Chapter-4

INTRODUCTION

Coordination chemistry of macrocyclic ligands has been a fascinating area of current research interest to the inorganic chemists all over the world. The continued interest and quest in designing new macrocyclic ligands arise mainly from their use as models for protein-metal binding sites in a substantial array of metalloproteins in biological systems, as synthetic ionophores, as models to study the magnetic exchange phenomena, as therapeutic reagents in chelate therapy for the treatment of metal intoxication, as cyclic antibiotics that owe their antibiotic actions to specific metal complexation, to study the host-guest interactions, and in phase transfer catalysis [1,2]. Macrocycles have important roles in a large number of applications such as: sensor technology, solvent extraction of cations, transport of cations through membranes, and models for biological structure and function [3]. The investigations on the coordination chemistry of new macrocyclic metal complexes could be helpful to develop the macrocycles which could comprehend selectively the above mentioned properties.

The family of complexes with azamacroyclic ligands has been a focus of scientific attention for many decades, and *in situ* one pot template condensation lies at the heart of macrocyclic chemistry [4,5]. Therefore, template reaction has been widely used for synthesis of macrocyclic complexes [6], where generally the transition metal ions are used as templating agents [7]. Transition metal macrocyclic complexes have received a lot of attention because of their biological activities, *viz.* antiviral, anticarcinogenic [8, 9], antifertile [10], antibacterial, antifungal [11], antioxidant [12] and many industrial applications [13]. Recently, the interest in the synthesis of macrocyclic complexes with N₄ and N₂O₂ donor ligands is rapidly growing on
account of their unique structural properties and biological activities [14].
Tetraazamacrocyclic ligands and their metal complexes have attracted interest among
coordination chemists. In particular, transition metal complexes of tetradeutate Schiff
base ligands are good candidates for application in catalysis and as biomimetic
enzyme models [15].

Transition metal complexes have been the subject of thorough investigation
because of their extensive applications in wide ranging areas from material sciences to
biological sciences [16]. Metal complexes are well-known to accelerate the drug
action and the efficacy of a therapeutic agent can often be enhanced upon
coordination with a metal ion [17]. The pharmacological activity has also been found
to be highly dependent on the nature of the metal ion and the donor sequence of the
ligands as different ligands exhibit different biological properties [18]. Studies have
shown that macrocyclic complexes can interact with DNA in different binding
fashions and exhibit effective nuclease activities [19]. In recent years, binding studies
of transition metal complexes have become very important in the development of
DNA molecule probes and chemotherapeutics [20]. Therefore, in view of the
aforesaid applications of transition metal complexes it was of worth interest to report
the synthesis, characterization and DNA binding studies of a few first row transition
metal macrocyclic complexes of the type [ML\textsubscript{2}Cl], \( [M = \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)} \text{and} \text{Zn(II)}] \) obtained by the template condensation reaction between o-phenaldehyde and
3,4-diaminotoluene.
EXPERIMENTAL

Materials

The chemicals 3,4-diaminotoluene, o-phthalaldehyde and ethidium bromide (E. Merck) were used as received. The metal salts MCl$_2$.6H$_2$O M = Co(II) and Ni(II), CuCl$_2$.2H$_2$O and ZnCl$_2$ (all E. Merck) were commercially available pure samples. Methanol (AR) was used as solvent. Highly polymerized calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma. Other chemicals were of reagent grade and used without further purification. Calf thymus DNA was dissolved to 0.5% w/w, (12.5mM DNA/phosphate) in 0.1M sodium phosphate buffer (pH 7.40) at 310K for 24 h with occasional stirring to ensure formation of homogeneous solution. The purity of the DNA solution was checked from the absorbance ratio $A_{260}/A_{280}$. Since the absorption ratio lies in the range $1.8 < A_{260}/A_{280} < 1.9$, therefore no further deproteinization of DNA was needed.

Physical Measurements

The elemental analyses were performed on Perkin-Elmer 2400 CHN Elemental Analyzer obtained from the Micro-analytical Laboratory of Central Drug Research Institute (CDRI), Lucknow, India. The FT-IR spectra (4000-200 cm$^{-1}$) of the complexes were recorded as KBr/CsI discs on a Perkin-Elmer Spectrum RX-I spectrophotometer. $^1$H and $^{13}$C-NMR spectra were recorded in DMSO-d$_6$ on Bruker Avance II 400 NMR spectrometer with Me$_4$Si as an internal standard. Electrospray mass spectra were recorded on a micromass quattro II triple quadrupole mass spectrometer from Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh, India. Metals and chloride were estimated volumetrically [21] and gravimetrically [22], respectively. The electronic spectra of the complexes in
DMSO were recorded on a Cary 5E UV-VIS-NIR spectrophotometer at room temperature from Indian Institute of Technology, Chennai, India. EPR spectrum was recorded at liquid nitrogen temperature on E-112 ESR spectrometer using TCNE as the g-marker from Sophisticated Analytical Instrument Facility, Indian Institute of Technology, Mumbai, India. Magnetic susceptibility measurements were carried out using a Faraday balance at 25 °C from Guru Nanak Dev University, Amritsar, Punjab, India. The molar conductivities of (10⁻³ M solutions in DMSO) were obtained on a Systronic type 302 conductivity bridge equilibrated at 25.00 ± 0.1 °C. Fluorescence measurements for the complexes were performed on Shimadzu Spectrofluorometer model RF-540 equipped with data recorder DR-3. A 1.00cm quartz cell was used for measurements. For the determination of binding parameters, (30 µM) of complexes solutions were taken in a quartz cell and increasing amounts of CT DNA was titrated. Fluorescence spectra were recorded at 310 K, in the scan range 300-850nm upon excitation at 308nm for [CoLCl₂], 280-540 nm upon excitation at 290 nm for [NiLCl₂], 280-450 nm upon excitation at 280 nm for [CuLCl₂] and 320-820 nm upon excitation at 315 nm for [ZnLCl₂].

Synthesis of the macrocyclic complexes:

**Dichloro[3,4,7,8,11,12,15,16-tetrabenzo-1,6,9,14-tetraazaacyclohexadecane-1,5,9,13-tetraene] metal(II), [MLCl₂]; [M = Co(II), Ni(II), Cu(II) and Zn(II)]**

To a magnetically stirred methanolic solution (~20 ml) of metal salts (0.01mol), methanolic solutions (~25 ml) of both 3,4-diaminotoluene (0.02 mol, 2.44g) and o-phthalaldehyde (0.02 mol, 2.68g) were added simultaneously with constant stirring. The reaction mixture was stirred for 12 h and then allowed to stand
at room temperature resulting in isolation of microcrystalline solid product in few days. The product was washed with methanol and dried in vacuum.

**The fluorescence studies of ethidium bromide bound to DNA in the presence of metal complexes**

The experiment was carried out at pH 7.0 in the buffer containing 50 mM NaCl and 5 mM Tris-HCl. DNA and ethidium bromide (EB) were dissolved in buffer at the concentrations of 3 and 1 µg/ml, respectively. The concentrations of the tested complexes were 50 µM. EB displays very weak fluorescence in aqueous solution. However, in the presence of DNA, it exhibits intense fluorescence because of the intercalation to base pairs in DNA. Complexes were added to EB bound with CT DNA and the intensity of fluorescence of EB was measured. Fluorescence spectra were recorded at excitation wavelength of 478 nm and the emission range set between 485 and 685 nm. Before examining the fluorescent properties of EB, it was checked that the complexes did not quench the EB fluorescence.

**Binding data analysis of the complexes**

To elaborate the fluorescence quenching mechanism the Stern-Volmer equation was used for data analysis [23].

\[
\frac{F_0}{F} = 1 + K_{SV} [Q]
\]

Where \( F_0 \) and \( F \) are the steady-state fluorescence intensities in the absence and presence of quencher (DNA), respectively, \( K_{SV} \) the Stern-Volmer quenching constant and \([Q]\) is the concentration of the quencher (DNA). The plot of \( F_0/F \) versus \([Q]\) exhibited a good linear relationship (Figure 1-4). The linearity of the Stern-Volmer plots for DNA bound complexes indicated that the interaction was purely static in
nature. The $K_{SV}$ value of the complexes, [CoCl$_2$], [NiCl$_2$], [CuCl$_2$] and [ZnCl$_2$] were calculated to be $7 \times 10^2$, $7.3 \times 10^3$, $1.07 \times 10^4$ and $1.5 \times 10^2$ M$^{-1}$, respectively. The $K_{SV}$ values suggest that these macrocyclic complexes exhibit different degree of affinity towards the DNA molecule. The highest binding affinity is in the case of Cu(II) complex and lowest for Zn(II) complex.
Figure 1. Fluorescence emission spectrum of Co(II) complex in presence of increasing amount of CT DNA. Inset shows the Stern-Volmer plot for the binding with CT DNA.
Figure 2. Fluorescence emission spectrum of Ni(II) complex in presence of increasing amount of CT DNA. Inset shows the Stern-Volmer plot for the binding with CT DNA.
Figure 3. Fluorescence emission spectrum of Cu(II) complex in presence of increasing amount of CT-DNA. Inset shows the Stern-Volmer plot for the binding with CT-DNA.
Figure 4. Fluorescence emission spectrum of Zn(II) complex in presence of increasing amount of CT DNA. Inset shows the Stern-Volmer plot for the binding with CT DNA.
RESULTS AND DISCUSSION

A novel series of 16-membered Schiff base tetraazamacrocyclic complexes have been synthesized by [2+2] metal template condensation of 3,4-diaminotoluene and o-phthalaldehyde in methanol (Scheme 1). The purity of the complexes was checked by running TLC on silica gel coated plates using benzene (85%) and methanol (15%) as eluent. All the complexes were microcrystalline in nature, stable at room temperature and soluble in most of the polar solvents. The formation of the Schiff base macrocyclic complexes has been confirmed on the basis of results of elemental analyses, molecular ion peak in the mass spectra (Table 1), the characteristic bands in the FT-IR and resonance signals in the $^1$H and $^{13}$C NMR spectra. The overall geometry of the complexes has been inferred from the observed values of magnetic moments and the positions of the bands in the electronic spectra. The molar conductance measurements of all the complexes recorded in DMSO, exhibit their non-electrolytic nature. However, all efforts failed to grow single crystal suitable for X-ray crystallography. The DNA binding study suggests that these macrocyclic complexes have good binding affinity towards DNA molecule.
Scheme 1. Synthesis and proposed structure of tetraazamacrocyclic complexes.

\[ M = \{ \text{Co(II), Ni(II) } n = 6, \text{ Cu(II) } n = 2, \text{ Zn(II)} \} \]

\[ \text{MX}_2.n\text{H}_2\text{O} \]

\[ \text{M} = \{ \text{Co(II), Ni(II) } n = 6, \text{ Cu(II) } n = 2, \text{ Zn(II)} \} \]
Table 1. Elemental analyses, m/z value, color, yield, molar conductance, and melting point values of complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>m/z found (calc.)</th>
<th>Color</th>
<th>Yield (%)</th>
<th>Found (calc.) (%)</th>
<th>$A_m$ (ohm$^{-1}$ cm$^2$ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{30}H_{24}N_4CoCl_2$</td>
<td>571.23</td>
<td>Black</td>
<td>52</td>
<td>10.75 12.12 63.58 4.95 9.93</td>
<td>14</td>
</tr>
<tr>
<td>$[CoLCl_2]$</td>
<td>(570.36)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{30}H_{24}N_4NiCl_2$</td>
<td>571.78</td>
<td>Dark</td>
<td>65</td>
<td>10.59 12.83 63.75 4.68 9.61</td>
<td>30</td>
</tr>
<tr>
<td>$[NiLCl_2]$</td>
<td>(570.12)</td>
<td>Brown</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{30}H_{24}N_4CuCl_2$</td>
<td>575.95</td>
<td>Black</td>
<td>61</td>
<td>11.51 12.69 62.89 4.76 9.89</td>
<td>33</td>
</tr>
<tr>
<td>$[CuLCl_2]$</td>
<td>(574.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{30}H_{24}N_4ZnCl_2$</td>
<td>577.81</td>
<td>Grey</td>
<td>57</td>
<td>11.12 12.06 62.08 4.50 9.33</td>
<td>21</td>
</tr>
<tr>
<td>$[ZnLCl_2]$</td>
<td>(576.10)</td>
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</table>
IR spectra

The preliminary identification regarding formation of the macrocyclic complexes, has been obtained from IR spectral findings (Table 2). The absence of the bands characteristic of the amino group $\nu(-\text{NH}_2)$ of the free 3,4-diaminotoluene and carbonyl group $\nu(\text{C}=\text{O})$ of o-phthalaldehyde suggest that complete condensation has taken place. A strong intensity band in the region 1624-1684 cm$^{-1}$ characteristic of azomethine group $\nu(\text{C}=\text{N})$, provides a strong evidence for the formation of macrocyclic framework [24,25], which is further confirmed by the appearance of medium intensity band in the region 480-500 cm$^{-1}$ assignable to $\nu(\text{M-N})$ vibration [26]. The presence of aromatic rings has been identified by their characteristic ring vibrations in 1445-1448, 1015-1196, 725-772 cm$^{-1}$ regions. A weak absorption band in the region 2920-2923 cm$^{-1}$ may be assigned [19] to $-\text{CH}_3$ stretching vibration. The presence of the bands in the region 250-290 cm$^{-1}$ may reasonably be assigned to $\nu(\text{M-Cl})$ vibration [27].
Table 2. IR spectral data (cm⁻¹) of complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>υ(C=N)</th>
<th>υ(C-H)</th>
<th>υ(M-N)</th>
<th>υ(M-Cl)</th>
<th>Phenyl ring vibrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CoLCl₂]</td>
<td>1684</td>
<td>2920</td>
<td>485</td>
<td>250</td>
<td>1448, 1083, 772</td>
</tr>
<tr>
<td>[NiLCl₂]</td>
<td>1626</td>
<td>2920</td>
<td>492</td>
<td>261</td>
<td>1445, 1056, 725</td>
</tr>
<tr>
<td>[CuLCl₂]</td>
<td>1682</td>
<td>2923</td>
<td>500</td>
<td>290</td>
<td>1446, 1077, 769</td>
</tr>
<tr>
<td>[ZnLCl₂]</td>
<td>1624</td>
<td>2920</td>
<td>480</td>
<td>274</td>
<td>1448, 1015, 729</td>
</tr>
</tbody>
</table>
**1H and 13C NMR spectra**

Another strong evidence for the formation of Schiff base macrocyclic complexes comes from 1H NMR spectrum. The 1H NMR spectrum of macrocyclic Zn(II) complex shows a signal at 8.46 ppm corresponding to azomethine protons (s, -CH=N; 4H) [28], indicating the condensation between primary amine and carbonyl group of 3,4-diaminotoluene and o-phthalaldehyde moiety, respectively (Figure 5). The multiplets appearing in the region 7.0-7.9 ppm (m, Ar-H) may reasonably be assigned to aromatic protons of macrocyclic Zn(II) complex. A sharp signal observed at 2.4 ppm may be ascribed to methyl protons [29].

The formation of macrocyclic framework is further confirmed by 13C NMR spectrum of Zn(II) complex which exhibits appropriate number of resonance signals (Figure 6). A sharp signal appearing at 157 ppm may be assigned to azomethine carbons [30], while the signals for aromatic carbons have been observed in the region 118-143 ppm [31]. The signal corresponding to methyl carbons appear at 21 ppm [32].
Figure 5. $^1$H NMR spectrum of Zn(II) complex.
Figure 6. $^{13}$C NMR spectrum of Zn(II) complex.
Mass Spectra

The mass spectra of all the synthesized macrocyclic complexes exhibited molecular ion peak \([M + H]^+\), \(m/z\) at 571, 571, 575 and 577 a.m.u corresponding to their molecular formulae \([\text{Co(C}_{30}\text{H}_{24}\text{N}_4]\text{Cl}_2]\), \([\text{Ni(C}_{30}\text{H}_{24}\text{N}_4]\text{Cl}_2]\), \([\text{Cu(C}_{30}\text{H}_{24}\text{N}_4]\text{Cl}_2]\) and \([\text{Zn(C}_{30}\text{H}_{24}\text{N}_4]\text{Cl}_2]\), respectively (Table 1). The mass spectrum of macrocyclic Co(II) complex shows a molecular ion peak at \(m/z\) 571, which corresponds to \([\text{C}_{30}\text{H}_{24}\text{N}_4\text{CoCl}_2 + H]^+\) as the calculated mass being 570. The series of peaks have been observed at \(m/z\) 476, 432, 339, 285, 267, 221 and 135 a.m.u corresponding to various fragments (Figure 7).
Figure 7. Mass spectrum of Co(II) complex.
EPR spectrum

The EPR spectrum of polycrystalline solid Cu(II) complex was recorded at liquid nitrogen temperature. The spectrum shows a single broad signal (Figure 8). The analysis of the spectrum gives $g_\parallel = 2.16$ and $g_\perp = 2.05$. The trend $g_\parallel > g_\perp > 2.0023$ ($g_e$), observed for the complex indicate that the unpaired electron is localized in $d_{x^2-y^2}$ orbital of the Cu(II) ion. These observations are characteristic of axially distorted octahedral geometry [33].

The parameter $G = (g_\parallel - 2) / (g_\perp - 2)$ which measures the exchange interaction between the metal centers in polycrystalline solid has been calculated. According to Hathaway if $G > 4$, the exchange interaction is negligible and if $G < 4$ considerable exchange interaction occurs in the solid complex [34]. The $G$ value of 3.2 for Cu(II) complex indicates considerable exchange interaction between the Cu(II) centers.
Figure 8. EPR spectrum of Cu(II) complex
Electronic spectra and magnetic moment

The electronic spectrum of the macrocyclic Co(II) complex exhibit three bands at 8,333, 15,384 and 20,833 cm$^{-1}$ attributable to $^{4}T_{1g}(F) \rightarrow ^{4}T_{2g}(F)$, $^{4}T_{1g}(F) \rightarrow ^{4}A_{2g}(F)$ and $^{4}T_{1g}(F) \rightarrow ^{4}T_{1g}(P)$ transitions, respectively consistent with the octahedral geometry around the Co(II) ion. The observed magnetic moment of 4.7 B.M. further complements the electronic spectral findings [35].

The proposed octahedral geometry around the Ni(II) ion has been confirmed by the position of absorption bands appearing at 9,756, 16,129 and 27,027 cm$^{-1}$ which may be attributed to $^{3}A_{2g}(F) \rightarrow ^{3}T_{2g}(F)$, $^{3}A_{2g}(F) \rightarrow ^{3}T_{1g}(F)$ and $^{3}A_{2g}(F) \rightarrow ^{3}T_{1g}(P)$ transitions, respectively [36]. The observed magnetic moment value of 3.1 B.M. further corroborates the electronic spectral data.

The electronic spectrum of macrocyclic Cu(II) complex show a broad band at 19,100 cm$^{-1}$ with a shoulder band at 16,300 cm$^{-1}$ assignable to $^{2}B_{1g} \rightarrow ^{2}E_{g}$ and $^{2}B_{1g} \rightarrow ^{2}B_{2g}$ transitions, respectively [35] corresponding to distorted octahedral geometry, which is further confirmed by its magnetic moment value of 1.9 B.M.

Fluorescence measurements

DNA binding of the complexes

The fluorescence quenching is a useful method to study the reactivity of chemical and biological systems since it allows non-intrusive measurements of substances in low concentration under physiological conditions [37,38]. It can reveal information about binding mechanisms to compounds and provides clues to the nature of the binding phenomenon. Fluorescence intensity of a compound can quench as a
result of variety of molecular interactions such as excited state reactions, molecular rearrangements, energy transfer, ground state complex formation and collisional quenching. The fluorescence spectroscopy provides insight in the changes taking place in the microenvironment of DNA molecule on binding to complexes. The binding of these complexes with calf thymus DNA was studied by monitoring the changes in the intrinsic fluorescence of these compounds at varying DNA concentration.

The quenching of the fluorescence clearly indicates that the binding of DNA to the macrocyclic complexes changed the microenvironment of fluorophore residue. The spectra illustrate that excess of DNA led to more effective quenching of fluorophore molecule fluorescence (Figure 1-4). There are three major modes of DNA interaction relevant to the metal complexes depending on the presence of charged atoms, hydrophobicity and structure of these complexes. As external binders the complexes with positive charges interact with the DNA backbone due to the electrostatic interaction with negatively charged phosphates. Groove binders interact with the DNA groove and hydrophobic interactions are usually important components of this binding process. Such a mode of interaction is also governed by geometric and steric factors such as non-planar structures and the presence of methyl groups that prevent intercalation. The third mode involves the insertion of a planar fused aromatic ring system between the DNA base pairs leading to intercalation [39-41]. Few studies on tetrazamacrocyclic complexes have proposed intercalation as a possible mode of DNA interaction [19,42]. In order to examine the possible mode of interaction of these macrocyclic complexes with DNA, ethidium bromide displacement assay has been performed. Ethidium bromide, a polycyclic aromatic dye, is the most widely
used fluorescence probe for DNA structure. It binds to DNA by intercalation within the stacked bases [43]. It has been reported that the enhanced fluorescence of the EB–DNA complex can be quenched at least partially by the addition of a second molecule and this could be used to assess the relative affinity of the molecule for DNA intercalation [44]. The emission spectra of EB bound to DNA in the absence and presence of complexes is given in Figure 9. The addition of these molecules to DNA being complexed with EB does not cause reduction in emission intensity, indicating that none of these complexes compete with EB in binding to DNA. The external interaction with DNA backbone is also ruled out as these complexes are non-cationic, lacking in a nucleophilic attracting centre. These aromatic complexes with methyl groups possibly interact with DNA within the grooves via stabilization through hydrophobic cohesion. This is presumably explained due to octahedral geometry and steric hindrance encountered by methyl groups, thus preventing the DNA base intercalation of these macrocyclic complexes.
Figure 9. Fluorescence emission spectra of ethidium bromide bound to DNA in absence and presence of Co(II), Ni(II), Cu(II) and Zn(II) complexes.
REFERENCES


