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

The chemicals used in the present investigation ranges from Lab Reagent (LR) grade to Analytical Reagent (AR) grade. Specifically spectral grade deuterated DMSO and CDCl₃ were used to record NMR spectra. Spectroscopic grade KBr were used to record FTIR. The chemicals were supplied by various makes viz., Sigma, Aldrich, Qualigens, Himedia, Merck, Sd-fine Chemicals etc.

Purity is a matter of degree. Contaminants such as dust, paper fibers, wax, cork, etc. that may have been incorporated into the sample during manufacture, all commercially available chemical substances are in some measure impure. Any amounts of unreacted starting material, intermediates, by products, isomers and related compounds may be present depending on the synthetic or isolation procedures used for preparing the substances.

Solvents and substances that are specified as pure for a particular purpose may, in fact, be quite impure for other uses. Absolute ethanol may contain traces of benzene, which makes it unsuitable for ultraviolet spectroscopy, or plasticizers, which make it unsuitable for use in solvent extraction.
The standard well-documented procedures available in Vogel [1] and Wilfred [2] were followed for the purification of all the reagents and solvents used irrespective of the grade of materials for assessing the degree of purity. The drying reagents employed at various stages viz., anhydrous sodium sulphate, anhydrous magnesium sulphate, calcium chloride, calcium hydride and the mineral acids such as hydrochloric acid, sulphuric acid and nitric acid were all of AR grade obtained from Sd-fine Chemicals. Distilled water was used throughout all the experimental processes.

Only complexes obtained analytically pure are reported in this thesis. Unless stated otherwise, metal complexes reported herein are stable and possess good keeping qualities. Reproducible measurements of physical properties were obtained with purified samples.

Analysis of complexes

The elemental analysis for metal and chloride was carried out by the following standard methods [3].

Determination of cobalt by EDTA complexometric titration

An accurately weighed (~ 0.100 g) complex was decomposed with a mixture of perchloric acid and concentrated hydrochloric acid (20 ml 1:1 v/v) on a sand bath. The solution was evaporated till the dense white fumes appeared and 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 till the colour changes from red to yellow-orange.

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

Where B.R.= burette reading.

**Determination of nickel as bis(2,3-butanedionedioximato)nickel(II) complex**

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. 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.

\[
\% \text{ Ni} = \frac{\text{Wt. of residue} \times 0.2031}{\text{Wt. of Complex}} \times 100
\]

**Determination of copper as bis(salicyldioximato)copper(II) complex**

An accurately weighed complex was decomposed as discussed in the estimation of cobalt. The resulting clear solution was diluted with distilled water (100ml) and then treated with sodium hydroxide solution (2N) dropwise to neutralize the mineral acid and then acidified with dilute acetic acid. 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(C₂H₆O₂N)₂.

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

Determination of zinc by EDTA complexometric titration

An accurately weighed (0.1g) 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 of 10 pH. Titration is carried out till the colour change is from wine-red to blue.

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

Where B.R.= burette reading.

Determination of chloride

An accurately weighed (~ 0.100 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 filtered through previously weighed sintered glass crucible (G-4) and washed with very dilute HNO₃ and dried at 130-140 °C.

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

**Physical measurements**

**Elemental analysis of the compounds**

All the compounds were analyzed for carbon, hydrogen, nitrogen and sulfur (in few cases) by Thermo quest elemental analyzer 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 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, $\Lambda_M =$ Molar conductance

K = cell constant

C = 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,

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

The effective magnetic moment was calculated from the expression, \( \mu = 2.828(\chi_m T)^{1/2} \), where \( \chi_m \) is the molar magnetic susceptibility per metal atom corrected for diamagnetism.

Electronic spectra

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

FT-IR spectra

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 spectrometer.

Nuclear Magnetic Resonance (NMR) spectra

Proton and carbon magnetic resonance spectra were recorded on a Bruker 300 MHz spectrometer in DMSO-\(d_6\) and CDCl\(_3\)-\(d_6\) using TMS as an internal standard.
Materials, Methods and instruments

Electron Paramagnetic Resonance (EPR)

The EPR spectra of Cu(II) complexes were recorded on a Varian E-4 X-band spectrometer using TCNE as g-marker.

Thermal measurements

Thermal analysis of the metal complexes were carried out in nitrogen atmosphere on Rigaku thermoflex instrument using limiting temperature of 800 °C and heating rate 10 °C / minute.

Fast Atomic Bombardment (FAB) mass spectra

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

Cyclic voltammetric measurements

The cyclic voltammetric experiments were carried out with a three electrode apparatus using a CHI110A electrochemical analyzer (USA). Cyclic voltammetric data were recorded using a glassy carbon working electrode (0.082 cm2), a platinum counter electrode, and an Ag/Ag+ reference electrode. Glassy carbon electrode surfaces were polished with 0.05-mm alumina, rinsed in water, and air-dried immediately before use. The electrochemical experiments were carried out and the positions of the waves were compared to the potential of the ferrocene/ferrocenium couple. The DMSO solution (containing 0.1 M Tetramethylammoniumchloride, 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 N$_2$(g) saturated with DMSO. A N$_2$ atmosphere was continuously maintained above 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 microviscometer.
References

