CHAPTER 4
MATERIALS AND METHODS

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Section (i) - Preparation of reagent and solutions

Preparation of 5,5-Dimethyl-1,3-cyclohexanedione-bis-thiosemicarbazone monohydrochloride

(5,5-DiMe-1,3-CHDT·HCl)

In a 250-ml round bottom flask, a hot solution of 5,5-dimethyl-1,3-cyclohexanedione (5 g, 0.031 moles), in 15 ml of ethanol was added dropwise to a stirred solution of thiosemicarbazide (6.5 g, 0.0714 moles) dissolved in 25 ml of water. Then 6 ml of conc. HCl was added and heated to reflux for one hour, on water bath. On cooling the reaction mixture, a white product was separated, it was collected by filtration and washed with cold ethanol, m.p. 208°C, yield 7.5 g (76%).

Preparation of reagent solution

The reagent solution (1 x 10^{-2} M) was prepared by dissolving 325 mg of the compound in 100 ml of distilled water (in 100 ml standard flask).

Cr(VI) solution (0.1 M)

2.942 g of dried K_{2}Cr_{2}O_{7} was dissolved in distilled water and diluted to 100 ml in a standard flask.
\[
\text{\begin{align*}
\text{[Diagram]} & \quad \overset{\text{HCl}}{\longrightarrow} \\
\text{[Structure 1]} & + 2 \ \text{NH}_2\text{NH-C-NH}_2
\end{align*}}
\]
Ph(II) solution (1 x 10^{-2} M)

380 mg of lead acetate is dissolved in distilled water and diluted to 100 ml in a standard flask.

Cd(II) solution (1 x 10^{-2} M)

270 mg cadmium acetate (BDH) is dissolved in distilled water and diluted to 100 ml in a standard flask.

Hg(II) solution (1 x 10^{-2} M)

270 mg of HgCl₂ (AR) dissolved in distilled water and diluted to 100 ml in a standard flask.

Co(II) solution (1 x 10^{-2} M)

75 mg of cobaltous nitrate [Co(NO₃)₂·6H₂O] dissolved in distilled water and diluted to 25 ml in a standard flask.

Ni(II) solution (1 x 10^{-2} M)

75 mg of nickel sulphate [NiSO₄·2H₂O] dissolved in distilled water and diluted to 25 ml in a standard flask.

Cu(II) solution (1 x 10^{-2} M)

65 mg of copper sulphate [CuSO₄·5H₂O] dissolved in distilled water and diluted to 25 ml in a standard flask.
Zn(II) solution (1 x 10^{-2} M)

75 mg of zinc sulphate (ZnSO_{4}·7H_{2}O) dissolved in distilled water and diluted to 25 ml in a standard flask.

Buffer solutions

Buffer solutions of various pH values are prepared from following solutions by making use of the pH meter.

<table>
<thead>
<tr>
<th>pH</th>
<th>Component solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 3</td>
<td>1.0 M sodium acetate + 1 M HCl</td>
</tr>
<tr>
<td>4 to 6</td>
<td>0.2 M sodium acetate + 0.2 M acetic acid</td>
</tr>
<tr>
<td>8 to 11</td>
<td>2 M ammonium hydroxide + 2 M ammonium chloride.</td>
</tr>
</tbody>
</table>

Solvents

Ethanol

Ethanol is purified by refluxing crude solvent in the presence of requisite quantity of potassium hydroxide. Then it is cooled and distilled to collect absolute ethanol.

Dimethylformamide (DMF)

It is of AlQualigen make and used without further purification.
Section (ii) - Characterization of 5,5-Dimethyl-1,3-cyclohexanenedione-bis-thiosemicarbazone monohydrochloride

The purity of the product was found to be 99.5% by direct titration with 0.05 M sodium hydroxide with phenolphthalein as indicator. **Elemental analysis:** Found C, 39.48%; H, 5.35%; and N, 25.53%. Calculated for C$_{10}$H$_{19}$N$_6$S$_2$Cl C, 37.09%; H, 5.56%; and N, 25.96%.

To obtain the 5,5-dimethyl-1,3-cyclohexanenedione-bis-thiosemicarbazone, a hot saturated solution of this reagent salt (5,5-DiMe-1,3-CHDT.HCl) was neutralized with 5 molar sodium hydroxide. On cooling, a yellow colour product was obtained.

**UV-Visible spectra**

The absorption spectra of 5,5-dimethyl-1,3-cyclohexanenedione-bis-thiosemicarbazone monohydrochloride at various pH values are shown in Fig. 4.1. UV-visible spectra of micromolar ($4 \times 10^{-5}$ M) solutions of this ligand at various pH values are recorded. This ligand shows two absorption maxima around 215 and 315 nm, which may be due $\pi \rightarrow \pi^*$ transition. In alkaline medium (pH 8-10), these bands are shifted to higher wavelengths due to the formation of conjugated negative anion. The shift of absorption maxima from lower
wavelength to higher wavelengths is known as the bathochromic shift, which is a common spectral property of thiosemicarbazones. A plot is prepared between absorbance and pH at different wavelengths of radiation employed by following Philips and Merritt method. The pK values are calculated by taking the arithmetic mean of the values obtained from the measurements at four different wavelengths. The values for the deprotonation of this ligand are found to be pK₁ = 6.5 and pK₂ = 9.3.

NMR spectra

In ¹H-NMR spectrum (Fig. 4.2) of 5,5-DiMe-1,3-CHDT.HCl recorded in DMSO solvent shows signals correspond to -CH₃ (gem), -CH₂- (ring), NH and NH₂ protons at 1.96 (s, 6H), 2.43 (d, 6H, J = 4), 5.47 (s, 2H) and 7.90 (broad, 6H) (It may be noted that the position of imino and amino proton signals depends on the concentration).

Mass spectra

Mass spectra of 5,5-Dimethyl-1,3-cyclohexanedione-bis-thiosemicarbazone monohydrochloride is given in Fig. 4.3. This ligand shows molecular ion peak in its mass spectra. The important peaks that are observed are given in Table 4.1.
Figure 4.1 UV-Visible spectra of 4 x 10^{-5} M ligand solution at different pH values.
Fig. 4.2 $^1$H-NMR spectrum of 5,5-Di-Me CHDT.HCl in DMSO solvent.
Figure 4.3 Mass spectra of ligand
**TABLE 4.1**

Mass spectral data of ligand

<table>
<thead>
<tr>
<th>S.No.</th>
<th>m/e</th>
<th>Assignment</th>
<th>Formulae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>322</td>
<td>Molecular ion</td>
<td>C(<em>{10}H</em>{18})N(_6)S(_2)HCl</td>
</tr>
<tr>
<td>2</td>
<td>226</td>
<td><img src="" alt="Chemical Structure" /></td>
<td>C(<em>{9}H</em>{16})N(_5)S</td>
</tr>
<tr>
<td>3</td>
<td>210</td>
<td><img src="" alt="Chemical Structure" /></td>
<td>C(<em>{9}H</em>{15})N(_4)S</td>
</tr>
<tr>
<td>4</td>
<td>179</td>
<td><img src="" alt="Chemical Structure" /></td>
<td>C(<em>{7}H</em>{8})N(_4)S</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
<td><img src="" alt="Chemical Structure" /></td>
<td>C(_{2})H(_5)</td>
</tr>
</tbody>
</table>
Reactions with cations

The reactions of some important cations were tested at different pH values. The samples were prepared in 25 ml volumetric flask by adding 10 ml of buffer, metal ion, 2.5 ml of dimethylformamide and 1 ml of $1 \times 10^{-2}$ M ligand solution. The reaction mixture was diluted to the mark with distilled water. The absorbance was measured in 350-600 nm range against the reagent blank. The most important results are summarised in Table 2.

The high absorbances are found in complexation with $\text{Pb}^{2+}$, $\text{Cd}^{2+}$, $\text{Cu}^{2+}$, $\text{Ni}^{2+}$, $\text{Co}^{2+}$, $\text{Pd}^{2+}$ and $\text{Os}^{3+}$. 
<table>
<thead>
<tr>
<th>Metal ion</th>
<th>pH</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon$ (1 mol$^{-1}$ cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr(VI)</td>
<td>1.5</td>
<td>360</td>
<td>$1.64 \times 10^4$</td>
</tr>
<tr>
<td>Pb(II)</td>
<td>10.5</td>
<td>435</td>
<td>$1.52 \times 10^4$</td>
</tr>
<tr>
<td>Cd(II)</td>
<td>10.5</td>
<td>435</td>
<td>$2.50 \times 10^4$</td>
</tr>
<tr>
<td>Hg(II)</td>
<td>11.0</td>
<td>450</td>
<td>$2.30 \times 10^3$</td>
</tr>
<tr>
<td>Fe(II)</td>
<td>10.0</td>
<td>400</td>
<td>$1.50 \times 10^3$</td>
</tr>
<tr>
<td>Co(II)</td>
<td>8.0</td>
<td>400</td>
<td>$2.69 \times 10^4$</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>8.0</td>
<td>405</td>
<td>$3.51 \times 10^4$</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>10.0</td>
<td>445</td>
<td>$6.22 \times 10^4$</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>10.0</td>
<td>367</td>
<td>$1.97 \times 10^4$</td>
</tr>
<tr>
<td>Pd(II)</td>
<td>8.0</td>
<td>380</td>
<td>$3.46 \times 10^4$</td>
</tr>
<tr>
<td>Os(VIII)</td>
<td>10.0</td>
<td>432</td>
<td>$3.77 \times 10^4$</td>
</tr>
<tr>
<td>Ru(III)</td>
<td>10.0</td>
<td>410</td>
<td>$1.93 \times 10^4$</td>
</tr>
</tbody>
</table>
Section (iii) - A brief description of the instruments employed in the present study

A. **Spectrophotometer**

UV-Visible spectrophotometer of model 160A, manufactured by Schimadzu, Japan was used in the present study.

The instrument comprises of (a) a high resolution grating monochromator to select the desired wavelength, (b) a light source (deuterium and tungsten lamps) compartment, (c) a cell compartment with a provision to accommodate two cuvettes, (d) a highly sensitive wide range photomultiplier tube detector and amplifier and (e) a computerised unit for recording and displaying the data. The instrument is calibrated to cover a wide wavelength range of 200 nm to 1100 nm.

This instrument is highly sophisticated device for recording spectra or for measuring the absorbance of absorbing species present in solution.

B. **Digital pH Meter**

ELICO Digital pH meter model LI-120 by M/s. ELICO Private Limited, Hyderabad, India is employed for measuring pH of buffer solutions used in the studies. The instrument
has temperature compensation arrangement. The accuracy (precision) of measurement is within ± 0.01 pH.

C. **Mass spectrometer**

Mass spectrum of ligand was recorded using Fanning Mat 8230 Mass spectrometer.

D. **NMR spectrometer**

In the present study $^1$H-NMR spectrum was recorded on a FT-80A high resolution NMR instrument in DMSO solvent at room temperature.