Chapter – 3

Synthesis, characterization of Schiff base cobalt(III) and nickel(II) complexes containing N/S and N/Se donor atoms : DNA-binding and cleavage studies

3.1. Introduction

The design of new ligands for the improvement of their performances in different fields, particularly in pharmacology is an active area of research. Coordination chemistry of ligands containing nitrogen and sulphur/selenium as potential donor site is a current interest. Most of the ligands reported have either the N atom or S atom as donor site. Nitrogen containing ligands are all hard bases whereas sulphur containing ligands are soft bases. The most widely studied containing both N and S donor sites are the Schiff bases [1-5]. The real imputes towards the complexes of these type of ligands with metal ions may have biological properties including antitumour [6], antimicrobial [7] properties as well as physicochemical effects [8,9].

A large number of Schiff bases containing different donor atoms have been studied because of their interesting and important properties, such as ability to reversibly bind oxygen [10], catalytic activity in hydrogenation of olefins [11] and transfer of an amino group [12], photochromic properties [13] and complexing ability towards metals [14,15]. The high affinity for the chelation of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes.

Polyaza heteroaromatic compounds such as pyrazole, imidazole, quinoline, tetrazole, as well as their derivatives are well-known for the importance in industry and are widely used as ligands in the neutral and anionic forms [16–22]. Especially, quinolines and its derivatives constitute an interesting class of heterocyclic, which gained
considerable attention in recent years. They possess a broad spectrum of biological activities including antimicrobial and antifungal activities [23–27], e.g., quinoline antifungal agents, fluconazole and itraconazole have been extensively used for the treatment of prophylaxis and a variety of fungal infections [28]. They also show a versatile coordinating ability towards the metal ion, especially when the quinoline nucleus is substituted with additional donor groups [27] and provide 1,2-bridging as well as 2,4-bridging form in case of unsubstituted quinolines. The 2,4-bridging quinolines form is similar to 1,3-imidazolate bridging found in enzymes [29], which has made quinolines and their metal complexes much sought after compounds to mimic natural processes, and they are also used to mimic imidazoles in model compounds for such processes [30]. A large number of reports have been appeared on their metal complexes involving d-block metals [31–35].

In the present work, keeping the importance of quinolines and their coordination in mind, in this chapter we have discussed the synthesis, characterization, DNA binding and oxidative cleavage properties of Schiff bases-cobalt and nickel complexes of the type [Co(pmt)₂(H₂O)₂]⁺ and [Ni(pms)₂(H₂O)₂] in their PF₆ salt and neutral form.

3.2. Experimental

2-Chloro-3-quinoline carbaldehyde was prepared by the reported procedures described in Chapter 2. Synthesis of 3-[(phenyl-2-ylimino)methyl] quinolin-2-thiol (pmtH), 3-[(phenyl-2 ylimino) methyl] quinolin-2-selenol(pmsH) and its corresponding metal complexes of Co(III) and Ni(II) are described below.

3.2.1. Synthesis of 3-[(phenyl-2-ylimino)methyl]quinolin-2-thiol/selenol (pmtH)/(pmsH)

To the ethanolic solution (25 ml) of 2-sulfanylquinoline-3-carbaldehyde (7.64 g, 0.04 mol) was added to (25 ml) ethanolic solution of o-phenylenediamine (0.94 g, 0.01 mol) in 1:1 molar ratio. The resulting solution was refluxed for 3-4 h, a pale brownish
product (pmtH) precipitated out. It washed with cold ethanol, dried under vacuum, and recrystallized from ethyl acetate/dichloromethane.

Same procedure used for synthesis of 3-[(phenyl-2-ylimino)methyl]quinolin-2-selenol(pmsH) using 2-selenoquinoline-3-carbaldehyde as starting material.

pmtH: Brown solid, Yield 83%, m.p.201-203 °C. IR (ν) (KBr) cm⁻¹: 3019 (C-H, Ar-H); 1637 (CHN); 753 (SH); ¹H NMR (DMSO-d₆), δ 7.24 -8.4 (m, 10H, Ar-H), 10.71 (s, ¹H, CHN), 12.23 (s, ¹H, SH), [m/z]=265⁺


Brown solid, Yield 83%, m.p.204-205 °C. IR (ν) (KBr) cm⁻¹: 760 (C-H, Ar-H); 1635 (CHN); 765 (SeH); ¹H NMR (DMSO-d₆), δ 7.23 -8.84 (m, 10H, Ar-H), 10.72 (s, 1H, CHN), 12.20 (s, 1H, SeH), [M/S]=312⁺

3.2.3. Synthesis of [Co(pmt)₂(H₂O)₂] PF₆ (I) and [Co(pms)₂(H₂O)₂] PF₆ (2)

To a 50 ml methanolic solution of CoCl₂.6H₂O (0.57 g, 1 mol) was added to a methanolic solution of respective ligands: 3-[(E)-(phenyl-2-ylimino)methyl]quinolin-2-thiol (pmtH) (0.245 g, 3 mol)/ 3-[(phenyl-2-ylimino)methyl]quinolin-2-selenol(pmsH). The mixture was refluxed for 1-2 hrs with constant stirring and further stirred for 4-5 hr under nitrogen. The Co(III) complex was precipitated by the addition of NH₄PF₆ solution whereas Ni(II) complex obtained after concentrating the mixture solution. Then, it was filtered and dried under vacuum before being recrystallized (acetone-ether) (Scheme-1).

3.2.4. Synthesis of [Ni(pmt)₂(H₂O)₂] (3) and [Ni(pms)₂(H₂O)₂] (4)

A mixture of methanolic solution containing NiCl₂.5H₂O (0.58 g, 1 mmol) and 3-[(E)-(phenyl-2-ylimino)methyl]quinolin-2-thiol (pmtH) (0.245 g, 3 mmol)/ 3-[(phenyl-2-ylimino)methyl]quinolin-2-selenol(pmsH) was taken in a round bottom flask. The mixture was stirred for 1 hr and refluxed for 4-5 hr under nitrogen. The obtained complex was filtered and dried under vacuum before being recrystallized (acetone-ether) (Scheme-1).

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The synthesis of complexes are presented in Scheme 1.

**Scheme 1.** Synthesis of Co(III) and Ni(II) metal complexes of Schiff base ligands derived from quinolines containing S/Se donor atoms
3.3. Results and Discussion

The nature of the ligands and their complexes was established using elemental analysis, IR, NMR and mass spectroscopy. The elemental analysis data conductometric values and magnetic moment values of the complexes are summarized in Table 3.1. Various attempts to develop the crystals suitable for X-ray diffraction studies such as slow diffusion, crystallization using mixtures of solvents and low temperature crystallization were unsuccessful.

The analytical data for the complexes together with some physical properties reveal that the formation of complexes of Co(III) and Ni(II) ions have the general formula \([ML_2(H_2O)]^{n+}\) where \(M=\text{Co(III)}\) or \(\text{Ni(II)}\), \(L=3-[E-(\text{phenyl-2-ylimino})\text{methyl}]\) quinolin-2-thiol (pmt) or \(3-[\text{(phenyl-2-ylimino)methyl}]\text{quinolin-2-selenol(pms)}, n=1\) for Co(III). The elemental results in Table 3.1 shows that the experimental data are agree well with metal to ligand stoichiometry 1:2 for all the complexes. All the new complexes are stable at room temperature and are soluble in DMF, DMSO and buffer solution. The conductometric measurement values (12-18.6 mohs.cm\(^2\) mol\(^{-1}\)) of the complexes in DMF at \(10^{-3}\)M concentration indicating non electrolytic nature [36].

Table 3.1. Physical properties of the pmtH, pmsH ligands and their Co(III) and Ni(II) complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield %</th>
<th>Colour</th>
<th>Found (Cal.)%</th>
<th>(\Omega_M) Mohs</th>
<th>(\mu_{eff}) BM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
<td>N</td>
</tr>
<tr>
<td>pmtH</td>
<td>78</td>
<td>brown</td>
<td>72.70 (72.65)</td>
<td>4.58 (4.48)</td>
<td>10.60 (10.68)</td>
</tr>
<tr>
<td>pmsH</td>
<td>72</td>
<td>redish brown</td>
<td>61.74 (61.79)</td>
<td>3.89 (3.82)</td>
<td>9.00 (9.12)</td>
</tr>
<tr>
<td>[Co(pmt)(_2)(H(_2)O)(_2)]^+</td>
<td>70</td>
<td>Blueish green brown</td>
<td>65.41 (65.43)</td>
<td>4.12 (4.03)</td>
<td>9.53 (9.63)</td>
</tr>
<tr>
<td>[Co(pms)(_2)(H(_2)O)(_2)]^+</td>
<td>72</td>
<td></td>
<td>65.43 (65.36)</td>
<td>4.12 (4.03)</td>
<td>9.54 (9.60)</td>
</tr>
<tr>
<td>[Ni(pms)(_2)(H(_2)O)(_2)]</td>
<td>82</td>
<td>greenish</td>
<td>66.40 (66.48)</td>
<td>3.55 (4.03)</td>
<td>9.53 (9.63)</td>
</tr>
<tr>
<td>[Ni(pms)(_2)(H(_2)O)(_2)]</td>
<td>82</td>
<td>redish</td>
<td>66.42 (66.36)</td>
<td>3.55 (3.63)</td>
<td>8.23 (8.30)</td>
</tr>
</tbody>
</table>
3.4. Spectral characterization

IR, $^1$H NMR and magnetic moment data of the complexes are summarized in Table 3.2 and 3.3.

3.4.1. Infrared spectra

The determination of the coordination atoms were made on the basis of the comparison of the IR spectra of the ligands and their metal complexes (Fig. 3.1 - 3.3; Table 3.2).

The spectrum of the ligands shows $\text{-C=N}$ bands in the region 1635–1637 cm$^{-1}$, which is shifted to lower frequencies in the spectra of all the complexes in the region 1612–1616 cm$^{-1}$ indicating the involvement of $\text{-C=N}$ nitrogen in coordination to the metal ion,[36,37]. The band at 752 and 762 cm$^{-1}$ appeared due to C-SH/C-SeH in the ligands are disappeared in the spectra of complexes suggested the bonding involvement of S/Se atom of quinolines moiety in bonding to the metal ion [37]. Accordingly, the ligand acts as a tetradeutate chelating agent, bonded to the metal ions via the two nitrogen ($\text{-C=N}$) atoms of the Schiff base and two sulfur/selenium atoms of the quinoline moiety. Assignment of the proposed coordination sites is further supported by the appearance of medium bands at 415–430 cm$^{-1}$ and 418-451 cm$^{-1}$ attributed to $\nu$(M–N) and (M-S/Se) vibration, respectively[38]. In addition to these bands, the spectra of the complexes exhibited a new broad band around 3384-3445 nm which is assigned to the water molecules $\nu$(OH) associated with in the complexes.

In the IR spectra of complexes, the appearance of various quinoline nitrogen (pyridine ring) vibrations revealed the non-coordination of the quinoline nitrogen. The in plane and out-of-plane deformations and ring breathing mode of the pyridine ring did not change their positions supports the non-participation of the quinoline nitrogen in the coordination. [37].
## Table 3.2: Some important IR stretching frequencies (cm\(^{-1}\)) of Co(II) and Ni(II) complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>(v(C=\text{N})) cm(^{-1})</th>
<th>(v(\text{OH})) cm(^{-1})</th>
<th>(v(\text{C-SH}))</th>
<th>(v(\text{C-SeH}))</th>
<th>(v(\text{M-N})) cm(^{-1})</th>
<th>(v(\text{M-S})) cm(^{-1})</th>
<th>(\text{M-Se})</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmtH</td>
<td>1637</td>
<td>--</td>
<td>752</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>pmsH</td>
<td>1635</td>
<td>--</td>
<td>--</td>
<td>762</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>([\text{Co(pmt)}_2(\text{H}_2\text{O})_2]^+)</td>
<td>1615</td>
<td>3346</td>
<td>--</td>
<td>--</td>
<td>415</td>
<td>418</td>
<td>320</td>
</tr>
<tr>
<td>([\text{Ni(pmt)}_2(\text{H}_2\text{O})_2])</td>
<td>1616</td>
<td>3348</td>
<td>--</td>
<td>--</td>
<td>451</td>
<td>450</td>
<td>445</td>
</tr>
<tr>
<td>([\text{Co(pms)}_2(\text{H}_2\text{O})_2]^+)</td>
<td>1614</td>
<td>3350</td>
<td>--</td>
<td>--</td>
<td>318</td>
<td>445</td>
<td>448</td>
</tr>
<tr>
<td>([\text{Ni(pms)}_2(\text{H}_2\text{O})_2])</td>
<td>1613</td>
<td>3352</td>
<td>--</td>
<td>--</td>
<td>430</td>
<td>435</td>
<td>443</td>
</tr>
</tbody>
</table>

**Figure 3.1** IR spectra of pmtH ligand
Synthesis, characterization of new Schiff base cobalt(III) and nickel(II)...

Figure 3.2  IR spectra of [Co(pmt)₂(H₂O)₂] complex

Figure 3.3  IR spectra of [Ni(pms)₂(H₂O)₂] complex
3.4.2. $^1H$ NMR spectra

The $^1H$ NMR spectra of the ligands pmtH/pmsH exhibited the signal at $\delta$ 12.23 ppm due to -SH and -SeH proton. The H-C=N proton peak of pmt appear at $\delta$ 10.71 ppm. A multiplet appeared in the region $\delta$ 7.24-8.49 ppm is assignable to aromatic protons. In the $^1H$ NMR spectra of the Co(III) complex, the peaks due to various protons of ligands were seen to be shifted in complexation with corresponding free ligands, suggesting complexation. Unlike the cobalt(III) complexes are diamagnetic, nickel(II) complex was found to be paramagnetic as expected for typical $d^8$ system.

Figure 3.4. $^1H$ NMR spectra of pmtH ligand
Table 3.3: $^1$H NMR spectral data (δ, ppm) of ligands and their Co(III) complex

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^1$H NMR Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmh</td>
<td>12.23 (s, 1H, SH), 10.71 (s, 1H, H-C=N), 7.24-8.49 (m, 10H, Ar-H)</td>
</tr>
<tr>
<td>pms</td>
<td>12.23 (s, 1H, SeH), 10.71 (s, 1H, H-C=N), 7.24-8.49 (m, 10H, Ar-H)</td>
</tr>
<tr>
<td>[Co(pmt)$_2$(H$_2$O)$_2$]</td>
<td>11.28 (s, 1H, SH), 10.42 (s, 1H, H-C=N), 7.13-8.29 (m, 20H, Ar-H)</td>
</tr>
<tr>
<td>[Co(pms)$_2$(H$_2$O)$_2$]</td>
<td>10.92 (s, 1H, SeH), 10.42 (s, 1H, H-C=N), 7.13-8.29 (m, 20H, Ar-H)</td>
</tr>
</tbody>
</table>

3.4.3. UV–Vis spectra and magnetic susceptibility measurement

The electronic spectra of the ligands and their metal complexes have been studied in the range of 200–800 nm using DMF as solvent. In the U.V. region, the shoulder band observed at 295 nm of ligands may be assigned to n-$\pi^*$ transition within the C=N group in the free ligands. Electronic spectra of Co(III) complexes exhibit two major peaks at 14360 cm$^{-1}$ and at 15982 cm$^{-1}$ which are assigned to the transitions $^4$t$_{1g}$($^F$) → $^4$T$_{2g}$($^F$) and $^4$T$_{1g}$($^F$) → $^4$T$_{1g}$($^P$), respectively. The third band observed at 342000 cm$^{-1}$ was due to M → L charge transfer bands. These spectral data suggest an octahedral geometry for Co(III) complex [37]. It also supported by the magnetic moment values found in the range of 3.12 and 3.2 B.M. respectively. The Ni(II) complexes also exhibit two peaks at 12650 cm$^{-1}$ and 13760 cm$^{-1}$ attributed for $^3$A$_{2g}$ ($^3F$) → $^3$T$_{2g}$ ($^3F$) (v), $^3$A$_{2g}$ ($^3F$) → $^3$T$_{1g}$ ($^3F$) (v), $^3$T$_{2g}$ ($^3F$) → $^3$T$_{1g}$ ($^3F$) (v) three spin allowed transitions. The wide third peak appeared in the region 25000–29000 cm$^{-1}$ as shoulder due to transition $^3$T$_{2g}$ ($^3F$) → $^3$T$_{1g}$ ($^3F$) (v) observed in the complexes. This assuming an octahedral coordination for Ni(II) complexes. The magnetic susceptibility measurements were carried out in the solid state at room temperature. The Ni(II) complexes exhibit magnetic moment values in the range of 2.1–2.8 BM which corresponds to two electrons are consistent with a weak field octahedral geometry as expected. However, this magnetic moment value is lower than the spin only value for two unpaired electrons in the nickel(II) complexes [39].
Figure 3.5. Electronic spectra of \([\text{Co(pmt)}_2(\text{H}_2\text{O})_2]^+, [\text{Co(pms)}_2(\text{H}_2\text{O})_2]^+\) and \([\text{Ni(pmt)}_2(\text{H}_2\text{O})_2]^+\) complexes

3.5. DNA-binding mode and affinity

Electronic absorption spectroscopy is an effective method to examine the binding mode of DNA with metal complexes \([40-42]\). If the binding mode is intercalation, the \(\pi^*\) orbital of the intercalated ligand can couple with the \(p\) orbital of the base pairs, thus, decreasing the \(\pi \rightarrow \pi^*\) transition energy and resulting in the bathochromism. On the other hand, the coupling \(\pi\) orbital is partially filled by electrons, thus, decreasing the transition probabilities and concomitantly resulting in hypochromism \([43]\).

The absorption spectra of the complexes \([\text{Co(pmt)}_2(\text{H}_2\text{O})_2]\)PF\(_6\), and \([\text{Ni(pmt)}_2(\text{H}_2\text{O})_2]\) in presence of various concentration of DNA are given in Fig 3.6 and Fig 3.7, respectively. Two well resolved bands exist at 302, and 340 nm for \([\text{Co(pmt)}_2(\text{H}_2\text{O})_2]\)PF\(_6\), two well resolved bands observed at 292 and 315 nm for \([\text{Co(pms)}_2(\text{H}_2\text{O})_2]\)PF\(_6\), two well resolved bands at 295 and 368 nm for \([\text{Ni(pmt)}_2(\text{H}_2\text{O})_2]\) and two well resolved bands at 248 and 302 nm for \([\text{Ni (pms)}_2(\text{H}_2\text{O})_2]\) with increasing DNA concentration. The results shows that the absorbance (hypochromism) decreased by the successive addition of CT-
DNA to the complex solution. The hypochromism observed up to 36.5% at 302 nm and 32.6% at 340 nm for [Co(pmt)$_2$(H$_2$O)$_2$]PF$_6$; 28.3% at 292 nm and 23.2% at 315 nm for [Co(pms)$_2$(H$_2$O)$_2$]PF$_6$; 30.4% at 295 nm and 34.6% at 368 nm for [Ni(pmt)$_2$(H$_2$O)$_2$] and 37.4% at 248 nm and 35.3% at 302 nm for [Ni(pms)$_2$(H$_2$O)$_2$] accompanied by a small shift in wavelength (bathochromic shift) of 4-6 nm. The observed hypochromism and (red shift) bathochromic shift may suggest that the binding of metal complex to DNA is intercalative mode [42]. After the complexes intercalate the base pairs of DNA, the $\pi^*$ orbital of the intercalated ligand on the complexes could couple with $\pi$-orbital of the base pairs, thus decreasing the $\pi$-$\pi^*$ transition energies. On the other hand, the coupled $\pi^*$ orbitals of the Co(III) and Ni(II) complexes were partially filled by electrons, thus decreasing the transition probabilities. Thus, these effects resulted in the hypochromism and bathochromism [43]. 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 302 nm for [Co(pmt)$_2$(H$_2$O)$_2$]PF$_6$, 292 nm for [Co(pms)$_2$(H$_2$O)$_2$]PF$_6$, 295 nm for [Ni(pmt)$_2$(H$_2$O)$_2$] and 248 nm for [Ni(pms)$_2$(H$_2$O)$_2$] with increasing concentrations of DNA by using equation 2.1 and the results are summarized in Table 3.4. The observed $K_b$ values (given values) for Co(III) and Ni(II) complexes are equal to the $K_b$ 3.7x10$^{-6}$ and 3.5x10$^{-5}$ indicates 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. These strongest binding affinity exhibited by these complexes are expected on the basis of the additional aromatic nature of new Schiff base ligand [44]. Above $K_b$ values indicates Co (III) complex binds more than Ni(II).
**Figure 3.6:** Absorption spectra of Co(III) complex of pmt in Tris-HCl buffer upon addition of DNA. [Co] = 0.5 μM, [DNA] = 0-100 μM. Arrow showing variation in absorption with increase in concentration of [DNA]. Inner plot of [DNA]/(ε_a-ε_d) versus [DNA].

**Figure 3.7:** Absorption spectra of Co(III) complex of pms in Tris-HCl buffer upon addition of DNA. [Co] = 0.5 μM, [DNA] = 0-100 μM. Arrow showing variation in absorption with increase in concentration of [DNA]. Inner plot of [DNA]/(ε_a-ε_d) versus [DNA].
3.6. Viscosity measurements

To further clarify the interactions between the studied compounds and DNA, viscosity measurements were carried out. A classical intercalation model results in lengthening the DNA helix as base pairs were separated to accommodate the binding complexes, leading to the increase of DNA viscosity. In contrast, a partial and/or nonclassical intercalation of complexes could bend (or kink) the DNA helix, reducing its effective length and concomitantly its viscosity [45,46]. The effects Co(III) and Ni(II) complexes on the viscosity of CT-DNA at 25.0 °C shown in Figure 3.8. Viscosity results clearly show that all the complexes intercalated between adjacent DNA base pairs, causing an extension in the helix, and thus increased the viscosity of DNA. The results indicated that the Co(III) and Ni(II) complexes of ligand containing selenium donor atoms bound more strongly with an increasing concentration of complexes.

![Figure 3.8](image)

**Figure 3.8.** Effect of increasing amounts of the complex Co(III) [----] and Ni(II) [-----] on the relative viscosities of CT-DNA at 25 (±0.1)°C.
3.7. Thermal denaturation studies

Thermal behaviour of DNA in the presence of complexes can give insight in to their conformational changes when temperature is raised and offer information about the strength of the complexes with DNA. As intercalation of the complexes into DNA base pairs causes stabilization of base stacking when temperature in the double-stranded DNA solution increases, the (ds) DNA gradually dissociates to single strand, and generates hypochromic effect on the absorption spectra of DNA bases ($\lambda_{\text{max}}=260$ nm). Thus, the DNA melting experiment is useful in establishing the extent of intercalation [47]. 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.

In the present study, the $T_m$ DNA was found to be $60\pm1^\circ\text{C}$ under experimental conditions. As shown in Fig. 3.9, addition of complex $[\text{Co(pmt)}_2(\text{H}_2\text{O})_2]\text{PF}_6$, $[\text{Co(pms)}_2(\text{H}_2\text{O})_2]\text{PF}_6$, $[\text{Ni(pmt)}_2(\text{H}_2\text{O})_2]$ and $[\text{Ni(pms)}_2(\text{H}_2\text{O})_2]$ increased $T_m (\pm1^\circ\text{C})$ by 4 $^\circ\text{C}$, 6 $^\circ\text{C}$, 3$^\circ\text{C}$, and 5 $^\circ\text{C}$, respectively. indicates the complexes stabilize the double helix of DNA. The increased $T_m$ value of the complexes are comparable to that of the $T_m$ value of reported classical intercalators [48]. So, from the above data it is concluded that the new Co(III) and Ni(II) complexes act as a new class of DNA intercalators. The $T_m$ of the DNA is in the order $[\text{Co(pms)}_2(\text{H}_2\text{O})_2]\text{PF}_6 > [\text{Co(pmt)}_2(\text{H}_2\text{O})_2]\text{PF}_6 > [\text{Ni(pms)}_2(\text{H}_2\text{O})_2] > [\text{Ni(pmt)}_2(\text{H}_2\text{O})_2]$. 

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Figure 3.9. Melting curves of CT-DNA in the absence and presence of complexes

Table 3.4 Results of binding constant and thermal melting experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K_b$ ($M^{-1}$)</th>
<th>$T_m°C (σ_{T})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>--</td>
<td>60 (20)</td>
</tr>
<tr>
<td>([Co(pmt)_2(H_2O)_2]PF_6)</td>
<td>$4.9 \times 10^5$</td>
<td>64 (21)</td>
</tr>
<tr>
<td>([Co(pms)_2(H_2O)_2]PF_6)</td>
<td>$3.8 \times 10^5$</td>
<td>66 (23)</td>
</tr>
<tr>
<td>([Ni(pmt)_2(H_2O)_2])</td>
<td>$3.2 \times 10^4$</td>
<td>63 (25)</td>
</tr>
<tr>
<td>([Ni(pms)_2(H_2O)_2])</td>
<td>$2.7 \times 10^4$</td>
<td>65 (22)</td>
</tr>
</tbody>
</table>

3.8. Oxidative cleavage

The oxidative DNA cleavage activity of the complexes was studied by gel electrophoresis. The complexes were incubated at different concentrations with super coiled (SC) pUC19 DNA (0.5 μg) in Tris–HCl buffer (pH, 7.2) using hydrogen peroxide ($H_2O_2$)/ascorbic acid activation. The cleavage activity results show that at different
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concentration the complexes were exhibit nuclease activity. The control experiment using DNA alone does not show any apparent cleavage (Fig. 3.10 (a) lane (1)). At the concentration of 40 μM, the complex [Co(pmt)2(H2O)2]PF6 was able to convert 60% of the initial SC (Form I) to NC (Form II) and linear (Form III) (Fig. 3.10 (a), lane 3) whereas the complex [Co(pms)2(H2O)2]PF6 was able to convert only 35% of the initial SC (Form I) to NC (Form II) (Fig. 3.10 (a), lane 5). While at lower concentration of 30 μM the complex [Co(pms)2(H2O)2]PF6 exhibit 24% conversion, (Fig. 3.10(a), lane 2). In the same concentration the complex [Co(pmt)2(H2O)2]PF6 exhibit only 15% conversion (Fig. 3.12 (a), lane 4).

In case of [Ni(pmt)2(H2O)2] and [Ni(pms)2(H2O)2] complexes the two concentrations i.e. 20 and 40 μM were used. The complex [Ni(pms)2(H2O)2] was able to convert 36% and 71% of the initial SC (Form I) to NC (nicked circular)(Form II) (Fig. 3.12 (a), lane 2 and 4) and the [Ni (pmt)2(H2O)2] complex was able to convert 32% and 66% of the initial SC (Form I) to NC (Form II) at the concentration of 20 μM (Fig. 3.12 (a), lane 4). In conclusion at higher concentration of 40 μM, the [Ni(pms)2(H2O)2] complex shows more cleavage activity compare to the [Ni (pmt)2(H2O)2] complex. From these results, we infer that the [Ni(pms)2(H2O)2] complex 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
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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 Figure 3.11 and Fig.3.13.

**Figure 3.10.** Cleavage of supercoiled pUC19 DNA (0.5 μM) by the Co(III) and Ni(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 [Co(pms)_2(H_2O)_2] (1); Lane 3, DNA + 40 μM of [Co(pms)_2(H_2O)_2] (2); Lane 4, DNA + 20 μM of [Co(pmt)_2(H_2O)_2] (2); Lane 5, DNA + 40 μM of [Co(pnit)_2(H_2O)_2] (2)

**Figure 3.11.** 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.
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Form II: (SC DNA)

Form I: (NC DNA)

Lane 1 2 3 4 5

Figure 3.12. Cleavage of supercoiled pUC19 DNA (0.5 μM) by the Co(III) and Ni(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 [Ni(pms)₂(H₂O)₂] (3); Lane 3, DNA + 40 μM of [Ni(pms)₂(H₂O)₂] (3); Lane 4, DNA + 20 μM of [Ni(pmt)₂(H₂O)₂] (4); Lane 5, DNA + 40 μM of [Ni(pmt)₂(H₂O)₂] (4)

Figure 3.13. 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 H₂O: doubly distilled water as background). See text and experimental section for details.
3.9. Conclusion

A newly synthesized Co(III) and Ni(II) complexes of Schiff base derived form quinolines were demonstrated to be a sensitive spectroscopic DNA intercalator and probing agent. The fused aromatic ligand was shown to be the intercalative moiety on the complex by the following points: the aromatic moiety based UV bands caused evident hypochromism upon binding the complex to the DNA; the DNA binding constant is of the same order of magnitude as those for proven planar aromatic related DNA intercalators (phen or dppz).
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