Chapter-2

Materials, methods and instruments
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This chapter deals with the brief description of materials used, methods adopted and instruments employed in the present research project. The analytical methods, physicochemical measurements and biochemical techniques employed for the characterization and exploration of biological applications of the free ligands and their transition metal complexes are also compiled.

Chemicals

Most of the chemicals used in the current study were of Laboratory Reagent (LR) grade and few were of Analytical Reagent (AR) grade. Spectral grade deuterated DMSO and CDCl$_3$ were used in the NMR spectral analysis. Spectroscopic grade KBr was used in FTIR study. Super dry Ethanol (AR grade) was used in many of the dry reactions. The chemicals were supplied by various makes viz., Sigma, Qualigens, Himedia, Merck, Loba, SD-Fine Chemicals etc.

All commercially available chemical substances specially solvents like ethanol, methanol, 1,4-dioxane, chloroform, carbon tetrachloride, dichloromethane etc., were purified according to the standard methods described in the literature to avoid the impurities if any [1, 2]. The mineral acids such as hydrochloric acid, sulphuric acid and nitric acid; the drying agents viz., anhydrous sodium sulphate, anhydrous magnesium sulphate, calcium chloride, calcium hydride and the other general chemicals like acetic acid and ammonia utilized at various stages of the work were all of AR grade obtained from S. D. Fine Chemicals. Distilled water was used throughout the experimental processes.

The ligands and transition metal complexes reported herein are non hygroscopic, and stable at room temperature. The compounds were purified by thorough washing with the appropriate solvents to remove the impurities, bi-products and unreacted precursors if any. Further recrystallisation of the compounds was conceded wherever necessary. Only the compounds which are analytically pure with reproducible measurements of physical properties are reported in this thesis.
Analysis of complexes
The elemental analysis for metal and chloride was carried out by the following standard methods [3].

Volumetric estimation of cobalt
An accurately weighed (~ 0.1 g) complex was decomposed on a sand bath by treating with mixture of perchloric acid and concentrated hydrochloric acid (20 ml 1:1 v/v). The solution was evaporated until the appearance of dense white fume and then cooled to room temperature. The solution was diluted with distilled water (~ 50 ml) and transferred into conical flask. Three drops of xylenol orange indicator were added followed by very dilute H₂SO₄ until colour changes from red to yellow. Powdered hexamine was added with shaking until the deep-red colour is restored (pH-6). The solution was warmed to 60 °C and titrated against standard EDTA. The end point (Burette reading) is noted when the solution colour changes from red to yellow-orange. Further the percentage of cobalt was determined by the formula given below.

\[
\% \text{Co} = \frac{\text{BR} \times \text{Molarity of EDTA} \times 0.05894}{\text{Wt. of Complex.}} \times 100
\]

Where BR = burette reading.

Gravimetric estimation of nickel
An accurately weighed complex was decomposed and diluted as discussed in the estimation of cobalt. The solution was heated to 70-80 °C and to the hot solution, a slight excess of 1% ethanolic solution of dimethylglyoxime was added followed by the drop wise addition of ammonia solution till the precipitation was complete (pH ~9). The resulting precipitate was digested on a water bath for about 30 minutes. After cooling for an hour, the solution was filtered through a dried and previously weighed sintered glass crucible (G-4). The precipitate was washed with water until free from chloride and dried to a constant weight at 110-120 °C for one hour. It was weighed as Ni(C₄H₇O₂N₂)₂ Further the percentage of nickel was determined by the formula given below.

\[
\% \text{Ni} = \frac{\text{Wt. of residue} \times 0.2031}{\text{Wt. of Complex.}} \times 100
\]
Gravimetric estimation of copper
An accurately weighed complex was decomposed as discussed in the estimation of cobalt. The resulting clear solution was diluted with distilled water (100 ml) and then treated with sodium hydroxide solution (2 N) drop wise to neutralize the mineral acid and then acidified with dilute acetic acid (pH ~2.6). Finally 1% aqueous solution of salicylaldoxime reagent was added slowly to it with constant stirring. The precipitate was allowed to stand for half an hour and filtered through a previously weighed sintered glass crucible (G-4) and washed thoroughly with water until the washings are free from chloride. It was dried to a constant weight at 100-105 °C and weighed as Cu(C₇H₆O₂N)₂. Further the percentage of copper was determined by formula given below.

\[
\% \text{Cu} = \frac{\text{Wt. of residue} \times 0.1893}{\text{Wt. of Complex}} \times 100
\]

Volumetric Estimation of zinc
An accurately weighed (~ 0.1 g) complex was taken and organic matter was destroyed as above. The resulting solution was diluted up to the mark in 100 ml volumetric flask. Then 10 or 25 ml of this homogeneous solution was pipetted into a clean conical flask and diluted with 30 ml of water and titrated against standard EDTA solution using Erichrom-black-T indicator with 2 ml of buffer (pH-10). The end point (Burette reading) is noted when the solution colour changes from wine-red to blue. Further the percentage of zinc was determined by the formula given below.

\[
\% \text{Zn} = \frac{\text{BR} \times \text{Molarity of EDTA} \times 0.06539}{\text{Wt. of Complex}} \times 100
\]

Where BR= burette reading.

Gravimetric estimation of chloride
An accurately weighed (~ 0.1 g) complex was treated with 30 ml of dilute HNO₃ (1:1 v/v) on water bath for 1h. The solution was filtered through Whatman 40 filter paper to remove unwanted organic matter. Thus obtained solution was diluted to 100 ml and treated with AgNO₃ solution. The solution was heated nearly to boiling and allowed to stand for 2 h for complete coagulation. The process of precipitation and coagulation were performed in subdued light. The precipitate
was filter through previously weighed sintered glass crucible (G-4) and washed with very dilute HNO₃ and dried at 130-140 °C. Further the percentage of chloride was determined by the formula given below.

\[
\% \text{Cl} = \frac{\text{Wt. of AgCl} \times 0.2474}{\text{Wt. of Complex.}} \times 100
\]

Physical measurements

Elemental analysis of the compounds

All the compounds were analyzed for carbon, hydrogen, nitrogen and sulfur by Thermo quest elemental analyzer (Milan, Italy) at STIC Cochin University of Science and Technology, Cochin.

Conductance measurements

The molar conductance measurements were made on an ELICO conductivity bridge type CM-82 (EITI Ltd. Hyderabad, India) provided with a dip type conductivity cell fitted with platinum electrodes. The cell constant was determined by measuring the conductance of aqueous KCl solution of known specific conductance. The value of the cell constant was found to be 0.51.

The conductance values of the complexes were determined by using 10⁻³ M solution in DMF/DMSO. The molar conductance is calculated as follows.

\[
\Lambda_M = 1000 \times K \times \text{observed conductance (in mhos)/C}
\]

Where, \( \Lambda_M = \text{Molar conductance} \)

\( K = \text{cell constant} \)

\( C = \text{Molar concentration (10⁻³ M).} \)

Magnetic susceptibility measurements

The magnetic susceptibility measurements were carried out at room temperature using VSM method at Institute Instrumentation Center, Indian Institute of Technology, Roorkee. The results were given as magnetic moments \( \times 10^{-2} \) emu.

The magnetic susceptibility was calculated by the relation,

\[
\chi_s = \frac{\text{magnetic moment (emu)/weight of the sample} \times H \text{ (applied field in Oersteds).}}{\}

The effective magnetic moment was calculated from the expression,

\[
\mu = 2.828(\chi_mT)^{1/2}
\]
Where $\chi_m$ is the molar magnetic susceptibility per metal atom corrected for diamagnetism.

The Faraday balance was used in few cases for the magnetic susceptibility measurements. The room temperature magnetic susceptibility values were obtained by the relation,

$$\chi_g = \beta \times \Delta W/W$$

Where $W = (C-A)$ and $\Delta W = (D-C) + (A-B)$

- A: Empty weight of Faraday tube (without field)
- B: Empty weight of Faraday tube (with field)
- C: Weight of sample taken + Weight of Faraday tube (without field)
- D: Weight of sample taken + Weight of Faraday tube (with field)

$\beta$ - Faraday tube constant (calculated to be $275 \times 10^{-6}$ in present case)

Further the molar susceptibility calculated by the formula,

$$\chi_m = \chi_g \times \text{Molecular weight of complex}.$$  

The effective magnetic moment was calculated from the expression,

$$\mu = 2.828(\chi_m T)^{1/2}$$

Hg[Co(SCN)$_4$] is used as standard for calibration.

Electronic spectral studies
The UV-visible electronic spectra of all the compounds in DMSO/DMF were recorded on a Varian Cary 50 Bio UV-Visible spectrophotometer (USA).

FT-IR spectral studies
Infrared spectra of the ligands and their metal complexes were recorded in KBr discs in the region 4000-400 cm$^{-1}$ on a Nicolet 170 SX FT-IR spectrophotometer (USA).

Nuclear Magnetic Resonance (NMR) spectral studies
Proton and carbon magnetic resonance spectra were recorded on a Bruker 300 M Hz spectrometer (USA) in DMSO-d$_6$ and CDCl$_3$ using TMS as an internal standard.

Electron Paramagnetic Resonance (EPR) spectral studies
The EPR spectra of Cu(II) complexes were recorded on a Varian E-4 X-band spectrometer (USA) using TCNE as $g$-marker at IIT Bombay.

Thermal measurements
Thermal studies (Thermo gravimetric and Differential Thermal analysis) of the metal complexes were carried out in nitrogen atmosphere on Rigaku thermoflex
and few on Universal V2.4F TA Instrument with limiting temperature of 1000 °C and heating rate 10 °C/min.

**Fast Atom Bombardment (FAB) mass spectra**

The FAB mass spectra were recorded on a JEOL EX 102/DA-6000 mass spectrometer / Data system (Tokyo, Japan) using Argon / Xenon (6kV, 10 mA) as the FAB gas and m-nitrobenzylalcohol as matrix at Central Drug Research Institute Lucknow.

**Cyclic voltammetric measurements**

The cyclic voltammetric experiments were carried out by using CHI1110A electrochemical analyzer (USA) with a three electrode assembly of a glassy carbon working electrode (0.082 cm²), a platinum counter electrode and an Ag/Ag⁺ reference electrode. Glassy carbon electrode surface was polished with 0.05 mm alumina, rinsed in water, and air-dried instantly before use. The electrochemical experiments were carried out and the positions of the waves were compared to the potential of the ferrocene/ferrocnium couple. The DMSO solution (containing 0.1 M tetrabutylammonium-perchlorate, as supporting electrolyte, 10⁻³ molar concentration of the ligand and each of the complexes) was placed in a single-compartment electrochemical cell and degassed by bubbling with N₂(g) saturated with DMSO. A nitrogen atmosphere was continuously maintained in the solution while the experiments were in progress.

**Electroconvulsiometer (Anticonvulsant activity)**

The action of reported ligands and the metal complexes against recurrent seizures in Wistar rats is measured by electroconvulsiometer NICO, Ambala provided with a pair of earclip electrodes.

**Glucometer (Antidiabetic activity)**

The US made laboratory glucometer is used in the measurement of blood glucose level and oral glucose tolerance test (OGTT).

In DNA binding/cleavage study, an electrophoresis chamber, Bio-Rad Trans UV illuminator and a Polaroid camera (a red filter and Polaroid film) were used. The Hydrodynamic experiments were carried out by using Oswald micro-viscometer.
References

