CHAPTER 3

SYNTHESIS, CHARACTERIZATION OF COMPLEXES OF Co(II), Ni(II), Cu(II) AND Zn(II) WITH 12-MEMBERED SCHIFF BASE TETRAAZAMACROCYCLIC LIGAND AND THE STUDY OF THEIR ANTIMICROBIAL AND REDUCING POWER
INTRODUCTION

Although the chemistry of polyazamacroyclic ligands and its complexes has been a fascinating area of research but tetraazamacrocycles are the most extensively studied due to their biological relevance [1,2]. A dramatic progress in the chemistry of tetraazamacrocyclic complexes has received particular attention due to their mimicry with biologically significant molecules invoking a variety of catalytic, biochemical and industrial applications [3-8]. Most of the imino macrocyclic ligands have been derived by condensation reaction between dicarbonyls and primary diamines [9]. The interactions of crown ethers and cyclophanes, especially the ones possessing aromatic moieties are known to form charge transfer complexes with a variety of guests [10-13], providing new insights into non-covalent interactions, chiefly cation-π interactions which led to the stabilization of a positive charge by the face of an aromatic ring [10]. These strong attractive non-covalent interactions between π-systems are a class of intermolecular forces to reckon within chemistry, biology, material sciences as they control a range of recognition, self assembly phenomena such as vertical base pair interactions which stabilize the DNA double helix and also intercalation of drugs [14,15] into DNA, tertiary structure of proteins, -porphyrin aggregation [16,17], packing of aromatic molecules in crystals [18], complexation in many host-guest systems especially with respect to conformational preferences and binding properties of polyaromatic macrocycles [19]. The
condensation of 9,10-phenanthrenequinone and o-phenylenediamine is a well
known identification reaction which leads to the formation of [1+1] product,
phenazine [20] and also that phenanthrenequinones have been used as pendant
groups in the porphyrin ring which serve as biomimetic models for
fundamental studies on photo-induced electron transfer [21] but not as basic
part of the macrocyclic framework. In view of the aforesaid applications, it
was thought worthwhile to synthesize tetraaza macrocycles bearing
polyaromatic groups by the [2+2] condensation reaction between 9,10-
phenanthrenequinone and o-phenylenediamine resulting in a 12-membered
Schiff-base macrocyclic ligand and its complexes with Co(II), Ni(II), Cu(II)
and Zn(II) ions.

EXPERIMENTAL

The metal salts MX₂₆H₂O, CuX₂nH₂O (M = Co(II) and Ni(II); X = Cl⁻ and
NO₃⁻ ; n = 2, 3), ZnCl₂, Zn(NO₃)₂₆H₂O (all E. Merck) were commercially
available and used as received. The chemicals o-phenylenediamine (Rachem),
9,10-Phenanthrenequinone, [K₃Fe(CN)₆], Ferric Chloride, Trichloroacetic acid,
NaH₂PO₄, Na₂HPO₄ (all E. Merck) were used as received. Methanol and
Acetonitrile (A.R grade) were used as solvents without further purification.
Synthesis of Ligand:

**Synthesis of (2,3:8,9-diphenanthrene-5,6:11,12-dibenzo-1,4,7,10-tetraazacyclododecane) (L)**

To a stirring solution of o-phenylenediamine (0.02 mol) in methanol (25 mL), hydrochloric acid (1-2 mL; 2M) and 9,10-phenanthrenequinone (0.02 mol) in methanol (25 mL) were added dropwise with constant stirring and the mixture was boiled under reflux for 5h affording a greenish precipitate. The product was filtered off, washed with methanol and dried in vacuo.

Synthesis of Complexes:

**Synthesis of dichloro / dinitrato (2,3:8,9-diphenanthrene-5,6:11,12-dibenzo-1,4,7,10-tetraazacyclododecane)metal(II) [MLX2]; (2,3:8,9-diphenanthrene-5,6:11,12-dibenzo-1,4,7,10-tetraazacyclododecane)copper(II) chloride nitrate [CuL]X2 (M = Co(II), Ni(II) and Zn(II); X = Cl, NO3)**

A solution of ligand (L) (0.01mol) in acetonitrile (25mL) was added dropwise to a stirred acetonitrile solution (25mL) of metal salts (0.01mol) at 50 °C for 3 h resulting a colored product. The product was filtered off, washed with acetonitrile, and dried under vacuum at room temperature.

Reducing Power

The complexes (25mM-800mM) in DMSO were mixed with 2.5 mL of phosphate buffer (0.2M, pH 6.6) and 2.5 mL potassium ferricyanide,
[K$_3$Fe(CN)$_6$] (1%). The mixture was incubated at 50°C for 30 minutes followed by the addition of 2.5 mL of trichloroacetic acid (10%) which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL distilled water and 0.5 mL FeCl$_3$ (0.1%), and the absorbance was measured at 700 nm.

**Physical Measurements**

The elemental analyses data were obtained by using Perkin Elmer-2400 C, H, N analyzer. Analytical thin layer chromatography was performed on silica gel coated glass plates. Electron impact mass spectrum was recorded on Jeol, JMS, DX-303 mass spectrometer. IR spectra were recorded on Perkin Elmer-2400 FTIR spectrophotometer with KBr/CsI pellets. $^1$H- NMR spectra were recorded on Jeol Eclipse 400 NMR spectrometer in DMSO-d$_6$. Metals and chloride were determined volumetrically [22] and gravimetrically [23] respectively. The electronic spectra of the complexes in DMSO were recorded over 200-1100nm range on Pye-Unicam 8800 spectrophotometer at room temperature. Magnetic susceptibility measurements were carried out using Faraday balance at 25°C. The electrical conductivities of 10$^{-3}$ M solutions in DMSO were obtained on a digital APX 185 conductivity bridge equilibrated at 25±°C.

**Results and Discussion**

A [1+1] condensation reaction between 9,10-phenanthrenequinone and o-phenylenediamine has long been known for the synthesis of dibenzo[a,c]phenazine (m.p.-156-172°C) [20]. A 12-membered Schiff base
tetraaza macrocyclic ligand, (L) (m.p. 182-187°C) was synthesized by [2+2] condensation reaction between 9,10-phenanathrenequinone and o-phenylenediamine by slight modification of reaction conditions. The formation and purity of the synthesized macrocyclic ligand was checked by comparing the TLC with the starting materials which resulted a single spot different from the starting materials. The mass spectrum of uncomplexed macrocyclic ligand did not show M⁺ peak rather peaks at m/z 105.3, 177.3, 205.3, 385.3, etc were observed which may be explained in terms of fragmentation of M⁺. The base peak was observed at m/z 281.3 which may be attributed to [M+2H]²⁺. The results of elemental analyses (Table 1), absorption bands and proton resonance peaks in FT-IR (Table 2) and ¹H NMR spectra, respectively, confirm the proposed stoichiometry and structure for the macrocyclic ligand.

The reaction of macrocyclic ligand (L) with transition metal ions in 1:1 molar ratio leads to the formation of the macrocyclic complexes of the types, [MLX₂] and [CuL]X₂ (M = Co(II), Ni(II), Zn(II); X = Cl⁻, NO₃⁻) (Figure 1). All the complexes are microcrystalline in nature, stable to the atmosphere and are soluble in almost all the polar solvents. All the attempts failed to develop a single crystal either of the ligand or of the complexes suitable for X-ray crystallography. The molar conductivity measurements in DMSO (ca. 1×10⁻³ M) at room temperature show non-electrolytic nature for all the complexes except that of copper(II) complexes which show 1:2 electrolytic nature.
Table 1. Formula weight, melting point, yield(%), color, analytical data and molar conductance for the macrocyclic ligand and its complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>F.W</th>
<th>M.P. (°C)</th>
<th>Yield (%)</th>
<th>Color</th>
<th>Found (Calc.) %</th>
<th>Molar Conductance (cm²Ω⁻¹mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L C₄₀H₂₄N₄</td>
<td>560.65</td>
<td>182-7</td>
<td>40</td>
<td>Green</td>
<td>85.70 (85.69)</td>
<td>9.97 (9.99)</td>
</tr>
<tr>
<td>[CoLCl₂] C₄₀H₂₄Cl₂CoN₄</td>
<td>690.48</td>
<td>210-13</td>
<td>50</td>
<td>Brown</td>
<td>8.49 (8.54) 10.24 (10.27) 69.60 (69.58) 3.53 (3.50) 8.13 (8.11)</td>
<td>20</td>
</tr>
<tr>
<td>[CoL(NO₃)₂] C₄₀H₂₄CoN₆O₆</td>
<td>743.59</td>
<td>211-13</td>
<td>52</td>
<td>Brown</td>
<td>7.90 (7.93) - 64.58 (64.61) 3.27 (3.25) 11.32 (11.30)</td>
<td>18</td>
</tr>
<tr>
<td>[NiLCl₂] C₄₀H₂₄Cl₂N₄Ni</td>
<td>690.24</td>
<td>218-20</td>
<td>56</td>
<td>Dark Green</td>
<td>8.52 (8.50) 10.29 (10.27) 69.64 (69.60) 3.48 (3.50) 8.11 (8.12)</td>
<td>22</td>
</tr>
<tr>
<td>[NiL(NO₃)₂] C₄₀H₂₄N₆NiO₆</td>
<td>743.35</td>
<td>215-19</td>
<td>54</td>
<td>Dark Green</td>
<td>7.92 (7.90) - 64.65 (64.63) 3.26 (3.25) 11.32 (11.31)</td>
<td>24</td>
</tr>
<tr>
<td>[CuL]Cl₂ C₄₀H₂₄Cl₂CuN₄</td>
<td>695.10</td>
<td>216-19</td>
<td>58</td>
<td>Dark Brown</td>
<td>9.17 (9.14) 10.23 (10.20) 69.15 (69.12) 3.50 (3.48) 8.03 (8.06)</td>
<td>115</td>
</tr>
<tr>
<td>[CuL(NO₃)₂] C₄₀H₂₄N₆O₆</td>
<td>748.20</td>
<td>216-18</td>
<td>56</td>
<td>Dark Brown</td>
<td>8.50 (8.49) - 64.18 (64.21) 3.25 (3.23) 11.22 (11.23)</td>
<td>117</td>
</tr>
<tr>
<td>[ZnLCl₂] C₄₀H₂₄Cl₂N₆Zn</td>
<td>696.94</td>
<td>220-22</td>
<td>62</td>
<td>Pale Green</td>
<td>9.40 (9.38) 10.20 (10.17) 68.95 (68.93) 3.50 (3.47) 8.07 (8.04)</td>
<td>23</td>
</tr>
<tr>
<td>[ZnL(NO₃)₂] C₄₀H₂₄N₆O₆Zn</td>
<td>750.05</td>
<td>220-23</td>
<td>60</td>
<td>Pale Green</td>
<td>8.68 (8.72) - 64.07 (64.05) 3.25 (3.23) 11.22 (11.20)</td>
<td>22</td>
</tr>
</tbody>
</table>
Figure 1. Synthetic scheme of the macrocyclic ligand (L) and its complexes.

where, $X = \text{Cl}^-, \text{NO}_3^-$
IR Spectra

The IR spectrum of the free ligand (Table 2) shows very strong intensity absorption band at 1590 cm\(^{-1}\) assigned to C=N stretching mode. The presence of aromatic rings has been identified by their characteristic ring vibrations in 1450-1400, 1100-1090 and 760-720 cm\(^{-1}\) regions. The absence of bands characteristic of \(\nu(C=O)\) and aromatic primary amine moiety \(\nu(N-H)\) expected to appear in free 9,10-phenanthrenequinone and \(\sigma\)-phenylenediamine, respectively, confirm the formation of the proposed macrocyclic framework. The infrared spectra of the complexes (Table 2) show the weak intensity absorption band in the frequency range 1530-1520 cm\(^{-1}\) for the coordinated imine function \(\nu(C=N)\) comparable to that expected for similar complexes [24,25]. The medium intensity band in the region 460-440 cm\(^{-1}\) may be due to \(\nu(M-N)\) vibrations. The coordination of the chloro and nitrato groups have been ascertained by the appearance of bands in 300-290 cm\(^{-1}\) and 240-230 cm\(^{-1}\) regions which may reasonably be assigned to \(\nu(M-Cl)\) and \(\nu(M-O)\) of O-NO\(_2\) groups in [MLCl\(_2\)] and [ML(NO\(_3\))\(_2\)] complexes, respectively. The spectra of the complexes, [ML(NO\(_3\))\(_2\)] show additional absorption bands in 1260-1230 cm\(^{-1}\), 1050-1020 cm\(^{-1}\) and 890-870 cm\(^{-1}\) regions consistent with monodentate coordination of the nitrato group [26].
Table 2. IR vibrational frequencies (cm⁻¹) of the macrocyclic ligand and its complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>v(C=Н)</th>
<th>v(M–N)</th>
<th>v(M–O)</th>
<th>v(M–Cl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>1590 s</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[CoLCl₂]</td>
<td>1520 w</td>
<td>460 m</td>
<td>-</td>
<td>300</td>
</tr>
<tr>
<td>[CoL(NO₃)₂]</td>
<td>1525 w</td>
<td>455 m</td>
<td>240</td>
<td>-</td>
</tr>
<tr>
<td>[NiLCl₂]</td>
<td>1522 w</td>
<td>445 m</td>
<td>-</td>
<td>285</td>
</tr>
<tr>
<td>[NiL(NO₃)₂]</td>
<td>1530 w</td>
<td>450 m</td>
<td>238</td>
<td>-</td>
</tr>
<tr>
<td>[CuL]Cl₂</td>
<td>1525 w</td>
<td>453 m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[CuL(NO₃)₂]</td>
<td>1527 w</td>
<td>460 m</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[ZnLCl₂]</td>
<td>1524 w</td>
<td>445 m</td>
<td>-</td>
<td>290</td>
</tr>
<tr>
<td>[ZnL(NO₃)₂]</td>
<td>1530 w</td>
<td>450 m</td>
<td>230</td>
<td>-</td>
</tr>
</tbody>
</table>

s = strong intensity band  
w = weak intensity band  
m = medium intensity band
**1H-NMR Spectra**

The 1H-NMR spectrum of the ligand shows six different signals integrating for four protons in the phenanthrene ring and two in the phenyl rings of condensed o-phenylenediamine with chemical shifts (δ) and coupling constant (J) values as δ, 9.31 (4H, d, J = 8.04 Hz), δ, 8.83 (4H, d, J = 7.32), δ, 8.37 (4H, m), δ, 8.01 (4H, m) and δ, 7.91 (4H, t, J = 7.32) and δ, 7.84 (4H, t, J = 7.32 Hz) (Figure 2). However, analogous spectra were recorded for [ZnLX₂] complexes with proton resonance peaks shifted to upfield by δ, 0.12-0.08.

**Electronic Spectra and Magnetic Moment Data**

The electronic spectra of all the complexes in DMSO exhibit essentially similar patterns in 400-250 nm region due to intraligand and charge transfer transitions. The low molar absorptivity values (Table 3) of the Co(II) and Ni(II) complexes suggest a slight deviation from the perfect octahedral geometry due to the possible vibrations that could occur from the mixing of d and p-orbitals of the complexes in order to relax the symmetry of the molecule. The molar absorptivity values of Cu(II) complexes have been found to be comparable with that of the reported [27] Cu(II) square planar complexes.

The electronic spectra of Cobalt(II) complexes (Table 3) showed two bands in 1110-1100 nm and 515-510 nm regions assignable to 4T₁g(F)→4T₂g(F) and 4T₁g(F)→4T₁g(P) transitions, respectively similar to that expected to the six
Figure 2. \(^1\)H-NMR spectra of the macrocyclic ligand (L) and its Zn(II) complexes.
Table 3. Magnetic moments, electronic spectral data, molar absorptivity values and assignments of the complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$\mu_{\text{eff}}$ (B.M)</th>
<th>Wavelength (nm) / $\varepsilon$ (L mol$^{-1}$cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CoLCl$_2$]</td>
<td>4.75</td>
<td>1110 / 9, 515 / 15</td>
<td>$^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (F) $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ (P)</td>
</tr>
<tr>
<td>[CoL(NO$_3$)$_2$]</td>
<td>4.80</td>
<td>1100 / 7, 510 / 18</td>
<td>$^4T_{1g}$ (F) $\rightarrow$ $^4T_{2g}$ (F) $^4T_{1g}$ (F) $\rightarrow$ $^4T_{1g}$ (P)</td>
</tr>
<tr>
<td>[NiLCl$_2$]</td>
<td>3.10</td>
<td>1050 / 9, 687 / 16, 410 / 23</td>
<td>$^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (F) $^3A_{2g}$ $\rightarrow$ $^3T_{1g}$ (F) $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (P)</td>
</tr>
<tr>
<td>[NiL(NO$_3$)$_2$]</td>
<td>3.14</td>
<td>1045 / 8, 685 / 13, 408 / 20</td>
<td>$^3A_{2g}$ (F) $\rightarrow$ $^3T_{2g}$ (F) $^3A_{2g}$ $\rightarrow$ $^3T_{1g}$ (F) $^3A_{2g}$ (F) $\rightarrow$ $^3T_{1g}$ (P)</td>
</tr>
<tr>
<td>[CuL]Cl$_2$</td>
<td>1.86</td>
<td>555 / 43</td>
<td>$^2B_{1g}$ $\rightarrow$ $^2E_g$</td>
</tr>
<tr>
<td><a href="NO$_3$">CuL</a>$_2$</td>
<td>1.87</td>
<td>550 / 45</td>
<td>$^2B_{1g}$ $\rightarrow$ $^2E_g$</td>
</tr>
</tbody>
</table>
coordinated octahedral Co(II) ion [27]. The observed magnetic moment values (4.75 and 4.80 B.M.) deviate from the calculated spin-only values. The slightly higher values may be explained in terms of orbital contribution expected for a high spin state of an octahedral Cobalt(II) ion [28].

The absorption spectra of Nickel (II) complexes (Table 3, Figure 3) exhibit three spin allowed transitions, $^3A_2g(F)\rightarrow^3T_2g(F)$, $^3A_2g(F)\rightarrow^3T_1g(F)$ and $^3A_2g(F)\rightarrow^3T_1g(P)$ appearing in the frequency regions, 1050-1045 nm, 687-685 nm and 410-408 nm, respectively, consistent with a typical of Ni(II) in octahedral environment [27]. The observed magnetic moments (3.10 and 3.14 B.M.) further confirm the above geometry [29].

In case of Copper(II) complexes (Table 3), a broad and less intense band appeared in 555-550 nm region, assignable [30] to $^2B_1g\rightarrow^2E_g$ transition characteristic of Cu(II) ion in a square planar disposition. The slightly higher values of observed magnetic moment of the Copper(II) complexes (1.86 and 1.87 B.M.) correspond to an orbitally non-degenerate ground state of Copper (II) ion [31].

**Reducing Power**

The reducing capacity of the complexes may serve as significant indicator of its potential antioxidant activity [32]. The Co(II) and Cu(II) complexes were tested for their relative reducing capacities (Figures 4 and 5). Increased absorbance of the reaction mixture indicated increased reducing power. The
Figure 3. The electronic spectrum of [NiL(NO$_3$)$_2$] complex.
Reducing Power

Figure 4. The comparison of the representative ISEM values of the three independent experiments that show reducing power of [CuL]Cl₂ and [CoLCl₂] as series 1 and series 2.
Figure 5. The comparison of the representative ISEM values of the three independent experiments that show reducing power of [CuL](NO₃)₂ and [CoL(NO₃)₂] as series 1 and series 2.
antioxidant activities of putative antioxidants may be attributed to various mechanisms, such as prevention of chain initiation, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging. Hence, it can be suggested that there is no correlation between total antioxidant activity and reducing power activity [33]. Thus, Co(II) complexes may have low reducing power and can have high total antioxidant activity than Cu(II) complexes. In view of the significant reducing power of these complexes they may be considered as potential antioxidants capable of generating metal(II) ligand radical.

Antifungal and Antibacterial Screening

The antifungal activity of ligand and its metal complexes was determined on *Candida albicans* and *Cryptococcus neoformans* using agar well diffusion method as performed by previous workers [34,35]. YPD or YPA agar plates were inoculated with the freshly grown culture by spreading method. Wells (5mm diameter) were punched in the agar and loaded with the 150μl (5mg/ml) samples of the ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes. Blanks containing neat solvent, DMSO (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 48 h and antifungal activity was determined by measuring the diameter of the zone of inhibition. The relative antifungal potency (Figures 6 and 7) of the given preparation was calculated by comparing its zone of inhibition with that of the blank. Similarly, antibacterial activity of ligand and its metal complexes was...
In-vitro antifungal and antibacterial activity

Figure 6. Anti-microbial activity of macrocyclic ligand (L) and its Co(II), Ni(II), Cu(II) and Zn(II) chloride complexes.
In-vitro antifungal and antibacterial activity

Figure 7. Anti-microbial activity of macrocyclic ligand (L) and its Co(II), Ni(II), Cu(II) and Zn(II) nitrate complexes.
performed on *Salmonella typhimurium* and *Escherichia coli* using above mentioned procedure with the difference of incubation conditions (37°C / 24 h) (Figures 6 and 7). These complexes exhibited potential activity at the concentration dose of 5mg/ml comparable to that of tetradeutate 12-membered macrocyclic compounds which have been shown to possess antimicrobial activity against various pathogens by disc diffusion technique [36].
REFERENCES


