CHAPTER II

Experimental Techniques

This chapter deals with the chemicals used, solvent purification, the analytical methods, various common physicochemical and biological techniques employed for the characterization of metal complexes at various stages. Details of the synthesis of the ligands and the individual complexes are described in the relevant chapters.

Chemicals

The following chemicals were used at various stages of this project work. The sources of the chemicals are also given.

1. Ruthenium trichloride monohydrate ---- Aldrich
2. Triphenyl phosphine ---- Aldrich
3. Thiphenyl arsine ---- Aldrich
4. Lithium chloride ---- Loba
5. Sodium perchlorate ---- Merck
6. 4-N, N’-Dimethylaminoaniline ---- Merck
7. 5-Chlorosalicylaldehyde ---- Aldrich
8. 5-Bromosalicylaldehyde ---- Aldrich
<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>5-Iodosalicylaldehyde</td>
<td>Aldrich</td>
</tr>
<tr>
<td>10</td>
<td>Silica gel</td>
<td>Merck</td>
</tr>
<tr>
<td>11</td>
<td>Salicylaldehyde</td>
<td>Aldrich</td>
</tr>
<tr>
<td>12</td>
<td>3,5-Dichlorosalicylaldehyde</td>
<td>Aldrich</td>
</tr>
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<td>13</td>
<td>3-Bromo-5-Chlorosalicylaldehyde</td>
<td>Aldrich</td>
</tr>
<tr>
<td>14</td>
<td>4-Fluoroaniline</td>
<td>Loba</td>
</tr>
<tr>
<td>15</td>
<td>Copper carbonate</td>
<td>Loba</td>
</tr>
<tr>
<td>16</td>
<td>Perchloric acid</td>
<td>Merck</td>
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<tr>
<td>17</td>
<td>Nickel acetate tetrahydrate</td>
<td>Loba</td>
</tr>
<tr>
<td>18</td>
<td>Cobalt acetate monohydrate</td>
<td>Loba</td>
</tr>
<tr>
<td>19</td>
<td>Silver nitrate</td>
<td>Merck</td>
</tr>
<tr>
<td>20</td>
<td>Sodium acetate</td>
<td>Merck</td>
</tr>
<tr>
<td>21</td>
<td>1,10-Phenanthroline</td>
<td>Merck</td>
</tr>
<tr>
<td>22</td>
<td>Lithium bromide</td>
<td>Merck</td>
</tr>
<tr>
<td>23</td>
<td>Anhydrous Calcium chloride</td>
<td>Merck</td>
</tr>
<tr>
<td>24</td>
<td>Potassium bromide</td>
<td>Merck</td>
</tr>
</tbody>
</table>
25. Silver chloride ---- Merck
26. Tetrabutyl ammonium perchlorate ---- Fluka
27. Tris hydrochloride ---- Himedia
28. Herring Sperm DNA ---- Himedia
29. Sodium chloride ---- Merck

These chemicals were used as purchased without any further purification.

Solvents

Common solvents benzene, acetone, ethanol, dry ethanol, 2-methoxy ethanol, methanol, chloroform, n-hexane, ether, dimethylsulphoxide, acetonitrile, petroleum ether and dimethylformamide used at various stages of this project were purified according to the standard procedures described either in Weiss Berger series [1] or in qualitative analysis by Vogel [2].

Instrumental methods

Electronic spectra

Electronic absorption spectral measurements were recorded in solution using JASCO V-550 UV-Vis spectrophotometer.
**FT-IR spectra**

Infrared spectra for all the complexes and ligands were recorded on a JASCO FT-IR 410 (4000-400) spectrophotometer. Potassium bromide disc was employed for sample preparation. The instrument was calibrated against polystyrene film.

**$^1$H NMR spectra**

$^1$H NMR spectra were recorded in CDCl$_3$ with TMS as an internal standard on a Joel GSX-400 / 500 MHz, FT NMR spectrophotometer.

**ESR spectra**

The ESR spectra in MeCN were obtained at 77K on a E-112 Varian ESR spectrometer. DPPH was used as an internal field marker.

**X-Ray Diffraction**

**Data collection**: Bruker APEX2 CCD diffractometer

**Absorption correction**: Multi-scan (SADABS; Bruker, 1999)

$T_{\text{min}} = 0.451$, $T_{\text{max}} = 0.573$

(expected range = 0.393–0.500)

16111 measured reflections

3975 independent reflections
2533 reflections with I > 2σ(I)

R_{int} = 0.027.

**Refinement:** \( R[F^2 > 2\sigma(F^2)] = 0.044 \)

\( wR(F^2) = 0.138 \)

S = 0.99

3975 reflections

164 parameters

H-atom parameters constrained

\( \Delta \rho_{\text{max}} = 0.72 \text{ e Å}^{-3} \)

\( \Delta \rho_{\text{min}} = -0.56 \text{ e Å}^{-3} \)

**Cyclic voltammetry**

Cyclic voltammetric measurements were carried out on a Bio-Analytical System (BAS) model CV-50W electrochemical analyzer. The three-electrode cell comprised a reference Ag/AgCl, counter electrode as platinum wire and working glassy carbon (GC) electrodes with surface area of 0.07 cm\(^2\). The GC was polished with 0.3 and 0.005 mm alumina before each experiment and if necessary the electrode was sonicated in distilled water for 10 min. Dissolved oxygen was removed by purging the solution with pure nitrogen gas for about 15 minutes before each experiment. Scanning the cyclic
voltammogram for a blank solution checked the purity of the supporting electrolyte and the solvent.

**Antibacterial Screening**

Antimicrobial activity was measured using the standard method of well diffusion on agar plates.

**Preparation of Nutrient Agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Peptone</td>
<td>0.5g</td>
</tr>
<tr>
<td>Beef extract</td>
<td>0.5g</td>
</tr>
<tr>
<td>Agar</td>
<td>2.0g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100cm³</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 2</td>
</tr>
</tbody>
</table>

**Sub culture of micro organisms**

The pure cultures of organisms were sub-cultured in nutrient broth. They were inoculated separately into nutrient broth and kept at 37 °C for 24h. After that they were kept into 4°C until use.

**Growth method**

- At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture of particular microorganism.
The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of nutrient broth medium.

The broth culture is incubated at 35°C for 8h.

After the incubation period broth culture became turbid.

Well diffusion method

Mueller-Hinton Agar Medium

Mueller-Hinton agar is considered to be the best for routine testing of bacteria for the following reasons.

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- Medium is transparent, so that the inhibition zone can be visualized clearly.
- It gives satisfactory growth of most non-fastidious pathogens.

Composition of Mueller Hinton Agar

- Beef, infusion form : 300 gm/lit
- Casein acid hydrolysate : 17.50 gm/lit
- Starch : 1.50 gm/lit
- Agar : 17 gm/lit
- pH : 7.3 ± 0.2 (at 25°C)

Preparation of Mueller-Hinton Agar
- Mueller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer’s instructions.

- Immediately after autoclaving allow it to cool in a 45 to 50°C water bath.

- Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed Petri dishes on a horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 40 to 45 ml for plates with a diameter of 100 mm.

- The agar medium should be allowed to cool to room temperature and unless the plate is used the same day, stored in a refrigerator.

- Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.

- A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24h or longer.

**Preparation of stock solution**

The stock solutions (3mg/lit) of the Schiff bases and the complexes were prepared in 10% MeCN / DMSO in methanol and were stored dry at room temperature. From the stock solution the various dilutions 0.15%, 0.20% and 0.25% are made.

**Antibiotic control**

Broad spectrum antibiotics, ampicillin was used as control drugs.
**Preparation of antibiotic stock solutions**

Powders of those two antibiotics (ampicillin) were bought from authorized medical shop. They were accurately weighed and dissolved in sterile distilled water and appropriate dilutions similar to that of newly synthesized test compounds 0.15%, 0.2% and 0.25% are made.

**Inoculation**

- A sterile cotton swab was dipped into the turbid culture suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will removes excess inoculum from the swab.

- The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar was swabbed.

- The lid may be left over for 3 to 5 minutes, but not more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug in to the wells.
DNA binding

Preparation of tris-hydrochloride buffer

Tris-hydrochloride (197 mg, 5 mm) and sodium chloride (730 mg, 50 mm) were accurately weighed and made up to 250 ml in a SMF using double distilled water. The pH of this solution was adjusted to 7.2 using 1 mm sodium hydroxide solution with the help of pH meter (EUTECH INSTRUMENT, pH 510) before making up to the mark. This buffer pH 7.2 was used for all DNA studies in the relevant chapters.

Absorption spectral titration

DNA binding experiment was recorded on a CARY 100 UV-Visible spectrometer at 25°C. Solution of Herring sperm DNA (SISCO) in the buffer (5 mM Tris and 50 mM NaCl, pH 7.2) gave the ratio of absorbance at 260 and 280 nm above 1.8, indicating that the DNA was sufficiently free of protein [3]. The concentration of DNA was determined using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm [4] and a stock of 100 µM is prepared. All experiments were carried out in Tris buffer at pH 7.2 in milli Q triply deionised water.
References


