CHAPTER IV

Complexation of Transition Metal ions with Monobasic Tridentate Ligand
Estelar
4.1. Introduction

Schiff base derivatives and their transition metal complexes, known from the 19th century, have made considerable contributions to the advances in coordination chemistry and have found a broad spectrum of applications in very diverse fields such as biochemical, analytical, catalytic, therapeutic and biomimetic activities (El-Khawaga O A, 2000). These applications include their uses as MRI contrast agents (Yang et.al, 2000), biological markers (Briggs et.al, 2002), antitumor agents (Tai et.al, 2003) etc. Copper(II) complexes are known to be effective against rheumatoid arthritis and they also show antiulcer activity (Abdel-Latif et.al, 2007). This is significant because gastrointestinal irritation often precludes treatment by other antiarthritic drugs. Symmetric and asymmetric transition metal complexes of Schiff bases have been used as catalysts in reactions, such as epoxidation, asymmetric synthesis, asymmetric sulfoxidation, asymmetric silylcyanation and many other applications (Shi M, Wang C J, 2006).

With the increasing incidence of deep mycosis, there has been increasing emphasis on the screening of new and more effective antimicrobial drugs with low toxicity. In order the need to find cheap and effective antibacterial agents, we synthesized new complexes of transition metals with novel Schiff base ligand. Furthermore, it was also intended to screen these complexes for antimicrobial activities against *Escherichia coli* (Gram- negative) and *Staphylococcus aureus* (Gram-positive) bacteria (Banjo et.al, 2006; Menichetti F, 2005). Thus, the present results will add new information in pharmaceutical industry to synthesize antibacterial drugs as the synthesized compounds showed promising antimicrobial activity. Heterocyclic compounds are used as pharmaceuticals, pesticides, herbicides, dyes and plastics. Recently the coordination chemistry of Schiff bases of heterocyclic carbaldehyde have received much attention. Here we report the synthseses, characterization and biological activities of transition metal complexes of tridentate Schiff base ligand (Naeimi H, Moradian M, 2008; Wu S N, Chang H D, 2006; Wahab Z H A E, 2007; Singh et.al, 2010).

4.2. Experimental

4.2.1. Material and Methodology

All the chemicals used were of analytical grade and used as procured. The stoichiometric analysis(C, H and N) of the complexes were performed using
Elementar vario EL III model. Metal contents were estimated on an AA-640-13 Shimadzu flame atomic absorption spectrophotometer in solutions prepared by decomposing the complex in hot concentrated HNO₃. The IR spectra were recorded on Perkin-Elmer FTIR spectrophotometer in KBr and polyethylene pellets in the region 4000-400 & 400-100 cm⁻¹ respectively. The UV-visible spectra were recorded in DMSO on Beckman DU-64 spectrophotometer with quartz cells of 1 cm path length and mass spectra (TOF-MS) were recorded on Waters KC-455 model with ES⁺ mode in DMSO. ¹H NMR spectra were recorded in DMSO-d₆ solvent on a Bruker Advance 400 instrument. X-band EPR spectra was recorded on a Varian E-112 spectrometer with a variable temperature liquid nitrogen cryostat (The error in g value is ±0.001) and g factors were quoted relative to the standard marker TCNE (g = 2.00277). Cyclic voltammetric measurements were carried out on a BAS CV 50W electrochemical analyzing system (accuracy ±1.0mV) at room temperature in three-electrode cell using nitrogen purged DMSO solution containing 0.1M TBAP and 10⁻³M of the complex. A Pt wire is used as a working electrode and Ag/AgCl as a reference electrode. Magnetic susceptibility measurements were carried out at room temperature in powder form on a vibrating sample magnetometer PAR 155 with 5000G-field strength, using Co[Hg(SCN)₄] as the calibrant (magnetic susceptibility ≈ 1.644 × 10⁻⁵ cm³g⁻¹).

4.2.2. Synthesis of Ligand, [2-aminophenol-pyrrole-2-carbaldehyde]

Pyrrole-2-carboxaldehyde (30 mmol) was dissolved in absolute ethanol (20 ml) added drop wise to a solution of 2-aminophenol (30 mmol) in absolute ethanol (20 ml) with constant stirring. Stirring was continued with heating at 80⁰C for 3h. A brown coloured powder was collected by vacuum filtration and dried overnight in vacuum. The yield and melting point of the product were determined.

4.2.3. Synthesis of Metal Complexes

2-[(1H-pyrrole-2-ylmethylene) aminophenol (figure 4.1) (5 mmol) in EtOH (20 mL) was added drop wise to a solution containing metal salts (5mmol) in EtOH (20mL). The mixture was stirred for 4 h at 60⁰C. The precipitate were filtered, washed with cold alcohol and dried under vacuum over silica gel. The yield and melting point
of each product was determined. The metal salts used were Copper(II) acetate, Cobalt(II) nitrate, Nickel(II) acetate, Manganese(II) Chloride.

4.2.4. Biological Activity: Antibacterial Screening

In vitro antibacterial activity of the compounds against *Escherichia coli* and *Staphylococcus aureus* were carried out using Muller Hinton Agar media (Hi media). The activity was carried out using paper disc method. Base plates were prepared by pouring 10 ml of autoclaved Muller-Hinton agar into sterilized Petri dishes (9 mm diameter) and allowing them to settle. Molten autoclaved Muller Hinton that had been kept at 48°C was incubated with a broth culture of the *Escherichia coli* and *Staphylococcus aureus* bacteria and then poured over the base plate. The discs were air dried and placed on the top of agar layer. The plates were incubated for 24 – 30 h and the inhibition zones (mm) were measured around each disc. As the organism grows, it forms a turbid layer, except in the region where the concentration of antibacterial agent is above the minimum inhibitory concentration, and a zone of inhibition is seen. The size of the inhibition zone depends upon the culture medium, incubation conditions, rate of diffusion and the concentration of the antibacterial agent. The solutions of all compounds were prepared in double distilled water and chloramphenicol was used as reference.

4.3. Results and Discussion

The synthesized compounds are crystalline and non-hygroscopic in nature. These are insoluble in water, partially soluble in ethanol but soluble in acetone, DMF and DMSO. Single crystals of the compounds could not be isolated from any organic solvents; thus no definite structures can be described. However, the analytical and spectroscopic data enables us to predict possible structures. The biological activities of the complexes were also tested against *Escherichia coli* and *Staphylococcus aureus* which were close to the standard drug. Molar conductance values of the complexes and Schiff bases show very low values of molar conductance indicating their non-electrolytic nature (Vale et.al,2006).
4.3.1. IR Spectra and Mode of Bonding

I.R. spectrum of the ligand shows a broad band of medium intensity at 3339 cm\(^{-1}\) due to ν(NH) (That may be overlapping with hydrogen bonded O...H stretching vibrations). The band due to N–H disappeared in the spectra of complexes. The involvement of deprotonated phenolic moiety in complexes is confirmed by the shift of ν(C-O) stretching band observed at 1283 cm\(^{-1}\) in the free ligand to a lower frequency to the extent 10-20 cm\(^{-1}\) (Omar M M, Mohamed G G, 2005). It indicates that nitrogen and phenolic oxygen are directly linked to metal ions. The shift of ν(C-O) band at 1283 cm\(^{-1}\) to a lower frequency suggests the weakening of ν(C-O) and formation of stronger M-O bond. The free Schiff base ligand showed a strong band at 1630 cm\(^{-1}\), which is characteristic of the azomethine(-HC=N) group (Ramesh R, Maheshwaram S, 2003). Coordination of the Schiff base to the metal ions through the nitrogen atom is expected to reduce electron density in the azomethine link and lower the νC=N absorption frequency. The band due to νC=N is shifted to lower frequencies and appears around 1610-1625 cm\(^{-1}\), indicating coordination of the azomethine nitrogen to metal ions (Ali et al., 2002). The weak to medium bands in the two ranges 580-615 and 430-560 cm\(^{-1}\), which could be assigned to the stretching frequencies of the ν(M-O) and ν(M-N) bands, respectively, supporting that the bonding of the ligand to metal ions is achieved by the phenolic oxygen and azomethine nitrogen atom of the ligand (Nakamoto K, 1997). The N-H stretching frequency at 3135-2900 cm\(^{-1}\) in the free ligand (having pyrrole ring) showed considerable shift in all the complexes, indicating participation of this N-H group in complexes (Chandra S, Kumar U, 2004). The bands in the range of 3352-3575 cm\(^{-1}\) which can be assigned to the stretching frequencies of the ν(OH) of water molecules associated to the complexes which are also confirmed by the elemental analyses. In addition to the modified slightly on account of coordination, infrared spectra of the cobalt(II) complex show absorption bands at 1270, 1095, 730, 1383, 965, 1035 cm\(^{-1}\) due to terminally bonded monodentate nitrato group (Addison C C, Sultons D, 1967; Addison et al., 1971). In addition to these two weak bands with a separation of ca. 12 cm\(^{-1}\) appear in the 1800-1700 cm\(^{-1}\) region indicating clearly the exclusive presence of terminal monodentate nitrato group (Lever et al., 1971; Choca et al., 1972). The Cu(II) and Ni(II) complexes have bands in the region 1620-1630(s) cm\(^{-1}\) and 1412-1418(s) cm\(^{-1}\) which can be assigned to
$\nu_{as}(\text{CO}_2)$ and $\nu_{s}(\text{CO}_2)$ fundamental stretching bands respectively, which are in agreement with the acetate groups being monodentate (Nakamoto K, 1997).

4.3.2. $^1$H NMR Spectra and EPR Spectra

The $^1$H NMR spectrum of the ligand and its Ni(II) complex have been recorded in DMSO-$d_6$ as solvent to confirm the binding of the Schiff base to the metal ions. The spectrum shows the stability of the complex in solution. The tridentate Schiff base ligand possess one phenolic, azomethine and pyrrole group. The $^1$H- nmr spectra of the ligand showed the aromatic protons as multiplet in the range 6.80-7.70 ppm and peaks in the region 6.2-6.7 ppm were assigned chemical shift of pyrrole hydrogen (Silverstein R M, Webster F X, 2007). The downfield shifting of the pyrrole hydrogen peak indicating its coordination with Ni(II) ion. In the spectra of the ligand, the OH proton of the phenolic ring at 11.76 ppm, but the Ni(II) complex did not shows phenolic proton, indicating deprotonation of the hydroxyl group. The azomethine proton appeared as a sharp singlet at 8.75 ppm. Moreover, the azomethine proton of the free ligand is shifted downfield to 7.92 ppm on complexation. These observations suggest that the ligand coordinate to the Ni(II) through the phenolic oxygen and nitrogen atom of the azomethine group. In complexes the peak in the region of 3.0-3.5 ppm were assigned for coordinated water and another peak at 4.5 ppm especially in DMSO solvent confirms the hydrogen bonded water molecules(Silverstein R M, Webster F X, 2007). A new peak in the region $\delta$ 2.0-3.0 ppm, characteristic of acetate groups are present in the spectrum of the complex are absent in the spectrum of the ligand.

The EPR spectrum of polycrystalline copper(II) complex samples shows similar features at room temperature and at liquid nitrogen temperature. Basic spectral features at both temperatures are same with slightly better resolution at liquid nitrogen temperature. The spectrum of powdered sample of Cu(II) complex showed normal features with $g_{||} > g_{\perp} > 2$ indicating that the unpaired electron lies in the dx$^2$-y$^2$ orbital (Bew et.al, 1972), characteristic of square planar geometry around Cu(II) ion. The exchange interaction parameter term $G$, estimated from the expression $G = (g_{||} - 2.0023) / (g_{\perp} - 2.0023) = 1.813$, indicating that the ligand has strong field (Hathaway B J, Billing D E, 1970). The value of $G < 4$, indicating considerable exchange coupling and the misalignment is appreciable. The value of $A_{||}(158)$, nuclear hyperfine constant
and $\alpha^2(0.69)$ degree of covalency support the structure (Manimekalai A, Shivkumar B S, 2004).

4.3.3. Mass Spectra

This method is particularly useful when a poorly crystalline nature of complexes prevents their x-ray characterization. Mass spectrometry has been successfully used to investigate molecular species in solution (Sanmartin et.al, 2006; Beloso et.al, 2003; Singh et.al, 2009). The pattern of mass spectrum gives an impression of the successive degradation of the target compound with the series of peaks corresponding to the various fragments. Their intensity gives an idea of stability of fragments. The recorded mass spectra of the ligand and their metal complexes of molecular ion peaks have been used to confirm the proposed formula given in figure 4.3-4.5. The mass spectrum of ligand having a molecular ion peak at 186\{100\% m/z\} and having prominent peak at 145\{34\% m/z\} that corresponds to the $[C_9H_8NO]^+$, confirming purity of the ligand. The molecular ion peaks of Cu(II), Co(II), Ni(II) and Mn(II) - ligand complexes were observed respectively at 344,342,339 and 330 m/z which confirm the stoichiometry of the metal complexes to be $[M(L)(X)].nH_2O$. All the synthesized complexes containing metal ion were confirmed by good agreement between the observed and calculated molecular formula (Swamy S J, Pola S, 2008). Elemental analysis values are in close agreement with the values calculated from molecular formulas assigned to these complexes, which is further supported by the mass studies. The molecular ion peak of the complex by the loss of water molecules gave a fragment ion $[M(L)(X)]^+$. All these fragments leading to the formation of the monomeric species $[M(L)]^+$ which undergoes demetallation to form the species $[HL]^+$ are usually present in the mass spectra of these systems (Yoshida et.al, 2000). The last two fragments at 185/186 and 146/145 are $[LH]^+$ and $[C_9H_8NO]^+$ of ligand peaks appear in all the complexes shows similar pattern of degradation.

4.3.4. Electronic Spectra and Magnetic Studies

The electronic spectra of the complexes were recorded in DMSO. The bands in the 200-380 nm region coincide with the bands observed in the free ligand. Two relatively less intense bands ($n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$) occur in the 380-450 nm region, these can be assigned to phenolic oxygen to metal and imine nitrogen to the metal.
charge transfer transitions, respectively. The electronic spectra of the Schiff base-Cu(II) complex shows very intense absorption bands in the UV region, attributed to ligand transitions (n→π* and π→π*), and a characteristic broad bands at 660 nm and 510 nm assignable to 2B1g→2Eg and 2B1g→2A1g and transitions respectively in visible region, which indicates the possibility of square planar geometry of copper complex. The Cu(II) complex has magnetic moment value 1.75 BM reveals square planar geometry around the metal ion (Nakamoto K, McCarthy S J, 1968). Cobalt (II) complex shows absorption bands at 665 nm assigned to 4A2→4T1(P) transitions. The existence of spin – orbit coupling also allows some quartet → doublet spin transition to occur. Another band at 704 nm is assigned to 4A2→4T1(F). The expected 4A2→4T2 transition appearing at a 4500 cm⁻¹ is overlapped by ligand vibration transitions (i.e., the infrared bands) suggests tetrahedral geometry of the complex (Nakamoto K, McCarthy S J, 1968), which is also corroborated by magnetic moment(The magnetic moment of Co(II) is 4.22 BM) value of the complex (Nakamoto K, McCarthy S J, 1968; Ciampolini M, 1969). The Ni(II) complex has diamagnetic character and shows the square planar geometry around the metal center. Spectra of the nickel complex shows an absorption band at 650 nm, assignable to a 1A1g→1A2g transition and a shoulder at 550 nm corresponding to a 1A1g→1B1g transition which are consistent with square planar stereochemistry about the nickel(II) ion. It can be explained with the planar ligand set causes one of the d-orbitals (dx²−y²) to be uniquely high in energy and the eight electrons can occupy the other four d-orbitals but leave this strongly antibonding one vacant. The Mn(II) complex show intense absorption at 340 nm assigned to ligand centered transition while two weak transitions are observed at 375nm and 460 nm may be referred to a combination of MLCT and d-d transition (4A1→4T1), suggesting tetrahedral geometry of the complex. The magnetic moment value of the manganese(II) complex is 5.60 BM, may have tetrahedral geometry (Lever A B P, 1968; Figgis B N, 1963). The tetrahedral geometry suggested by electronic spectra of manganese(II) complex supported magnetic moment, a high spin tetrahedral complex.

4.3.5. Cyclic Voltametric Studies

The electrochemical behaviors of the copper(II), cobalt(II), and nickel(II) complexes were studied using cyclic voltametric(CV) technique in DMSO solution containing 0.1 M TBAP. The CVs are presented in figure 4.6-4.7. The Cu(II)
complex(1×10⁻³M) exhibit a reversible oxidation and reversible reduction peaks at the scan rate 100 mVs⁻¹. The copper complex (Figure 4.6) showed reversible behavior, with ΔEₚ(Cu^{II}-Cu^{I}) = - 0.21V in DMSO solution containing tetra butyl ammonium perchlorate (TBAP) (0.10 mol dm⁻³). The ΔEₚ = - 0.21V value is consistent with a coordination geometry intermediate between square planar and tetrahedral around the Cu(II). Besides, unsaturated nitrogen as the donor atom in the ligand stabilizes more a low oxidation state, such as Cu(I) ion, than a saturated one, by π- back bonding between the metal and the nitrogen atom. The other spectroscopic studies confirms square planar geometry around Cu(II). Cyclic voltamograms of the Co(II) complex (1×10⁻³M) exhibit reversible oxidation and reduction peaks at 50 and 500 mVs⁻¹. At 50 mVs⁻¹, the Co(II) complex shows reversible oxidation peak at 1.88V. The peak has been changed to -0.98 V for oxidation and -0.52V for reduction processes, respectively. The CV of the nickel complex (figure 4.7) which exhibited a quasi-reversible one - electron reduction at100 mVs⁻¹ scan rate with a corresponding anodic wave. This reduction is referred to the Ni(II)/ Ni(I) couples. The quasi-reversibility of the reduction process are confirmed with the corresponding peak separation value between cathodic peak potential and anodic peak potential (ΔEₚ = 0.121V) and almost the unity of the ratio of the anodic peak current to cathodic peak current. The half wave potential of the nickel complex was located at E₁/₂ = -1.38V verses Ag/ AgCl. Cyclic voltammetric experiment on the Mn(II) complex reveals the presence of an irreversible, anodic process (Eₚ = + 0.78V) and quasi-reversible reduction (E₁/₂ = -1.05V), which regenerates in the reverse scan of the oxidation of the original complex. The oxidation may be attributed to the Mn(II)/ Mn(III) couple, where the Mn(III) complex after its formation, being quite unstable in the original geometry, evolves to an unidentified differently coordinated species (fragmentation can not be ruled out), which however upon reduction regenerates the Mn(II) complex.

4.3.6. Biological Studies

The bacteriological effect of tridentate Schiff base and its M(II) complexes were determined against two bacteria under different concentration, as described in the experimental section. The agar well – diffusion method was employed for the bacteria with respect to chloramphenicol as standard drug. The results showed that some compounds are very effective on some microorganisms (Figure 4.8-4.9). The
complex III showed better activity than other metal complexes for both microorganisms. The activity of any compound is a complex combination of steric, electronic and pharmacokinetic factors. A possible explanation for the toxicity of the complexes has been postulated in the light of chelation theory. It was suggested that the chelation considerably reduces the charge of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the whole chelate ring. This increases the lipophilic character of the metal chelate which favors its permeation through lipid layer of cell membranes. Furthermore, the mode of action of the compounds may involve the formation of a hydrogen bond through –N=C group of the chelate or the ligand with the active centers of the cell constituents resulting in interference with the normal cell process. The higher bacteriotoxicity experienced by the compounds may be ascribed to the fact that the metal ions are more susceptible towards the bacterial cells than ligands (Phaniband M A, Dhumwad S D, 2007).

**Conclusion**

With the help of various physico-chemical techniques, geometries of the newly synthesized compounds have been proposed (Figure 4.9). Metal complexes were found to be monomer and involved coordination through nitrogen and oxygen atoms of the ligand giving tetrahedral/square planar geometry. The ligand is so flexible to satisfy tetrahedral/square planar geometry has been confirmed with the help of different spectral studies. The Ni(II) complex was found to be most active against *Escherichia coli* and *Staphylococcus aureus* bacteria.
Table 4.1: Analytical data of ligand and their metal complexes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound (Empirical Formula)</th>
<th>Color</th>
<th>MP (°C)</th>
<th>Yield (%)</th>
<th>Elemental Analysis: Found (Calc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>L (C₁₁H₁₀N₂O)</td>
<td>Brown</td>
<td>130</td>
<td>84</td>
<td>70.93 (70.96)</td>
</tr>
<tr>
<td>2</td>
<td>[Cu(L)Ac].2H₂O I (C₁₃H₁₆N₂O₅Cu)</td>
<td>Light Blue</td>
<td>185</td>
<td>82</td>
<td>45.41 (45.40)</td>
</tr>
<tr>
<td>3</td>
<td>[Co(L)NO₃].2H₂O II (C₁₃H₁₃N₃O₆Co)</td>
<td>Brown</td>
<td>200</td>
<td>85</td>
<td>38.57 (38.60)</td>
</tr>
<tr>
<td>4</td>
<td>[Ni(L)Ac].2H₂O III (C₁₃H₁₆N₂O₅Ni)</td>
<td>Dark Brown</td>
<td>175</td>
<td>82</td>
<td>46.05 (46.05)</td>
</tr>
<tr>
<td>5</td>
<td>[Mn(L)Cl].3H₂O IV (C₁₃H₁₅N₂O₅Mn)</td>
<td>Yellow</td>
<td>180</td>
<td>80</td>
<td>40.07 (40.09)</td>
</tr>
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</table>
Table 4.2: Infrared spectroscopic data and magnetic moment of ligand and their metal complexes

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Comp.</th>
<th>Infrared (cm$^{-1}$)</th>
<th>Magnetic Moment $\mu_{\text{eff}}$ (BM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\nu$ (C=N) $\nu$ (M-N) $\nu$ (M-O) others</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>L</td>
<td>1630 (s)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>I</td>
<td>1625 (s) 490(m) 580(m) $\nu_{\text{as}}$(CO$<em>2$):1620(m), $\nu</em>{\text{s}}$(CO$_2$):1418(s)</td>
<td>1.75</td>
</tr>
<tr>
<td>3</td>
<td>II</td>
<td>1620 (s) 513(m) 613(m) $\nu$(NO$_3$):1270($\nu_1$),1090($\nu_2$),730($\nu_3$), 1384($\nu_4$), 965($\nu_5$), 1035($\nu_6$)</td>
<td>4.22</td>
</tr>
<tr>
<td>4</td>
<td>III</td>
<td>1610 (s) 465(m) 590(w) $\nu_{\text{as}}$(CO$<em>2$):1630(m), $\nu</em>{\text{s}}$(CO$_2$):1412(s)</td>
<td>Diamagnetic</td>
</tr>
<tr>
<td>5</td>
<td>IV</td>
<td>1618 (s) 501(m) 598(m) $\nu$(M-Cl):280</td>
<td>5.60</td>
</tr>
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</table>
Figure 4.1: Structure of the ligand
Figure 4.2: Mass spectrum of the complex I
Figure 4.3: Mass spectrum of the complex III
Figure 4.4: Mass spectrum of the complex IV
Figure 4.5: The cyclic voltamogram of the complex I
Figure 4.6: The cyclic voltamogram of the complex III
Figure 4.7: Effect of different concentration of ligand and complexes with *Escherichia coli*
Figure 4.8: Effect of different concentration of ligand and complexes with *Staphylococcus aureus*
Where, $M = \text{Cu(II)}$, $X = \text{CH}_3\text{CO}_2$, $n = 2$
$M = \text{Co(II)}$, $X = \text{NO}_3$, $n = 2$
$M = \text{Ni(II)}$, $X = \text{CH}_3\text{CO}_2$, $n = 2$
$M = \text{Mn(II)}$, $X = \text{Cl}$, $n = 3$

Figure 4.9: Proposed structure of the complexes