Metal complexes of multidentate azo dye ligand derived from 4-aminoantipyrine and 2,4-dihydroxybenzoic acid; Synthesis, characterization and biological activity

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ABSTRACT

A series of metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) have been synthesized with a new azo dye ligand 4-(2',4'-dihydroxy-5'-carboxyphenylazo)antipyrine derived from 4-aminoantipyrine and 2,4-dihydroxybenzoic acid. The metal complexes along with the ligand have been characterized by analytical, molar conductance, magnetic susceptibility measurement, IR, NMR, electronic, esr, mass spectra and thermal study. The analytical, spectral data and computational study predict tetradentate nature of the ligand, distorted octahedral geometry for Co(II), Ni(II), Cu(II) complexes and tetrahedral geometry for Zn(II) complex. Computational study of the ligand and the metal complexes has been made to determine the geometrical parameters and the global reactive descriptors. The XRD(powder pattern) indicates triclinic crystal system for the Cu(II) complex and cubic crystal system for the Ni(II) complex, thermal study reveals thermal stability of the complexes and the fluorescence study predicts photoactive properties of the azo compounds. The SEM image of the Zn(II) complex provides its information about its surface morphology. The Biological study indicates the antibacterial properties and DNA binding activity of the newly synthesized compounds.

Keywords: Fluorescence study, Computational study, Biological study, Non-linear optical properties, SEM image

INTRODUCTION

Azo compounds are known for widespread applications in various fields and have been attracting the attention of synthetic and theoretical chemists. They are the subject of large research works due to their applications as textile dyes[1], pharmaceuticals[2], indicators[3]. Azo dyes are the important class of dyes accounting for about 50% of all commercial dyes. Azo dyes derived from heterocyclic amines containing nitrogen in the aromatic rings and their metal complexes have been receiving the attention of research groups due to their biological activities as antitumor[4], antibacterial[5], antifungal[6]. These compounds are now in good demands as optical and conducting organic materials[7]. Azo dyes derived from Antipyrine moiety and its metal complexes have a variety of applications in biological, clinical, analytical and pharmacomological areas[8,9,10,11]. These observations encouraged me to synthesize a new azodye ligand from 4-aminoantipyrine and 2,4-dihydroxybenzoic acid, their Co(II), Ni(II), Cu(II), Zn(II) complexes for evaluation of their biological activities.
MATERIALS AND METHODS

All the solvents, the metal salts and other chemicals used are of either analytical grade or high purity supplied by Merck and BDH. Doubly distilled water is used in all experiments.

Equipment’s
Elemental analysis of the ligand and complexes is carried out by Perkin –Elmer elemental analyser, cobalt, nickel, copper contents is determined by Perkin –Elmer2380 atomic absorption spectroscopy and chloride contents is estimated by standard procedure, Systronic conductivity bridge 30 is used to measure molar conductance of the complexes, Magnetic susceptibility of the complexes is measured by Guoy’s balance using Hg[Co(NCS)_4] as a calibrant at room temperature and diamagnetic correction have been made by pascal’s constants, IR spectra of the ligand and metal complexes are recorded on using KBr pellets by perkin elmer FT- IR spectrometer within the range 4000- 450 cm⁻¹, UV-Visible spectra of the complexes are collected using a THERMO SPECTRONIC 6 HEXIOS α and fluorescence spectra are recorded in a Fluorescence spectrometer, ^1H NMR spectra of the ligand and the Zn(II) complex are obtained from Bruker AV III 500 MHZ FT NMR spectrometer using TMS as reference, ESR spectrum of the Cu(II) complex is recorded on JES-FA 200 ESR spectrometer, mass spectra of the ligand and its complexes are recorded through JEOL GC Mate GC-MS Mass Spectrometer, thermal study of the metal complex is done by NETZSCH STA 449 F3 JUPITOR, SEM image of the complexes are taken in JES FA 200, the XRD powder pattern of the Cu(II) complex is collected using a Philips X’Pert Pro diffractometer.

Computational study
Molecular modeling is now considered as an important tool for calculating certain physic-chemical and geometrical parameters of the chemical compounds. In order to get the better picture about reactivity and geometrical parameters, computational study of the ligand and the metal complexes are performed by Gaussian 03 software package[12]. The molecular geometry of the ligand and its complexes are fully optimised using B3LYP level of theory along with 6-331G(d,p) basis set. The B3LYP provides better results than the Hartree-Fock method and reproduce better geometrical parameters comparable to the experimental values.

Biological Activity
The DNA binding study of the azo compounds is made by Gel electrophores method[13]. 10 µL of the metal complexes are taken along with 15 µL of CT DNA solution dissolved in Tris-EDTA in centrifuge tubes. The tubes are incubated at 37°C for 1 hour. After incubation, the tubes containing solution are kept in a refrigerator at 0°C for few minutes, 5 µL gel loading buffer with tracking dye (0.25% bromo phenol) is taken in the tubes for electrophoresis. The electrophoresis is continued under constant voltage (50 V) and photographed under UV illumination.

Hydrodynamic volume change[14] is observed by Ostwald Viscometer immersed in a thermostatic bath maintained at 37°C. A digital stopwatch is used to measure the flow time, mixing of complexes under investigation with CT-DNA is carried out by bubbling nitrogen. Data are presented by plotting a graph indicating (ɳ/ɳ₀)^{-1/3} verses [complex]/[DNA] where ɳ is the viscosity of DNA in presence of complexes and ɳ₀ represents the viscosity of DNA alone. Viscosity values are collected by following the equation ɳ =t-t₀ where t is the flow time of the DNA containing solutions and t₀ is the flow time of DNA alone.

The antibacterial activity of the ligand and its metal complexes is studied in vitro by the cup-plate method[15] against the *Escherichia coli* (MTCC-40)and *Staphylococcus aureus* (MTCC-87)using agar nutrient as the medium by the cup plate method[15]. The investigated ligand and its complexes are dissolved in DMF. The sterilised agar plates are swabbed with the bacteria culture and filled with test solutions, then incubated at 37 °C for 24 hours. The activity is evaluated by measuring the zone of inhibition with respect to the standard drug Tetracycln

Synthesis of ligand
The ligand 4-(2',4'-dihydroxy-5'-carboxyphenylazo) is synthesized by the coupling reaction between the diazonium chloride produced from 4-aminoantipyrine) and alkaline solution 2,4-dihydroxybenzoicacid. This can be achieved by diazotization of 4-aminoantipyrine by dissolving 0.01 mol, 2.03 gm of it in hydrochloric acid, cooling it to 0-5°C and adding equivalent amount of ice cooled sodium nitrate solution with stirring. The cooled diazonium chloride is then made to react with the alkaline solution of 2,4-dihydroxybenzoicacid(0.01 mol,1.54 gm). The colored azo dye ligand is formed which is recrystallised from ethanol.(Reaction scheme-1).
Synthesis of Metal complexes
The Metal (Co(II), Ni(II), Cu(II) and Zn(II)) chlorides (0.01 mol) in ethanolic solution are added to the ethanolic solution of the ligand in 4:1 ratio separately. The reaction mixtures are refluxed for about one hour at 60°C to get the respective solid metal-ligand complexes. The refluxed solutions are allowed to cool and concentrated Ammonia solution was added drop by drop till the formation of precipitates of Co(II), Ni(II), Cu(II) and Zn(II). The precipitates of azo metal complexes as given in Figure-1, Figure-2 are collected by filtration, washed with ethanol and dried in vacuum. This is represented in reaction Scheme-2.

\[
\text{Reflux 1 hour, 60}^0\text{C} \quad \begin{align*}
2 \text{MCl}_2 + \text{LH}_3 & \quad \text{NH}_4\text{OH} \\
\text{M} = \text{Co(II)}, \text{Ni(II)}, \text{Cu(II)} & \quad \text{[M}_2\text{LCl}_2(\text{H}_2\text{O})_6] + 3\text{NH}_4\text{Cl}
\end{align*}
\]

\[
\text{Reflux 1 hour, 60}^0\text{C} \quad \begin{align*}
2 \text{MCl}_2 + \text{LH}_3 & \quad \text{NH}_4\text{OH} \\
\text{M} = \text{Zn(II)} & \quad \text{[M}_2\text{LCl}_2(\text{H}_2\text{O})_2] + 3\text{NH}_4\text{Cl}
\end{align*}
\]
RESULTS AND DISCUSSION

The ligand and all its metal complexes are coloured and stable to air. The complex compounds are soluble in solvents like DMF and DMSO but remain insoluble in ethanol, acetone, ether and carbon tetrachloride. The high melting point of all complexes than the melting point of ligand indicates more thermal stability of the complexes than the ligand (Table-1).

3.1. Elemental analysis
The elemental analysis (Table-1) of the compounds is in good agreement with the composition of the suggested formula. The analytical data of the complexes indicate their formula as [M₂LCl₂(H₂O)₆], where M represents Co(II), Ni(II), Cu(II) ions, [M₂LCl₂(H₂O)₂] where M stands for Zn(II) ion and L stands for the ligand.

3.2. Conductance measurements
The Conductance measurements of the solutions of the metal complexes (10⁻³ M in DMF) show the value in the range of 10⁻¹⁵ Ohm⁻¹ cm² mol⁻¹. These values suggest that all metal complexes are non-electrolyte in nature[16].

Table-1 Analytical data

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>Yield(%)</th>
<th>M.P (°C)</th>
<th>% Found(calcd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>M C H N Cl</td>
</tr>
<tr>
<td>1. LH₃, 2.[Co₂LCl₆(H₂O)₁₆], 3.[Ni₂LCl₆(H₂O)₁₆], 4.[Cu₂LCl₆(H₂O)₁₆], 5.[Zn₂LCl₆(H₂O)₁₆]</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 Red</td>
<td>67</td>
<td>65</td>
<td>-</td>
<td>58.53 (58.69)</td>
</tr>
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<td></td>
<td></td>
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<td>3.62 (3.81)</td>
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<td>8.56 (8.46)</td>
</tr>
<tr>
<td>2 Brick red</td>
<td>52</td>
<td>&gt;300</td>
<td>17.65</td>
<td>32.42 (32.65)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.72 (3.81)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.16 (8.46)</td>
</tr>
<tr>
<td>3 Scarlet red</td>
<td>56</td>
<td>&gt;300</td>
<td>17.38</td>
<td>32.52 (32.67)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>3.54 (3.81)</td>
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<td></td>
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<td></td>
<td></td>
<td>8.23 (8.47)</td>
</tr>
<tr>
<td>4 Reddish brown</td>
<td>53</td>
<td>&gt;300</td>
<td>18.61</td>
<td>31.89 (32.20)</td>
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<td></td>
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<td>3.54 (3.75)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8.21 (8.34)</td>
</tr>
<tr>
<td>5 Brown</td>
<td>53</td>
<td>&gt;300</td>
<td>21.39</td>
<td>35.48 (35.85)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>2.65 (2.84)</td>
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<td></td>
<td></td>
<td>8.94 (9.29)</td>
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<td></td>
<td></td>
<td></td>
<td>11.34 (11.76)</td>
</tr>
</tbody>
</table>

Spectral study

IR Spectra and mode of bonding
The IR spectrum of the ligand (Graph-1) is compared with the spectra of metal complexes (Graph-2) in order to examine the mode of bonding between ligand and complexes (Table-2). The IR spectrum of the ligand shows a broad band at 3434 cm⁻¹ which is missing from the spectra of the metal complexes that indicates deprotonation of phenolic (-OH) and bonding of metal atoms with oxygen atom of the –OH group. The C-O frequency vibration band observed at 1270 cm⁻¹ in ligand is shifted to ~1236 cm⁻¹ in complexes confirming bonding of metal atoms with oxygen atom of –OH group[17]. Two bands appear at 1411 and 1632 cm⁻¹ corresponds to ʋsym and ʋasym respectively in the spectrum of the ligand which are observed at 1386 cm⁻¹ and 1621 cm⁻¹ due to ʋsym and ʋasym that indicates monodentate nature of the carboxylate group and bonding of carboxylic oxygen with metal atoms[18]. A band appears at 1493 cm⁻¹ corresponds to –N=N- group in the ligand is shifted to ~1456 cm⁻¹ in metal complexes that suggests bonding of azo nitrogen with the metal ions[19]. The spectra of complexes reveal the presence of band at ~3418 cm⁻¹ due to the vibrational frequency of O-H of coordinated/ lattice held water. The presence of coordinated water is further confirmed by the rocking band at ~833 cm⁻¹ and twisting band at ~732 cm⁻¹[20]. The vibrational frequencies of M-O and M-N bonds which appear at ~606 cm⁻¹ and ~585 cm⁻¹ respectively confirm the bonding between metal ions with the ligand through phenolic oxygen and azo nitrogen atoms[21].
The peak at δ 3.38 ppm Electronic spectra and magnetic moment

The electronic spectra and magnetic measurements are taken in to account to establish the geometric structures of the complexes since we failed to synthesize single crystal of all the complexes. The electronic spectrum of Co(II) complex(Table-3, Graph-3) exhibits four bands at 10500, 16200 and 17450 cm\(^{-1}\) assignable to \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)T\(^{2,3}_{2g}(F)(\nu_1)\), \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)A\(^2\)g\( (F)(\nu_2)\), \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)T\(^{2,3}_{2g}(P)(\nu_3)\) transitions which are characteristic of octahedral geometry[22]. The fourth band is a CT band and the electronic parameters such as D\(_q\), B, \(\beta\), \(\nu_2/\nu_1\) and \% \(\sigma\) are calculated by using following equations and given in the table-

Table-2 IR data of the investigating compounds

<table>
<thead>
<tr>
<th>compound</th>
<th>(\nu(C-O)) cm(^{-1})</th>
<th>(\nu(N=N)) cm(^{-1})</th>
<th>(\nu(COO^{\text{sym.}})) cm(^{-1})</th>
<th>(\nu(COO^{\text{asym.}})) cm(^{-1})</th>
<th>(\nu(M-O)) cm(^{-1})</th>
<th>(\nu(M-N)) cm(^{-1})</th>
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<td>1270</td>
<td>1493</td>
<td>1411</td>
<td>1632</td>
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</tr>
<tr>
<td>2</td>
<td>1236</td>
<td>1456</td>
<td>1386</td>
<td>1621</td>
<td>606</td>
<td>585</td>
</tr>
<tr>
<td>3</td>
<td>1234</td>
<td>1456</td>
<td>1385</td>
<td>1588</td>
<td>605</td>
<td>585</td>
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<tr>
<td>4</td>
<td>1235</td>
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<td>1385</td>
<td>1587</td>
<td>604</td>
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<td>1236</td>
<td>1454</td>
<td>1384</td>
<td>1586</td>
<td>604</td>
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</tr>
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</table>

1-LH, 2-Co(II) complex, 3-Ni(II)complex, 4- Cu(II) complex and 5- Zn(II) complex

The electronic spectra and magnetic measurements are taken in to account to establish the geometric structures of the complexes since we failed to synthesize single crystal of all the complexes. The electronic spectrum of Co(II) complex(Table-3, Graph-3) exhibits four bands at 10500, 16200 and 17450 cm\(^{-1}\) assignable to \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)T\(^{2,3}_{2g}(F)(\nu_1)\), \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)A\(^2\)g\( (F)(\nu_2)\), \(^4\)T\(^{1,2}_{1g}(F) \rightarrow ^4\)T\(^{2,3}_{2g}(P)(\nu_3)\) transitions which are characteristic of octahedral geometry[22]. The fourth band is a CT band and the electronic parameters such as D\(_q\), B, \(\beta\), \(\nu_2/\nu_1\) and \% \(\sigma\) are calculated by using following equations and given in the table-

Table-2 IR data of the investigating compounds

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<tr>
<th>compound</th>
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<th>(\nu(COO^{\text{sym.}})) cm(^{-1})</th>
<th>(\nu(COO^{\text{asym.}})) cm(^{-1})</th>
<th>(\nu(M-O)) cm(^{-1})</th>
<th>(\nu(M-N)) cm(^{-1})</th>
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<td>1270</td>
<td>1493</td>
<td>1411</td>
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<td>585</td>
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<td>1586</td>
<td>604</td>
<td>585</td>
</tr>
</tbody>
</table>

1-LH, 2-Co(II) complex, 3-Ni(II)complex, 4- Cu(II) complex and 5- Zn(II) complex
\[ \text{Dq} = \nu_2 - \nu_1/10 \]
\[ B = \nu_2 + 3\nu_1/15 \]
\[ \beta_{35} = B/971 \]
\[ \beta_{35}\% = (1 - \beta_{35}) \times 100 \]

Similarly, the Ni(II) complex (Graph-3) also exhibits three d-d transition three bands at 12100, 15150, 23500 cm\(^{-1}\) corresponding to \( ^3A_{2g}(F) \rightarrow ^3T_{2g}(F) \), \( ^3A_{2g}(F) \rightarrow ^3T_{1g}(F) \), \( ^3A_{2g}(F) \rightarrow ^3T_{1g}(P) \) transitions, these suggests octahedral geometry for the Ni(II) complex [23,24]. The fourth band is a CT band and parameters like Dq, B, \( \nu_2/\nu_1 \) and \% \( \beta_{35} \) are calculated by using following equations:

\[ \text{Dq} = \nu_2/10 \]
\[ B = \nu_2 + 3\nu_1/15 \]
\[ \beta_{35} = B/1041 \]
\[ \beta_{35}\% = (1 - \beta_{35}) \times 100 \]

The Racah parameter (B) of the Co(II) and Ni(II) complexes are found to be less than the free ion values and Nephelauxetic (\( \beta_{35} \)) parameter for both the complexes is less than one. All these observations suggest that the metal ligand bonds in the complexes are covalent in nature.

The spectrum of Cu(II) complex (Graph-4) shows a CT bands at \( 15384 \) cm\(^{-1}\) and \( 19607 \) cm\(^{-1}\) and one d-d transitions band at \( 15384 \) cm\(^{-1}\) which may be assigned to \( ^2E_g \rightarrow ^2T_{2g} \) transition supporting a distorted octahedral configuration for the complex [25].

The magnetic moment of the Co(II), Ni(II) and Cu(II) complexes are found to be 3.71 B.M., 2.69 B.M. and 1.68 B.M. respectively. All these values indicate octahedral geometry for the metal complexes [26,27]. The Zn(II) complex is found to be diamagnetic, hence tetrahedral geometry is suggested from the analytical and magnetic data.

### Table-3 Electronic data of the metal complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \lambda_{max} ) (cm(^{-1}))</th>
<th>Transitions</th>
<th>B</th>
<th>( \beta_{35} )</th>
<th>% of ( \beta_{35} )</th>
<th>( \nu_2/\nu_1 )</th>
<th>Geometry</th>
<th>( \mu_{eff} ) B.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12820, 16447, 22739, 32258</td>
<td>( ^3T_{1g}(F) \rightarrow ^3T_{2g}(F) )</td>
<td>48.4</td>
<td>0.049</td>
<td>95.1</td>
<td>1.28</td>
<td>octahedral</td>
<td>3.71</td>
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<td></td>
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<td>( ^3T_{1g}(F) \rightarrow ^3A_{2g}(F) )</td>
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<tr>
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<td></td>
<td>( ^3T_{1g}(F) \rightarrow ^3T_{1g}(P) )</td>
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<tr>
<td></td>
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</tr>
<tr>
<td>2</td>
<td>15625, 22220, 25974, 32786</td>
<td>( ^3E_g \rightarrow ^3T_{2g} )</td>
<td>87.9</td>
<td>0.084</td>
<td>91.6</td>
<td>1.42</td>
<td>octahedral</td>
<td>2.69</td>
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<tr>
<td></td>
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<td>( ^3A_{2g}(F) \rightarrow ^3T_{1g}(F) )</td>
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<td>( ^3A_{2g}(F) \rightarrow ^3T_{1g}(P) )</td>
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</tr>
<tr>
<td>3</td>
<td>15350</td>
<td>( ^3E_g \rightarrow ^3T_{2g} )</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Distorted octahedral</td>
<td>1.68</td>
</tr>
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</table>

1-Co(II) complex, 2-Ni(II) complex, 3- Cu(II) complex
ESR Spectra

The room temperature solid state of the Cu(II) spectrum (Graph-4) exhibits an axially symmetric g-tensor having $g_{ll}$ value 2.23 and $g_{ll}$ value 2.04. As $g_{ll} > 2.0023$, the copper site has a $d_{x^2-y^2}$ ground state [28]. The G is related to $g_{ll}$, $g_{ll}$ by the expression $G = g_{ll} - 2/ g_{ll} - 2$ and it is found to be 5.75 that indicates negligible exchange interaction between the Cu$^{2+}$ centres in the solid state [29]. The $g_{av}$ value of the complex is calculated as 2.10 by using the relation $g_{av} = 1/3 (g_{ll} + 2g_{ll}^{-1})$. The spin-orbit coupling constant is also calculated by using the equation $g_{av} = 2(1 - 2A/10Dq)$ and it is found to be -769.2 cm$^{-1}$. This value is found to be less than the free ion value (-830 cm$^{-1}$) that indicates overlapping of metal-ligand orbitals [30]. The covalent bonding between metal ion and orbitals of the ligand is also confirmed from the $g_{av}$ value. According to Kivelson and Neiman covalent bonding can be predicted for a Cu(II) complex with $g_{av}$ value less than 2.3 [31]. Hence, distorted octahedral geometry may be proposed for the Cu(II) complex.

Graph-4 ESR Spectrum of Cu(II) complex

$^1$H NMR spectra

The spectra of the ligand (Graph-5) and Zn(II) complex (Graph-6) are recorded in DMSO-d$_6$ solvent. The $^1$H NMR spectrum of the ligand shows multiplet at $\delta$ 5.75-8.08 ppm which may be assigned to aromatic protons. A single peak is observed at $\delta$ 12.38 ppm corresponds to phenolic(-OH) and –COOH due to exchange between (-OH) and –COOH protons. The peak observed at $\delta$ 3.38 ppm confirms the presence of –CH$_3$ group [32].
The spectrum of Zn(II) complex is compared with the azo dye ligand and it is observed that the peak due to –OH and -COOH group found in the ligand was absent in the complex. This indicates deprotonation of –OH group and formation of metal-O bond [33] in accordance with the data by revealed IR.

Mass Spectra
The mass spectra of the ligand(Graph-7) and its Co(II)(Graph-8) complexes are recorded to confirm their molecular mass and stoichiometric composition since mass spectra of the compounds provides vital information for their structural elucidations. The spectrum of the ligand shows the molecular ion peak at m/z 366.3715 corresponding to the molecular mass of \([\text{C}_18\text{H}_{16}\text{N}_4\text{O}_5]\). The spectrum of the Co(II) complex of the ligand gives molecular ion peak at m/z 661.2198 which confirms its proposed molecular formula as \([\text{Co}_2\text{LCl}_2(\text{H}_2\text{O})_6]\)
XRD study
The XRD (powder pattern) study of the Cu(II) complex as given in Graph-9, Table-4 and Ni(II) complex given in Graph-10, is made to determine their crystal system. The X-ray powder diffraction diagram is collected from the X’Pert diffractometer and the recording conditions are 40 kV and 40 mA for CuKα with λ = 1.542Å between $2\theta$ to 80° with a step size of 0.0089°.
The XRD powder pattern is processed in X’pert high score software package. The search matching procedure is adopted for the PXRD pattern for the Cu(II) complex and revealed a match with a copper compound corresponding JCPDS powder diffraction file with PDF No 181618. The pattern can be indexed to be a triclinic crystal system with
S. N. Chaulia

$a=8$, $b=8.11$, $c=7.53$, $\alpha=99.18^\circ$, $\beta=99.52^\circ$, $\gamma=100.23^\circ$. Lattice-primitive and space group is $P1(2)$. Similarly, the search matching procedure is repeated for Ni(II) complex that provided a match with a Nickel compound corresponding JCPDS powder diffraction file with PDF No 481769 and the pattern is indexed to be a cubical crystal system $a=b=c=11.48$, lattice-face centered and space group Fm3m(225).

Thermo gravimetric analysis

The Co(II) complex is subjected to thermo gravimetric study as a representative example to examine the thermal stability. The thermo gravimetric study of the Ni(II)(Graph-11,12) complex as a representative member of the investigating complexes is carried out by the simultaneous TG, DTG and DSC techniques in the atmosphere of nitrogen at a rate of $10^\circ$C per minute from the ambient temperature to $1400^\circ$C. The TG/DTG curves show that the complex suffers mass loss in a number of stages. The complex loses a mass of 16.94% at $83.3^\circ$C in the first stage, with an endothermic peak at $90^\circ$C in the DSC curve. In the second stage, it suffers a mass of 9.06% at $306.2^\circ$C with an endothermic peak at $238.9^\circ$C. The complex compound loses a mass of 13.52% at $409^\circ$C and it suffers a mass loss of 11.55% at $725^\circ$C with an endothermic peak at $707^\circ$C. The complex suffers a total mass of 71.56% and its residual mass is 28.44% at $1399^\circ$C. This study indicates thermal stability of the complex.
Fluorescence study
The fluorescence study of the ligand (Graph-13) and its Cu(II) complex (Graph-14) are carried out to study the photoconductive nature of the investigated compounds and the emission spectra of the compounds are given here. The ligand shows a emission maximum wavelength at 340 nm but the Cu(II) complex depicts the emission maximum wavelength at 480 nm. The red shift of the $\lambda_{\text{max}}$ value of the complexes may be due to the deprotonation of the (-OH) group and complexation. The fluorescence intensity of the complex is more than the ligand due to complexation as it enhances conformational rigidity and non-radiative energy loss[34]. The emission in the complexes may be due to intraligand $\pi - \pi^*$ transition. These findings suggest that both the ligand and its complex are fluorescent in nature[35].
Graph-14 Emission graph of the Cu(II) complex

**Scanning electron micrography**

The surface morphology study of the Zn(II) complex (Image no-1) of the ligand as a representative of all complexes is undertaken to evaluate its morphology and particle size. It is seen from the SEM image of the complex as given in image no1 that the size of the particles is 500 nm with the formation of single phase morphology. It is also noticed that there is a uniform matrix of the synthesized complex and the complex shows a flower like shape that indicates that the complex is polycrystalline with nanosized grain.

**Computational study**

A computational study of the investigating compounds is made to examine their reactivities and to evaluate geometrical parameters. Gauss view 4.1[36] and chemcraft software[37] are used to draw the structures. The structures of the ligand and its all metal complexes are optimised at B3LYP[38] level of theory using 6-311++G(d,p)
basis set. 6-311++G(d,p) is a large basis set which include diffused and polarised wave functions to take into account the characteristics associated with ionic species having heavy atoms like N. The harmonic frequency calculation is also carried out at the same level of theory to ensure that the structures are true minima. Optimised was performed without any symmetry constraint using the default convergence criteria provided in the software.

Conceptual DFT defines chemical potential $\mu$ as the first derivative of energy with respect to number of electrons

$$\mu = \left( \frac{\partial E}{\partial N} \right) \vartheta(r)$$

where $E$= energy, $N$= number of electrons of the system at constant external pressure $\vartheta(r)$.

and chemical hardness $n$ as the half of the second derivative of energy with respect to number of electrons, so chemical hardness will the first derivative of energy with respect to number of electrons

$$n = \frac{1}{2} \left( \frac{\partial^2 E}{\partial N^2} \right) \vartheta(r)$$

But chemical potential($\mu$) and chemical hardness($n$) are also calculated in most cases in terms ionisation potential(IP) and electron affinity(EA) and therefore

$$\mu = -\left( \frac{IP+EA}{2} \right) \quad \text{and} \quad n = \left( \frac{IP-EA}{2} \right)$$

According Koopman’s theorem, IP and EA are related to energies of the Highest occupied molecular orbital($E_{\text{HOMO}}$) and Lowest occupied molecular orbital($E_{\text{LUMO}}$) in this way—$IP = -E_{\text{HOMO}}$ and $EA = -E_{\text{LUMO}}$

$$\mu = \left( \frac{E_{\text{LUMO}}-E_{\text{HOMO}}}{2} \right) \quad \text{and} \quad n = \left( \frac{E_{\text{LUMO}}+E_{\text{HOMO}}}{2} \right)$$

and Parr and coworkers proposed electrophilicity[39] as a measure of electrophilic power of a compound the electrophilicity can be represented as

$$\omega = \frac{\mu^2}{2n}$$

The chemical potential($\mu$) and chemical hardness($n$), electrophilicity and dipole moment of the ligand and complexes are given in the table-6. The reactivity of the ligand and its metal complexes can be predicted by considering the minimum electrophilicity principle. According to minimum electrophilicity principle, compound having minimum electrophilicity will have maximum stability. The chemical potential($\mu$), chemical hardness($n$), electrophilicity($\omega$) were calculated from the HOMO and LUMO value of the ligand(Fig-1,2) and its complexes(Fig-3-10) and presented in the table-5. The Co(II), Ni(II) and Cu(II) complexes are more reactive than the ligand and the order of reactivity of the complexes is $[\text{Co}_{2}\text{LCl}_{2}(H_{2}O)]_{6} > [\text{Ni}_{2}\text{LCl}_{2}(H_{2}O)]_{6} > [\text{Cu}_{2}\text{LCl}_{2}(H_{2}O)]_{2} > [\text{Zn}_{2}\text{LCl}_{2}(H_{2}O)]_{2}$. 

<table>
<thead>
<tr>
<th>compound</th>
<th>HOMO</th>
<th>LUMO</th>
<th>$\mu$(eV)</th>
<th>$\omega$</th>
<th>$\mu$(D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>-0.30132</td>
<td>0.06621</td>
<td>0.1837</td>
<td>-0.1175</td>
<td>0.037</td>
</tr>
<tr>
<td>$[\text{Co}<em>{2}\text{LCl}</em>{2}(H_{2}O)]_{6}$</td>
<td>-0.18385</td>
<td>0.02051</td>
<td>0.1021</td>
<td>-0.1633</td>
<td>0.130</td>
</tr>
<tr>
<td>$[\text{Ni}<em>{2}\text{LCl}</em>{2}(H_{2}O)]_{6}$</td>
<td>-0.22991</td>
<td>-0.05716</td>
<td>0.0863</td>
<td>-0.1435</td>
<td>0.119</td>
</tr>
<tr>
<td>$[\text{Cu}<em>{2}\text{LCl}</em>{2}(H_{2}O)]_{2}$</td>
<td>-0.25916</td>
<td>-0.00192</td>
<td>0.1286</td>
<td>-0.1305</td>
<td>0.066</td>
</tr>
<tr>
<td>$[\text{Zn}<em>{2}\text{LCl}</em>{2}(H_{2}O)]_{2}$</td>
<td>-0.04696</td>
<td>-0.04296</td>
<td>0.0448</td>
<td>-0.0021</td>
<td>0.00005</td>
</tr>
</tbody>
</table>
The geometrical parameters of the investigating compounds were also collected from their optimised geometry and presented in the table no-6. It is seen from the table that the bond angles around the metal ion in case of Co(II), Ni(II) and Cu(II) complexes are close to $90^\circ$ and in case of Zn(II) it is close to $109^\circ$. Therefore, distorted octahedral
geometry for Co(II), Ni(II) and Cu(II) complexes and distorted tetrahedral geometry may be suggested for the Zn(II) complex.

Table-6 Selected bond length and bond angle

<table>
<thead>
<tr>
<th>Compound</th>
<th>Bond length(Å)</th>
<th>Bond angle(°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.234</td>
<td>1.442</td>
</tr>
<tr>
<td>2</td>
<td>1.352</td>
<td>1.469</td>
</tr>
<tr>
<td>3</td>
<td>1.307</td>
<td>1.429</td>
</tr>
<tr>
<td>4</td>
<td>1.375</td>
<td>1.321</td>
</tr>
</tbody>
</table>

1. LH₃  2. [Co₂LCl₂(H₂O)₆]  3. [Ni₂LCl₂(H₂O)₆]  4. [Zn₂LCl₂(H₂O)₂]

Non-linear optical properties
The electronic properties of chemical compounds are related to their non-linear optical activities. Easy electron transition between molecular orbitals is the basic requirement for good nonlinear optical materials. It is seen from the Table-5 that all metal complexes have higher dipole moment than the free ligand. The energy gap between the HOMO and LUMO of the ligand is found to be higher than the complexes. All these findings indicate that complexes have better nonlinear optical properties[40] than the free organic ligand. The Zn(II) complex will be the good nonlinear optical material due to small energy gap between its HOMO and LUMO.

Biological study
DNA binding study

Gel electrophoresis
The interaction between complex and metal complexes is also studied by gel electrophoresis as given in image-2. The electrophoresis study shows that intensity of the DNA-complex bands are less than the DNA control and the intensity decreases in the order of [Co₂LCl₂(H₂O)₆]> [Ni₂LCl₂(H₂O)₆]> [Cu₂LCl₂(H₂O)₆]. Due to the intercalation of the metal complexes in to the DNA base pairs, intensity decreases

![Image-2 From left 1. DNA control 2. Cu(II) 3.Ni(II), 4.Co(II) complexes](image)

Viscosity measurement test
All the metal complexes are subjected to viscosity measurement study to confirm the DNA binding abilities of the metal complexes with the CT DNA. The increase in viscosity of DNA occurs when the complexes intercalate between the base pairs due to extension in the helix[41]. The effects of all the synthesized complexes on the viscosity of DNA are shown in the graph-15. The graph shows that viscosity of DNA increases with increase in the concentration of complexes and the order of increase of viscosity is Co₂LCl₂(H₂O)₆]> [Ni₂LCl₂(H₂O)₆]> [Cu₂LCl₂(H₂O)₆]> [Zn₂LCl₂(H₂O)₂]
Antibacterial study

All the test compounds are screened against the gram-positive and gram negative bacteria (Table-7, Graph-16). The ligand and some complexes have moderate effect on the growth of the microorganism. The complexes have more antibacterial abilities than the free ligand and the enhanced ability of the complexes may be explained by considering overtone’s concept and chelation theory [42].

<table>
<thead>
<tr>
<th>comp</th>
<th>concentration</th>
<th>E. coli</th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LH</td>
<td>500 µg/ml</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>2. [CoLCl₂(H₂O)₁₂]</td>
<td>500 µg/ml</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>3. [NiLCl₂(H₂O)₁₂]</td>
<td>500 µg/ml</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>4. [CuLCl₂(H₂O)₁₂]</td>
<td>500 µg/ml</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>5. [ZnLCl₂(H₂O)₁₂]</td>
<td>500 µg/ml</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>6. Tetracycline</td>
<td>500 µg/ml</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Graph-15 Viscosity measurement Series-1-Zn(II), 2-cu(II),3- Ni(II),4-Co(II)

Graph-16 Antibacterial screening of the ligand and its complexes
CONCLUSION

The newly synthesized ligand is suggested to be a tetradentate ligand and its Co(II), Ni(II), Cu(II) complexes are proposed to have distorted octahedral and Zn(II) has tetrahedral geometry on the basis of analytical, spectral and computational study. The photoluminescence study reveals that both ligand and its complexes are found to possess photo conducting properties. The computational study indicated more reactivity of the metal complexes than the ligand except Zn(II) complex. The investigating compounds are found to have DNA binding abilities and potential antibacterial properties.

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