CHAPTER III

Synthesis, spectral characterization, electrochemical, thermal, antimicrobial, DNA binding and cleavage studies of new binuclear Schiff base metal complexes derived from 3, 3’diaminobenzidine.

3.1 Introduction

An oxime is a chemical compound belongs to the class of imines, with general formula R₁R₂C=NOH, where R₁ is an organic side chain and R₂ may be hydrogen, forming an aldoxime, or another organic group, forming a ketoxime. O-Substituted oximes form a closely related family of compounds. The chemistry of oxime/oximato metal complexes has been widely investigated since the time of their first synthesis, e.g. preparation of nickel(II) dimethylglyoximato and recognition of the five-member chelate character of this complex by Chugaev [1]. Extensive studies of cobalt oximes begun in the 1960s made use of Co(DMGH)₂ as a substitute for the naturally occurring cobalt core ring system [2, 3]. Vic-dioximes have received considerable attention as model compounds due to the fact that they mimic biofunctions such as reduction of vitamin B₁₂ [4]. The exceptional stability and unique electronic properties of the vic-dioxime complexes can be attributed to their planar structure stabilized by hydrogen bonds. These compounds were tested as liquid crystals, gas sensor, and inhibitors for chemical warfare agents[5]. Coordination chemistry of the oxime ligands has been extensively studied with the 3d metal ions [6–8]. The oxime-imines represent an important class of ligands capable of stabilizing the higher oxidation states of the metal ion through strong ligand to metal (L →M) σ-donation. The tetradeinate vic-dioxime ligands behave
similarly by enveloping themselves around metal ions in a planar geometry, forming a hydrogen bond between two oxime groups by removing one hydrogen ion. The strength of the hydrogen bond between the two oxime groups, which is represented by the O–O distance, depends on the size of the metal ions and chemical environment around the metal ions. The oxidation states of the central metals, number of donor atoms and core structures of the complexes are major factors in determining structure–activity relations of transition metal complexes [9]. The nature of the ligands around the metal has been found to dramatically affect the energy conversion process. Particularly, the introduction of electronic effects via electron-donor substituent on 4, 5-diazafluoren-9-one, cyclopenta dipyridine-2, 5-dione, 3, 3-dicarboxy-2, 2-bipyridine and 1, 10-phenanthroline (phen) ligands notably improved the absorption of light in the visible region for efficient sunlight collection [10–12]. Different oxime and their metal complexes have shown notable bioactivity as chelating therapeutics, as drugs, as inhibitors of enzymes and as intermediates in the biosynthesis of nitrogen oxides [13]. Also some oxime acts as important analytical reagents for the gravimetric and colorimetric determination of transition metals [14]. Examples of oxime ligands are shown below.

![Examples of oxime ligands](image)
Fig. 3.1.1 Examples of oxime ligand.

3.2 Results and discussion

The color, melting point, elemental analysis and empirical formulae of the prepared complexes are listed in table 1. The results of the elemental analysis are in good agreement with the calculated values. The metal contents of the complexes were determined according to literature methods [15]. The binuclear complexes are stable in air, non-hygroscopic, insoluble in water and most organic solvents, but are easily soluble in DMF and DMSO.

3.2.1 Molar conductivity measurements

The electrolytic nature of the complexes was measured in DMF at $10^{-3}$ M. The conductivity $\Lambda_m$ lies between 13 to $7 \Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$. This result shows that the complexes are non-electrolyte in nature, and anions are coordinated inside the
coordination sphere [16]. In binuclear nickel(II) acetate complex, the molar conductivity \(\Lambda_m\) was 260 \(\Omega^{-1}\) cm\(^2\) mol\(^{-1}\). This shows that the binuclear nickel(II) acetate complex is 1:4 electrolytes in nature.

### 3.2.1 IR spectral studies

IR spectra are much useful to give information on the nature of bonding of a ligand to the metal in coordination complexes. The IR spectra of metal complexes and ligand were recorded in the range of 100 cm\(^{-1}\) to 4000 cm\(^{-1}\) (Table No.2). In the IR spectra of ligand, -OH band was observed at 3200 cm\(^{-1}\), >C=N band at 1608 cm\(^{-1}\), and >N-O at 1470 cm\(^{-1}\). In metal complexes the broad singlet bands were observed at 1720, 1710, 1705, 1722, and 1716 cm\(^{-1}\) for Cu(II), Ni(II), Co(II) and Mn(II) complexes respectively (Fig 3.1 to 3.10). A broad band due to O-H…O intra molecular hydrogen bond was observed [17-18]. In binuclear nickel(II) acetate complex(3), the bands were observed at 1370 cm\(^{-1}\) and 1552 cm\(^{-1}\) were attributed to symmetric stretching frequency and asymmetric frequency of acetate ions. The difference in two frequencies is 130 cm\(^{-1}\). This shows that the acetate ions present outside the coordination sphere [19]. The shift in >C=N and -O-H in complexes predict the concept of co-ordination of ligand through nitrogen atoms. A shift in >N-O frequency of complexes proved the oxime group of nitrogen is coordinated. The bands at 470-420 cm\(^{-1}\) are due to coordination of metal and the oximino or imino nitrogen of complexes (M-N) [20]. The bands at 310-330 cm\(^{-1}\) are due to the coordination of metal and the chloride ions (M-Cl) [21].
Electronic absorption spectral studies

Electronic spectra of all the complexes were recorded in DMF medium (Table 3). The electronic spectra of metal complexes the wide range of bands are due to transition of -CH=N-, charge transfer results from electronic interaction between the metal and the ligand which involves either a metal to ligand or ligand to metal electron transfer[22]. The bands were observed in 240 to 280 nm are due to \( \pi \rightarrow \pi^* \) transition of benzene ring and \( \geq C=N \) group [23]. The bands were shifted to higher wavelength, due to the coordination of metal ion. The absorption bands were observed in the range of 320 to 400 nm due to \( n \rightarrow \pi^* \) transition of an imine group corresponding to the ligand or metal complexes (Fig 3.11 to 3.16). The copper(II) binuclear complex shows a broad absorption band at 664 nm due to the d-d transition \( ^2\text{Eg} \rightarrow ^2\text{T}_{2g} \) of Cu(II) ion suggest that the copper ion exhibits an octahedral geometry [24-25]. Electronic spectra of the nickel (II) binuclear complex shows bands at 555, 600 nm which are assigned to \(^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{P}), ^3\text{A}_{2g}(\text{F}) \rightarrow ^3\text{T}_{1g}(\text{F}) \) transitions, respectively suggesting an octahedral arrangement around the nickel (II) complex [24-25]. In binuclear nickel(II) acetate complex, the band was observed at 510 nm is attributed to \(^1\text{A}_{1g} \rightarrow ^1\text{A}_{2g} \) transistion. This shows that the geometry of nickel(II) ion is square planar in nickel(II) binuclear complex. The electronic spectra of binuclear cobalt (II) complexes exhibit absorption at 604, 666 nm are assigned to \(^4\text{T}_{1g}(\text{F}) \rightarrow ^4\text{T}_{1g}(\text{P}), ^4\text{T}_{1g} \rightarrow ^4\text{A}_{2g} \) transitions, respectively corresponding to cobalt(II) octahedral complex [24-25]. The manganese(II) binuclear complex shows bands at 552, 597 nm, respectively are corresponding to
${}^{6}A_{1g} \rightarrow {}^{4}E_{g} (4D)$, $6A_{1g} \rightarrow {}^{4}T_{2g} (4G)$ transitions which are compatible with an octahedral geometry around manganese(II) ion [24-25].

3.2.4 NMR Spectral studies

The structure of ligand was confirmed by $^1$H NMR (Fig 3.17). A singlet at 8.13 ppm is attributed to CH=N- proton. The multiplets observed around 7.4 to 8.0 ppm are due to aromatic protons. The singlet appeared at 11.37 ppm was due to proton of >N-OH. The structure of ligand was also confirmed by $^{13}$C NMR spectra (Fig 3.18). The oxyimino carbon appears at 168.9 ppm and imine carbon appears at 169.6 ppm. The peaks between 123.3 to 134.7 ppm are assigned to the aromatic carbons.

3.2.5 ESR and magnetic moments studies

ESR spectrum of copper(II) binuclear complex using powder sample was recorded at room temperature, which showed a single line correspond to an isotropic system with $g$ value of 2.157 (Fig 3.19). The value of $g_{\text{iso}}$ shows that the copper(II) ion is octahedral environment in copper(II) binuclear complex. The magnetic moments of Cu(II), Ni(II), Co(II) and Mn(II) are 1.84, 2.80, 4.82 and 5.85 B.M respectively. The values are almost equal to spin only value. This indicates that the two metal centers are equivalent and there is no anti-ferromagnetic interaction between two metal centers. The pairing of electron is prevented by greater distance between two metal centers [26]. The higher spin only value of cobalt(II) binuclear complex is due to the spin-orbital coupling contribution. The zero magnetic moment was confirmed the square planar geometry of binuclear nickel(II) acetate complex.
3.2.6 Electrochemical Studies

Electrochemical properties mainly depend upon the chelating ring/ size, distribution of unsaturation and substitution pattern in the chelating ring [27-29]. The electrochemical properties were studied by using cyclic voltammetry in DMF solution containing TBAP as supporting electrolyte in the potential range of 1.2 to -2.0 V. The data were shown in the Table 4. The quasi-reversibility of reduction process is confirmed with the corresponding peak to peak (ΔEp) separation value between the cathodic peak potential and anodic peak potential, the anodic peak current to cathodic peak current ratio(Ipa/ Ipc) is almost unity.

In copper(II) binuclear complex the first redox potential appears at 487 mV and 295 mV. The ΔEp₁ is 192 mV and the second redox potential appears at –674 mV, –500 mV. The ΔEp₂ is –174 mV. The ΔEp value shows that the copper(II) binuclear complex has followed quasi-irreversibility with one electron transfer reaction. The ratio between anodic peak current to cathodic peak current is almost unity this also confirmed the reaction is followed quasi-irreversible with one electron transfer reaction. Based on above redox potential, the copper complex may involve step wise reduction/ oxidation process as follows,

\[ \text{Cu}^{II} \leftrightarrow \text{Cu}^{I} \]

In nickel(II) binuclear complex the first redox potential appears at 370 mV and 475 mV. The ΔEp₁ is 105 mV and the second redox potential appears at –600 mV, –475 mV. The ΔEp₂ is –125 mV. The ΔEp value shows that the nickel(II) binuclear complex is followed by quasi-irreversibility with one electron transfer. The ratio between anodic peak current to cathodic peak current was almost unity. In binuclear
nickel(II) acetate complex the first redox potential appears at 770 mV and 912 mV. The \( \Delta E_{p1} \) is 142 mV and the second redox potential appears at \(-1345\) mV, \(-1150\) mV. The \( \Delta E_{p2} \) is \(-195\) mV. The \( \Delta E_p \) values shows that the binuclear nickel(II) acetate complex is followed by quasi-reversibility reaction. The stepwise reduction/oxidation process of nickel(II) complex as follows,

\[
\text{Ni}^{II} \rightleftharpoons \text{Ni}^{II} \rightarrow \text{Ni}^{I} \rightleftharpoons \text{Ni}^{I}
\]

In cobalt(II) binuclear complex the first redox potential appears at 325 mV and 200 mV. The \( \Delta E_{p1} \) is 125 mV and the second redox potential appears at \(-1300\) mV, \(-1200\) mV. The \( \Delta E_{p2} \) is \(-100\) mV. The ratio between anodic peak current to cathodic peak current is almost equal to one. In manganese(II) binuclear complex the first redox potential appears at 85 mV and 185 mV. The \( \Delta E_{p1} \) is 100 mV and the second redox potential appears at \(-850\) mV, \(-725\) mV. The \( \Delta E_{p2} \) is \(-125\) mV. The ratio between anodic peak current to cathodic peak current is almost equal to one. From the results of Co(II), Mn(II) binuclear complex shows that the reactions have followed by quasi-reversibility with one electron transfer (Fig. 3.20 to 2.24).

### 3.2.7 Thermal studies

Thermal analysis such as thermo gravimetric analysis (TGA) and Differential Thermal analysis (DTA) was widely applied in studying the thermal behavior of metal complexes [30-31]. The data (Table 4) provides information concerning thermal stability and thermal decomposition of these compounds in solid state. In copper(II) binuclear complex an endothermic peak observed at 125 °C was assigned to loss of four hydroxyl ions 7.48 (6.75) % from 30 °C to 140 °C. An endothermic peak observed at 190 °C which is due to the loss of four
chloride ions from 141 °C-245 °C. Above 245 °C the decomposition of the complex occurred (Fig 3.25). In cobalt(II) binuclear complex an endothermic peak was observed at 100 °C which is the elimination four hydroxyl ions 7.22 (6.81) % at 30-110 °C. Another endothermic peak was observed at 155 °C is assigned to the loss of four chloride ions 14.51(14.23) % at 111 °C to 190 °C. Above 190 °C the decomposition of the complex occurred (Fig 3.26). In nickel(II) binuclear complex an endothermic peak was observed at 75 °C is assigned to the loss of for acetate ions 21.90 (21.63) % at 30 to 250 °C. An exothermic peak observed at 255 °C was attributed to the loss of four hydroxyl ions 6.25% at 251 to 300 °C. Above 300 °C the decomposition of the metal complex was observed (Fig 3.27).

3.2.8 Anti-microbial studies

The ligand and its metal complexes [Cu(II), Ni(II), Co(II) and Mn(II)] were screened for antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes* as gram positive bacteria and *Escherichia coli* and *Klebsiella pneumoniae* as gram-negative and the fungi *Fusarium oxysporum* and *Aspergillus fumigatus* by disc diffusion method. From table(6), the Gram positive bacteria on all metal complexes were found to inhibit all tested bacteria at different rates and the activity as following order Co > Ni > Cu > Mn. In Gram negative bacteria also follows the same order and all complexes have higher bacterial activity than ligand. In fungal activity, the ligand showed activity against *Fusarium oxysporum* and *Aspergillus fumigatus* and metal complexes showing activity in the following order Cu > Co = Ni > Mn. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or on differences in ribosome of microbial cells [32-36]. The binuclear metal(II)
complexes show more activity and the ligands have less activity against same microorganisms under identical experimental conditions. This would suggest that, the chelation could facilitate the ability of a complex to cross a cell membrane and can be explained by Tweedy’s chelation theory [37]. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with donor groups and possible electron delocalization over the whole chelate ring. Such a chelation could enhance the lipophilic character of the central metal atom, which subsequently favors its permeation through the lipid layer of the cell membrane [Fig 3.28 to 3.30].

3.2.9 DNA binding studies

3.2.9.1 Electronic absorption studies

Electronic absorption spectroscopy is universally employed to determine the binding characteristics of metal complex with DNA. The absorption spectra of copper(II) binuclear complex in absence and presence of CT-DNA are shown in figure (3.31). In UV region two intense bands were observed at 269, 390 nm. 390 nm is attributed to the ligand to metal charge transfer absorption and another at 269 nm which is assigned to the π→π* transition of aromatic chromophores. It has been reported that the intercalating ability of the complex depends on the planarity of ligands, the coordination geometry, ligand donor atom type and the metal ion type. Intercalative mode of binding usually results in hypochromism and red shift due to the strong stacking interaction between aromatic chromophores and the base pairs of DNA [38]. The extent of red shift and hypochromism are commonly found to correlate with the intercalative binding strength. But, metal complexes which bind non-intercalative or electrostatically with DNA may result in hyperchromism
or hypochromism. In general, the absorption spectra of metal complexes bound to DNA through intercalation exhibit significant hypochromism and red shift due to the strong $\pi \rightarrow \pi^*$ stacking interaction between the aromatic chromophore ligand of metal complex and the base pairs of DNA. In copper(II) binuclear complex the decrease in absorption intensity (hypochromism) with a slight red shift is due to the intercalative binding between DNA and metal complex. The absorption bands of copper(II) binuclear complex at 269 nm shifted to red nearly 5, 8, 11 nm respectively, with increasing concentrations (40, 60, 80 µM) [39].

3.2.9.2 Viscosity measurements

Viscosity titration measurements were undertaken to identify the mode of interaction between the investigated compounds and CT-DNA. Optical photophysical probes provide necessary, but not sufficient, clues to support a binding model. To further prove the nature interactions between the metal complexes and DNA, viscosity measurements were carried out. Viscosity measurements are sensitive to change in length of DNA chain and are regarded as the least ambiguous and the most critical tests of a binding model in solution in the absence of crystallographic structural data [40]. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding drug, leading to the increase of DNA viscosity. In contrast, a partial intercalation could bend (or kink) the DNA helix, reduce its effective length and, concomitantly, its viscosity. The effect of the copper complex on the viscosity of CT-DNA is shown in Figure (3.32). On increasing the amounts of copper(II) binuclear complex, the relative viscosity of DNA increases apparently.
The result suggests that the binuclear copper(II) complex can bind to DNA by the intercalation mode.

3.2.9.3 Cyclic voltammetry in DNA binding studies

The application of cyclic voltammetry (CV) to the study of binding of metal complexes to DNA provides a useful complement to the above methods of investigations. Typical cyclic voltammograms of the complex in the absence and presence of DNA are shown in figure (3.33). In the absence of DNA, the first redox potential of copper(II) binuclear complex appears at 487 mV and 295 mV. The \( \Delta E_{p1} \) was 192 mV and the second redox potential appears at \(-674 \) mV, \(-500 \) mV. The \( \Delta E_{p2} \) was 174 mV. In these two redox couples, the ratio of \( \text{ipc/ ipa} \) is approximately unity. This indicates that the reaction of the complex on the platinum electrode surface is a quasi-reversible redox process. During the addition of DNA to the complex, the first redox couple causes an increase in \( \Delta E_{p1} \) from 192 mV to 200 mV and in the second redox couple causes an increase in \( \Delta E_{p2} \) from 174 mV to 200 mV. The shift of the redox potential of the complexes in the presence of DNA to more positive values indicates a binding interaction between the complex and DNA that makes the complexes less readily reducible. The changes of the voltammetric currents in the presence of CT-DNA can be attributed to diffusion of the metal complex bound to the large, slowly diffusing DNA molecule [41-42]. The changes of the peak currents observed for the complexes upon addition of CT- DNA may indicate that and the copper(II) binuclear complex possess a DNA-binding affinity.
3.2.10 DNA cleavage Studies by Gel electrophoresis method

Gel electrophoresis experiments were performed using pBR322-DNA with ligand and complexes in presence of H$_2$O$_2$. Complexes exhibited cleavage ability at low concentration (40µM). The ligand exhibits no significant activity in the presence of oxidant. The activity was much higher for the complexes in presence of H$_2$O$_2$. When DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact super coil form (Form I). If scission occurs on one strand (nicking), the super coil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Form-I and Form-II will be generated [43]. From the figure (3.34) the complexes shows more activity in the presence of oxidant which may due to reaction of hydroxyl radical with DNA. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety followed by hydroxyl cleavage of sugar phosphate backbone. The results of DNA cleavage studies were shown in figure [3.34]. In control experiment using DNA lane (1) does not show any significant cleavage of DNA even after long exposure time. The copper(II) and cobalt(II) binuclear complexes (lane 2 and 4) Form I and Form II were observed. In nickel(II) and manganese(II) binuclear complex, Form I, FormII and form III were observed. The different cleavage efficiency of the complexes is due to the different binding efficiency of the complexes to DNA.
3.3 Conclusion

The analytical and physico-chemical analysis confirmed the composition and structure of the newly synthesized ligand and its complexes. The spectroscopic data of binuclear metal(II) complexes indicate that the Schiff base ligand coordinated to the metal ions through nitrogen of oxime and imine groups. The complex exhibits different geometry around metal(II) ion. The metal(II) ion in binuclear complex 1, 2, 4 and 5 possessed an octahedral structure whereas in binuclear complex 3 a square planar geometry was observed. Cyclic voltammograms result showed that all the binuclear metal(II) complexes followed by quasi-reversibility involving one electron transfer reactions. The observed magnetic moment values of the binuclear complexes were found to be equal to spin only value and hence there was no anti-ferromagnetic interaction occurred between the two metal centers. Further these binuclear metal(II) complexes were screened for anti-bacterial and anti-fungal studies. The results showed that the complexes are more active than the free ligand but less active than standards. Among the metal complexes the cobalt(II) binuclear complex exhibited better anti-bacterial activity and copper(II) binuclear complex showed higher anti-fungal activity than other complexes. The DNA binding experiments did reveal that the binuclear copper(II) complex displayed interactive mode of interaction and all metal(II) binuclear complexes have significant ability to cleave the DNA base pairs.
Table 3.1
Elemental analysis and physical parameters of the ligand and its complexes.

<table>
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<tr>
<th>Sl. No</th>
<th>Compound</th>
<th>Color</th>
<th>% Yield</th>
<th>M.p (°C)</th>
<th>$\Lambda_m$ $\Omega^{-1}$ cm$^2$ mol$^{-1}$</th>
<th>Found(Calc) %</th>
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<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
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<td>1</td>
<td>$[\text{Cu}_2(L)\text{Cl}_4]$</td>
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<td>80</td>
<td>278</td>
<td>12.9</td>
<td>52.2(52.4)</td>
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<tr>
<td>2</td>
<td>$[\text{Ni}_2(L)\text{Cl}_4]$</td>
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<td>75</td>
<td>287</td>
<td>8.2</td>
<td>52.8(52.9)</td>
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<td>70</td>
<td>284</td>
<td>260</td>
<td>57.4(57.2)</td>
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<td>85</td>
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<td>12.4</td>
<td>52.9(52.9)</td>
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<td>5</td>
<td>$[\text{Mn}_2(L)\text{Cl}_4]$</td>
<td>Pink</td>
<td>80</td>
<td>280</td>
<td>7.7</td>
<td>54.0(53.3)</td>
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Table 3.2
IR spectral data of the ligand and its complexes ($\nu$ in cm$^{-1}$).

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<th>M–N</th>
<th>O–H….O</th>
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<td>464</td>
<td>1720</td>
<td>311</td>
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<td>1710</td>
<td>324</td>
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<td>[Ni$_2$(L)]$^{4+}$4Ac$^-$</td>
<td>3372</td>
<td>1571</td>
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<td>440</td>
<td>1697</td>
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<td>423</td>
<td>1722</td>
<td>315</td>
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<td>447</td>
<td>1716</td>
<td>329</td>
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Table 3.3
Electronic spectral data and magnetic moment values of the ligand and its complexes.

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<th>$\lambda_{\text{max}}$ in nm (nm)</th>
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<td>[Ni$_2$(L)Cl$_4$]</td>
<td>2.80</td>
<td>268 393 555,600</td>
<td>$^3\text{A}<em>{2g}$ (F) $\to$ $^1\text{T}</em>{1g}$ (P), $^3\text{A}<em>{2g}$ (F) $\to$ $^3\text{T}</em>{1g}$ (F) (Octahedral)</td>
</tr>
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<td>3</td>
<td>[Ni$_2$(L)]$^{4+}$4Ac$^-$</td>
<td>---</td>
<td>258 380 510</td>
<td>$^1\text{A}<em>{1g} \to ^1\text{A}</em>{2g}$ (Square planner)</td>
</tr>
<tr>
<td>4</td>
<td>[Co$_2$(L)Cl$_4$]</td>
<td>4.82</td>
<td>270 398 604,666</td>
<td>$^4\text{T}<em>{1g}$ (F) $\to$ $^4\text{T}</em>{1g}$ (P), $^4\text{T}<em>{1g}$ $\to$ $^4\text{A}</em>{2g}$ (Octahedral)</td>
</tr>
<tr>
<td>5</td>
<td>[Mn$_2$(L)Cl$_4$]</td>
<td>5.85</td>
<td>257 385 552,597</td>
<td>$^6\text{A}<em>{1g} \to ^4\text{E}</em>{g}$ (4D), $^6\text{A}<em>{1g} \to ^4\text{T}</em>{2g}$ (4G) (Octahedral)</td>
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<tr>
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<td>Compound</td>
<td>Epa (mV)</td>
<td>Epc (mV)</td>
<td>ΔEp (mV)</td>
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<td>125</td>
</tr>
<tr>
<td>5</td>
<td>[Mn₂(L)Cl₄]</td>
<td>85</td>
<td>185</td>
<td>100</td>
</tr>
</tbody>
</table>

Supporting electrolyte: Tetra butyl ammonium perchlorate (0.05 M)
Complex concentration: 0.01M
Solvent: DMF
Scan rate: 100 mVs⁻¹
ΔEp = Epa-Epc (Epa and Epc are anodic and cathodic potentials respectively)
Table 3.5
Thermal analysis of the metal complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Range  °C</th>
<th>DTA  °C</th>
<th>Estimated loss(Cal) %</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mass loss %</td>
<td>Total loss %</td>
</tr>
<tr>
<td>[Cu₂(L)Cl₄]</td>
<td>30-160</td>
<td>Endo-125 °C</td>
<td>7.48(6.75) %</td>
<td>21.00(20.85)%</td>
</tr>
<tr>
<td></td>
<td>161-245</td>
<td>Endo-180 °C</td>
<td>13.52(14.10)%</td>
<td></td>
</tr>
<tr>
<td>[Co₂(L)Cl₄]</td>
<td>30-110</td>
<td>Endo-100 °C</td>
<td>7.22(6.81) %</td>
<td>21.73(21.03)%</td>
</tr>
<tr>
<td></td>
<td>111-190</td>
<td>Endo-155 °C</td>
<td>14.51(14.23) %</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Above 190</td>
<td>Exo-525 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ni₂(L)]₄⁺Ac⁻</td>
<td>30-250</td>
<td>Endo-75 °C</td>
<td>21.90(21.63) %</td>
<td>28.15(27.86)%</td>
</tr>
<tr>
<td></td>
<td>251-300</td>
<td>Exo-255 °C</td>
<td>6.25(6.23) %</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exo-750 °C</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.6
Anti-microbial activities of the ligand and its metal complexes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive</td>
<td>Gram-negative</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>S. pyogenes</td>
</tr>
<tr>
<td>Ligand</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>[Cu₂(L)Cl₂]</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>[Ni₂(L)Cl₂]</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>[Ni₂(L)]⁺⁴Ac⁻</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>[Co₂(L)Cl₂]</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>[Mn₂(L)Cl₂]</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Zone of inhibition in mm.
Concentration 100 µg/ mL.
Fig. 3.1 Infrared spectra of Ligand.

Fig. 3.2 Infrared spectra of [Cu₂(L)Cl₄].
Fig. 3.3 Far Infra red spectra of [Cu$_2$(L)Cl$_4$].

Fig. 3.4 Infrared spectra of [Ni$_2$(L)Cl$_4$].
Fig. 3.5 Far Infrared spectra of $[\text{Ni}_2(\text{L})\text{Cl}_4]$.

Fig. 3.6 Infrared spectra of $[\text{Ni}_2(\text{L})]^{4+}4\text{Ac}^-$. 
Fig. 3.7 Infrared spectra of $[\text{Co}_2(\text{L})\text{Cl}_4]$. 

Fig. 3.8 Far Infrared spectra of $[\text{Co}_2(\text{L})\text{Cl}_4]$. 

150
Fig. 3.9 Infrared spectra of $[\text{Mn}_2(\text{L})\text{Cl}_4]$.  

Fig. 3.10 Far Infrared spectra of $[\text{Mn}_2(\text{L})\text{Cl}_4]$.  

151
Fig. 3.11 Electronic absorption spectra of Ligand.

Fig. 3.12 Electronic absorption spectra of [Cu₂(L)Cl₄].
Fig. 3.13 Electronic absorption spectra of $[\text{Ni}_2(\text{L})\text{Cl}_4]$. 

Fig. 3.14 Electronic absorption spectra of $[\text{Ni}_2(\text{L})]^+$ $4\text{Ac}^-$. 
Fig. 3.15 Electronic absorption spectra of $[\text{Co}_2(L)\text{Cl}_4]$.  

Fig. 3.16 Electronic absorption spectra of $[\text{Mn}_2(L)\text{Cl}_4]$.  

Fig. 3.17 $^1$H NMR Spectrum of Ligand.

Fig. 3.18 $^{13}$NMR Spectrum of Ligand.
Fig 3.19 ESR spectrum of $[\text{Cu}_2\text{(L)Cl}_4]$ at RT.

Fig. 3.20 Cyclic voltammogram of $[\text{Cu}_2\text{(L)Cl}_4]$. Scan rate 100 mV/s.
Fig. 3.21 Cyclic voltammogram of [Ni$_2$(L)Cl$_4$]. Scan rate 100 mV/s.

Fig. 3.22 Cyclic voltammogram of [Ni$_3$(L)$_2$]$^{4+}4$Ac$^-$ . Scan rate 100 mV/s.
Fig. 3.23 Cyclic voltammogram of $[\text{Co}_2(\text{L})\text{Cl}_4]$. Scan rate 100 mV/s.

Fig. 3.24 Cyclic voltammogram of $[\text{Mn}_2(\text{L})\text{Cl}_4]$. Scan rate 100 mV/s.
Fig. 3.25 Thermal analysis of [Cu$_2$(L)Cl$_4$].

Fig. 3.26 Thermal analysis of [Co$_2$(L)Cl$_4$].
Fig. 3.27 Thermal analysis of $[\text{Ni}_2(\text{L})]^4\text{Ac}^-$.  


Fig. 3.28 Anti-bacterial studies (Gram-positive) of Schiff base ligand and its metal complexes. Zone of inhibition in mm.

**Fig. 3.29** Anti-bacterial studies (Gram-negative) of Schiff base ligand and its metal complexes. Zone of inhibition in mm.


**Fig. 3.30** Anti-fungal studies of Schiff base ligand and its metal complexes. Zone of inhibition in mm.
**Fig. 3.31** Absorption spectra of copper(II) complex in the absence and in the presence of CT-DNA. (a) Metal complex only, (b) Complex with CT-DNA (40 µM), (c) Complex with CT-DNA (60 µM) and (d) Complex with CT-DNA (80 µM).

**Fig. 3.32** Viscosity measurements: The effect of the increasing amount of [Cu₂(L)(Cl)₄] complex on the relative viscosity of DNA at 27±0.1°C (20 µM, 40 µM, 60 µM, 80 µM).
Fig 3.33 DNA binding studies in cyclic voltammogram. a= [Cu$_2$(L)Cl$_4$] without DNA, b= [Cu$_2$(L)Cl$_4$] with DNA.

Fig. 3.34 Cleavage of pBR322-DNA (30µM) by the metal complexes (40 µM) in the presence of reducing agent H$_2$O$_2$ 50 µM, in 50mM of tris-Hcl buffer (pH 7.2).

From left to right Lane 1 Control; Lane 2 DNA+ Copper(II) complex+H$_2$O$_2$; Lane 3. DNA+ Nickel(II) complex+H$_2$O$_2$; Lane 4. DNA+ Cobalt(II) complex+H$_2$O$_2$; Lane 5. DNA+ Manganese(II) complex+H$_2$O$_2$. 
3.6 References

37. B.G. Tweedy, Phytopathology 55 (1964) 910.