CHAPTER VII
RESULTS AND DISCUSSION
ACENAPHTHENEQUINONE-1,8-DIAMINONAPHTHALENE-BENZYLAMINE
(L⁴) SYSTEM

7.1 Characterization of Macroyclic Schiff base ligand L⁴

7.1.1 Elemental Analysis

The macrocyclic Schiff base ligand, Acenaphthenequinone-1,8-diaminonaphthalene-benzylamine, L⁴, was synthesized by the condensation of Acenaphthenequinone, 1,8-diaminonaphthalene and benzylamine. The synthesized macrocyclic Schiff base ligand L⁴ was checked by comparing the TLC with the starting materials, which results a single spot different from the starting materials, confirms the formation and the purity of the ligands. The sharp melting points further confirm the purity of the ligand. The macrocyclic Schiff base ligand L⁴ was found to be stable towards air, and is soluble in common organic solvents like chloroform, ethanol, methanol etc.

The elemental analytical data of the macrocyclic Schiff base ligand L⁴ is given in the Table 7.1.1. The values obtained are closer to the theoretically calculated values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol. formula</th>
<th>% C</th>
<th>% N</th>
<th>% H</th>
</tr>
</thead>
<tbody>
<tr>
<td>L⁴</td>
<td>C_{48}H_{32}N_{4}</td>
<td>86.63 (86.72)</td>
<td>8.37 (8.43)</td>
<td>4.80 (4.85)</td>
</tr>
</tbody>
</table>
7.1.2 $^{13}$C NMR spectral analysis

The $^{13}$C NMR spectrum of the macroacyclic Schiff base ligand ($L^4$) is shown in Figure 7.1.2.

The $^{13}$C NMR spectrum of $L^4$ exhibited signals at $\delta$ 116.19, 119.86, 123.51, 124.38, 126.33, 127.23, 127.60, 127.96, 128.05, 129.61, 131.27, 133.80, 138.44, 152.39 were due to aromatic carbons (Ar-C). The imino carbon ($\text{C}=\text{N}$) atoms\textsuperscript{[1]} are observed at $\delta$ 162.95. The peak observed at $\delta$ 58.08, 57.94 were due to methylene carbon (-CH$_2$-) of the benzylamine moiety. The $^{13}$C NMR spectrum confirms the formation of Schiff base ligand by the condensation of acenaphthenequinone, 1,8-diaminonaphthalene and benzylamine.

![13C NMR spectrum of the macroacyclic Schiff base ligand ($L^4$)](image)

**Fig 7.1.2** $^{13}$C NMR spectrum of the macroacyclic Schiff base ligand ($L^4$)
**7.1.3 Electron Impact Mass spectral analysis**

The Electron Impact mass spectrum of the macrocyclic Schiff base ligand ($L^4$) is shown in Figure 7.1.3.

The EI mass spectrum of $L^4$ shows the molecular ion peak at $m/z = 664 \ [M]^+$ ($C_{48}H_{32}N_4)^+$. It confirms the formation of the macrocyclic Schiff base ligand ($L^4$). The peaks at $m/z = 558, 430, 344, 320, 259, 244, 189, 153$ and 126 corresponds to the fragments $C_{40}H_{26}N_4$, $C_{31}H_{19}N_3$, $C_{25}H_{16}N_2$, $C_{23}H_{16}N_2$, $C_{19}H_{13}N$, $C_{18}H_{13}N$, $C_{14}H_{10}N$, $C_{10}H_6N_2$ and $C_{10}H_6$ respectively. This confirms the molecular structure of the macrocyclic Schiff base ligand ($L^4$).

![Fig 7.1.3 EI mass spectrum of the macrocyclic Schiff base ligand ($L^4$)](image-url)
7.1.4 FT-IR spectral analysis

The FT-IR spectrum of the Schiff base ligand L⁴, formed by the condensation of Acenaphthenequinone, 1,8-diaminonaphthalene and benzylamine, is shown in Figure 7.1.4. The $\nu_{(C=O)}$ band of the acenaphthenequinone expected at 1675 cm$^{-1}$, as well as the $-\text{NH}_2$ stretching vibrations of benzylamine (likely at 3432 and 3328 cm$^{-1}$), are absent in the IR spectrum of L⁴. Instead, a significant band at 1637 cm$^{-1}$ has emerged, and this can be assigned$^{[2]}$ to the absorption due to the imino group (C=N). Hence, we can conclude that the macroacyclic Schiff base ligand, L⁴, has been formed by the condensation reaction. The spectrum also shows a strong band in the 1597 cm$^{-1}$ region, which is assigned to aromatic ring -C=C- stretching vibration. The other strong band at 2980 cm$^{-1}$ is related to (-C-H) modes of vibrations.

Fig 7.1.4 FT-IR spectrum of the macroacyclic Schiff base ligand (L⁴)
7.1.5 Structure of Macroacyclic Schiff base ligand (L$^4$)

From the above discussed analytical and spectral data, the structure of the macroacyclic Schiff base ligand L$^4$, has been confirmed and is given in Figure 7.1.5. The macroacyclic Schiff base contains four imino nitrogen atoms, and is potentially a tetradentate ligand.

Colour: Pale brown compound

Yield: 56 %.

M.p. : 237 °C.

Mol Formula: C$_{48}$H$_{32}$N$_4$

% Elements: C, 86.63; H, 4.80; N, 8.37.

IR (cm$^{-1}$): 1637 [s, (ν (C=N)].

EI-MS m/z : 664.60 [M$^+$].

$^{13}$C NMR (CDCl$_3$): δ 58.08, 57.94 (CH$_2$), δ 162.95 (C=N).
7.2 Characterization of Mononuclear Co(II), Ni(II), Cu(II) and Zn(II) complexes [ML₄]

7.2.1 Elemental Analysis

The mononuclear complexes of L₄ with Co(II), Ni(II), Cu(II) and Zn(II) have been synthesized. All the four macroacyclic Schiff base complexes [ML₄] are found to be stable towards air, and they are soluble in DMSO and DMF but insoluble in common organic solvents.

The elemental analytical data of the macroacyclic Schiff base complexes are given in the Table 7.2.1. The values obtained agree well with the theoretically values.

Table 7.2.1

Elemental analytical data of the macroacyclic Schiff base complexes [ML₄]

(Theoretical values in parentheses)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Mol. formula</th>
<th>% M</th>
<th>% C</th>
<th>% N</th>
<th>% H</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoL₄</td>
<td>[Co(C₄₈H₃₂N₄)]Cl₂</td>
<td>8.05 (8.14)</td>
<td>79.58 (79.66)</td>
<td>7.71 (7.74)</td>
<td>4.42 (4.46)</td>
</tr>
<tr>
<td>NiL₄</td>
<td>[Ni(C₄₈H₃₂N₄)]Cl₂</td>
<td>8.08 (8.11)</td>
<td>79.63 (79.69)</td>
<td>7.69 (7.74)</td>
<td>4.41 (4.46)</td>
</tr>
<tr>
<td>CuL₄</td>
<td>[Cu(C₄₈H₃₂N₄)]Cl₂</td>
<td>8.67 (8.72)</td>
<td>79.11 (79.15)</td>
<td>7.66 (7.69)</td>
<td>4.35 (4.42)</td>
</tr>
<tr>
<td>ZnL₄</td>
<td>[Zn(C₄₈H₃₂N₄)]Cl₂</td>
<td>8.89 (8.96)</td>
<td>78.83 (78.95)</td>
<td>7.59 (7.67)</td>
<td>4.34 (4.42)</td>
</tr>
</tbody>
</table>
7.2.2 FT-IR spectral analysis

The infrared spectra of the mononuclear Schiff base complexes CoL⁴, NiL⁴, CuL⁴ and ZnL⁴ are provided respectively in Figures 7.2.2.1, 7.2.2.2, 7.2.2.3 and 7.2.2.4. The IR spectral data of the complexes are tabulated in Table 7.2.2. In order to study the binding mode of the Schiff base ligand (L⁴) to the metal in the complexes, the IR spectrum of the free ligand was compared with those of the complexes. The band at 1637 cm⁻¹ for the imino group of the ligand (L⁴) has been shifted to lower frequencies 1613 cm⁻¹, 1619 cm⁻¹, 1609 cm⁻¹ and 1611 cm⁻¹ respectively in CoL⁴, NiL⁴, CuL⁴ and ZnL⁴ complexes. This clearly indicates the coordination of the imino nitrogen to metal centers, viz. Co(II), Ni(II), Cu(II) and Zn(II). Further, the IR spectra of metal complexes also show some new sharp bands in the region 424 cm⁻¹, 456 cm⁻¹, 460 cm⁻¹ and 453 cm⁻¹ for Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively which is due to the formation of coordinate bond between the imino nitrogen and the metal. These observations confirm the formation of Schiff base complexes [ML⁴].

Table 7.2.2

<table>
<thead>
<tr>
<th>Complexes</th>
<th>υ C-H (cm⁻¹)</th>
<th>υ C=N (cm⁻¹)</th>
<th>υ C=C (cm⁻¹)</th>
<th>υ M—N (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L⁴</td>
<td>2980</td>
<td>1637</td>
<td>1597</td>
<td>-</td>
</tr>
<tr>
<td>CoL⁴</td>
<td>2949</td>
<td>1613</td>
<td>1579</td>
<td>424</td>
</tr>
<tr>
<td>NiL⁴</td>
<td>2921</td>
<td>1619</td>
<td>1583</td>
<td>456</td>
</tr>
<tr>
<td>CuL⁴</td>
<td>2926</td>
<td>1609</td>
<td>1575</td>
<td>460</td>
</tr>
<tr>
<td>ZnL⁴</td>
<td>2851</td>
<td>1611</td>
<td>1569</td>
<td>453</td>
</tr>
</tbody>
</table>
Fig 7.2.1 FT-IR spectrum of the mononuclear Co(II) complex (CoL⁴)

Fig 7.2.2 FT-IR spectrum of the mononuclear Ni(II) complex (NiL⁴)
Fig 7.2.3 FT-IR spectrum of the mononuclear Cu(II) complex (CuL₄)

Fig 7.2.4 FT-IR spectrum of the mononuclear Zn(II) complex (ZnL₄)
7.2.3 Electronic spectral analysis

The electronic spectra of the complexes have been measured in the range 200-800 nm in DMSO. The UV-Vis absorption bands of the complexes CoL⁴, NiL⁴ and CuL⁴ are obtained in the range of 200-500 nm is given in Figure 7.2.3a, and the absorption bands obtained in the range of 500 to 800 nm is shown in Figure 7.2.3b.

The ligand shows absorption between 220-280 nm which is intra-ligand charge transfer transitions.[⁵] The absorption band in the region 350-450 nm is attributed to the metal - ligand charge transfer.

The electronic absorption spectrum of cobalt complex shows only one d–d band at 390 nm (⁴A₂g → ⁴T₁g) which reveals that it exists in square-planar geometry.[⁶]

The absorption spectrum of the Ni(II) complex show one d-d band at 655 nm which is assigned as ¹A₁g → ¹B₁g transition,[⁷] indicating square planar geometry. This complex shows the diamagnetic behavior suggesting the square planar environment around the Ni(II) ion.

The absorption spectrum for Cu(II) complex show one d-d band at 621 nm which is assigned to ²B₁g → ²E_g transition[⁸] characteristic of square planar geometry with dₓ²−ᵧ² ground state.

Zn(II) ion which has a completely filled d¹⁰ electronic configuration is not expected to show any d-d electronic transition, and the complex is expected to have tetrahedral geometry[⁹] with sp³ configuration.
The electronic spectral data of the complexes, the corresponding transitions and the proposed geometry are provided in Table 7.2.3.

**Table 7.2.3 Electronic spectral data of the complexes [ML⁴]**

<table>
<thead>
<tr>
<th>No</th>
<th>Complex</th>
<th>λ_max (nm)</th>
<th>Transition</th>
<th>Geometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CoL⁴</td>
<td>390</td>
<td>⁴A₂g → ⁴T₁g</td>
<td>Square planar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>π-π*(ligand)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>NiL⁴</td>
<td>655</td>
<td>¹A₁g → ¹B₁g</td>
<td>Square planar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>420</td>
<td>M-L CT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>247</td>
<td>π-π*(ligand)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>CuL⁴</td>
<td>621</td>
<td>²B₁g → ²E₉</td>
<td>Square planar</td>
</tr>
<tr>
<td></td>
<td></td>
<td>417</td>
<td>M-L CT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>π-π*(ligand)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ZnL⁴</td>
<td>No bands in Vis region</td>
<td>No d-d transition</td>
<td>Tetrahedral</td>
</tr>
</tbody>
</table>
Fig 7.2.3a Electronic spectra of Co$^{4+}$, Ni$^{4+}$ & Cu$^{4+}$ complexes (200 to 500 nm)

Fig 7.2.3b Electronic spectra of Co$^{4+}$, Ni$^{4+}$ & Cu$^{4+}$ complexes (500 to 800 nm)
7.2.4 Molar conductance measurements

The molar conductance of $10^{-3}$ M solutions of the complexes in DMSO was measured at room temperature. The values are tabulated in Table 7.2.4. The complexes showed molar conductance values in the range 98–105 S cm$^2$ mol$^{-1}$, which indicates the 1:2 electrolytic nature of the complexes. Hence, it is concluded that the chloride ions are present outside the coordination sphere. This is further proved by the positive silver nitrate test for the complexes.\textsuperscript{10} Therefore, these complexes may be formulated as [ML$^4$]Cl$_2$, where M = Co(II), Ni(II), Cu(II) and Zn(II).

Table 7.2.4 Molar conductance values of the complexes [ML$^4$]

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Molar conductance (S cm$^2$ mol$^{-1}$)</th>
<th>Nature</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoL$^4$</td>
<td>98.21</td>
<td>1:2 electrolyte</td>
</tr>
<tr>
<td>NiL$^4$</td>
<td>104.86</td>
<td>1:2 electrolyte</td>
</tr>
<tr>
<td>CuL$^4$</td>
<td>105.47</td>
<td>1:2 electrolyte</td>
</tr>
<tr>
<td>ZnL$^4$</td>
<td>101.51</td>
<td>1:2 electrolyte</td>
</tr>
</tbody>
</table>
7.2.5 EPR spectral analysis

The X-band EPR spectrum of the mononuclear copper(II) complex CuL$^4$ at liquid nitrogen temperature (LNT) is shown in Figure 7.2.5. The EPR spectra of mononuclear Cu(II) complex show four lines with nuclear hyperfine spin 3/2 due to hyperfine splitting i.e., four well resolved peaks$^{[28]}$ of low intensities in the low-field region and one intense peak in the high-field region resulting from the coupling of the unpaired electron with the nuclear spin of Cu(II). The observed $g_{\|}$ and $g_{\perp}$ values are 2.168 and 2.083 respectively. The fact that $g_{\|} > g_{\perp}$ confirms a square planar geometry with a $d_{x^2 - y^2}$ as a ground state$^{[29]}$ in Cu(II) complex. Relatively speaking $g_{\|} < 2.3$ is characteristic of anionic environment and $g_{\|} < 2.3$ of a covalent environment in M-L bonding. The fact that $g_{\|}$ less than 2.3 is an indication of considerable covalent character to the metal-ligand bond.

![X-band EPR spectrum of (CuL$^4$) recorded in frozen DMSO at 77 K](image)

Fig 7.2.5 X-band EPR spectrum of (CuL$^4$) recorded in frozen DMSO at 77 K
7.2.6 Electrochemical studies

The cyclic voltammogram of the mononuclear copper(II) complex $\text{CuL}^4$ is shown in Figure 7.2.6. The cyclic voltammogram of the Cu(II) complex shows one irreversible cathodic peak at $E_{pc} = -0.4 \text{ V}$ attributed to the reduction process Cu(II)/Cu(I) couple. The quasi-reversible anodic peak at $E_{pa} = 0.6 \text{ V}$ is due to oxidation of Cu(II) to Cu(III) ion and the corresponding cathodic peak at $E_{pc} = 0.4 \text{ V}$.

![Cyclic voltammogram of CuL^4](image)

**Fig 7.2.6 Cyclic voltammogram of CuL^4**
7.2.7 Structure of [ML₄] Schiff base complexes

From the analytical and spectral data discussed above, the structures of the complexes, CoL₄, NiL₄, CuL₄ and ZnL₄, have been elucidated. They are briefly described here, and the structures are provided in Figures 7.2.7.1, 7.2.7.2, 7.2.7.3 and 7.2.7.4 respectively. In all the four complexes, the Schiff base Acenaphthenequinone-1,8-diaminonaphthalene-benzylamine (L₄) binds the metal ions through the imino nitrogen atoms forming four coordinate complexes. CoL₄, NiL₄ and CuL₄ complexes exhibit square planar geometry, while ZnL₄ complex show tetrahedral geometry.
7.2.7.1 CoL₄ Complex

Colour: Dark brown compound

Yield: 80 %

Mol Formula: C₄₈H₃₂CoN₄

% Elements: C, 79.66; H, 4.42; Co, 8.05; N, 7.71

IR (cm⁻¹): 1613 [s, ν(MC=N)], 424 [s, ν(M-N)]

UV (nm): 390 (²A₂g → ⁴T₁g), 250 (π-π*)

Conductance (Λₘ, S cm² mol⁻¹) in DMSO: 98.21

Geometry: Square planar

7.2.7.2 NiL₄ Complex

Colour: Brown compound

Yield: 83 %

Mol Formula: C₄₈H₃₂N₄Ni

% Elements: C, 79.63; H, 4.41; N, 7.69; Ni, 8.08

IR (cm⁻¹): 1619 [s, ν(MC=N)], 456 [s, ν(M-N)]

UV (nm): 655 (¹A₁g → ¹B₁g), 420 (M-L CT), 247 (π-π*)

Conductance (Λₘ, S cm² mol⁻¹) in DMSO: 104.86

Geometry: Square planar
7.2.7.3 CuL$_4$ Complex

Colour: Dark green compound  
Yield: 83 %  
Mol Formula: C$_{48}$H$_{32}$CuN$_4$  
% Elements: C, 79.11; H, 4.35; Cu, 8.67; N, 7.66  
IR (cm$^{-1}$): 1609 [s, $\nu_{(\text{M-C=N})}$], 460 [s, $\nu_{(\text{M-N})}$]  
UV (nm): 621 ($^2\text{B}_{1g} \rightarrow ^2\text{E}_{g}$), 417 (M-L CT), 250 ($\pi-\pi^*$)  
Conductance ($\Lambda_m$, S cm$^2$ mol$^{-1}$) in DMSO: 105.47  
Geometry: Square planar

![Fig 7.2.7.3 Structure of CuL$_4$]

7.2.7.4 ZnL$_4$ Complex

Colour: Pale brown compound  
Yield: 78 %  
Mol Formula: C$_{48}$H$_{32}$N$_4$Zn  
% Elements: C, 78.83; H, 4.34; N, 7.59; Zn, 8.89  
IR (cm$^{-1}$): 1611 [s, $\nu_{(\text{M-C=N})}$], 453 [s, $\nu_{(\text{M-N})}$].  
Conductance ($\Lambda_m$, S cm$^2$ mol$^{-1}$) in DMSO: 101.51  
Geometry: Tetrahedral

![Fig 7.2.7.4 Structure of ZnL$_4$]
7.3 DNA BINDING STUDIES

7.3.1 Absorption spectral studies

The binding of metal complexes to DNA was monitored classically through absorption titration method. Metal complexes bound to DNA through intercalation which is characterized by the change in absorbance (hypochromism) and red shift in wavelength, due to a strong stacking interaction between the DNA base pairs. The electronic absorption spectra of the complexes CoL₄, NiL₄, CuL₄ and ZnL₄ are significantly perturbed by the addition of increasing amounts of DNA. With increasing concentration of CT-DNA (0 – 250 µM), hypochromism in the absorption bands around about 220-280 nm for the Co(II), Ni(II), Cu(II) and Zn(II) complexes was observed accompanied by a red shift of not more than 4-9 nm, suggesting of stabilization of the DNA Helix as represented in Figures 7.3.1.1, 7.3.1.2, 7.3.1.3 and 7.3.1.4 respectively.

Further, a plot of DNA]/(€ₐ₋€ᵣ) versus [DNA] was drawn to elucidate the DNA binding affinities of the complexes, and is provided in Figure 7.3.1.5.

In order to quantitatively compare the binding affinity of complexes CoL₄, NiL₄, CuL₄ and ZnL₄ with CT-DNA the intrinsic binding constants Kₐ of the complexes were determined. The binding constants (Kₐ) of the metal complexes were given in Table 7.3.1. Although the electronic absorption studies have confirmed that the complexes can bind to DNA by intercalation, it is necessary to carry out other experiments to prove the binding mode.
Fig 7.3.1.1 Absorption spectra of complex CoL₄ (1 x 10⁻⁵ M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10⁻⁵ M) at room temperature in 50 mM Tris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations.

Fig 7.3.1.2 Absorption spectra of complex NiL₄ (1 x 10⁻⁵ M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10⁻⁵ M) at room temperature in 50 mM Tris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations.
Fig 7.3.1.3 Absorption spectra of complex CuL₄ (1 x 10⁻⁵ M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10⁻⁵ M) at room temperature in 50 mM Tris-HCl/NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations.

Fig 7.3.1.4 Absorption spectra of complex ZnL₄ (1 x 10⁻⁵ M) in the absence and presence of increasing amounts of CT-DNA (0-25 x 10⁻⁵ M) at room temperature in 50 mM Tris-HCl / NaCl buffer (pH = 7.5). Arrow shows the absorbance changing upon increasing DNA concentrations.
Fig 7.3.1.5 The plots of $[\text{DNA}]/(\epsilon_a - \epsilon_l)$ versus $[\text{DNA}]$ for the titration of DNA with mononuclear CoL$_4$, NiL$_4$, CuL$_4$ and ZnL$_4$ complexes.

Table 7.3.1 Binding constant ($K_b$) of the complexes [ML$_4$]

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Binding Constant ($K_b$) M$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoL$_4$</td>
<td>$4.91 \times 10^4$</td>
</tr>
<tr>
<td>NiL$_4$</td>
<td>$1.83 \times 10^5$</td>
</tr>
<tr>
<td>CuL$_4$</td>
<td>$8.62 \times 10^5$</td>
</tr>
<tr>
<td>ZnL$_4$</td>
<td>$1.7 \times 10^4$</td>
</tr>
</tbody>
</table>
7.3.2 Fluorescence spectral studies

EB emits intense fluorescence around 612 nm in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs.\textsuperscript{[30]} No luminescence was observed for the complexes $\text{CoL}^4$, $\text{NiL}^4$, $\text{CuL}^4$ and $\text{ZnL}^4$ at room temperature in aqueous solution or in the present of CT-DNA, so the binding of the complexes cannot be directly presented in the emission spectra. Fluorescence intensities at 612 nm (526 nm excitation) were measured for different complex concentrations. The results (Figures 7.3.2.1, 7.3.2.2, 7.3.2.3 and 7.3.2.4) showed that the fluorescence intensity of EB bound CT-DNA decreased with the addition of the complexes $\text{CoL}^4$, $\text{NiL}^4$, $\text{CuL}^4$ and $\text{ZnL}^4$ which indicated that the complexes could bind to CT-DNA and replace EB from the EB-bound CT-DNA system. Two mechanisms have been proposed to account for this reduction in the emission intensity: the replacement of molecular fluorophores and/or electron transfer.\textsuperscript{[31]}

According to the classical Stern–Volmer equation, the quenching plot illustrates that the quenching of EB bound to CT-DNA by complex is in agreement with the linear Stern–Volmer equation, which also indicates that the complexes bind to DNA as shown in Figure 7.3.2.5. It indicates that the $\text{CuL}^4$ complex shows higher binding ability compared to the other three complexes.

The extent of reduction in the emission intensity gives a measure of the binding propensity of the complexes $\text{CoL}^4$, $\text{NiL}^4$, $\text{CuL}^4$ and $\text{ZnL}^4$ to DNA. The apparent binding constants ($K_{\text{app}}$) of the complexes were calculated and given in Table 7.3.2. The maximum value obtained for copper complex is consistent with the absorption spectral measurements.
Fig 7.3.2.1 Emission spectrum of EB bound to DNA in the presence of CoL⁴ ([EB] = 3.3 μM, [DNA] = 40 μM, [complex] = 0-30 μM, λ<sub>ex</sub> = 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations.

Fig 7.3.2.2 Emission spectrum of EB bound to DNA in the presence of NiL⁴ ([EB] = 3.3 μM, [DNA] = 40 μM, [complex] = 0-30 μM, λ<sub>ex</sub> = 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations.
Fig 7.3.2.3 Emission spectrum of EB bound to DNA in the presence of CuL⁴
([EB] = 3.3 μM, [DNA] = 40 μM, [complex] = 0-30 μM, λ_ex= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations.

Fig 7.3.2.4 Emission spectrum of EB bound to DNA in the presence of ZnL⁴
([EB] = 3.3 μM, [DNA] = 40 μM, [complex] = 0-30 μM, λ_ex= 430 nm). Arrow shows the absorbance changing upon increasing complex concentrations.
Fig 7.3.2.5 The plots of emission intensity $I_o/I$ vs [DNA] / [complexes]

Table 7.3.2 Apparent Binding constant ($K_{app}$) of the complexes [ML$^4$]

<table>
<thead>
<tr>
<th>Complex</th>
<th>Apparent binding constant ($K_{app}$) M$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoL$^4$</td>
<td>$1.1 \times 10^5$</td>
</tr>
<tr>
<td>NiL$^4$</td>
<td>$3.8 \times 10^5$</td>
</tr>
<tr>
<td>CuL$^4$</td>
<td>$6.4 \times 10^5$</td>
</tr>
<tr>
<td>ZnL$^4$</td>
<td>$9.4 \times 10^4$</td>
</tr>
</tbody>
</table>
7.3.3 CD spectral studies

Circular dichroism (CD) spectroscopy is useful in diagnosing changes in DNA morphology during drug–DNA interactions,\textsuperscript{[32]} since the positive band due to base stacking (270 nm) and the negative one due to right handed helicity (231 nm) are quite sensitive to the mode of DNA interactions with small molecules. Incubation of the DNA with the complexes Ni\textsubscript{L}^4, Cu\textsubscript{L}^4 and Zn\textsubscript{L}^4 induced considerable changes in CD spectrum as shown in Figure 7.3.3. The result reveals that the changes induced by Cu\textsubscript{L}^4 and Ni\textsubscript{L}^4 are more significant than Zn\textsubscript{L}^4, which suggests that Cu\textsubscript{L}^4 and Ni\textsubscript{L}^4 have higher affinities for CT-DNA than the Zn\textsubscript{L}^4 complex. Groove binding and electrostatic interaction of small molecules show less or no perturbation on the base stacking and helicity bands, whereas intercalation mode can induce the intensity changes of both the bands, thus stabilizing the right-handed conformation of DNA.\textsuperscript{[33]}

![CD spectra](image)

Fig 7.3.3 CD spectra recorded over the wavelength range 220-320 nm for solutions containing 2:1 ratio of CT-DNA (200 μM) and mononuclear Ni\textsubscript{L}^4, Cu\textsubscript{L}^4 & Zn\textsubscript{L}^4 complexes (100 μM).
7.3.4 Viscosity measurements

Viscosity studies of CT-DNA have been carried out with the metal complexes of different concentrations for further identification and affirmation of interaction mode between complex and DNA. Viscosity of DNA with complex of particular concentration has been calculated on the basis of DNA flow rate through a capillary viscometer. From the obtained flow rates, the specific viscosity contribution due to the DNA in the presence of metal complexes (binding agent) was calculated. From the viscosity measurements, it was observed that there is an increase in the relative viscosity of the DNA solution by adding the complexes NiL$_4$, CuL$_4$ and ZnL$_4$ as shown in the Figure 7.3.4. The obtained result confirms that the binding mode is classical intercalative mode. The increase in relative viscosity is expected to correlate with the compound’s DNA–intercalating potential$^{[34]}$ following the order CuL$_4$ > NiL$_4$ > ZnL$_4$.

![Fig 7.3.4 Viscosity measurements of the complexes NiL$_4$, CuL$_4$ & ZnL$_4$](image)