This chapter deals with synthesis of ligands, their metal complexes and elemental analysis. It also includes a brief description of various physico-chemical techniques employed in characterizing the ligands and their metal complexes. These physico-chemical techniques are useful in interpreting the nature of bonding between the metal ion and the coordination sites of the ligands and their metal complexes. All the chemicals and solvents used in this work were of analytical reagent grade (BDH / Aldrich) and used without further purification.

2 A-1. Preparation of diethylbromomalonate:

Place 160g (170mL) diethylmalonate and 150mL of CCl₄ in a three necked R.B. flask fitted with a stirrer, condenser and dropping funnel, the condenser was connected to rubber tubing which was immersed in water to dissolve the HBr liberating during bromination. In the dropping funnel 165gm (53mL, 1.03 mol) of dry Br₂ was taken and is added slowly. A large electric bulb was held near the flask until the reaction starts, and then bromine was added gradually so as to keep reaction mixture cold, gently.

It was then refluxed on water bath until no more HBr was liberated (about 1 hrs). The reaction mixture was cooled and washed 5 times with 50% Na₂CO₃ solution. It was then distilled under reduced pressure, the fraction being taken up from 130°C to 150°C/40mm of Hg, and the lower boiling fraction is not necessary to distill as it becoming dark. Yield =20 gm (78%), Density =1.402, B. P = 231-233°C Mol. Wt. =239.

\[
\begin{align*}
\text{H}_2\text{C} & \quad \text{COOC}_2\text{H}_5 \\
\text{COOC}_2\text{H}_5 & \quad \xrightarrow{\text{Br}_2} \\
\text{Br} & \quad \text{CH} \quad \text{COOC}_2\text{H}_5 \\
\text{COOC}_2\text{H}_5 & \quad \xrightarrow{-\text{HBr}} \\
\end{align*}
\]

Fig.2 (1)

2 A-2. Preparation of 2-carboethoxy benzofuran¹

A solution of salicylaldehyde (10g, 0.082mol) and diethyl bromomalonate (20g, 0.083mol) in ethyl methyl ketone (CH₃ CH₂COCH₃) 40mL was treated with anhydrous potassium carbonate (K₂CO₃) (10g). The reaction mixture was heated for about 8 hrs on a water bath. Solvent was (removed) distilled off under reduced
The residual salt was diluted with water (200ml), cooled in ice bath and acidified with dilute H$_2$SO$_4$.

The product was extracted with ether and ethereal layer was washed with saturated solution of sodium bicarbonate (NaHCO$_3$) solution and dried over anhydrous CaCl$_2$. Removal of solvent gave semi solid compound. Yield = 78%, M.P. = 27°C, B.P. = 274°C

![Fig. 2(2)](image)

**2 A-3. Preparation of benzofuran-2-carbohydrazide:**

To the solution of 2-carboethoxy benzofuran (10g, 0.053 mol) in ethanol (30mL) hydrazine hydrate 99% (5mL), was added and mixture was magnetically stirred at room temperature for about two hours. The colorless crystalline carbohydrazide started separating within few minutes and is complete in two hours. The reaction mixture is cooled thoroughly in ice bath and filtered. Recrystallised from ethanol as colorless needles. Yield= (78%), M. P 184°C

![Fig. 2 (3)](image)

**2 A-4. Synthesis of Schiff's base:**

The Schiff's base ligands were synthesized by refluxing the mixture of hot ethanolic solution (30mL) of benzofuran-2-carbohydrazide (0.01mol) and hot ethanolic solution (20mL) of 2-acetylpyridine / 2-acetylthiophene / acetophenone / p-methoxyacetophenone / p-chloroacetophenone / 3-methyl-2-thiophenecarboxaldehyde / 4-methylthiobenzaldehyde and 3-ethoxy-4-hydroxybenzaldehyde (0.01mol) for 4-5 hours on water bath. The Schiff's base was separated on removal of 50% of solvent and cooling to room temperature. The product filtered out and washed with ethanol
and recrystallised from ethanol. The results are summarized in the following Table 2 (1).

![Fig: 2 (4)](image1)

![Fig: 2 (5)](image2)
Refluxed for 4-5h ethanol

Fig: 2 (6)

Refluxed for 4-5h ethanol

Fig: 2 (7)
Refluxed for 4-5h ethanol

Fig: 2 (8)

Refluxed for 4-5h ethanol

Fig: 2 (9)
Fig: 2 (10)

Fig: 2 (11)
<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Ligands</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>M.P. °C</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BCAP</td>
<td>C₁₆H₁₃O₂N₃</td>
<td>279</td>
<td>163</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>BCAT</td>
<td>C₁₅C₁₂O₂N₃S</td>
<td>284</td>
<td>154</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>BCACP</td>
<td>C₁₇H₁₄O₂N₂</td>
<td>278</td>
<td>177</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>BCMeOACP</td>
<td>C₁₈H₁₆O₃N₂</td>
<td>308</td>
<td>157</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>BCCIACP</td>
<td>C₁₇H₁₅O₂N₂Cl</td>
<td>312</td>
<td>144</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>BCMeTPC</td>
<td>C₁₅C₁₂O₂N₂S</td>
<td>284</td>
<td>238</td>
<td>70</td>
</tr>
<tr>
<td>7</td>
<td>BCMeTB</td>
<td>C₁₇C₁₄O₂N₂S</td>
<td>310</td>
<td>207</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>BCEtHB</td>
<td>C₁₈C₁₅O₃N₂</td>
<td>324</td>
<td>198</td>
<td>75</td>
</tr>
</tbody>
</table>

1. BCAP: N’-(1-(pyridin-2-yl)ethylidene) benzofuran-2-carbohydrazide
2. BCAT: N’-(1-(thiophen-2-yl)ethylidene)benzofuran-2-carbohydrazide
3. BCACP: N’-(1-phenylethylidene) benzofuran-2-carbohydrazide
4. BCMeOACP: N’-(1-(4-methoxyphenyl)ethylidene)benzofuran-2-carbohydrazide
5. BCCIACP: N’-(1-(4-chlorophenyl) ethylidene) benzofuran-2-carbohydrazide.
6. BCMeTPC: N’-(3-methylthiophen-2-yl)methylenebenzofuran-2-carbohydrazide.
7. BCMeTB: N’-(4-(methylthio)benzylidene)benzofuran-2-carbohydrazide.
8. BCEtHB: N’- (3-ethoxy-4-hydroxybenzylidene) benzofuran-2-carbohydrazide.

2 A-5. Metal chlorides used for the preparations of metal complexes

The following metal salts of BDH make AR grade chemicals were used for the preparation of various metal complexes.

1. Copper chloride [CuCl₂.2H₂O]
2. Cobalt chloride [CoCl₂.6H₂O]
3. Nickel chloride [NiCl₂.6H₂O]
4. Zinc chloride [ZnCl₂]
5. Cadmium chloride [CdCl₂.2.5H₂O]
6. Mercury chloride [HgCl₂]
2 A-6. General procedure for the preparation of metal complexes with the following benzofuran Schiff’s base.

1. BCAP.
2. BCAT.
3. BCACP.
4. BCMeOACP
5. BCClACP
6. BCMeTPC
7. BCMeTB
8. BCEtHB

The metal complexes were prepared by adding ethanolic solution (20mL) of Cu(II), Co(II), Ni(II), Zn(II), Cd(II) and Hg(II) chloride (0.01mol) to Schiff’s base ligands (0.01mol) in an alcoholic medium (25mL). The reaction mixture was refluxed for about three hours on water bath and then aqueous alcoholic solution of sodium acetate was added to the mixture to adjust the pH 6-7 and further refluxed for about an hour. The complexes were precipitated by adding distilled water in some cases. The complex separated was filtered washed with distilled water, then with alcohol and finally dried over fused calcium chloride in vacuum. The analytical data of the Schiff base ligands and their metal complexes were listed in Table 3 (1-8).

2 B: Elemental analysis

2 B-1. Estimation of Cu(II), Co(II), Ni(II), Zn(II) and Cd(II) metals in the complexes

Accurately weighed 150mg of complex was decomposed with concentrated nitric acid (about 10mL) and perchloric acid (5mL). The residue was diluted with 150mL of distilled water and Cu(II), Co(II), Ni(II), Zn(II) and Cd(II), were estimated gravimetrically, Copper as Copper salicylaldoximate [Cu(saldox)²], Cobalt as Cobaltoximate [Co(OX)².2H₂O], Nickel as Nickel dimethylglyoximate [Ni(DMG)²], Zinc as Zinc ammonium phosphate [Zn(NH₄PO₄)] and Cadmium as Cadmium oximate [Cd(OX)²] respectively by standard methods. The conversion factors are [Cu-18.92%, Co-16.99%, Ni-20.32%, Zn-30.65% and Cd-28.05%].

2 B-2. Estimation of Chloride

Accurately weighed sample (0.02g) metal complex was boiled separately with aqueous ammonia to effect hydrolysis and finally boiled with aqueous ammonia. The precipitate was filtered out. It was washed thoroughly with water and washing were collected in the same beaker. Total bulk of the solution was acidified with concentrated nitric acid and heated to boiling. The solution was treated with 0.1N silver nitrate solution and the precipitate of silver chloride was boiled for a while and
filtered through previously weighed sintered glass crucible No.4, washed several times with water and dried at 120°C and weighed as AgCl which contains 24.74% chloride.

2 B-3. Estimation of Sulphur:

The Sulphur was determined by titrating the aqueous suspension of the chelates with bromine water and heating on a water bath for complete oxidation followed by treatment with BaCl₂ in dilute HCl medium till the precipitate was completed and filtered ignited in a previously weighed silica crucible as weighed as BaSO₄.

\[
S\% = \frac{\text{wt of ppt} \times 0.13737 \times 100}{\text{wt of complex}}
\]

2 C: Physico-Chemical Measurements:

2 C-1. CHN analysis.

Carbon, hydrogen and nitrogen analysis were carried out microanalytically on perkin-Elmer 240C model at Central Drug Research Institute (CDRI) Lucknow.

2 C-2. Electronic spectral measurements.

Electronic spectra of the Co(II), Ni(II) and Cu(II) complexes in DMF (10⁻³ m) solution were recorded on Elico SL-159 double beam UV-visible spectrophotometer in the range of 200-1100 nm.

2 C-3. Conductance measurements

The conductance measurements were made on Elico CM-180 conductivity using a dip type conductivity cell fitted with platinum electrode. The cell constant (K) was determined by measuring the conductance of aqueous KCl solution of known specific conductance. The value of cell constant was found to be 1.01 cm⁻¹. The conductance (C) values of complexes were determined by using 10⁻³M (DMF) solution. The molar conductance (\(\Lambda_M\)) is calculated as follows.

\[
\Lambda_M = \frac{1000 \times \text{observed conductance}}{\text{Concentration}} \text{ Ohm}^{-1}\text{cm}^2\text{mol}^{-1}
\]

The observed conductance of a substance in a particular solvent depends upon various factors like viscosity, polarity, mobility etc of the solvent.
2 C-4. Infrared spectral measurements

The IR-spectra of the ligands and their complexes were recorded in KBr pellets using Perkin-Elmer 783 FT-IR model IR spectrometer in the range of 4000-400 cm\(^{-1}\).

2 C-5. Nuclear magnetic resonance measurements

The proton magnetic resonance spectra of Schiff base ligands and their complexes were recorded on NMR-spectrometers at IISc Bangalore and CDRI Lucknow using TMS as internal standard and DMSO-d\(_6\) as a solvent.

2 C-6. Mass spectra

FAB mass spectra were recorded on a Jeol-SX 102/Da-600 mass spectrophotometer using Argon 6 kv, 10mA as the FAB gas, m-nitro benzyl alcohol (NBA) was used as matrix and spectra were obtain at CDRI Lucknow and Electrospray ionization mass spectrophotometer were obtain at the Indian Institute of Technology Kanpur.

2 C-7. Electronic spin resonance spectral measurements

The electron spin resonance spectra of all Cu(II) complexes in polycrystalline state (powder) were recorded on varian E-4X-band ESR spectrometer at SAIF, IIT Bombay, using tetracyanoethylene (TCNE) as ‘g’ marker (g=2.00277) at room temperature.

2 C-8. Magnetic susceptibility measurements:

Magnetic susceptibility of the complexes were determined at room temperature by Gouy balance using mercury (II) tetrathiocyanatocobaltate (II, Hg[Co(SCN)\(_4\)]) as the calibrant.

2 C-9. Powder X-ray diffraction measurement:

The powder X-ray diffraction data were recorded on a Bruker AXS D8 advance model X-ray powder Diffractometer using K-alpha 1-54056A\(^\circ\) radiations at SAIF / STIC Cochin University of science and technology Cochin.

2.C-10. Thermal measurement:

Thermogravimetry (TG) and differential thermal analysis (DTA) studies were made for some complexes in the range of 28-800°C temperature with heating rate of 10°C/min. in N\(_2\) atmosphere on Perkin Elmer thermal analyzer at SAIF / STIC Cochin University of science and technology Cochin.
2 C-11. Evaluation of antibacterial and antifungal activity:

In the light of interesting antimicrobial activities, the synthesized ligands and their metal complexes in the present investigation were screened for their antibacterial and antifungal activities by agar diffusion method\textsuperscript{3-5} at Biogenics Hubli.

2.C-12. DNA cleavage activities:

Some of the ligands and their metal complexes were screened for their DNA cleavage activities by agarose gel electrophoresis method at Biogenics Hubli\textsuperscript{6}.
References