Synthesis, spectral aspects and biological activities of 5-hydroxy-2-nitrobenzaldehydethiosemicarbazone and their Mn(II), Co(II) and Ni(II) complexes

M. Sivasankar Reddy^a, B. Prathima^a, M. Saraswathi^b, S. Babu^c, Y. Sarala^d, A. Varada Reddy^e

^aDepartment of Chemistry, Sri Venkateswara University, Tirupati-517 502, India. ^bDepartment of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati-517502, India. ^cDepartment of Physics, Sri Venkateswara University, Tirupati-517 502, India. ^dDepartment of chemical engineering, CBIT, Gandipet, Hyderabad-500 075, India.

ABSTRACT

The ligation behavior of 5-hydroxy-2-nitrobenzaldehydethiosemicarbazone (L) and its Mn(II), Co(II) and Ni(II) complexes has been synthesized and characterized by Elemental analysis, Electronic, FT-IR, Raman, EPR spectral techniques and Cyclic voltammetric study. The ligand (L) belongs to triclinic system with P1 space group. The IR spectral data of ligand indicate the taking part of sulphur and azomethine nitrogen in coordination to the central metal ion. The electronic, FT-IR and EPR spectral studies reveals all the complexes are distorted octahedral geometry. The synthesized ligand and its metal(II) complexes were tested for their antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method and antibacterial activity against E.coli, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi and Klebsiella pneumonia. The antibacterial and antioxidant activities of the ligand and its metal complexes had shown moderate to good activity, among all the compounds Ni(II) complex had shown more activity.

INTRODUCTION

Thiosemicarbazones belongs to a large group of thiourea derivatives, it is an important structural motif that has been potential to display chemical functionality in biologically active molecules (Lobana et al., 2009). The recent review (Narayana et al., 2012) discussed the analytical and biological applications of thio and phenyl thiosemicarbazones, but mainly focused on the analytical applications (Wood et al., 2008). Thiosemicarbazones usually act as chelating ligands with transition metal ions, bonding through the sulphur and hydrazine nitrogen atoms. Schiff bases derived from thiosemicarbazide and their metal complexes are of great significance for their pharmacological properties such as antibacterial, antifungal, antitumoral, antiviral and anticancer (Patil et al., 2010; Sharma et al., 2010; Al-Amiery et al., 2011; Wieeck et al., 2010; Ferraz et al., 2009; Graminha et al., 2008; Raja et al., 2010). Manganese and Cobalt are essential trace elements, forming the active sites of a number of metalloproteins; Mn shows many oxidation states (Sreekanth et al., 2006). Cobalt-60 is used as an efficient radioactive tracer and an anticancer treatment agent in medicine, cobalt such as vitamin B12 are imported for biological activities (Praveen Kumar et al., 2007).

Now we reported the synthesis and spectral characterization of spectral aspects by using CHN analyzer, FT-IR, Raman, EPR, Electronic absorption spectra, and cyclic voltammetric techniques for the ligand and its Mn(II), Co(II) and Ni(II) complexes. Further, the ligand characterized by NMR and HRMS techniques. Hence above synthesized compounds were screened for their antimicrobial and antioxidant activities.

© 2016 M. Sivasankar Reddy et al. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlikeUnported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).
EXPERIMENTAL

Chemicals
All the chemicals used in the study were of analytical grade unless reported. The organic chemicals 5-hydroxy-2-nitrobenzaldehyde was purchased from AVRA synthesis, thiosemicarbazide and nickel chloride were purchased from SdFine chemicals. Mn(II), Co(II) chlorides and organic solvents were used of GR grade without further purification.

Physical measurements
Elemental analyses (CHN) was performed by using Flash EA 1112 series. IR spectra were recorded as KBr pellets in the 4000-4000 cm⁻¹ region using a Thermo scientific Nicolet 380 spectrophotometer. Shimadzu 3600 UV-Vis spectrophotometer equipped with quartz cell is used for the absorbance studies. The NMR spectrum of L was recorded on a Bruker(400 MHz) spectrometer at 300°K, using DMSO-d₆ as a solvent and tetramethylsilane(TMS) as an internal reference compound. A mass spectrum of the ligand was recorded in ESI mode using Bruker HRMS at University of Hyderabad.

Synthesis of ligand (L)

The ligand was synthesized by slightly modifying the previously reported procedure (Ferrari et al., 2000), an ethanolic solution of thiosemicarbazide (0.015 M) was added to an ethanolic solution of 5-hydroxy-2-nitrobenzaldehyde (0.015 M). The mixture was refluxed for 3-4 h. During which period a yellow color precipitate was formed. The reaction was cooled to room temperature and the solid compound was filtered, precipitate was washed with methanol and ether, dried under vacuum P₂O₅. The purity of the complexes was checked by TLC. Elemental and chemical analysis data are shown in Table 1.

Antibacterial activity

The ligand and its metal complexes were screened against two Gram-positive of Staphylococcus aureus and Bacillus subtilis and three Gram-negative of Escherichia coli, Salmonella typhi and Klebsiella pneumonia bacteria using agar-well diffusion method (Raman et al., 2003). To each agar well 25, 50, 100 and 150 µL of the compound reconstituted in DMSO in concentration of 1.0 mg/ml is added.

Table 1: Analytical data for the ligand and its Mn(II), Co(II) and Ni(II) complexes.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compounds</th>
<th>Color</th>
<th>M.Wt.</th>
<th>M.P(°C)</th>
<th>Yield (%)</th>
<th>Elemental analyses data (%) (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ligand</td>
<td>Yellow</td>
<td>240.2391</td>
<td>240</td>
<td>84</td>
<td>C: 40.36(40.00) H: 3.41(3.36) N: 23.45(23.32)</td>
</tr>
<tr>
<td>2</td>
<td>Mn(II)</td>
<td>Cream</td>
<td>637.3993</td>
<td>&gt;300</td>
<td>68</td>
<td>C: 33.98(33.92) H: 3.66(3.64) N: 17.60(17.58)</td>
</tr>
<tr>
<td>3</td>
<td>Co(II)</td>
<td>Red</td>
<td>641.3944</td>
<td>&gt;300</td>
<td>65</td>
<td>C: 33.74(33.71) H: 3.62(3.61) N: 17.51(17.47)</td>
</tr>
<tr>
<td>4</td>
<td>Ni(II)</td>
<td>Brown</td>
<td>641.1546</td>
<td>&gt;300</td>
<td>58</td>
<td>C: 33.81(33.72) H: 3.63(3.62) N: 9.18(9.15)</td>
</tr>
</tbody>
</table>

DMSO is used as a negative control and streptomycin used as positive control. All the plates were incubated at 37°C for 24 h, and observed for the zone of inhibition. The diameter of zone of inhibition was calculated in millimeters. The well diameter was deducted from the zone diameter to get the actual zone of inhibition diameter. All the experiments were done twice independently for each of the compounds.

Antioxidant activity

DPPH radical scavenging activity of the ligand and its metal complexes were determined by using DPPH according to the literature (Baraca et al., 2001). The principle for the reduction of DPPH free radicals are that the antioxidants reacts with stable free radical DPPH and it converts in to 1,1-diphenyl-2-picrylhydrazine. The ability to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm.

Synthesis of complexes

A mixture of hot methanolic solution of free ligand (0.002 M) and hot methanolic solution of (0.001 M) of the metal chloride solutions (MnCl₂.4H₂O/CoCl₂.6H₂O/ NiCl₂.6H₂O) was refluxed for 4 h. Precipitate was observed, the resulting solutions are allowed to stand at room temperature and upon slow evaporation gives cream, red and brown colored crystals of Mn(II), Co(II) and Ni(II) complexes, respectively. Then filter and washed with methanol and ether, dried under vacuum P₂O₅. The purity of the complexes was checked by TLC. Elemental and chemical analysis data are shown in Table 1.

Scheme 1.

Table 1: Analytical data for the ligand and its Mn(II), Co(II) and Ni(II) complexes.
RESULTS AND DISCUSSION

Infrared and Raman spectra

The FT-IR and Raman spectral assignments of ligand and their metal complexes are listed in Table 2. The bonding sites of the thiosemicarbazones to the metal ion have been deduced by comparing the spectra of the complexes with the spectrum of the ligand. IR spectrum of the free ligand is compared with the spectra of the metal complexes shows many characteristic absorption bands. In the IR spectrum of ligand \( \nu(N-H) \) band at 3224 cm\(^{-1}\) is present, the stretching frequency of the C=N function was observed at 1598 cm\(^{-1}\) in IR and 1602 cm\(^{-1}\) in Raman spectrum of the ligand. For the same compound, one more important stretching band at 848 cm\(^{-1}\) in IR and 850 cm\(^{-1}\) in Raman, that can be attributed for the stretching vibrations of the thione function (C=S) of the free ligand. The NH band was disappeared upon the complexation in all complexes. While the stretching vibrations of C=N in Mn(II), Co(II) and Ni(II) complexes showed bands at 1567, 1543, 1620 cm\(^{-1}\) in IR and 1570, 1564, 1585 cm\(^{-1}\) in Raman, respectively. The reduce in frequency of this band in the spectra of the complexes is an confirmation for the coordination via the azomethine nitrogen atoms (Kovala Demertzı et al., 2000). A similar pattern of red shift was observed with the stretching frequency of C=S bond show bands at 827, 829, 770 cm\(^{-1}\) and same bands are observed at 824, 827, 782 cm\(^{-1}\) in FT-IR and Raman respectively. These bands were shifted to lower frequencies, this indicates the coordination of sulfur atom (Kovala Demertzı et al., 2001).

NMR spectra

\(^1\text{H}\) NMR (Fig. 1) and \(^{13}\text{C}\) NMR (400 MHz, \( \delta \) (ppm)) spectrum of ligand in DMSO-\(d_6\) exhibit following signals: \( \delta \) 11.69 (s, 1H, OH); \( \delta \) 10.94 (s, 1H, \(^3\text{NH}\)); \( \delta \) 8.56 (-CH=); \( \delta \) 8.31 (s, 1H, \(^3\text{NH}\)) \( \delta \) 6.9-8.3 (m, aromatic) (Bindu et al., 1999)\(^{18}\). \(^{13}\text{C}\) NMR: \( \delta \) 140 (C=S); \( \delta \) 179 (C=N); \( \delta \) 114-140 (aromatic). The electronic impact mass spectrum of ligand (Fig. 2), owed a molecular ion peak at \( m/z = 241.0396 \) corresponding to species (C\(_8\)H\(_8\)N\(_4\)O\(_3\)S) which confirms the proposed structure of the formulae.

### Table 2: Assignments of the characteristic FT-IR (cm\(^{-1}\)) and Raman (cm\(^{-1}\)) spectral bands of the ligands and their Mn (II), Co(II) and Ni (II) complexes.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Mn(II)</th>
<th>Co(II)</th>
<th>Ni(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT-IR</td>
<td>FT-IR</td>
<td>Raman</td>
<td>FT-IR</td>
</tr>
<tr>
<td>3224</td>
<td>3398</td>
<td>-</td>
<td>3414</td>
</tr>
<tr>
<td>1598</td>
<td>1602</td>
<td>1567</td>
<td>1570</td>
</tr>
<tr>
<td>1305</td>
<td>1324</td>
<td>1226</td>
<td>1235</td>
</tr>
<tr>
<td>848</td>
<td>850</td>
<td>827</td>
<td>824</td>
</tr>
</tbody>
</table>

Table 2: Assignments of the characteristic FT-IR (cm\(^{-1}\)) and Raman (cm\(^{-1}\)) spectral bands of the ligands and their Mn (II), Co(II) and Ni (II) complexes.

Fig. 1: \(^1\text{H}\) NMR spectrum of the ligand.
Electronic absorption spectra

Electronic absorption spectrum (Fig. 3) of Mn(II) complex exhibit four weak intensity bands at 18621, 22123, 28409 and 33112 cm$^{-1}$. These bands may be assigned to the transitions: $^6A_{1g} \rightarrow ^4T_{1g}$, $^6A_{1g} \rightarrow ^4E_g$ (G), $^6A_{1g} \rightarrow ^4T_{2g}$ (D) and $^4T_{1g} \leftarrow ^4A_{2g}$, respectively. These bands are characteristics of distorted octahedral geometry (Chandra et al., 2009). The electronic spectra of cobalt(II) complex exhibit absorption bands at 14880, 19047 and 28325 cm$^{-1}$. These bands may be $^4T_{1g} \rightarrow ^4A_{2g}$ and $^4T_{1g}(F) \rightarrow ^4T_{1g}(P)$ respectively. The third band may be due to charge transfer. The position of above bands indicates that these complexes have distorted octahedral geometry (Chandra et al., 2004).

Electron paramagnetic resonance (EPR) spectrum

The X-band EPR spectrum of Mn(II) complex is obtained at room temperature for the polycrystalline sample and is shown in Fig. 4. The EPR spectrum exhibits a broad signal without fine or hyperfine structure.

The effective g value is found to be 2.04. This signal suggests that dipolar interactions between manganese ions exist. Mn(II) ions belongs to 3d$^5$ electron configuration for $^{55}$Mn nucleus, the nuclear spin $I = 5/2$ and hence EPR spectrum of Mn(II) complex at room temperature exhibits a characteristic six line hyperfine splitting. If the symmetry around Mn(II) is distorted...
due to complexation, the resonances become anisotropic and a randomly oriented sample may exhibit a broad line (Batista et al., 2010). The observed broad signal in the present work suggests that the symmetry around Mn(II) ion in the complex is distorted one.

**Cyclic voltammetric studies**

Thiosemicarbazone metal complexes of electrochemical properties with sulphur donor atoms have been studied in order to ensure spectral and structural changes associated electron transfer (Bond et al., 1984). The cyclic voltammograms of the synthesized ligand and its Mn(II), Co(II) and Ni(II) complexes of 0.1 mM are carried in 1 M KCl at 25 ± 0.1°C with 0.1 mM of tetrabutyl ammonium hexafluoro phosphate (TBAHFP) as an electrolyte. The redox properties of the ligand and their complexes were performed by using a conventional three-electrode system and at a scan rate of 50 mV/S by using glassy carbon electrode (GCE). The cyclic voltammogram of substituted thiosemicarbazone was investigated at the potential range (-1000 to 1500 mV). The ligand and metal complexes of the cyclic voltammograms were shown in Fig. 5. The ligand related voltammogram contain two redox peak currents and potentials found to be 0.1049, -0.6044 and 0.0530, -0.8370 V with respectively. The Co(II), Ni(II) and Mn(II) complexes voltammograms were shows the potential differences between two anodic peaks and cathodic peak potential. These potentials are shifted towards negative direction for Co(II), Ni(II) and Mn(II) complexes (ΔEpa = Epa1 - Epa2) 0.0731, 0.0807 and 0.0519 V potentials differences with respectively and in cathodic peak potential are also shifted towards negative direction with 0.6044, 0.3359 and 0.0227 V potentials differences with respectively. Coordination of the ligands shifts redox potential of the ligands to negative potentials due to the electron deficient character of the metal centre and also the obtained potentials differences shows the formation of ligand to metal linkage were observed (Chikate et al., 2005).

These are the complexes show more activity compared to the parent ligand, but less than the standard antibiotic of streptomycin. The ligand did not show any activity against the *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Klebsiella pneumonia* and except for *E.coli*, whereas Mn, Co and Ni complexes are inhibited the growth of organisms, respectively. Among the three complexes, Ni showing more active against all the bacterial isolates in all concentrations than the Mn(II) and Co(II) complexes. And where as Co complex showing the more activity for *Bacillus subtilis* and *Salmonella typhi* with increasing of concentration. The reason for greater activity of complexes can be explained by Chelation theory (Naz et al., 2005). From the chelation theory, the polarity of the metal ion is found to be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups especially with sulphur and nitrogen donors. Finally, the growth of antibacterial activity for all compounds following in the order of Ni(II) > Co(II) > Mn(II) > ligand.

**Antioxidant activity**

5-Hydroxy-2-nitrobenzaldehydethiosemicarbazone and its metal complexes are tested for *in vitro* antioxidant activity at 37°C by DPPH free radical scavenging assay method (Sivassankar Reddy et al., 2015). DPPH is a stable free radical used for studying radical scavenging activity in chemical analysis (Bukhari et al., 2009). The DPPH radical shows a strong absorption band at 517 nm in visible spectrum due to the presence of an odd electron. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1,1-diphenyl-2-picrylhydrazine as shown in Fig. 6.

Fig. 6: DPPH scavenging activity of ligand and its Mn(II), Co(II) and Ni(II) complexes.

The change in the absorbance produced in this reaction is used to measure antioxidant properties. All compound solutions are prepared at different concentrations in DMSO solvent. 1 ml of every sample solution having different concentrations and 4 ml of 0.1 mM DPPH solution are taken in different test tubes and the mixture is shaken vigorously for 2 min, after these tubes are incubated for 30 min at room temperature (37°C). The absorbance
is measured at 517 nm against blank samples lacking scavenger. The percent of DPPH radical scavenging effect is calculated using the following equation: All the compounds are show good antioxidant activity against DPPH method especially.

DPPH Scavenging effect (\%) = \left[ \frac{(A_0 - A_t)}{A_0} \right] \times 100

\(A_0\) is the absorbance of the control and \(A_t\) is the absorbance of the test solution.

### Table 3: Anti bacterial activity of ligand and their Mn(II), Co(II) and Ni(II) complexes measured in mm.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Compound</th>
<th>Zone of inhibition in mm</th>
<th>Concentration in g/ml</th>
<th>Std. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>Ligand</td>
<td>-</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Mn(II)</td>
<td>4</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Co(II)</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td><strong>Klebsiella pneumonia</strong></td>
<td>Ligand</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mn(II)</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Co(II)</td>
<td>3</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>5</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td><strong>Salmonella typhi</strong></td>
<td>Ligand</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mn(II)</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Co(II)</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><strong>Bacillus cereus</strong></td>
<td>Ligand</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Mn(II)</td>
<td>2</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Co(II)</td>
<td>2</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>Ligand</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Std. Streptomycin; Standard error: ± 0.3 mm

### CONCLUSIONS

Syntheses of Mn(II), Co(II) and Ni(II) complexes with the ligand of 5-hydroxy-2-nitro-benzaldehydethiosemicarbazone, and were characterized by different spectral methods. Infrared and electronic spectral studies were revealed a distorted octahedral geometry for all the metal complexes. The antibacterial and antioxidant activities of the ligand and its metal complexes were shows good activity, among the all compounds Ni(II) complex showing more activity.

### ACKNOWLEDGEMENTS

One of the authors M.Sivasankar Reddy is grateful to the UGC, Government of India, New Delhi for financial assistance in the form of a meritorious research fellowship. The authors thank the School of Chemistry, University of Hyderabad, for providing the instrumentation facility. The authors sincerely thank Prof. J. Lakshmana Rao, Department of Physics, S.V. University, Tirupati, for his help in EPR study.

### REFERENCES


How to cite this article: