CHAPTER III
SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF Cu (II), Zn (II) and Ni(II) SCHIFF BASE METAL COMPLEXES DERIVED FROM 2, 4-DIHYDROXYBENZALDEHYDE AND P-PHENYLENEDIAMINE/P-AMINOPHENOL

EXPERIMENTAL

3.1. Materials and physical measurements

All the chemicals and solvents used were of analytical grade. Metal salts, 2,4-dihydroxy benzaldehyde and p-phenylenediamine/p-aminophenol were purchased from Aldrich and were used without further purification. Ethanol, methanol, dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were used as solvents. Ethanol was distilled with a good fractionating column. The elemental analysis was performed using Carlo-Eraba 1106 instrument. Molar conductances of the complexes in DMF solution were measured with ELICO CM 185 conductivity Bridge. The infrared spectra were recorded on the Perkin Elmer FT-IR-8300 model spectrometer using KBr disc in the region 4000–400 cm⁻¹. ESR spectra were recorded on a Varian JEOL-JESTE100 ESR spectrophotometer at X-band microwave frequencies for powdered samples at room temperature. Magnetic susceptibility data were collected on powdered sample of the compounds at room temperature with PAR155 vibrating sample magnetometer. Thermal studies were carried out in the 100–900°C range using an SETSYS Evolution-1750 model thermal analyzer. The ¹H-NMR spectrum of the ligand was recorded on Varian Mercury plus 400 MHz spectrometer using TMS as internal standard and (D₆) DMSO as a solvent.
3.2. Synthesis of ligand

The ligand was synthesized by the following general procedure [1]. The Schiff bases L³ and L⁴ were synthesised by ethanolic solution of and p-phenylenediamine (0.01 mmol, 1.08 g)/ p-aminophenol (0.01 mmol, 1.09 g) with 2,4-dihydroxy benzaldehyde (0.01 mmol, 1.38 g) in 50 ml ethanolic medium and then refluxed in a water bath for 3 hours. The solid products were removed by filtration and recrystallised from ethanol. Synthetic pathway is shown in Scheme 3.2.1 and 3.2.2

Scheme 3.2.1: Synthesis of Schiff base Ligand (L³)
Scheme 3.2.2: Synthesis of Schiff base Ligand (L$^4$)

3.3. Synthesis of Schiff base metal complexes

To the stirred solution of Schiff bases(s) (1 mmol), the metal starting compounds Cu(CH$_3$COO)$_2$.H$_2$O, Zn(CH$_3$COO)$_2$.2H$_2$O and Ni(CH$_3$COO)$_2$.4H$_2$O (1 mmol) was added in an ethanolic solution. Then the reaction mixture was reflux for six hours. Then the metal complexes were separated by filtration and washed thoroughly with ethanol and dried over fused CaCl$_2$. Synthesis pathway is shown in Scheme 3.3.1 and 3.3.2
Scheme 3.3.1: Synthesis of Schiff base Complex derived from L3
Scheme 3.3.2: Synthesis of Schiff base Complex derived from L$^4$

3.4. RESULTS AND DISCUSSION

Schiff base metal complexes were synthesized by General methods. The synthetic route represented in Figures 3.3.1 and 3.3.2, were synthesized and characterized by various physico chemical methods. This is also authenticated by elemental analysis data and conductance data, which show that the complexes are non ionic in nature. All the complexes are dark coloured with high melting points >230°C. They are quite stable at room temperature and can be stored for a long period.
3.4.1. Elemental composition

The carbon, hydrogen, nitrogen and metal contents of the complexes have been
determined. The elemental analysis data were compared with that of the formulation, which
gives good agreement with the proposed formula.

Table 3.1 Analytical data for Schiff base metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Calculated (Found) (%)</th>
<th>ΛM (Ohm⁻¹ cm² mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>L³</td>
<td>68.96</td>
<td>4.59</td>
</tr>
<tr>
<td></td>
<td>(68.97)</td>
<td>(4.57)</td>
</tr>
<tr>
<td>L⁴</td>
<td>68.12</td>
<td>4.80</td>
</tr>
<tr>
<td></td>
<td>(68.13)</td>
<td>(4.82)</td>
</tr>
<tr>
<td>[CuL³]</td>
<td>58.53</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>(58.54)</td>
<td>(3.45)</td>
</tr>
<tr>
<td>[ZnL³]</td>
<td>58.39</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>(58.40)</td>
<td>(3.42)</td>
</tr>
<tr>
<td></td>
<td>(59.38)</td>
<td>(3.45)</td>
</tr>
<tr>
<td>[CuL⁴]</td>
<td>60.00</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>(60.04)</td>
<td>(3.80)</td>
</tr>
<tr>
<td>[ZnL⁴]</td>
<td>59.88</td>
<td>3.83</td>
</tr>
<tr>
<td></td>
<td>(59.85)</td>
<td>(3.80)</td>
</tr>
<tr>
<td>[NiL⁴]</td>
<td>60.70</td>
<td>3.89</td>
</tr>
<tr>
<td></td>
<td>(60.74)</td>
<td>(3.87)</td>
</tr>
</tbody>
</table>

3.4.2. Conductivity studies

The molar conductances of the synthesised complexes were performed to establish the
non electrolyte nature of the complexes. The complexes were dissolved in DMF, and the
molar conductivities of 10⁻³ M of their solutions at 29°C were measured. The observed molar
conductance values are given in Table 1. The measurements were made in DMF solvent. The
conductivity values are in the range of 10-30 ohm⁻¹ mol⁻¹ cm². These values are too low to
account for any dissociation of the complexes in DMF and the obtained values were taken as
a good evidence for the existence of a non electrolytic in nature of the complexes²⁷.
3.4.3. Infrared spectra

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. The important IR spectral bands of the synthesised ligands L$_3$ and L$_4$ as well as the complexes [ML$_3$] and [ML$_4$] are presented in Table 3.2. The IR spectra of the metal complexes show significant changes compared to the ligands. The IR spectra of the ligand L$_3$ and L$_4$ showed a broad band in the region 3200–3600 cm$^{-1}$, assignable to –OH groups. The appearance of this peak in all the spectra of the complexes [ML$_3$] and [ML$_4$] indicates that the free –OH groups are present in the complexes. The spectrum of the ligand L$_3$ and L$_4$ shows sharp band at 1615 cm$^{-1}$ and 1645 cm$^{-1}$, which is shifted to lower frequencies in the spectra of all the complexes (1590–1599 cm$^{-1}$) indicating the involvement of –C=N nitrogen in coordination to the metal ion and the aromatic C=C between 1400-1550 cm$^{-1}$, Accordingly, the ligand acts as a tetradeinate chelating agent, bonded to the metal ion is shown in (Scheme 3.3.1 and 3.3.2). This is further substantiated by the presence of a new band at 450-478 cm$^{-1}$ assignable to (M–N) $^9$. The complexes [ML$_3$] and [ML$_4$] also showed medium intensity bands in the region 500-550 cm$^{-1}$, which could be attributed to (M-O) band, appeared in the region.
Figure 3.1 IR spectrum of ligand $[L^3]$.

Figure 3.2 IR spectrum of ligand $[L^4]$. 
Figure 3.3 IR spectrum of Cu(L$^3$)

Figure 3.4 IR spectrum of Zn(L$^3$)
Figure 3.5 IR spectrum of Ni(L³)

Figure 3.6 IR spectrum of Cu(L⁴)
Figure 3.7 IR spectrum of Zn(L₄)

Figure 3.8 IR spectrum of Ni(L₄)
Table 3.2 Infrared spectral data for Schiff base metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>(C=N) (cm⁻¹)</th>
<th>C=C (cm⁻¹)</th>
<th>(M−N) (cm⁻¹)</th>
<th>(M−O) (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L³</td>
<td>1615</td>
<td>1525</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L⁴</td>
<td>1645</td>
<td>1578</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>[CuL³]</td>
<td>1592</td>
<td>1478</td>
<td>456</td>
<td>515</td>
</tr>
<tr>
<td>[ZnL³]</td>
<td>1596</td>
<td>1511</td>
<td>478</td>
<td>519</td>
</tr>
<tr>
<td>[NiL³]</td>
<td>1590</td>
<td>1495</td>
<td>459</td>
<td>512</td>
</tr>
<tr>
<td>[CuL⁴]</td>
<td>1596</td>
<td>1458</td>
<td>460</td>
<td>514</td>
</tr>
<tr>
<td>[ZnL⁴]</td>
<td>1597</td>
<td>1495</td>
<td>462</td>
<td>520</td>
</tr>
<tr>
<td>[NiL⁴]</td>
<td>1599</td>
<td>1452</td>
<td>450</td>
<td>519</td>
</tr>
</tbody>
</table>

3.4.4. Electronic spectral studies

The electronic spectra of the Schiff bases L³ and L⁴ and their complexes [ML³] and [ML⁴] are summarized in Table 3.3. Electronic spectra all the complexes were obtained in DMF medium and also exhibit three main features. One or two peaks in the range of 250–295 nm were assigned to the intra ligand charge transfer transition π→π*. The peaks obtained in the range of 300-370 nm was assigned to n→π* transition. An intense peak in the range of 410–460 nm was due to ligand-to-metal charge transfer transition. The absorption band appeared in the range 500-700 nm was assigned to d-d transition.¹⁰⁻¹²

The electronic absorption spectra of the Schiff bases L³ and L⁴ and the complexes [CuL³], [NiL³], [CuL⁴] and [NiL⁴] were recorded at 300 K. The absorption band appeared in 200-700 nm region, the spectral data reviled that the proposed geometry of the complexes are square–planar.

The ligand L³ showed absorption bands at 255, 312 and 425 nm. These bands are assigned to π→π*, n→π* and L→M transition. The electronic absorption spectrum of the
[CuL$^3$] complex showed weak bands at 260, 308, 427 nm. These bands are assigned to $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$, $L\rightarrow M$ transition further, the d-d transition showed a strong band at 625 nm for [CuL$^3$] complex, this is due to $^2B_1g\rightarrow^2A_1g$. The electronic absorption spectrum of the [NiL$^3$] complex showed weak bands at 262, 343, 435 and (512,684) nm. These bands are assigned to $\pi\rightarrow\pi$, $n\rightarrow\pi^*$, $L\rightarrow M$ transition further, the d-d transition showed a strong bands at (514, 612) nm for [NiL$^3$] complex, this is due to $^1A_1g\rightarrow^2A_1g$ and $^1A_1g\rightarrow^1B_1g$.

The ligand L$^4$ showed absorption bands at 257, 312 and 436 nm. These bands are assigned to $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$ and $L\rightarrow M$ transition. The electronic absorption spectrum of the [CuL$^4$] complex showed weak bands at 256, 341, 456 nm. These bands are assigned to $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$, $L\rightarrow M$ transition further, the d-d transition showed a strong band at 613 nm for [CuL$^4$] complex, this is due to $^2B_1g\rightarrow^2A_1g$. The electronic absorption spectrum of the [NiL$^4$] complex showed weak bands at 267, 324, 423 nm. These bands are assigned to $\pi\rightarrow\pi^*$, $n\rightarrow\pi^*$, $L\rightarrow M$ and d-d transition further, the d-d transition showed a strong bands at (536, 643) nm for [NiL$^4$] complex, this is due to $^1A_1g\rightarrow^2A_1g$ and $^1A_1g\rightarrow^1B_1g$.

The spectral data reviled that the proposed geometry of the Cu (II) and Ni (II) complexes are square planar.$^{13,14}$

The [ZnL$^3$] and [ZnL$^4$] complexes showed weak bands only and does not provide (d-d transition) spectral line in the visible region.
Figure 3.9 Electronic spectrum of $[L^3]$}

Figure 3.10 Electronic spectrum of $[\text{CuL}^3]$}

Figure 3.11 Electronic spectrum of $[\text{ZnL}^3]$}
Figure 3.12 Electronic spectrum of [NiL₃]

Figure 3.13 Electronic spectrum of [L⁴]
Figure 3.14 Electronic spectrum of $[\text{CuL}_4]$  

Figure 3.15 Electronic spectrum of $[\text{ZnL}_4]$  

Figure 3.16 Electronic spectrum of $[\text{NiL}_4]$
### Table 3.3 UV–Visible data of Schiff base metal complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Absorption (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\pi \rightarrow \pi^*$</td>
</tr>
<tr>
<td>L$^3$</td>
<td>255</td>
</tr>
<tr>
<td>L$^4$</td>
<td>257</td>
</tr>
<tr>
<td>[CuL$^3$]</td>
<td>260</td>
</tr>
<tr>
<td>[ZnL$^3$]</td>
<td>274</td>
</tr>
<tr>
<td>[NiL$^3$]</td>
<td>262</td>
</tr>
<tr>
<td>[CuL$^4$]</td>
<td>256</td>
</tr>
<tr>
<td>[ZnL$^4$]</td>
<td>275</td>
</tr>
<tr>
<td>[NiL$^4$]</td>
<td>267</td>
</tr>
</tbody>
</table>

### 3.4.5. Magnetic properties

Magnetic susceptibility is quite useful technique for determining unpaired electrons in paramagnetic complexes which in turns help in suggesting structure of the complexes and the nature of the ligand. Ligands were strong field ligands and this fact that was supported by magnetic moment values for metals.

The magnetic moments of the solid–state complexes were measured at room temperature. The measured magnetic moments of mononuclear [CuL$^3$] and [CuL$^4$] complexes are 1.60 and 1.65 B.M. Magnetic susceptibility measurements showed that these complexes are paramagnetic, which corresponds to the +2 oxidation state of copper(II) complexes.$^{15,16}$ This indicates that the Cu(II) complexes exhibit square planer geometry.

The [NiL$^3$] and [NiL$^4$] complexes have diamagnetic properties using Gouy balance. The observed zero magnetic moments also confirms the square planar environment for the
Ni(II) complex, it conformity with the fact that all the known square planar complexes of Ni(II) complexes are diamagnetic.\textsuperscript{15}

The [ZnL\textsuperscript{3}] and [ZnL\textsuperscript{4}] complexes are diamagnetic having d\textsuperscript{10} system which is tetrahedral as per expected.

**Table 3.4:** Analytical data for Schiff base metal complexes

<table>
<thead>
<tr>
<th>compounds</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Yield %</th>
<th>Melting point (^\circ)C</th>
<th>Magnetic moment (\mu)(B.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L\textsuperscript{3}</td>
<td>[C\textsubscript{20}H\textsubscript{16}N\textsubscript{2}O\textsubscript{4}]</td>
<td>348</td>
<td>73</td>
<td>&gt;240</td>
<td>---</td>
</tr>
<tr>
<td>L\textsuperscript{4}</td>
<td>[C\textsubscript{13}H\textsubscript{11}NO\textsubscript{3}]</td>
<td>213</td>
<td>78</td>
<td>&gt;240</td>
<td>---</td>
</tr>
<tr>
<td>[CuL\textsuperscript{3}]</td>
<td>[CuC\textsubscript{20}H\textsubscript{14}N\textsubscript{2}O\textsubscript{4}]</td>
<td>410</td>
<td>62</td>
<td>&gt;240</td>
<td>1.60</td>
</tr>
<tr>
<td>[ZnL\textsuperscript{3}]</td>
<td>[ZnC\textsubscript{20}H\textsubscript{14}N\textsubscript{2}O\textsubscript{4}]</td>
<td>411</td>
<td>65</td>
<td>&gt;240</td>
<td>---</td>
</tr>
<tr>
<td>[NiL\textsuperscript{3}]</td>
<td>[NiC\textsubscript{20}H\textsubscript{14}N\textsubscript{2}O\textsubscript{4}]</td>
<td>404</td>
<td>69</td>
<td>&gt;240</td>
<td>---</td>
</tr>
<tr>
<td>[CuL\textsuperscript{4}]</td>
<td>[CuC\textsubscript{26}H\textsubscript{20}N\textsubscript{2}O\textsubscript{6}]</td>
<td>520</td>
<td>65</td>
<td>&gt;240</td>
<td>1.65</td>
</tr>
<tr>
<td>[ZnL\textsuperscript{4}]</td>
<td>[ZnC\textsubscript{26}H\textsubscript{18}N\textsubscript{4}O\textsubscript{8}]</td>
<td>521</td>
<td>61</td>
<td>&gt;240</td>
<td>---</td>
</tr>
<tr>
<td>[NiL\textsuperscript{4}]</td>
<td>[NiC\textsubscript{26}H\textsubscript{18}N\textsubscript{4}O\textsubscript{8}]</td>
<td>514</td>
<td>66</td>
<td>&gt;240</td>
<td>---</td>
</tr>
</tbody>
</table>

**3.4.6. EPR spectra**

The ESR studies of paramagnetic transition metal (II) complexes yield information about the distribution of unpaired electrons and tell about the nature of the bonding between the metal ion and its ligands. These have been many reports concerning the application of ESR to square-planar or distorted octahedral complexes of Cu (II) and of the interpretations of the ESR parameters in terms of covalency factors, i.e. d-orbital mixing coefficients, there have been only a few molecular orbital calculations for either four or six coordinate Cu (II) systems.

The EPR spectrum of the Cu complex was recorded in DMSO at room temperature. The frozen solution spectrum shows a well-resolved spectrum and no features characteristic
for a dinuclear complex. This is also supported by the magnetic moment of copper complex which confirms the mononuclear nature of the complex. This band is well defined for [CuL$^1$] complex at “g” values of around 2. At room temperature, the spectrum of complex is anisotropic, the fitting result of Cu (II) complex is the following: $g_{iso} = 2.1856^{17}$. In the spectrum of Cu (II) complex, the intensity pattern of the line is complex. Also, the resonance line at the higher magnetic field shows an increased broadness due to some intramolecular interaction between the metal complexes.

In square-planar complexes, the unpaired electron lies in the $dx^2-y^2$ orbital giving $g_{ll} > g_l > 2$. From the observed values it is clear that $g_{ll} > g_l > 2$ which suggested that the complex is square planar geometry is shown in figure 3.17.

![Figure 3.17 EPR Spectra of Cu(II) schiff base metal complexes](image)

3.4.7. Cyclic Voltammetry

The electrochemical behaviour was studied by cyclic voltammetry in DMF containing $10^{-1}$ M tetrabutylammonium perchlorate over the range of 1.2 to −2.0 V. A three-electrode configuration was used, comprising a glassy carbon electrode as the working electrode, a Pt-wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The
electrochemical data such as cathodic peak potential \(E_{pc}\) and anodic peak potential \(E_{pa}\) were measured.

The cyclic voltammogram of the \([\text{CuL}_3]\) complex in DMF showed one quasireversible reduction waves Figure 3.18. Controlled potential electrolysis was carried out at 100 mVs\(^{-1}\) and the experiment reports that each couple correspond to one electron transfer process. So, the process is assigned as \(\text{Cu (II) / Cu (I)}\).

The reduction potentials are attributed to the reduction of copper (II) in the complex. The complex has \(E_{pc} = -1.0 \text{ V} (E_{pa} = -0.5 \text{ V}, E_{pc} = -1.0 \text{ V}, \Delta E_p = -0.5 \text{ V}, E_{1/2} = -0.75 \text{ V})\). It is observed that the copper (II) complex get reduced at relatively high negative potential. The \([\text{CuL}_3]\) complex showed a quasireversible oxidation waves, Figure 3.18. Which is assigned as \(\text{Cu (II)/Cu (III)}\) couple. The complex \([\text{CuL}_3]\) has \(E_{pa} = 0.95 \text{ V} (E_{pc} = 0.95 \text{ V}, E_{pa} = 0.60 \text{ V}, \Delta E_p = 0.35 \text{ V}, E_{1/2} = 0.77 \text{ V})\). The \(\Delta E_p\) values suggest that the each couple was quasireversible.

The cyclic voltammogram of the \([\text{CuL}_4]\) complex in DMF exhibits quasireversible at \(E_{pa} = 0.80 \text{ V} (E_{pa} = 0.80 \text{ V}, E_{pc} = 0.42 \text{ V}, \Delta E_p = 0.38 \text{ V}, E_{1/2} = 0.40 \text{ V})\) corresponding to \(\text{Cu (II)/Cu (III)}\) couple is shown in figure 3.20. However in the negative region another quasi-reversible peak was obtained at \(E_{pc} = -0.7 \text{ V} (E_{pa} = -0.4 \text{ V}, E_{pc} = -0.7 \text{ V}, \Delta E_p = -0.3 \text{V}, E_{1/2} = -0.55 \text{ V})\) which is characteristics of \(\text{Cu (II) / Cu (I)}\). The \(\Delta E_p\) values suggests that the each couple was quasireversible.

The cyclic voltammogram of the \([\text{NiL}_3]\) complex in DMF showed one quasireversible reduction waves Figure 3.19. Controlled potential electrolysis was carried out at 100 mVs\(^{-1}\) and the experiment reports that each couple correspond to one electron transfer process. So, the process is assigned as \(\text{Ni (II) / Ni (I)}\).

The reduction potentials are attributed to the reduction of nickel (II) in the complex. The complex has \(E_{pc} = -1.32 \text{ V} (E_{pa} = -0.56 \text{ V}, E_{pc} = -1.32 \text{ V}, \Delta E_p = -0.76 \text{ V}, E_{1/2} = -0.94 \text{ V})\).
V). It is observed that the nickel (II) complex get reduced at relatively high negative potential. The [NiL₃] complex showed a quasireversible oxidation waves, Figure 3.19. Which is assigned as Ni (II)/Ni (III) couple. The complex [NiL₃] has Epₐ = 1.20 V (Epₑ = 1.20 V, Epₖ = 0.64 V, ΔEp = 0.52 V, E₁/₂ = 0.92 V). The ΔEp values suggest that the each couple was quasireversible.

The cyclic voltammogram of the [NiL₄] complex in DMF exhibits quasireversible at Epₐ = 0.84 V (Epₑ = 0.84 V, Epₖ = 0.58 V, ΔEp = 0.26 V, E₁/₂ = 0.71 V) corresponding to Ni (II)/Ni (III) couple is shown in figure 3.21. However in the negative region another quasi-reversible peak was obtained at Epₖ = −1.13 V (Epₑ = −0.45 V, Epₖ = −1.13 V, ΔEp = −0.68 V, E₁/₂ = −0.79 V) which is characteristics of Ni (II) / Ni (I). The ΔEp values suggests that the each couple was quasireversible

![Figure 3.18 Cyclic voltammogram of the [CuL₃]]
Figure 3.19 Cyclic voltammogram of the [NiL₃]

Figure 3.20 Cyclic voltammogram of the [CuL₄]
3.4.8. Thermal studies

Thermo gravimetric analyses data for complexes \([\text{CuL}^3]\), \([\text{CuL}^4]\) and \([\text{ZnL}^3]\) are given in figure 3.22, 3.23 and 3.24. Thermal analyses for the mononuclear Schiff base metal complexes were carried out within the temperature range from ambient temperature up to 900°C. The correlation between the different decomposition steps of the compounds with the corresponding weight losses are discussed in terms of the proposed formula of the compounds.

The complex \([\text{CuL}^3]\) with the molecular formula \([\text{CuC}_{20}\text{H}_{14}\text{N}_{2}\text{O}_{4}]\) is thermally decomposed in a single step. The estimated mass loss of 83.62% (calculated mass loss = 84.87%) within the temperature range 235–670°C may be attributed to the loss of aromatic ligand groups.

The thermal decomposition of the complex \([\text{CuL}^4]\) with the molecular formula \([\text{CuC}_{28}\text{H}_{20}\text{N}_{2}\text{O}_{6}]\) proceeds with only one degradation steps. The step occurs within the
temperature range 210-670°C with an estimated mass loss 39.31% (calculated mass loss = 40.96%) which is reasonably accounted for the loss of loss of aromatic ligand groups. The last step did not finish completely. Therefore, last decomposition residue was not determined.

The thermal decomposition of the complex [ZnL₄] with the molecular formula [ZnC₂₀H₂₀N₂O₆] proceeds with only one main degradation steps. The first step occurs within the temperature range 180–678°C with an estimated mass loss of 39.56% (calculated mass loss = 41.43%), which is reasonably accounted for the loss of aromatic ligand groups.

The results of thermal analysis showed good agreement with the theoretical formula as suggested from the elemental analysis.

![Graph](image)

**Figure 3.22** Thermal analysis of [CuL³]
Figure 3.23 Thermal analysis of [CuL₄]

Figure 3.24 Thermal analysis of [ZnL₄]
3.4.9. NMR spectra

$^1$H-NMR spectra of the Schiff base $L^4$ was recorded in DMSO-d$_6$. The Schiff base $L^4$ shows peaks at 7.0 and 7.1 ppm which are attributable to the aromatic protons. The Schiff base $L^4$ shows peak at 13.6 ppm which is attributable to the phenolic OH proton. The Schiff base $L^4$ shows peak at 8.51 ppm which is attributable to the azomethine group. The formation of azomethine group in Schiff base $L^4$ was confirmed by $^1$H-NMR spectrum is shown in figure 3.25 and 3.26.

![NMR Spectra of Schiff base $L^4$](image)

**Figure 3.25** NMR Spectra of Schiff base $L^4$
3.4.10. Cleavage of plasmid pUC18 DNA

DNA cleavage is controlled by relaxation of supercoiled circular conformation of pUC18 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between.

The results of DNA cleavage are given in Figure 3.27. All the metal complexes were able to convert supercoiled DNA (Form I) into open circular DNA (Form II). The standard DNA cleavage reactions were performed in a total volume of 10 μL consisting of 2 mM tris-HCl buffer (pH=7.2), CT-DNA (500μM) and metal complexes (0.0001M). The reaction were
carried out at $32^0\ C$ for 120min followed by addition of gel loading buffer containing bromophenol blue as dye and the directly loaded on 1% agarose gel for analysis by gel electrophoresis at 50V. The gels were stained with ethidiumbromide (0.1 $\mu\text{mL}^{-1}$) and photographed on a uv transilluminator\textsuperscript{26}.

The general oxidative mechanisms proposed account of DNA cleavage by hydroxyl radicals via. Abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from while the hydrogen atom is removed\textsuperscript{27}. It has been shown that earlier\textsuperscript{28} that the cleavage is inhibited by free radical scavengers implying the hydroxyl radical or epoxy derivatives mediate the cleavage reaction. The reaction is modulated by a metallo complexes bound hydroxyl radical or peroxo species generated from the co-reactant $\text{H}_2\text{O}_2$. This results in oxidative attack on the deoxyribose moiety at C-1 hydrogen leading to a series of elimination reactions that ruptures the phosphodiester back-bone and yields 3and 5 phosphodiester terminal free base and 5-methelene-2-furanone.

The greater cleavage efficiency of the complexes compared to that of the control is due to their efficient DNA –binding ability. Figure 3.27.illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC18 DNA induced by the complexes. The control experiments did not show any apparent cleavage of DNA (lane-1). This results revealed the damage of DNA in $[\text{CuL}^3], [\text{ZnL}^4]$ and $[\text{NiL}^4]$ system could be attributed to the cleavage of DNA. The other metal complexes showed same electrophoretic behaviour and also cleavage activity against CT-DNA. The complex $[\text{NiL}^4]$ in the presence of $\text{H}_2\text{O}_2$ (lane 4) at higher concentration (50$\mu$M) shows more cleavage activity compared to complexes $[\text{CuL}^3]$ and $[\text{ZnL}^3]$. The supercoiled plasmid DNA was completely degraded (100% cleavage efficiency was observed). This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on
The result indicates the important role of metal complexes in the CT-DNA cleavage reaction. The oxidative DNA cleavage by singlet oxygen likely to proceed via oxidation of guanine nucleobase\textsuperscript{30,31}.

**Figures 3.27** DNA Cleavage studies of Cu(L\textsuperscript{3}) (Lane 2), Zn(L\textsuperscript{4}) (Lane 3), Ni(L\textsuperscript{4}) (Lane 4) Schiff base metal complexes.

### 3.4.11. Antimicrobial assay

Mainly the aim of production and synthesis of antibacterial compared is to inhibit the casual microbe without any side effects on the patients. In addition, it is worthy to stress here on the basic idea of applying any chemotherapeutic agent which depends essentially on the specific control of only one biological function and not multiple ones. [CuL\textsuperscript{3}], [ZnL\textsuperscript{3}], [NiL\textsuperscript{3}], [CuL\textsuperscript{4}], [ZnL\textsuperscript{4}] and [NiL\textsuperscript{4}] complexes showed a remarkable biological activity against staphylococcus aureus, Bacillus substilis, Escherichia Coli and Pseudomonas putita. The importance of this unique property of the investigated Schiff base complexes lies in the fact treatment of infections and some common diseases eg. Specticaemia, Gastreoteritis, Urinary tract infections. The ligand and their complexes have been tested for in vitro growth inhibitory activity against staphylococcus, Bacillus sp, E.Coli and Pseudomonas by using well-diffusion method. As the test biological activity also increases. The minimum inhibitory concentration MIC values of the investigated compounds are summarized in Table 3.5. From this table, the observed MIC values indicates that the metal complexes [CuL\textsuperscript{3}], [ZnL\textsuperscript{3}], [NiL\textsuperscript{3}], [CuL\textsuperscript{4}], [ZnL\textsuperscript{4}] and [NiL\textsuperscript{4}] have higher antimicrobial activity than the ligands as
shown in figure 3.28. The increase in antibacterial activity is due to the faster diffusion of metal complexes as a whole. Such increased activity of the copper complex can be explained on the basis of overtone concept32 and the Tweedy’s chelation theory33. On chelation, the ligand with N and O donor system might have inhibited enzyme production, since enzymes which require a free hydroxyl group for their activity appear to be especially susceptible to deactivation by the ions of the complexes. Mainly chelation reduces the polarity of the central metal ion to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Furthermore, it increases the delocalization of π-electrons around the whole chelate ring and enhances the penetration of the complexes into lipid membranes34 and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms35.

Table 3.5 Antibacterial activity of the Schiff base metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E.Coli (mm)</th>
<th>Bacillus sp (mm)</th>
<th>Pseudomonas (mm)</th>
<th>Staphylococcus (mm)</th>
</tr>
</thead>
<tbody>
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<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>L₄</td>
<td>5</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
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<td>17</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Zn(L₃)</td>
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<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Ni(L₃)</td>
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<td>7</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
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<td>18</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Zn(L₄)</td>
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</tr>
<tr>
<td>Ni(L₄)</td>
<td>9</td>
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<td>10</td>
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</table>
The new Schiff base complexes were synthesized from 2, 4-dihydroxy benzaldehyde and p-phenylenediamine/p-aminophenol containing N₂O₂ donors set in different environments. The involvement of the azomethine nitrogen atoms of the ligand in binding with the metal has been inferred from IR spectra. The electronic absorption spectral data of the isolated complexes confirmed the electronic transition and geometry. The molar conductivity measurements reveal that the metal complexes are non-electrolyte in nature. The magnetic moment and EPR spectra of copper complexes suggest that the complexes are monomer and square planar. Electrochemical studies shows the redox potential for Cu(II) and Ni(II) ions, thus confirming the presence of these metal ions in the mononuclear complexes. The thermal results allowed acquiring the information concerning the stoichiometry of all the complexes. Gel electrophoresis experiment suggests that the complex cleaves DNA in the presence of H₂O₂. All the transition metal complexes have activity than the control CT-DNA. Further, these complexes have higher antibacterial activity than the free ligands. ¹H-NMR spectra of the Schiff base and its complexes suggest that the general formula of the complexes are [ML].
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