ASIAN JOURNAL OF CHEMISTRY

Synthesis of Schiff Base (E)-2-((3-Hydroxyphenyl)imino)methyl)-6-methoxyphenol Containing N and O Donors and its Metal Complexes: Spectral, Thermal, Redox Behaviour, Fluorescence Quenching, Antimicrobial and Anticancer Studies

G. VALARMATHY¹*, R. SUBBALAKSHMI¹, R. RENGANATHAN² and R. KOKILA¹

¹PG and Research Department of Chemistry, Seethalakshmi Ramaswami College, Tiruchirappalli-620 002, India
²School of Chemistry, Bharathidasan University, Tiruchirappalli-620 024, India

*Corresponding author: E-mail: valarchola@gmail.com

Convenient synthesis of new octahedral complexes from 3-aminophenol and 2-hydroxy-3-methoxybenzaldehyde with the molecular formula [M(L2-2H)] (where M = Mn²⁺ and Co²⁺) have been described. The Schiff base behaves as a tridentate ligand with O and N donors and coordinates to metal ion via phenolic oxygens and azomethine nitrogen. The compositions of the ligand and its metal complexes have been established by elemental analysis and metal estimation. The ionic nature of the metal complexes has been deduced from conductance measurements. Structural features and bonding mode of Schiff base have been proposed by magnetic properties and spectral methods. Both the complexes exhibit d-d transitions, photo emissive and redox behaviour. The Schiff base ligand and its metal complexes have been screened for antimicrobial evaluation towards some clinically important microorganisms. The quenching studies were carried out for the [Co(L2-2H)] complex by alizarin red S dye in DMSO using fluorescence measurements. The evaluated highly biologically active [Co(L2-2H)] complex shows excellent cytotoxicity towards (MCF-7) cancer cell line.

Keywords: Schiff base ligand, 3-Amino phenol, 2-Hydroxy-3-methoxybenzaldehyde, Fluorescent quenching, Biological activities.

INTRODUCTION

Schiff base ligands containing N and O donors are unique and multifaceted, known for their selectivity and sensitivity towards the metal ions. The biomedical properties of free organic molecules depend upon chelation with suitable metal ion led to the implementation of metal complexes for several biomedical applications as therapeutically active analgesic, antipyretic, antiinflammatory, cytotoxic, antiviral, antitumorous and antitubercular activity besides their applications as antimicrobial agents [1-5]. Now a days there is an increasing interest in the development of fluorescence sensors for the enantioselective recognition of chiral organic compounds and drug molecule [6]. In recent years, there have been numerous reports of their use in homogeneous and heterogenous catalyst. An interesting application of Schiff bases, is their use as an effective corrosion inhibitor, which is based on their ability to spontaneously forms a monolayer on the surface to be protected [7]. They have played a seminal role in the development of modern coordination chemistry, as well as they can also be found at key points in the development of inorganic biochemistry, catalysis and optical materials [8].

Previously a number of biologically important complexes have been reported by our group [9-13]. In order to broaden the scale of investigations on the Schiff bases, the present paper records the synthesis and characterization of Mn(II) and Co(II) complexes derived from 2-aminophenol and 2-hydroxy-3-methoxybenzaldehyde. The structures of the ligand and its metal complexes were characterized by elemental analysis, IR, ¹H NMR, UV, molar conductance and magnetic susceptibility measurements. The biological activities are also studied against gram positive and gram negative bacterial and fungi organisms for Schiff base ligand and their complexes. The Co(II) complex shows excellent cytotoxicity towards human breast cancer cell (MCF-7). The fluorescence quenching studies were carried out for the [Co(L2-2H)] complex by the alizarin red S dye in DMSO using fluorescence measurements.

EXPERIMENTAL

All the chemicals used were of analytical reagent grade (AR) and of highest purity available. Solvents were purified and dried according to the standard procedures. All metal(II) compounds were used as chloride salts. IR spectra of the complexes
Synthesis of Schiff base ligand (E)-2-(((3-hydroxyphenyl)imino)methyl)-6-methoxyphenol: The Schiff base was prepared by the condensation of equimolar amounts of 3-aminophenol (0.01 mol) and 2-hydroxy-3-methoxybenzaldehyde (0.01 mol) in minimum quantity of ethanol. The resulting mixture was then refluxed for 4-5 h. An orange coloured solid mass separated out on cooling was filtered, washed and dried over anhydrous CaCl₂ in desiccator. The purity of the ligand was checked out on cooling is filtered, washed and dried over anhydrous CaCl₂ in desiccator. The purity of the ligand was checked by TLC and spectral data. The structure of Schiff base ligand was confirmed by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm⁻¹ is shifted to 1606 cm⁻¹ in Co(II) complex suggesting the coordination of the N and O atoms of the Schiff base with the metal through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal was shown by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm⁻¹ is shifted to 1606 cm⁻¹ in Co(II) complex and 1614 cm⁻¹ Mn(II) complex suggesting the coordination through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal atom was shown by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm⁻¹ is shifted to 1606 cm⁻¹ in Co(II) complex and 1614 cm⁻¹ Mn(II) complex suggesting the coordination through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal atom was shown by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm⁻¹ is shifted to 1606 cm⁻¹ in Co(II) complex and 1614 cm⁻¹ Mn(II) complex suggesting the coordination through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal atom was shown by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.

The shift of frequency is due to the coordinate bonding of hydroxyl oxygen to the metal. The azomethine peak in ligand at 1603 cm⁻¹ is shifted to 1606 cm⁻¹ in Co(II) complex and 1614 cm⁻¹ Mn(II) complex suggesting the coordination through >C=N- group (Table-3). Further evidence for the coordination of the N and O atoms of the Schiff base with the metal atom was shown by the appearance of a new weak band in the IR spectra: ν(>C=N-) [15] whereas the broad band at 3474 cm⁻¹ is characteristic of hydrogen bond (O-H) [16] stretching vibration. On comparison of the IR spectrum of ligand with the metal complexes, it is observed that the strong and intense peaks observed in the range 3500-3400 cm⁻¹ which indicates the presence of O-H stretching frequency [17]. The peak at 3474 cm⁻¹ in the ligand and 3360 cm⁻¹ the Co(II) complex corresponds to O-H stretching.
region 740-734 cm\(^{-1}\) which may be assigned to \(\nu(M-O)\) and band at around 540-558 cm\(^{-1}\) may be due to \(\nu(M-N)\) \[18\].

**Electronic spectra and magnetic susceptibility measurements:** The ligand is orange in colour and shows three absorption bands at 210 nm (47431 cm\(^{-1}\)), 228 nm (43767 cm\(^{-1}\)), 310 nm (32235 cm\(^{-1}\)) accounts for \(\pi-\pi^*\) and \(n-\pi^*\) transitions \[19\]. The Co(II) complex is reddish brown in colour exhibits three absorption bands. The bands at 39384 and 36847 cm\(^{-1}\) may be due to the intra ligand charge transfer and 30137 cm\(^{-1}\) may be due to the transition from \(^4A_{2g} \rightarrow ^4T_{1g}\) \[20\]. The Mn(II) complex is brown in colour exhibits two absorption bands (Fig. 2). The band at 36182 cm\(^{-1}\) may be due to the intra ligand charge transfer and 30120 cm\(^{-1}\) band is assigned to \(^6A_{1g} \rightarrow ^2T_{2g}\) transition \[21\]. The magnetic moment value of Co(II) complex is 5.14 BM confirms the octahedral geometry whereas Mn(II) complex is 5.82 BM is confirms the distorted octahedral geometry \[22\] (Table-4).

**1H NMR:** 1H NMR spectra of free ligand and its metal complexes were recorded in DMSO-\(d_6\). The multiplet which extends from \(\delta 6.7\) to \(\delta 7.3\) corresponds to the 7 protons of the aromatic rings \[23\]. The ligand NMR spectrum has a singlet peak at \(\delta 8.8\) ppm suggest the presence of \(CH=N-\) linkage (Fig. 3). The peak at \(\delta 13.2\) ppm indicates the phenolic hydroxyl proton signal at \(\delta 3.81\) ppm corresponds to \(-OCH_3\) proton \[24\]. The position of phenolic OH peak at \(\delta = 13.2\) ppm in the ligand is unchanged in the complexes indicating the involvement of phenolic OH group in coordination with the metal without deprotonation \[25\]. In addition, the number of protons calculated from the integration curves and those obtained from the values of the CHN analysis, agree well with each other.

**Thermal analysis:** Thermogravimetric curves of the complexes were recorded heating rates of 10 °C/min under nitrogen atmosphere in the temperature range from room temperature to 800 °C. The complex is stable upto 350 °C as shown in Fig. 4. This excludes the possibility of lattice as well as coordinated water molecules in the complex. Above this temperature, decomposition begins slowly. A gradual weight loss starts from 300 °C which corresponds to the decomposition of the Schiff base ligand from the metal chelates. The horizontal thermal curve observed above 540 °C corresponds to the step by step removal of the ligand and the conversion of metal to a metal oxide residue. Thus the overall thermogravimetric results were consistent with the formulation of the complex along with the conclusion derived from infrared and mass spectral studies.

---

**TABLE-3**

<table>
<thead>
<tr>
<th>Schiff base &amp; complexes</th>
<th>(\nu(O-H)) (cm(^{-1}))</th>
<th>Phenolic (\nu(C-O))</th>
<th>(\nu(&gt;C=N-))</th>
<th>(\nu(M-O))</th>
<th>(\nu(M-N))</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>3474</td>
<td>1460</td>
<td>1603</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Co(L(_2)-2H)]</td>
<td>3360</td>
<td>1471</td>
<td>1606</td>
<td>734</td>
<td>540</td>
</tr>
<tr>
<td>[Mn(L(_2)-2H)]</td>
<td>3421</td>
<td>1471</td>
<td>1614</td>
<td>740</td>
<td>558</td>
</tr>
</tbody>
</table>

**TABLE-4**

<table>
<thead>
<tr>
<th>Schiff base &amp; complexes</th>
<th>Electronic spectral data</th>
<th>Magnetic Susceptibility Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (cm(^{-1}))</td>
<td>Transition</td>
</tr>
<tr>
<td>L</td>
<td>47431</td>
<td>(\pi-\pi^*)</td>
</tr>
<tr>
<td></td>
<td>43767</td>
<td>(\pi-\pi^*)</td>
</tr>
<tr>
<td>[Co(L(_2)-2H)]</td>
<td>39384, 36847</td>
<td>Intra ligand</td>
</tr>
<tr>
<td></td>
<td>30137</td>
<td>(^4A_{2g} \rightarrow ^4T_{1g})</td>
</tr>
<tr>
<td>[Mn(L(_2)-2H)]</td>
<td>36182</td>
<td>(^6A_{1g} \rightarrow ^2T_{2g})</td>
</tr>
</tbody>
</table>
Based on the above interpretation the probable structure of complexes proposed in the present work is given in Fig. 5.

**Cyclic voltammetry measurements:** Electrochemical cyclic voltammetry measurements were performed for all the complexes at room temperature in an air tight three electrode cell by using Glassy carbon electrode with 0.071 cm² surface area as a working electrode, a platinum wire served as the counter electrode and a Ag/AgCl in a saturated KCl solution as reference electrode. The electrochemical reaction cell was charged with DMF solution of each complex (1 × 10⁻⁴ M) and tetra butyl ammonium perchlorate (0.1 M) as supporting electrolyte [26]. Cyclic voltammetric measurements were made over a potential range between -2.7 V to +2.1 V for all the complexes with a scan rate of 0.2 Vs⁻¹. The important parameters are calculated from cyclic voltammogram and are shown in Table-5. The cyclic voltammogram obtained for Mn(II) complex (Fig. 6) inferred that the redox potential value is metal centered, one electron quasi reversible, since the ΔEp value greater than 100 mV (160 mV). The cyclic voltammogram obtained for Co(II) complex (Fig. 7), inferred that the redox potential value is metal centered, one electron irreversible.

**Antimicrobial activities of the complexes:** Antibacterial and antifungal activity of Schiff base and its metal complexes (Table-6) have been tested by disc diffusion technique [27,28]. Various gram positive and gram negative bacterial organisms such as gram negative bacteria (*Klebsiella aurogenes*) and fungi (Mucor) are used to find out the antimicrobial activity. The results were compared with standard drug ciprofloxacin 5 mg/disc and nystatin 100 mg/disc for fungi. It is evident that overall potency of the ligand is enhanced on coordination with metal ion. Both metal complexes showed a remarkable biological activity against bacteria and fungus. Hence it is evident that overall potency of uncoordinated ligand is enhanced on coordination with metal ion. Thus, both Mn(II) and Co(II) complexes were observed to be more active against *Mucor* and *Klebsiella aerogenes*. The enhancement in antimicrobial activity of the metal complexes as compared to Schiff base may be explained on the basis of Tweedy’s chelation theory.

### TABLE-5

<table>
<thead>
<tr>
<th>Electrochemical Data of Schiff Base Metal Complexes</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex</td>
<td>E_p (mv)</td>
</tr>
<tr>
<td>[Co(L₂-2H)]</td>
<td>-300</td>
</tr>
<tr>
<td>[Mn(L₂-2H)]</td>
<td>-300</td>
</tr>
</tbody>
</table>

### TABLE-6

<table>
<thead>
<tr>
<th>in vitro CYTOTOXICITY ASSAY OF [Co(L₂-2H)] AGAINST MCF-7 CELL LINE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (µm)</td>
<td>Cell inhibition (%)</td>
</tr>
<tr>
<td>0.25</td>
<td>0.200535</td>
</tr>
<tr>
<td>2.5</td>
<td>2.339572</td>
</tr>
<tr>
<td>25</td>
<td>12.36631</td>
</tr>
<tr>
<td>50</td>
<td>66.24332</td>
</tr>
<tr>
<td>100</td>
<td>85.36096</td>
</tr>
</tbody>
</table>

IC₅₀ = 42.67 µg/mL; R² = 0.9841
According to Tweedy’s chelation theory, chelation reduces the polarity of the metal ion mainly because of the partial sharing of the positive charge with the donor groups present in the ligand and possibly the electron delocalization within the whole chelate ring system thus formed during coordination.

The metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The variation in the activity of different complexes against different organisms restricts further growth of the organism. The variation in the cell and thus block the synthesis of proteins, which restricts further growth of the organism. The variation in the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Cytotoxic activity of Co(II) complex: The metal complexes may also be a vehicle for activation of the ligand as the cytotoxic agent. The in vitro cytotoxicity of the newly-synthesized complexes with remarkable antimicrobial activity were carried out in three different human tumor cell lines. Cell line namely human breast cancer cell line (MCF-7) were assayed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay [29]. The complexes were initially dissolved in neat dimethyl sulfoxide to make a stock solution and additional five series of dilutions (6.25, 12.5, 25, 50 and 100 µM) were made to provide a total of five sample concentrations. The concentration of the compounds at % cell inhibition growth (IC<sub>50</sub>) was calculated. The percentage cell inhibition was determined using the following formula:

\[
\text{Cell inhibition (\%) = } \left( 1 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \right) \times 100
\]

Non-linear regression graph was plotted between % cell inhibition and log concentration and IC<sub>50</sub> was determined using GraphPad Prism software [30].

The Co(II) complex derived from (E)-2-(((3-hydroxyphenyl)imino)methyl)-6-methoxyphenol shows an excellent cytotoxicity towards human breast cancer cell (MCF-7). The complex exhibit considerable IC<sub>50</sub> value 42.67 µg/mL. The concentration of the complex at 50% cell growth was inhibited and IC<sub>50</sub> was calculated (Table-6). The better cytotoxicity activity of Co(II) complex against (MCF-7) may play a significant role in metallodrug formulation.

Fluorescence quenching studies of [Co(L<sub>2</sub>-2H<sub>2</sub>): The excited state interaction of [Co(L<sub>2</sub>-2H<sub>2</sub)] with alizarin red S dye was carried out through fluorescence measurements. Fig. 8 shows the emission spectrum of [Co(L<sub>2</sub>-2H<sub>2</sub]) complex measured in DMSO and the complex was effectively quenched by increasing the concentration of alizarin red S dye (0-5 × 10<sup>-5</sup> M). The observed quenching is entirely due to interaction of complex with alizarin red S and not due to inner filter effect or re absorption. Hence the binding constant for this type of interaction was calculated using fluorescence quenching data using the formula [31]:

\[
\frac{1}{(F_0 - F)} = \frac{1}{(F_0 - F^*)} + \frac{1}{K(F_0 - F)[Q]}
\]

where K is the binding constant, F<sup>0</sup> is the initial fluorescence intensity of the complex, F* is the fluorescence intensity of alizarin red S adsorbed Co(II) complex and F is the observed fluorescence intensity at its maximum. The plot of 1/(F<sup>0</sup>-F) versus 1/[Q] gives a straight line (inset Fig. 8) and from the slope the calculated binding constant (K<sub>b</sub>) are found to be 7.6 × 10<sup>3</sup> for the complex. Thus there is a good linear relationship [R = 0.997] between 1/(F<sup>0</sup>-F) and the reciprocal concentration of alizarin red S. The fluorescence quenching can be enlightened by a number of possible mechanisms such as electron transfer, energy transfer, proton transfer or hydrogen atom transfer. For energy transfer, there should be an overlap between emission spectrum of donor complex and absorption spectrum of the acceptor (alizarin red S). Since there is no wavelength overlap between absorption of acceptor (alizarin red S) and emission of donor (complex), energy transfer between donor and acceptor is negligible. The free energy change can be calculated for the above mechanisms which can be used to predict the feasibility of the reaction [32].

Conclusion

Synthesis, spectral characterization, electrochemical, photochemical behaviour and in vitro biological evaluation of transition metal complexes containing Schiff base of 2-aminophenol and 2-hydroxy-3-methoxybenzaldehyde has been described. The low molar conductance values indicate the absence of anion outside the sphere. Schiff base behaves as a tridentate N and O donors. The presence of ν(M-O) and ν(M-N) bonds in the complexes have been confirmed by IR spectra. The absence of water molecules has been confirmed by thermal analysis. Based on spectral studies, octahedral geometry has been proposed for the metal complexes. The comparison of antimicrobial activities of Schiff base and its metal complexes indicates that the Schiff bases have been found to be biologically active and most of their metal complexes exhibit significantly enhanced antimicrobial activities against the microorganisms. It is observed that the potency to act against the cancerous cell is highly influenced by the nature of metal ion present in the chelate. The feasibility of the reaction has been established from fluorescence quenching studies.

REFERENCES