Chapter-5

Synthesis, Spectroscopic characterization, DNA interaction and antibacterial study of metal complexes of tetraazamacrocyclic Schiff base
INTRODUCTION

In recent years, macrocyclic chemistry has received great attention for their unique properties such as biomimic functions [1,2], ionic and molecular recognition [3,4], as building block in supramolecular chemistry [5,6]. There is a continued interest in synthesizing macrocyclic complexes because of their potential applications in fundamental and applied sciences and importance in the area of coordination chemistry [7]. The macrocyclic complexes have potential applications in a variety of areas such as metal selective extraction, stabilization of unusual oxidation states, sensor technology, magnetic resonance imaging, contrast enhancing agents, catalysts and models for biological structures and functions [8]. The chemical properties of macrocyclic complexes can be tuned to force metal ions to adopt unusual coordination geometry. The stability of macrocyclic metal complex depends upon a number of factors, including the number and type of donor atoms present in the ligand and their relative positions within the macrocyclic skeleton, as well as the number and size of the chelate rings formed on complexation [9]. The chemistry of Schiff base complexes has been an important and popular area of research due to their simple synthesis, versatility and diverse range of applications [10]. A considerable number of Schiff-base complexes have potential biological interest, being used as more or less successful models of biological compounds [11]. Macrocyclic Schiff base nitrogen donor ligands have received special attention because of their mixed hard-soft donor character, versatile coordination behavior [12,13], and for their biological activities i.e., toxicity against bacterial growth [14], anticancerous [15] and other biochemical properties [16]. Polyaza macrocyclic Schiff bases have been studied as potential inorganic and organic cation receptors [17], electron transfer agents, homogeneous
catalysts and DNA, RNA interacting agents [18]. Nitrogen donor macrocycles are an important class of compounds due to their prominent behavior of forming highly stable complexes with a variety of transition metal ions [19,20]. Macrocyclic compounds with basic nitrogen donors could also form protonated moieties, which can interact with simple as well as with more complex inorganic and organic anions [21]. The family of complexes with aza-macrocyclic ligands has remained a focus of scientific attention for many decades [22]. A precise understanding of the DNA-binding properties of metal complexes are driven by numerous motivations, which include therapeutic approaches, study of nucleic acid conformations and new tools for nanotechnology [23]. Studies on the interaction of small molecules with DNA continue to attract considerable attention due to their importance in cancer therapy and molecular biology. In this respect, transition metal complexes have attracted special attention due to their unique spectroscopic and electrochemical signatures [24]. Transition metal complexes with their varied coordination environment and versatile redox and spectral properties offer immense scope for designing species that are suitable to bind and cleave DNA [25]. Recently, there has been tremendous interest in studies related to the interaction of transition metal ions with nucleic acid because of their relevance in the development of new reagents for biotechnology and medicine [26].

This chapter deals with the synthesis and characterization of 12-membered tetraazamacrocyclic complexes of type, [MLCl₂] formed by [2+2] template condensation reaction of benzil and 3,4-diaminotoluene with Co(II), Ni(II), Cu(II) and Zn(II) ions. Interaction of the complexes with calf thymus DNA has been studied by fluorescence measurements. The macrocyclic complexes have been tested for their

**EXPERIMENTAL**

**Materials**

The chemicals, 3,4-diaminotoluene and benzil (E. Merck) were used as received. The metal salts MCl₂·6H₂O M = Co(II) and Ni(II), CuCl₂·2H₂O and ZnCl₂ (all E. Merck) were commercially available pure samples. Methanol (AR) was used as solvent. The solid media namely nutrient agar no.2 (NA) (M 1269S-500G, Himedia Labs Pvt. Ltd, Bombay, India) was used for preparing nutrient plates, while nutrient broth (NB) (M002-500G, Himedia Labs Pvt. Ltd, Bombay, India) was used for the liquid culture media. 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 310 K 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 carried out on Perkin-Elmer 2400 CHN Elemental Analyzer, FAB mass spectra were recorded on Joel SX-102 spectrometer from 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 from Sophisticated Analytical Instrumentation Facility, Punjab University, Chandigarh, India. Metals and chloride were estimated volumetrically [27] and gravimetrically [28], respectively. The electronic spectra of the complexes in DMSO were recorded on Cary 5E UV-VIS-NIR spectrophotometer at room temperature. EPR spectrum was recorded at liquid nitrogen temperature on E-112 ESR spectrometer using TCNE as the g-marker from Indian Institute of Technology, Mumbai, India. Magnetic susceptibility measurements were carried out on a Lakeshore VSM 7410 magnetometer from Indian Institute of Technology, Chennai, India. The molar conductivities of complexes (10\(^{-3}\) M solutions in DMSO) were obtained on a Systronic type 302 conductivity bridge equilibrated at 25.00 ± 0.1 °C. Fluorescence measurements were performed on spectrofluorophotometer Model RF-5301PC (Shimadzu, Japan) equipped with a data recorder DR-3. A fixed concentration of complex solution (30 µM) was taken in a quartz cell and increasing amounts of calf thymus DNA solution was titrated. The excitation wavelength and emission range for [CoLCl\(_2\)], [NiLCl\(_2\)], [CuLCl\(_2\)], [ZnLCl\(_2\)] were (350 nm, 355-520 nm), (365 nm, 370-430 nm) (250 nm, 340-490 nm) and (250 nm, 375-480 nm), respectively. The path length of the sample was 1 cm with 5 nm slit at 310 K.
Synthesis of the complexes:

*Dichloro[2,3:8,9-tetraphenyl-5,6:11,12-tetrabenzo-1,4,7,10-tetraazacyclododeca-1,3,7,9 tetraene] metal(II), [MLCl₂] [M = Co(II), Ni(II), Cu(II) and Zn(II)]*

To a methanolic solution (20ml) of metal salts (1 mmol), the methanolic solutions of both 3,4 diaminotoluene (2 mmol, 0.244g) and benzil (2 mmol, 0.420g) were added simultaneously with constant stirring. The reaction mixture was then refluxed for 4-5 h, resulting in a clear solution. The resultant solution was kept for evaporation at room temperature leading to the isolation of a microcrystalline product after a few days. The product was washed with methanol and dried in vacuum.

**Antibacterial Activity**

The antibacterial activity of the macrocyclic complexes was evaluated by agar well diffusion method [29]. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately $1.5 \times 10^8$ cfu/ml [30]. 20 ml of agar media was poured into each petri plate and plates were swabbed with a colony from the inoculums of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 6 mm diameter, wells were bored into the seeded agar plates and these were loaded with 50µl volume with concentration of 10 mg/ml of each compound reconstituted in dimethylsulphoxide (DMSO). All the plates were incubated at 37 ºC for 24 h. Antibacterial activity of all the complexes was evaluated by measuring the diameter of zone of inhibition in mm. The medium with dimethylsulphoxide (DMSO) as solvent was used as a negative control whereas media with ciprofloxacin (standard antibiotic for Gram positive) and gentamicin (standard
antibiotic for Gram negative) were used as positive control. The experiments were performed in triplicates.

**Determination of minimum inhibitory concentration (MIC)**

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial compound which inhibit the visible growth of microorganisms after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to antimicrobial agents and also to monitor the activity of new antimicrobial agents. The MIC of the macrocyclic complexes was tested against bacterial strains through a broth dilution method. In this method, the test concentrations of all the complexes were made from 2.5 to 0.01 mg/ml in the sterile wells of the micro-titer plates. In sterile microtitre plates (96-u-shaped wells) 50 µl of the sterile nutrient broth was poured in each well in three rows, then from fresh inoculums so formed (10⁸ cfu/ml diluted with 100µl Nutrient broth to have 10⁶cfu/ml) 50 µl of the suspension was poured in each well in the first and third row, second row was again filled with 50 µl of nutrient broth, finally the drug sample 50µl was added in the first row diluting uniformly from 2.5 to 0.01 mg/ml till the 8th well. All the microtitre plates were incubated at 37 °C for 18-24 hours. MIC was expressed as the lowest dilution, which inhibited the growth of bacteria observed by lack of turbidity in the well.

**Binding data analysis of the complexes**

To determine the DNA-binding ability of the complexes, fluorescence intensity data was analyzed by the Stern-Volmer equation [31].

\[ \frac{F_0}{F} = 1 + K_{sv} [Q] \]
Where, $F$ and $F_0$ are the fluorescence intensity with and without the quencher
(complex-DNA), $K_{SV}$ the Stern-Volmer quenching constant, and $[Q]$ the concentration
of the quencher. The $K_{SV}$ value of the complexes $[\text{CoLCl}_2]$, $[\text{NiLCl}_2]$, $[\text{CuLCl}_2]$ and
$[\text{ZnLCl}_2]$ were calculated to be $1.74 \times 10^4$, $1.5 \times 10^5$, $3.83 \times 10^5$ and $3.6 \times 10^5 \text{ M}^{-1}$,
respectively. A higher $K_{SV}$ value of Cu(II) complex suggests its stronger quenching
ability than other complexes. This implicates the higher binding affinity of Cu(II)
complex toward the DNA than other complexes.

The state of the quenching due to molecular interaction between luminophore
and quencher can be purely static or purely dynamic. However, these two states are
the extremes and in most cases the upward curvature of Stern-Volmer plot represents
a combination of both static and dynamic quenching as stated above which occur
simultaneously. The data plotted as emission intensity against quencher concentration
(Figure 1-4) gives an upward curve, thus indicating the incidences of both static and
dynamic quenching.
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

The tetraaza Schiff base macrocyclic complexes of the type \([\text{MLCl}_2]\) \([\text{M} = \text{Co(II), Ni(II), Cu(II), Zn(II)}]\) have been synthesized by \([2+2]\) condensation reaction between 3,4 diaminotoulene and benzil (Scheme 1). The purity of the complexes was checked by thin layer chromatography (TLC) run in 1:1 benzene-methanol. However, inspite of all possible efforts a single crystal suitable for X-ray crystallography could not be successfully isolated. All the complexes were stable at room temperature and soluble in DMSO. The molar conductance values of the \(10^{-3}\) M solution of the complexes measured in DMSO indicate the non-electrolytic nature of all the complexes. The analytical data along with some physical properties of the complexes are summarized in Table 1. The formation of Schiff base complexes and bonding modes have been inferred from positions of characteristic bands in FT-IR spectra and resonance signals in \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra. The overall geometry of all the complexes has been deduced from the observed values of magnetic moments and the position of the bands in electronic spectra.
\[ M = [\text{Co(II), Ni(II)} \ n = 6, \text{Cu(II)} \ n = 2, \text{Zn(II)}] \]

**Scheme 1.** Synthesis and proposed structure of tetraazamacrocyclic complexes.
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>Molar Conductivity (ohm⁻¹ cm² mol⁻¹) / m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M</td>
<td>Cl</td>
</tr>
<tr>
<td>C₄₂H₃₂N₄CoCl₂</td>
<td>722.29 (722.55)</td>
<td>Dark brown</td>
<td>42</td>
<td>8.22</td>
<td>9.62</td>
</tr>
<tr>
<td>C₄₂H₃₂N₄NiCl₂</td>
<td>722.15 (722.32)</td>
<td>Brown</td>
<td>48</td>
<td>8.25</td>
<td>9.55</td>
</tr>
<tr>
<td>C₄₂H₃₂N₄CuCl₂</td>
<td>727.10 (727.56)</td>
<td>Light green</td>
<td>50</td>
<td>8.50</td>
<td>9.52</td>
</tr>
<tr>
<td>C₄₂H₃₂N₄ZnCl₂</td>
<td>729.45 (729.01)</td>
<td>Pale white</td>
<td>51</td>
<td>8.75</td>
<td>9.40</td>
</tr>
</tbody>
</table>
IR spectra

The preliminary identification regarding formation of the complexes has been obtained from the IR spectral findings (Table 2). The IR spectra of all the complexes do not show bands corresponding to free amino or carbonyl group rather a strong intensity band appeared in the region 1618-1622 cm\(^{-1}\) characteristic of azomethine group \(\nu(C=N)\) [32]. The presence of medium intensity band in the region 440-460 cm\(^{-1}\) assignable to \(\nu(M-N)\) vibration [33] confirms the coordination of azomethine nitrogen to metal ions. The bands characteristic of aromatic ring vibrations appeared in the 1440-1447, 1026-1061 and 700-770 cm\(^{-1}\) regions in all the complexes. A weak absorption band in the region 2906-2942 cm\(^{-1}\) may be assigned to –CH\(_3\) stretching vibration. The coordination of the chloro group has been ascertained by bands in 255-290 cm\(^{-1}\) region which may be assigned to \(\nu(M-Cl)\) vibration [34].
<table>
<thead>
<tr>
<th>Complexes</th>
<th>( \nu(\text{C=(\text{N})}) )</th>
<th>( \nu(\text{M-N}) )</th>
<th>( \nu(\text{M-Cl}) )</th>
<th>( \nu(\text{C-H}) )</th>
<th>Phenyl ring vibrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>[CoLCl₂]</td>
<td>1621</td>
<td>439</td>
<td>272</td>
<td>2925</td>
<td>1442, 1060, 776</td>
</tr>
<tr>
<td>[NiLCl₂]</td>
<td>1620</td>
<td>436</td>
<td>255</td>
<td>2942</td>
<td>1447, 1062, 701</td>
</tr>
<tr>
<td>[CuLCl₂]</td>
<td>1618</td>
<td>460</td>
<td>275</td>
<td>2919</td>
<td>1440, 1026, 770</td>
</tr>
<tr>
<td>[ZnLCl₂]</td>
<td>1622</td>
<td>430</td>
<td>290</td>
<td>2906</td>
<td>1445, 1061, 700</td>
</tr>
</tbody>
</table>
1H and 13C NMR spectra

The 1H NMR spectrum gives some important information regarding the formation of proposed macrocyclic moiety (Figure 5). The 1H NMR spectrum of macrocyclic Zn(II) complex recorded in DMSO-d6 shows a sharp signal at 2.08 ppm which may reasonably be assigned to the methyl protons (-CH3; 6H) [35]. Multiplets observed in the region 7.23-7.96 ppm may be attributed to aromatic ring protons [36].

The 13C NMR spectrum of the Zn(II) complex shows a number of sharp peaks corresponding to various carbon atoms in the proposed structure, resulting due to the non-equivalence of the various carbon atoms (Figure 6). A sharp signal observed at 153 ppm may be assigned to azomethine carbons [37]. The resonance signals for aromatic carbons appear in the 127-141 ppm region [38]. A sharp signal observed at 21 ppm corresponds to methyl carbons [39].
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 macrocyclic compounds, show molecular ion peaks, (M⁺) at m/z 722.29 [C₄₂H₃₂N₄CoCl₂], 722.15 [C₄₂H₃₂N₄NiCl₂], 727.10 [C₄₂H₃₂N₄CuCl₂] and 729.45 [C₄₂H₃₂N₄ZnCl₂] which are in good agreement with the respective molecular formulae.

EPR spectrum

The EPR spectrum of polycrystalline copper (II) complex was recorded at liquid nitrogen temperature. The spectrum do not show any hyperfine splitting, it exhibit only a single signal (Figure 7). The analysis of the spectrum gives g∥ = 2.12, g⊥ = 2.03. The observed g∥ value is less than 2.3 which is in agreement with the covalent character of the metal-ligand bond as per the criterion of Kivelson and Neiman [40]. The trend g∥ (2.12) > g⊥ (2.03) > 2.0023 observed for the complex indicates that the unpaired electron is localized in dₓ₂₋₄ᵧ₂ orbital of Cu(II) ion, characteristic of the axial symmetry. Tetragonally elongated structure is thus confirmed for the Cu(II) complex [41].

The g values are related by the expression G = (g∥ - 2) / (g⊥ - 2) which measure the exchange interaction between the copper centers in the polycrystalline solid. If G > 4, the exchange interaction is negligible and if G < 4 considerable exchange interaction occurs in the solid complexes [42]. The calculated G value for the complex is 3 (G < 4) which indicates considerable exchange interaction present between the Cu(II) centers.
Figure 7. The EPR spectrum of Cu(II) complex
Electronic spectra and magnetic susceptibility data

The electronic spectra of the macrocyclic Co(II) complex exhibits three spin allowed bands at 8500, 14,260 and 21,500 cm$^{-1}$ corresponding to $^4T_{1g}(F) \rightarrow ^4T_{1g}(F)$, $^4T_{1g}(F) \rightarrow ^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ transitions, respectively consistent with the octahedral geometry around the cobalt(II) ion which is further supported by a magnetic moment value of 4.49 B.M [43].

The electronic spectrum of Ni(II) complex exhibits three bands at 9000, 15,400 and 22,550 cm$^{-1}$ attributed to three spin allowed transitions viz. $^3A_{2g}(F) \rightarrow ^3T_{2g}(F)$, $^3A_{2g}(F) \rightarrow ^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow ^3T_{1g}(P)$, respectively corresponding to an octahedral geometry for the Ni(II) complex. The observed magnetic moment value of 3.12 B.M further complements the electronic spectral findings [43, 44].

The electronic spectrum of the copper (II) complex shows bands at 16,250 and 20,500 cm$^{-1}$ which may be ascribed to $^2B_{1g} \rightarrow ^2E_g$ and $^2B_{1g} \rightarrow ^2B_{2g}$ transitions, respectively. A distorted octahedral geometry around Cu(II) ion is suggested on the basis of electronic spectral findings and magnetic moment value of 1.83 B.M. [43].

Antibacterial activity

Antibacterial activity of the synthesized complexes has been studied against some bacterial strains viz. *Staphylococcus aureus, Streptococcus mutans, Streptococcus pyogenes, Staphylococcus epidermidis, Bacillus cereus, Corynebacterium xerosis, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and *Pseudomonas aeruginosa*. Preliminary screening for all the complexes has been performed at fixed concentration of 10 mg/ml. The results obtained were compared with standard antibiotics: ciprofloxacin (for gram-positive) and gentamicin (for gram-negative)
bacterial strains. All the complexes are found to be active on both type of bacterial strains. On the basis of the data obtained for diameter of zone of inhibition Cu(II) and Ni(II) complexes are found to be very effective (Figure 8a and 8b). In addition all the complexes are found to be effective at different dilutions based on the activity. The Minimum inhibitory concentrations of these complexes have been determined by broth dilution method in which the effectiveness is observed at lower concentrations (Table 3). It has been observed from the MIC values that Cu(II) complex is quite effective against S. mutans (0.156 mg/ml), S. pyogenes (0.312 mg/ml), C. xerosis (0.312 mg/ml), K. pneuomoniae (0.312 mg/ml), E. coli (0.625 mg/ml) and P. vulgaris (0.625 mg/ml). The MIC for Ni(II) complex is found to be 0.156 mg/ml for K. pneuomoniae, 0.312 mg/ml for S. mutans and S. pyogenes and 0.625 mg/ml for S. aureus, S. epidermidis, B. cereus and P. vulgaris. For Co(II) complex MIC is highest for S. aureus and K. pneuomoniae (0.312 mg/ml) and then 0.625 mg/ml for S. mutans and S. pyogenes. The Zn(II) complex shows comparatively low activity against most of the bacterial strains.

It is concluded that all the complexes show antibacterial activity but they are found to be more potent inhibitors against gram positive bacterial strains (Table 3). The Cu(II) complex shows comparatively more inhibition against K. pneuomoniae as compared to the commercial antibiotic (Figure 8b).
Figure 8a. Comparison of zone of inhibition (in mm) of macrocyclic complexes with standard antibiotic against gram positive bacterial stains. Ciprofloxacin-standard antibiotic.
Figure 8b. Comparision of zone of inhibition (in mm) of macrocyclic complexes with standard antibiotic against gram negative bacterial stains. Gentamicin-standard antibiotic.
Table 3. Minimum inhibitory concentration (MIC in mg/ml) of the macrocyclic complexes.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Bacterial Strains</th>
<th>Complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[CoLCl₂]</td>
</tr>
<tr>
<td>1.</td>
<td>S. aureus</td>
<td>0.312</td>
</tr>
<tr>
<td>2.</td>
<td>S. mutans</td>
<td>0.625</td>
</tr>
<tr>
<td>3.</td>
<td>S. epidermidis</td>
<td>1.250</td>
</tr>
<tr>
<td>4</td>
<td>S. pyogenes</td>
<td>0.625</td>
</tr>
<tr>
<td>5</td>
<td>B. cereus</td>
<td>2.500</td>
</tr>
<tr>
<td>6</td>
<td>C. xerosis</td>
<td>1.250</td>
</tr>
<tr>
<td>7</td>
<td>E. coli</td>
<td>2.500</td>
</tr>
<tr>
<td>8</td>
<td>K. pneuomoniae</td>
<td>0.312</td>
</tr>
<tr>
<td>9</td>
<td>P. aeruginosa</td>
<td>2.500</td>
</tr>
<tr>
<td>10</td>
<td>P. vulgaris</td>
<td>1.250</td>
</tr>
</tbody>
</table>
Fluorescence measurements

DNA binding of complexes

Fluorescence quenching is a useful method to monitor the molecular interactions of chemical and biological systems because of its high sensitivity [45, 46]. 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. Dynamic quenching results from collision between fluorophore and quencher whereas static quenching is due to ground state complex formation between fluorophore and quencher [47]. In the present study the interactions of synthesized macrocyclic complexes with calf thymus DNA have been investigated by fluorescence spectroscopy. Fluorescence quenching is usually classified as dynamic quenching and static quenching [48]. However, the characteristic Stern-Volmer plot of combined quenching (both static and dynamic) is an upward curvature. The binding of macrocyclic complexes with calf thymus DNA has been studied by monitoring the changes in the intrinsic fluorescence of different complexes at varying DNA concentrations. The representative fluorescence emission spectra of the complexes upon excitation at different wavelengths are given in Figure 1-4. The spectra illustrate that excess of DNA causes a gradual decrease in the fluorescence emission intensity of the complexes suggesting changes in the microenvironment of the fluorophore upon interaction with DNA, indicating binding of complexes with DNA. Over all, it is concluded that all the complexes show remarkable binding with DNA which is one of the main molecular targets in the design of numerous therapeutic compounds like anti-cancer drugs [49].
REFERENCES


22. G. A. Melson (Ed), *Coordination Chemistry of Macrocyclic Complexes*, Plenum Press, New York, **1979**.


