CHAPTER II

EXPERIMENTAL
This chapter deals with the details of the methods of purification of solvents. Preparation of ligands and their metal complexes, elemental analysis and the various physicochemical techniques and methods of biological evaluations employed in the present investigations.

A. Solvents employed and their purification

1) Benzene

Reagent grade benzene was mixed with 15% its volume of concentrated sulphuric acid. The mixture was mechanically stirred for 20-30 minutes in a three necked flask. The process was repeated until the acid layer was almost colourless indicating the complete removal of thiophene. The mixture was transferred to a separating funnel, the acid layer was separated and rejected. Benzene was then shaken and washed with water followed by aqueous solution of sodium carbonate to remove traces of acid. It was again washed with water to remove the carbonate. The benzene so washed was stored over anhydrous calcium chloride. The dried benzene was distilled and stored over fresh sodium wire. It was then freshly distilled in an all-glass apparatus whenever required. The fraction distilled at 76-77°C was collected.

2) Ethanol

About 50-80 ml of distilled commercial absolute alcohol was
taken in a two litre round bottom flask provided with a double walled reflux condenser and calcium chloride guard tube. Clean magnesium (~ 5 gms) and iodine (~ 0.5 gms) were added to the flask and contents were refluxed on a heating mantle until all the magnesium was converted into ethylate, with the evolution of hydrogen and decolouring the solution. Absolute alcohol (~ 900 ml) was added and refluxion was continued for 30 min. The alcohol was then distilled off in allglass apparatus with careful exclusion of moisture directly into a clean flask.

3) **Methanol**

This was purified in a similar manner as ethyl alcohol by treatment with magnesium and iodine. The fraction distilling at 61-62°C was collected.

4) **Acetone**

The commercial grade acetone was refluxed with successive small quantities of potassium permanganate until the violet colour persists. It was then dried over anhydrous potassium carbonate filtered and distilled before use.

5) **Dimethylformamide (DMF)**

B.D.H. or reagent grade DMF was kept over potassium hydroxide pellets for two days in a stoppered bottle and distilled
under reduced pressure (just before use) using all glass apparatus 
(B.P. 58-59°C e/25 mm sp. conductance 10^{-7} \text{ohm}^{-1} \text{cm}^{-1}).

6) **Dimethylsulphoxide** (DMSO)

Reagent grade dimethyl sulfoxide was allowed to stand over 
ignited calcium oxide for two or three days, and then distilled 
under reduced pressure (10 mm). The middle fraction boiling in 
the range 65-70°C was collected in a dry bottle (sp. conductance 
3 \times 10^{-7} \text{ohm}^{-1} \text{cm}^{-1}).

7) **Chloroform**

Chloroform (C.P) was shaken 5-6 times with half its volume 
of water to remove the added alcohol as a stabiliser and the 
aqueous layer was rejected. The chloroform was stored over 
anhydrous calcium chloride for several hours. It was then distilled 
using an all glass apparatus under anhydrous conditions. The fraction 
distilling at 57-58°C was collected and stored in an amber coloured 
bottle with the glass stopper.

8) **Ether**

Ether (C.P) was shaken thoroughly with 15% ferrous 
sulphate solution (to remove peroxide) and the aqueous layer was 
drawn off. Ether was then stirred over anhydrous calcium chloride
for several hours then distilled using calcium chloride guard tube. It was stored in ambered coloured bottle over sodium wire and freshly distilled before use. The fraction distilling at 34-35°C was collected.

9) **Petroleum ether**

Petroleum ether used in the present studies had a boiling range 40-60°C. It is distilled before use.

**B. Material employed**

1. **Aldehydes**

Benzaldehyde (BDH analar grade), salicylaldehyde, furfuryl aldehyde, (Bush grade) were distilled before use. o-Vanillin was Fluke make. o-Methoxybenzaldehyde was of BDH grade.

2. **Amino pyridines**

2,6-Diaminopyridine and 2-aminopyridine of BDH grade were used. 2-Amino-3-methylpyridine was also of BDH grade.

3. **Aminothiozole**

2-Aminothiozole of Sarabhai make was recrystallised in ethanol before use.
4. **Metal salts**

Uranyl nitrate, thorium nitrate of BDH grade were used. Nickel chloride, cobalt chloride, copper chloride of Emerck grade were used. Zinc chloride, cadmium chloride and mercuric chloride of high grade quality were used.

5. **Hydrazine hydrate of BDH grade was used in preparation of hydrazides.**

C. **Preparation of ligands**

The following ligands were synthesised and used in our present investigation.

I, $R^1 = H, R^2 = H$

II $R^1 = \text{OH}, R^2 = H$

III $R^1 = \text{OH}, R^2 = -\text{OCH}_3$

![Chemical structure of ligands](image-url)
I. \( R^1 = H, R^2 = H, R^3 = H \)

II. \( R^1 = \text{OH}, R^2 = H, R^3 = H \)

III. \( R^1 = \text{OH}, R^2 = \text{OCH}_3, R^3 = H \)

IV. \( R^1 = \text{OCH}_3, R^2 = H, R^3 = H \)

V. \( R^1 = H, R^2 = H, R^3 = \text{-CH}_3 \)

VI. \( R^1 = \text{OH}, R^2 = H, R^3 = \text{-CH}_3 \)

VII. \( R^1 = \text{OH}, R^2 = \text{OCH}_3, R^3 = \text{-CH}_3 \)

VIII. \( R^1 = \text{OCH}_3, R^2 = H, R^3 = \text{CH}_3 \)

I. \( R^1 = \text{OH}, R^2 = H \)

II. \( R^1 = \text{OH}, R^2 = \text{OCH}_3 \)

I. \( R = H \)

II. \( R = \text{-CH}_3 \)

LV

2-Furfurylidene-2-aminothiazole
D. The method employed for synthesis of the ligands

I. Calculated quantity of benzaldehyde, or salicyaldehyde, or o-vanilline (0.02M) and 2,6-diaminopyridine (0.01M) were taken in ethanol and refluxed for about 1.5 hours using magnetic stirrer. The Schiff base formed was solidified rapidly on cooling in ice cold water. The solid was separated by filtration, it was then washed with little alcohol and ether. This was recrystallised using suitable solvents like ethyl or methyl alcohol.

II. Calculated quantity of benzaldehyde or salicylaldehyde or o-vanilline, or o-methoxybenzaldehyde (0.01M) and 2-aminopyridine or 2-amino-3-methylpyridine (0.1M) in ethanol were taken in round bottom flask and the mixture was refluxed for 2 hours using magnetic stirrer. It was cooled in ice for long time. Ligand formed was separated by filtration and was washed with little alcohol, and ether. Finally recrystallised using suitable solvents like ethyl or methyl alcohol.

III. Calculated quantity of salicylaldehyde or o-vanillin (0.01M) in ethanol and 2-aminothioazole (0.01M) in ethanol were taken in
round bottom flask and few drops of piperidine was added. The mixture was prefluxed for 2 hours on a magnetic stirrer. The flask was cooled in ice till solid was separated. This was recrystallised using methanol and pet ether.

IV. A calculated quantity of (0.01M) of furfuraldehyde in ethanol was taken in 100 ml round bottom flask and solution was warmed. To the warmed solution 2-aminopyridine or 2-amino-3-methylpyridine (0.01M) in ethanol was added and mixture was refluxed for about 3 hrs. The ligand formed was distilled and fraction distilling at 150°C and 152°C respectively were collected.

V. A calculated quantity of (0.01M) furfuraldehyde in warm ethanol was taken in 100 ml round bottom flask. To this warm solution 2-aminothiazole (0.01M) in ethanol along few drops piperidine was added and mixture was refluxed for 3-4 hours on waterbath and flask was cooled in ice. The ligand formed was separated by filtration and crystallised using suitable solvents.

**Preparation of 2-furoic acid hydrazide**

A solution of methyl-2-furate (5.15 gms) and hydrazine hydrate (2.48 gm) and water 2.5 ml, and ethyl alcohol (5 ml) was heated under reflux for 2 hours. Then excess of solvent was removed under reduced pressure by which solid hydrazide separated out.
It was kept in vacuum desiccator for two days to get hard solid. It was recrystallised from dry benzene. This was colourless solid having 78-79°C m.p.

**Preparation of hydrazones**

0.01M of furoic acid hydrazide in ethanol was taken in a 100 ml round bottom flask, and solution was warmed. To this warm solution 0.01M salicylaldehyde or o-vanillin in ethanol was added and mixture was refluxed for half an hour and cooled. The solid base formed was separated by filtration. It was then washed with little ether. White and yellow coloured ligands were obtained having m.p. 230° and 260°C respectively.

**Preparation of complexes**

**C₁ Preparation of uranium (VI) and thorium (IV) complexes**

In the preparation of these complexes a calculated quantity of Schiff base (0.02M) in ethanol and (0.01M) uranyl nitrate or thorium nitrate in ethanol were refluxed using magnetic stirrer for about 2 hours. The mixture was cooled in ice. The solid complex formed was separated by filtration and it was washed with alcohol and then with ether. Finally it was dried in vacuum over fused calcium chloride. For preparation of the complexes the ligands used were:

1) benzalidene-2,6-diaminopyridine,
2) salicylidene-2,6-diaminopyridine,
3) o-vanillidene 2,6-diaminopyridine and
4) benzalidene-2-aminopyridine,
5) o-methoxybenzalidene-2-
C II. Preparation of nickel(II), cobalt(II) and copper(II) complexes of Schiff bases

In preparation of these complexes, calculated quantity (0.02M) ligand in ethanol was taken in a round bottom flask and it was warmed. To the hot solution of Schiff base, calculated quantity (0.01M) of salt solution in ethanol was added in fraction and refluxed using magnetic stirrer for 2 hours. During refluxation few drops of potassium hydroxide solution (0.1M) was added. Solution is concentrated by removing excess of solvent. The flask was cooled in ice cold water for 1 hour. The complex formed was separated by filtration then it was washed with alcohol and ether. The complex obtained was dried in a vaccum over calcium chloride. The ligands used in preparation of complexes were salicylidene-2,6-diaminopyridine, o-vanillidene-2,6-diaminopyridine.

C III. Preparation of tin(IV) complexes

These complexes were prepared according the procedure noted elsewhere. In the preparation of complexes, a calculated quantity of (0.01M) ligand in dry benzene was taken in 250 ml round bottom
flask and to this solution a stannic chloride solution (0.01M) in dry benzene was added dropwise with constant stirring using magnetic stirrer. This reaction was carried out strictly in atmosphere of nitrogen. After the completion of the reaction, the flask with content was kept over night. So formed complex was separated by filtration, washed with dry alcohol and dry benzene. It was dried in vaccum deciccator for two days.

The ligands used in the preparation of complexes were-
(i) salicylidene-2-aminopyridine (ii) o-vanillidene-2-aminopyridine,
(iii) salicylidene-2-amino-3-methylpyridine (iv) o-vanillinidene-2- 
       amino-3-methylpyridine (v) salicylidene-2-aminothiazole, (vi) o-vanill-
       idene-2-aminothiazole.

C IV  Preparation of Zn(II), Cd(II) and Hg(II) complexes of Schif bases

These complexes were prepared according to the method given elsewhere. In the procedure a calculated quantity (0.02M) of the ligand solid/liquid, in ethanol was taken in a round bottom flask. Solution was warmed, to this warm solution, a metal salt solution (0.01M) in ethanol was added in fraction along with few drops of potassium hydroxide solution (0.01M). The mixture was refluxed for 2 hours, using magnetic stirrer. The flask with content was cooled in ice cold water for 3-4 hours. The complex formed was
separated by filtration and washed with alcohol and ether. The complex was recrystallised using methanol and pet. ether. Crystals were dried on $\text{H}_2\text{SO}_4$ in a vacuum deciccator.

When 2-furfurylidene-2-aminothiazole was used in the preparation of complex, few drops piperidine was added during the reaction.

The ligands used in preparation of complexes were:

(i) 2-furfurylidene-2-aminopyridine (ii) 2-furfurylidene-2-amino-3-methylpyridine (iii) 2-furfurylidene-2-aminothiazole.

C V. Preparation of Zn(II), Cd(II), Hg(II) complexes of hydrazones

These complexes were prepared according to the method detailed elsewhere$^8$. In the preparation calculated quantity of ligand (0.02M) in ethanol was taken in a round bottom flask and warmed. To this warm solution of ligand a metallic salt solution of (0.01M) in ethanol was added slowly during the reaction little quantity sodium acetate was added. The mixture was refluxed for 1 hour using a magnetic stirrer. The excess of solvent was removed and flask with content was cooled in cold water for half an hour. Complex was separated by filtration, and washed with little alcohol then dried over $\text{P}_2\text{O}_5$ in vacuum deciccator. The complexes formed were of bright yellow and yellow colour. The ligands used in preparation
of complexes were (i) salicylidene-2-furoylhydrazide, (ii) o-vanillidene-2-furoylhydrazide.

F. Analytical methods

The elemental analysis of complexes for metal, nitrogen, chloride and sulphur was carried out by the following standard methods.

Estimation of metals

Accurately weighed complex (about 0.15 gm) was decomposed with concentrated nitric acid or mixture of perchloric acid and conc. HCl (10-15 ml) in a beaker covered with a watch glass. It was digested on a sand bath at low temperature and heating was continued for complete decomposition. Then it was treated with concentrated H$_2$SO$_4$ or HCl (5 ml). The heating was continued till the last traces of nitric acid was removed (confirmed by the evaluation of white fumes of SO$_3$). The above procedure was repeated 2-3 times. Then the resulting solution was cooled to room temperature and diluted with distilled water (100-150 ml). This solution was used for the quantitative estimation of cobalt nickel, copper, zinc, cadmium, mercury tin, uranium and thorium ions.

Estimation of uranium

Decomposed uranium solution was taken, metal hydroxide was precipitated by the addition of ammonia with stirring. Ammonium nitrate
was added in order to effect the coagulation. The precipitate was filtered using whatman filter paper (41) after allowing for sometime to settle, and washed with distilled water and weighed as $U_3O_8$ after ingition.

**Estimation of thorium**

The thorium solution of the decomposed complex was cooled to room temperature and diluted with 100-120 ml of distilled water. Thorium in the solution was precipitated as thorium hydroxide with the addition of sufficient quantity of 1:1 ammonia. The precipitate was digested for half an hour and filtered through quantitative filter paper (No. 41) washed until free from $SO_4^{2-}$ ions and dried on hot air cone. Finally it was ignited in previously weighed silica crucible and weighed as thorium oxide $ThO_2$.

**Estimation of cobalt**

The cobalt solution of the decomposed complex was diluted to about 100 ml with distilled water. The mineral acids were neutralised with excess of ammonia solution and the pH was adjusted to 5-6 with acetic acid. To the above solution, sodium acetate (5 gm) was added and warmed to 70°C. The cobalt(II) was precipitated by adding a slight excess of 2% alcoholic oxine solution. Then it was allowed to settle, the precipitated complex was filtered off through a previously weighed sintered
glass crucible and washed with hot water. The precipitate was
dried to constant weight at 125-135°C and weighed as Co(C₆H₅ON)₂.

Estimation of nickel

A few drops of concentrated hydrochloric acid was added to the nickel solution of decomposed complex, and heated to 70-80°C. The nickel(II) was precipitated as nickel(II) dimethylglyoxime by addition of slight excess of 1½ ethanolic solution of dimethylglyoxime, followed by immediate addition of dilute ammonia solution (1:4) dropwise with constant stirring, until the precipitation started and then in slight excess. The resulting complex precipitated was allowed to stand on water bath for 20-30 min, and the supernatent solution was tested for completion of precipitation. The solution was cooled to room temperature and filtered through previously weighed sintered glass crucible. The precipitate was washed with distilled water until free from chloride. It was dried at 110-120°C for about one hour and weighed as Ni(C₄H₇N₂O₂)₂.

Estimation of copper

The copper solution of the decomposed complex was treated with 2N sodium hydroxide to neutralise the mineral acids, until a slight permanent precipitate was formed, and then precipitate was just dissolved by adding dilute acetic acid. To this solution salicylaldoxime reagent was added dropwise with constant and
vigorous stirring, in slight excess at room temperature. The precipitated complex thus obtained was filtered through previously weighed sintered glass crucible, then it was dried at 100-105°C to a constant weight for about one hour and weighed as Cu(C$_7$H$_6$O$_7$N)$_2$.

Estimation of zinc

The zinc solution of the decomposed complex was treated with concentrated hydrochloric acid (1 ml). It was then neutralised by ammonium hydroxide solution (1:1) using methyl red indicator. To the nearly boiling solution of zinc, diammonium hydrogen-phosphate (A.R. Grade) solution (10-25 ml) was added slowly. A flocculant precipitate of zinc ammonium phosphate was formed, which was digested on water bath for about an hour to form crystalline precipitate. This was allowed to cool to room temperature and kept over night. This was filtered through previously weighed sintered glass crucible. The precipitate was washed successively with diammonium hydrogen phosphate solution (1%) and then with aqueous ethanol (50%) to remove chloride and phosphate respectively, it was dried at 100-105°C to constant weight and weighed as ZnNH$_4$PO$_4$.

Estimation of cadmium

The cadmium solution of the decomposed complex was treated with slight excess of sodium carbonate solution to get turbidity.
The turbidity was just removed by addition of dilute acetic acid. The resulting solution was warmed to 60°C and sodium acetate (3-5 gm) was added. The cadmium in the solution was precipitated as pale yellow cadmium oxinate \([\text{Cd(C}_9\text{H}_6\text{NO})_2\text{]}_2\text{H}_2\text{O}\) by adding slight excess of ethanolic solution (2%) oxine. The mixture was heated nearly to boiling, kept for few minutes and was filtered through previously weighed sintered glass crucible. The precipitate was washed with hot water and it was dried at 130°C and weighed to a constant weight as \(\text{Cd(C}_9\text{H}_6\text{NO})_2\).

**Estimation of mercury**

The mercury solution of the decomposed complex was heated to about 40°C. The nitric acid (2N) was added dropwise to adjust the pH of the solution to 1.8. To this solution, ethanolic solution of \(-\text{nitroso-} -\text{nepthal (2\%)}\) was added dropwise with constant and vigorous stirring till the supernatent solution was distinctly yellow and the precipitate was granular. The mixture was allowed to stand at room temperature for half an hour and filtered through previously weighed sintered glass crucible. The residue was washed several times with cold distilled water till the washings were colourless. It was dried at 110°C and weighed to a constant weight as \(\text{Hg(C}_10\text{H}_6\text{NO}_2)_2\).

**Estimation of tin**

The tin solution of the decomposed complex was cooled to
room temperature and diluted with 100-200 ml of distilled water and paper pulp was added. Tin was precipitated as tin hydroxide with the addition of sufficient quantity of (1:1) ammonia. The precipitate was digested for half an hour and filtered through quantitative filter paper. It was then washed until free from sulphate ion and dried on hot air cone. Finally, it was ignited in a previously weighed silica crucible and weighed as SnO₂.

**Estimation of chloride**

The analysis for chloride in the complexes was carried out by fusing the accurately weighed amount of compound (150 mg) with a mixture of KOH and KNO₃ ('8:1 parts by weight 3 gm) followed by extraction of the fused mass with demineralised water. The solution was acidified with dilute nitric acid and slow addition of AgNO₃ solution in slight excess with constant stirring, until the precipitate co-agulated and the clear supernatent liquid was tested for complete precipitation with silver nitrate reagent. All these operations were carried out in subdued light. The beaker was kept in a dark place and a solution was allowed to stand for about one hour before filtration. The precipitate was filtered through previously weighed sintered glass crucible, washed with very dilute nitric acid (say 0.5 ml of concentrated acid in 200 ml of water) until the filtrate was free from silver ions. The precipitate was dried in an oven at 130-150°C to a constant weight. Thus the percentage of chloride was calculated using factor (0.24737).
Estimation of sulphur

The decomposed solution of sulphur containing complex was taken and diluted by adding 200 ml of distilled water. The solution was heated to boiling and treated with warm barium chloride solution (5%), adding dropwise with constant stirring till the precipitation was complete. The supernatant solution was tested for completion of precipitation. The solution was allowed to stand over night. It was then filtered through Whatman filter paper No. 40, washed with hot water, dried and ignited in silica crucible at red hot for 10-15 minutes. The crucible was allowed to cool and the residue was weighed as \( \text{BaSO}_4 \).

Estimation of nitrogen in hydrazone complexes

Pre-reductive treatment of hydrazone complexes

0.2 to 0.4 gm of the hydrazone complex was dissolved in 20 ml of alcohol and formaldehyde, contained in a round bottom flask of 100 ml capacity, after the addition of finely powdered zinc (90% pure) and concentrated HCl, the contents were heated at boiling point temperature for 30 minutes to reduce the hydrazone complex. A little SnCl\(_2\) was added, after 15 minutes to hasten the reaction between zinc and HCl. The solution was finally diluted with equal volume of water and nitrogen was estimated by kjeldhal's method.
Nitrogen in the Schiff base complex was estimated micro-
analytically by Dumas method.

G. Physico-chemical techniques employed

a) Electrical conductivity

Conductance measurements of complexes in (DMF) solution were
made to ascertain the nature of the complexes. The molar conductivity
of a solution was obtained from the formula.

\[
M = \frac{1000 \times \text{cell constant} \times \text{sp. conductance}}{\text{Molar concentration}}
\]

In the present study an Elico conductivity bridge, provided
with dip type platinised platinum electrode was employed for the
measurement of conductance of solutions. The cell constant was
determined by measuring the conductance of standard KCl solutions
whose specific conductance were known accurately. Usually
the solution of the order of $10^{-3}$ M concentration were employed for
the conductance measurements.

The molar conductance of the electrolyte in a particular
solvent depends upon various factors like viscosity, polarity and
donor property of the solvent. To assist the interpretation of
the experimental molar conductivity of the co-ordination-compounds, the molar conductivities of various types of electrolytes in a number of common solvents listed by Geary\textsuperscript{12} are given in the Table II a.

Magnetic susceptibility measurements

Magnetic susceptibility measurements of the paramagnetic complexes were made at room temperature by the Gouy method\textsuperscript{13}. The Gouy tube was packed uniformly with the complex without leaving space for the air gap, up to the mark. The tube was suspended vertically by means of an aluminium chain connected to the pan of a single pan mettler balance. The magnetic poles used, have the following significations.

(i) Pole gap = 3.5 cm  (ii) Diameter of the pole faces = 8 cms
(iii) Field applied = 10,000 gauss.

Under the magnetic field, the complex in the cylindrical shape experiences a magnetic gradient. This will naturally cause variations in the weight of the sample. Readings were noted, with and without the magnetic field, and the observation was repeated for three times. The mean of the observations is used to determine the apparent change in the weight ($F$). From this total Pull ($F'$) on the sample was calculated using equation
<table>
<thead>
<tr>
<th>Solvents</th>
<th>Type of electrolyte</th>
<th>Molar conductance (ohm$^{-1}$ cm$^2$ mole$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1:1</td>
<td>100-120</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>250-280</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>330-380</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>1:1</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>80</td>
</tr>
<tr>
<td>Nitrobenzene</td>
<td>1:1</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>45-60</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>80</td>
</tr>
<tr>
<td>Acetone</td>
<td>1:1</td>
<td>170-180</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>240-290</td>
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<tr>
<td>Dimethylformamide</td>
<td>1:1</td>
<td>75-85</td>
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<tr>
<td></td>
<td>1:2</td>
<td>140-170</td>
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<tr>
<td></td>
<td>1:3</td>
<td>200-260</td>
</tr>
<tr>
<td>Dimethyl sulphoxide</td>
<td>1:1</td>
<td>37-48</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>70-80</td>
</tr>
<tr>
<td></td>
<td>1:3</td>
<td>110</td>
</tr>
</tbody>
</table>
\[ F' = F = \delta \] where \( \delta \) is the pull (negative quantity) on the Gouy tube. The gram molar susceptibility \( \gamma_g \) of the sample was found using the relation

\[ \gamma_g = \frac{\alpha + \beta F'}{W} \]

where \( \alpha \) and \( \beta \) are constants for a particular Gouy tube.

\[ \alpha = 0.029 \times \text{specimen volume} \]
\[ \beta = \text{Tube calibration constant} \]
\[ W = \text{Weight of the sample taken}. \]

The product of gram susceptibility and the molecular weight of the sample yields the molar susceptibility \( \gamma_m \) which is used to calculate the magnetic moments. The molar susceptibility was corrected for diamagnetism of the constituents of the complex using Pascal's constants. The effective magnetic moment \( \mu_{\text{eff}} \) (BM) was calculated using the derivation.

\[ \mu_{\text{eff}} = 2.84 \sqrt{\gamma_m x T} \]

where
\[ m = \text{corrected molar susceptibility} \]
\[ T = \text{absolute temperature at which the values were recorded}. \]

Visible and near infrared spectra

Visible and near infrared spectra of copper(II) nickel(II) and
cobalt(II) the complexes were scanned on a Beckmann DMR-21 spectrometer (USA) in region 200-1200 nm in nujol mull.

Infrared spectra

Infrared spectra of samples were scanned using the following instruments.

i) Perkin Elmer-257 spectrometer in the region 4000-650 cm\(^{-1}\) in KBr pellets.

ii) Perkins-Elmer-577 grating spectrometer in the 650-200 cm\(^{-1}\) region in nujol mull.

Electron spin resonance

The ESR spectra of polycrystalline sample at room temperature were scanned for copper samples on EPR-E-4 spectrometer operating in the X-band region with 100 KHz modulation frequency using diphenyl-picryl-hydrazyl free radical (DPPH) as a g-marker.

Nuclear magnetic resonance spectra

The nuclear magnetic resonance spectra of ligands and their complexes were recorded on Varian T-60 spectrometer in DMSO-d\(_6\) solvent using TMS as an internal reference.
Some samples were also recorded on WH.270.FT.NMR spectrometer.

X-ray diffraction method

Powder X-ray patterns were recorded on Philips PW-1140/90, X-ray diffractometer attached with digital graphical and photographic assemblies, using CuKα (1.5418 Å) radiation at 12 mA and 32 KV.

Introduction to biological activity

One of the aspects of the use of coordination compounds is, their application as biologically active substances. The involvement of metal ions and their complexes in biological processes has been described in a number of monographs. Recently the attention of investigators has been attracted increasingly by biocoordination compounds which can be used as biologically active preparations in medicine and agriculture. The employment of complexes of microelements with biologically active substances, such as, vitamins, hormones, nucleic acids etc. is of interest in medical practice. Many medicinal drugs behave as ligands which interact specifically with the ions of particular metal or group of metals. A series of preparations with anticancer, antibacterial, and antiviral activities have been obtained in recent years on the basis of metal complexes. Some of these are used in clinical and experimental medicine.
In view of their toxicity and high phytocidal activity, inorganic salts of micro elements have not found extensive application in agriculture. However, complex containing microelements and organic compounds are as a rule more active biologically and less toxic. In assortment of chemical plant-protecting agents throughout the world, metal containing compounds occupy an important place. Thus, one of the groups of fungicides consist of various copper compounds which are widely employed in the fight against plant diseases.

The complexes proposed for use in agriculture are as a rule more active even at lower concentrations than, metal ions and organic molecules which enter into their compositions. They provide the micro-elements lacking by the plants, are not phytotoxic, have a broad spectrum of activity, are resistant to the action of light and heat, and, what is most important, are not toxic to warm blooded animals, or their toxicity is low. The complexes make it possible to combine in one molecule several biometals, or several different molecules with biological activity and acting synergistically, which results in an appreciable enhancement of the overall effect. The complexes act more slowly than salts, but their activity is more prolonged and involve no risk for agriculture-crops.

The use of complexes and salt mixed with other pesticides to prepare biocidal paints for the coating of the bottoms of ships and
other underwater construction is effective. The rapid development of biocoordination chemistry is directly related also to the study of the role of metal complexes in processes important in living organisms. One of such problems involves the study of the mechanism of the action of physiologically active substances on biological membranes, enzymes, etc. Here membrane-active compounds, promoting the selective transfer of metal ions through biological and artificial membranes which is basically related to the complex formation processes are of considerable importance.

Among the various classes of biologically active coordination compounds, Schiff base complexes containing -NNNO- and NNO, ONO, SNO, system as ligands have attracted attention, which play an important role in biological processes and have also found applications in pharmacological preparations and as an effective plant protecting agents are promising in this sense.

The Schiff bases are known to possess tuberculostic, fungicidal, bacteriostatic activities. The phenolic compounds in which azomethine group is situated ortho to the hydroxy group are known to yield chelates which possess fungidal properties. Salicyaldehyde derivatives are also known to possess these activities. The hydrazones are known to exhibit bacteriostatic and tuberculostic activities and are useful in therapy.
Co(II), Ni(II), and Cu(II) compounds are known to possess some biological activities. Tin(IV) compounds are reported to exhibit some biological activities fungicidal activities. Similarly Zn(II), Cd(II) and Hg(II) are found to be toxic to micro-organisms.

With this background of information we have screened most of the ligands and their complexes embodied in the thesis for their microbial activities.

**Material and methods**

Antimicrobial activity of test compounds was assessed against *Escherichia coli* (E. coli), *Bacillus cirraflagellous* (B.C) bacteria and *Candida albicans* (C.A), *Aspergillus niger*, (A.N). Fungi (as these are known phinogens of human body) by cup-plate method.

The following materials were used.

i) Nutrient agar.

ii) Sterilised petridishes and pipettes of 0.1 to 0.2 ml capacity.

iii) Cultures in nutrient broth.

iv) Sterilized test tubes containing solutions of the test compounds at known concentration.

**Preparation of media**

Nutrient agar was prepared by dissolving bacteriological
peptone (1.0%) meat extract (0.5%). Sodium chloride (0.5%) in distilled water and pH of the solution was adjusted to 7.4 by using sodium hydroxide solution (40%). This solution was filtered and agar was added (2%) and then sterilised for 30 min at 15 lbs pressure.

Preparation of sub-culture

The organisms used in the present study for the testing of antibacterial activity of the compounds were obtained from the biochemical laboratory stock. On the day of testing the organisms were sub-cultured in to sterile nutrient broth. After incubating the same for 3 hrs, the growth thus obtained was used as inoculum for the test. Glasswares pipettes and syringe and needles as well as test tubes were sterilized in autoclave at 15.16 lb pressure for 20 min.

Preparation of solutions of test compounds

10 mg of the test compound was dissolved in 1.0 ml of DMF in labelled sterile test tube thus giving final concentration of 1 gm (w/v) and these solutions were kept for 60 min.

Method of testing

The nutrient agar was melted in hot water and cooled at 45°C with gentle shaking to bring about uniform cooling. This was poured on to the petridishes 20-25 ml each and then allowed to
solidify. To this 0.5-0.6 ml of 18-24 hours old culture was added aseptically and the inoculum was spread evenly by using spreader.

Thereafter the cups were made by punching in to set-agar with sterile cork borer and scooping out punched part of the agar.

The diameter of each cup was 10 mm. To these cups, solution of test compounds in DMF were added with help of sterile pipette. The drug quantity in each cup was 0.1 ml. It was allowed to diffuse for about 1 hr. Then the plates were incubated at 37°C. The extent of inhibition was measured by the zone of inhibition produced in millimeter after 48 hours.

Aqueous phenol was used as a positive control and the solvent DMF was also run to know its activity as a control.

The results are summarised in individual chapters.
Preliminary studies on the effect of some ligands and complexes on the liver of an Indian Skipper frog "Rana cyanophlyctis"

Metals and other elements are fundamental to the basic survival of the animals including the human being. Many of the metals and specific metal complexes are essential for physiological and biochemical functions (e.g. Cu, Zn, Fe, Co, Mn). Some metals like Pb, Cd, Hg, As, have proven toxic to animals and human beings at exposure levels which have occurred in the environment. Metal exposures from many exposure routes inhalation, ingestion, skin transfer, transplacental, parenteral, do occur and health effects in humans have been reported for almost all metals (Kjellstrom et al.35). The metals in excess or lack of the metals constitutes a health hazard. (Fowler et al.36).

The present study was thus undertaken to investigate the effect of ligands and their complexes with different metals, on the physiology of animals.

The Schiff base studies have shown that the C=N- group has a considerable biological importance as it has a lone pair of electrons in either a \( \Pi \) or an sp\(^2\) hybridized orbital on trigonally hybridized nitrogen in the C=N- group. This is of fundamental biological importance. The variability of angles of hybridization makes possible the formation of nitrogen containing molecules with
all the delicate differences in physico-chemical properties necessary to produce the various phenomena of life (Dey 1974). The Schiff bases and some of their metabolic complexes have fungicidal properties (Dey 1974).

Material and methods

The frogs *R. cyanophlyctis* were collected from local ponds of Dharwar area. They were acclimatized to the laboratory conditions, before they were subjected to experimentation.

The frogs were divided into different groups each group containing six frogs. Each group of the frogs were kept in rectangular glass aquarium tanks (75 cm x 37.5 cm x 50 cm) containing tap water. The frogs were fed on alternate days with minced frog thigh muscles. The water of aquarium changed every day. The frogs of one of the groups were considered as a control without treatment. The frogs of remaining groups received 2 mg of the testing compounds (compounds were dissolved in little acetone and to this olive oil 1 ml is added as a carrier and solution was stirred with glass rod) by injection intraperitonially every day for five days. The total compound being 10 mg/animal.

The frogs were killed 24 hours after the last injection. The liver of control frogs and frogs of remaining groups were dissected out and weighed to the nearest mg. The liver of each frog was
The homogenised tissue with saline was kept for 24 hours at room temperature. Each sample was centrifuged for 20 minutes at 3000 rpm to get clear solution. Then 0.2 ml of this clear solution was taken for the estimation of total protein by the method of Lowery et al. using folin - (clear reagent i.e. FCR).

The standard solution of BAS protein was taken and optical density of the BAS solution with FCR was recorded using spectrophotometer. A graph of optical density against different concentrations of BAS was plotted. Similarly optical densities of different liver solutions were recorded at 660 nm on photospectrometer. By comparing the OD of samples with standards, (BAS values) the protein content in the liver were calculated.

The results were analysed according to students 't' test, (steel and Torrie) and the results were considered significant only when the computed values were more than the tabulated values at P = 0.05.

Experimental results and discussions are given in individual chapters.
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