more cleavage activity. From these results we infer that the cobalt(III) and nickel(II) complexes at higher concentrations act as a potent nuclease agent.

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. A plot of relative intensities is presented in Fig. 7 (For oxidative cleavage).

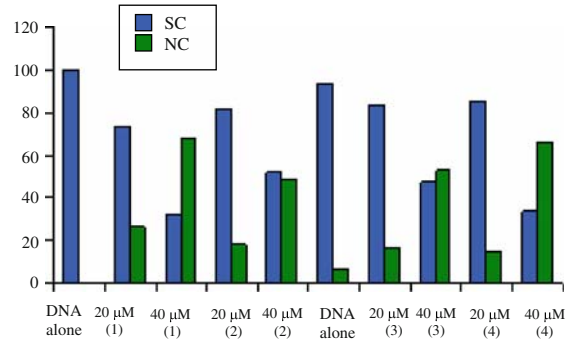

Fig. 7 Quantification of gel electrophoresis bands originating from SC and NC DNA in our cleavage experiments. The sum of intensities of both bands is standardized to 100% for each individual lane. Metal complexes and concentrations are annotated (dd H_2O: doubly distilled water as background). See text and experimental section for details

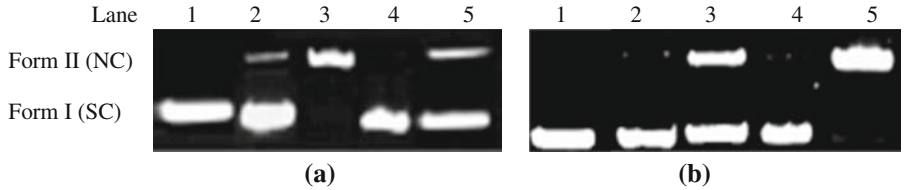

Fig. 6 (**a**) Cleavage of supercoiled pUC19 DNA (0.5 μg) by the cobalt(III) and nickel (II) complexes in a buffer containing 50 mM Tris-HCl and 50 mM NaCl at 37°C. Lane 1 DNA alone; Lane 2, DNA + 20 μM of complex (1); Lane 3, DNA + 40 μM of complex (1); Lane 4, DNA + 20 μM of complex (2); Lane 5, DNA + 40 μM of complex (2). Forms I-II are supercoiled and nicked circular DNA, respectively. (**b**) Cleavage of supercoiled pUC19 DNA (0.5 μg) by the cobalt(III) and nickel (II) complexes in a buffer containing 50 mM Tris-HCl and 50 mM NaCl at 37°C. Lane 1 DNA alone; Lane 2, DNA + 20 μM of complex (3); Lane 3, DNA + 40 μM of complex (4); Lane 4, DNA + 20 μM of complex (3); Lane 5, DNA + 40 μM of complex (4). Forms I-II are supercoiled and nicked circular DNA, respectively

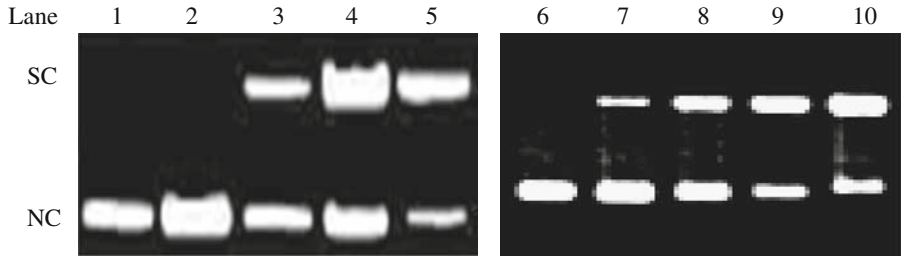

Fig. 8 Gel electrophoresis diagram of the control experiments using SC DNA (05 μg), $[Co(phen)_2(qt)]^{3+}$ and $[Ni(phen)_2(qt)]^{2+}$ and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; Lane 2, DNA + NaN_3 (38 μM) + complex (**1**); Lane 3, DNA + DMSO (4 μl) + complex (**1**); Lane 4, DNA + D_2O (14 μl) + complex (**1**); Lane 5, DNA + complex (**1**) : Lane 6, DNA Control; Lane 7, DNA + NaN_3 (38 μM) + complex (**2**); Lane 8, DNA + DMSO (4 μl) + complex (**2**); Lane 9, DNA + D_2O (14 μl) + complex (**2**); Lane 10, DNA + complex (**2**)

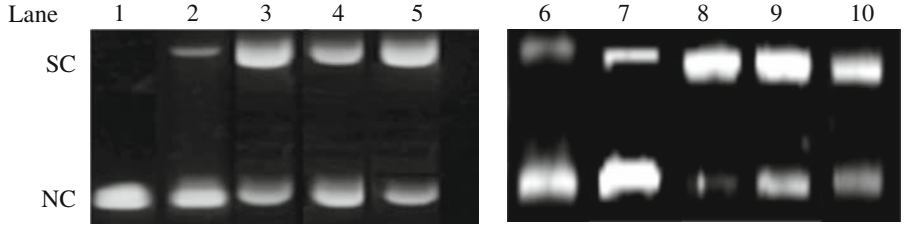

Fig. 9 Gel electrophoresis diagram of the control experiments using SC DNA (05 μg), $[Co(bpy)_2(qt)]^{3+}$ and $[Ni(bpy)_2(qt)]^{2+}$ and other additives at 365 nm for an exposure time of 1 h. Lane 1, DNA Control; Lane 2, DNA + NaN_3(38 μM) + complex (**3**); Lane 3, DNA + D_2O (14 μl) + complex (**3**); Lane 4, DNA + DMSO (4 μl) + complex (**3**); Lane 5, DNA + complex (**3**); Lane 6, DNA Control; Lane 7, DNA + NaN_3 (38 μM) + complex (**4**); Lane 8, DNA + D_2O (14 μl) + complex (**4**); Lane 9, DNA + DMSO (4 μl) + complex (**4**); Lane 10, DNA + complex (**4**)

Photocleavage studies

Irradiation of pUC19 DNA containing Co(III) and Ni(II) complexes was carried out in the presence and in absence of various 'inhibitors' by using gel electrophoresis method.

All the complexes cleave the DNA from its SC to NC form even in the absence of inhibitors on irradiation with UV light at 365 nm (Fig. 8, Lane 5 & 10; Fig. 9, Lane 5 &10*)*. In the presecnce of inhibitors D_2O and DMSO, to assess the possibility of photoactivated change involves the formation of singlet oxygen and hydroxyl radical respectively, which are responsible for the cleavage of DNA. Singlet oxygen would be expected to induce more strand scission in D_2O than in H_2O, due to its longer life time in the former solvent (Fig. 8, Lane 4 & 9; Fig. 9, Lane 3 & 8*)*. The same results were observed in the presence of hydroxyl radical ($OH^{\bullet}$) scavenger DMSO (Fig. 8, Lane 3 & 8; Fig. 9, Lane 4 & 9). Studies with singlet oxygen quencher NaN_3 were also carried out. The cleavage is slightly inhibited (Fig. 8, Lane 2 & 7; Fig. 9, Lane 2 & 7) in presence of NaN_3, which further confirmed that singlet oxygen may be the reactive species. These results indicate that, besides the presence of inhibitors, the complexes shows cleavage activity upon irradiating with UV light at 365 nm.

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