3.1 INTRODUCTION:-

Ruthenium complexes draw attention of coordination chemists due to its vast application in different fields. Not only the complexes show antifungal and antibacterial activity but also they posses potential anticancer activities[1,2] Beside that the following factors attract interest towards the metal for sustaining research on its coordination chemistry:-

1. Mixed ligand complexes of thiosemicarbazone derived from thiophen-2-carboxaldehyde and cyclooctadiene with ruthenium(II)[3] shows significant antiamoebic property and they are more effective than metrodinazole against HK-9 strain of Entamoeba histolytica.

2. Certain complexes of ruthenium, specially [Ru(NH$_3$)$_6$]Cl$_3$ are used as accelerators of development of photographic plates[4].

3. The most important activity of ruthenium complexes is probably their catalytic activity. Wilkinson and co-workers had shown that [RuCl$_2$(PPh$_3$)$_3$] and complexes derived from it, are very good hydrogenating catalyst[5] towards aldehydes, ketones, alifinic and other unsaturations. Several ruthenium complexes like [Ru(CO)$_3$(PPh$_3$)$_2$] etc. are significantly efficient as hydroformylating catalyst[6-8].
4 Ruthenium complexes like \([\text{RuI}_2(\text{CO})_4]\) and \([\text{Ru}(\text{acac})_3]\) behave as catalyst precursors with NaI, HI and MeI as promoter in the carbonylation of methanol, dimethyl ether and methyl acetate[9]. The major products are ethanol from methanol, methyl acetate from dimethyl ether and acetic anhydride with acetic acid from methyl acetate. The complexes \([\text{RuCl}_2(\text{CO})_2\text{py}]\) and \([\text{RuCl}_2(\text{py})_4]\) catalyze the reduction of nitrobenzene by carbon monoxide and water[10].

5 Ruthenium chloride solution in aqueous 5M hydrochloric acid can catalyze the hydration of acetylene to produce acetaldehyde or propyne to give acetone. The complexes \([\text{RuCl}_4(\text{H}_2\text{O})_2]\) and \([\text{RuCl}_6(\text{H}_2\text{O})]^2\) are responsible for this catalytic activity[11].

6 Highly selective ruthenium catalyzed oxidation of alcohols can be achieved. For example, primary alcohols can be oxidized selectively to aldehydes stage using \(\text{RuCl}_2(\text{PPh}_3)_3\) as catalyst and iodosylbenzene diacetate as oxygen source[12].

7 Tris(2,2' -bipyridyl) ruthenium(II) dication \((\text{[Ru(bipy)})_3]^{+2}\) is one of the most extensively studied coordination compounds because of its potential use in cyclic photochemical water decomposition[13]. The visible spectrum of the complex shows an intense MLCT band at 452 nm which is close to the emission maximum of the sun. Furthermore, its photochemically excited state has a long lifetime and is thermodynamically capable of both oxidizing and reducing water at pH 7. In practice, however, the complex itself is incapable of splitting water, but its electron-transfer properties have been used for hydrogen production in a number of reactions involving co-catalysts.
The ruthenium red is used as a stain for electron microscopy and in optical microscopy, it is the preferred stain for pectins[14].

Ruthenium has an atomic number of 44, with electronic configuration [Kr] 4d⁷ 5s¹. The metal exhibits a wide range of oxidation states from VIII to –II. The most common oxidation states are II and III while the least commons are VII, V, I and –I. Small π donor ligands such as F, O²⁻, N³⁻ etc stabilize the higher oxidation states (e.g. Ru⁷⁺O₄⁴⁻, [Ru⁶⁺NC₃]⁺ etc.) on the other hand π acceptor ligands like CO, PR₃ stabilize the lower oxidation states ( e.g. Ru⁴⁺(CO)₄⁺², Ru⁰(CO)₂ etc.). The ligands which are good σ donors but not π acceptors or donors (e.g. H₂O, NH₃) are usually associated with Ru⁴⁺ and Ru⁶⁺.

PRESENT STUDY:-

From the discussion stated above it is obvious that the coordination complexes of ruthenium(II) are of importance, particularly those with ruthenium-nitrogen and ruthenium sulphur coordination. So a lot of study has been done with ruthenium complexes with nitrogen and sulphur as coordination centers as discussed earlier in chapter 1. But the ruthenium(II) complexes with aromatic thiohydrazide as ligands have not been explored till this study. So in this chapter an attempt has been made to synthesis the corresponding complexes, determine their structure and point of coordination of the ligands with ruthenium(II) and to study their characteristics. The studies include electronic, infra red and nmr spectral analyses along with cyclic
voltammetry and other routine physical studies. Antibacterial activity of one of the complexes along with activity of the complexes against HEla cell line were studied.

**EXPERIMENTAL:**

**Apparatus:** Carbon, hydrogen and nitrogen analyses and magnetic moment measurements were carried at I.A.C.S. Kolkata, electronic spectra were taken with Hitachi-U3210 spectrophotometer, $^1$H nmr with bruker DRX 500 instrument and infrared spectra were taken using KBr disks between 4000-300 cm$^{-1}$ on Unicam 300S and FTIR were taken on Jasco 680 plus instrument.

**Reagents:** RuCl$_3$.xH$_2$O (J.M, U.K.) was used to prepare solution of ruthenium(III). All reagents used were of analytical grade and were employed without further purification. Solvents for spectrophotometric studies were used after proper purification. *Escherichia coli* ($K_{12}$) strain was obtained from the Department of Botany, Presidency College, Kolkata. Fresh cultures were prepared on MacConkey Agar (Himedia, India) every weak. Broth was prepared using Mueller Hinton Broth (Himedia, India). HEla was obtained from National Center for Cell Sciences, Pune, India.

**Preparation of the Ruthenium complexes:**

The ruthenium(II) complexes of substituted thiohydrazide ligands were prepared according to the following procedure:-
Ruthenium complex of thiobenzhydrazide Ru(thb)₂(Hthb) :-
RuCl₃ solution in dilute HCl (10.0 mL) containing 18 mg ruthenium/10 mL was evaporated nearly to dryness. To this was added ~160 mg of thiobenzhydrazide ligand in 6 N hydrochloric acid. The resulting mixture was digested on water bath for about half an hour and a beautiful violet complex was obtained. It was thoroughly washed with hot water to remove any excess ligand followed by cold distilled water to remove all adhered hydrochloric acid. The compound was dried in vacuum desiccator. Yield:- 80% based on starting ruthenium. The purity of the compound was examined by TLC.

On the basis of TLC result, the micro crystalline complex was further purified by column chromatography. Silica gel supported column was used to absorb the complex which was eluted by ethyl acetate-petroleum ether (boiling range 60-80°C) mixture. A white band comprising oxidized product of the ligand was eluted first followed by an intense violet band of desired complex. The main complex eluted at 1:10 ethyl acetate-petroleum ether (boiling range 60-80°C) of the eluent.

Ruthenium complex of ortho-hydroxythiobenzhydrazide Ru(o-thb)₂(o-Hthb) :-
RuCl₃ solution in dilute HCl (10.0 mL) containing 18 mg ruthenium/10 mL was evaporated nearly to dryness. To this was added ~180 mg of ortho-hydroxythiobenzhydrazide ligand in 6N hydrochloric acid medium. The resulting mixture was digested on water bath for about half an hour to get violet coloured complex. It was thoroughly washed with hot water to remove any excess ligand. All adhered hydrochloric acid was removed by washing with cold distilled water. The
compound was dried in vacuum desiccator. Yield: 85% based on starting ruthenium.

TLC experiment was done to examine the purity of the compound.

TLC experiment showed a secondary spot along with the main spot with sufficient difference in \( R_f \) values. Based on that experiment the micro crystalline complex was further purified by column chromatography. Silica gel supported column was used to absorb the complex. It was eluted by ethyl acetate- petroleum ether (boiling range 60-80°C) mixture. A white band comprising oxidized product of the ligand was eluted first followed by an intense violet band of desired complex. The main complex eluted at 1:10 ethyl acetate- petroleum ether (boiling range 60-80°C) of the eluent.

**Ruthenium complex of furan-2-thiohydrazide** \( \text{Ru} \text{(ffth)}_2 \text{(ffth)} \) :-

\( \text{RuCl}_3 \) solution in dilute HCl (10.0 mL) containing 18 mg ruthenium/10 mL was evaporated nearly to dryness. To this was added ~150 mg of *furan-2-thiohydrazide* ligand in 6N hydrochloric acid medium. A beautiful violet complex was obtained on digestion of the resulting mixture on water bath for about half an hour. It was collected and thoroughly washed with hot water to remove any excess ligand followed by washing with cold distilled water to remove all adhered hydrochloric acid. The compound was dried in vacuum desiccator. Yield: 80% based on starting ruthenium.

The purity of the compound was examined by TLC.

On the basis of TLC result, the micro crystalline complex was further purified by column chromatography. Complex solution in chloroform was absorbed in silica gel
supported column. The complex was eluted by ethyl acetate-petroleum ether (boiling range 60-80°C) mixture. A white band comprising oxidized product of the ligand was eluted first at 3:100 ethyl acetate: pet ether composition of the eluent. It was then followed by an intense violet band of desired complex. The main complex eluted at 1:10 ethyl acetate- petroleum ether (boiling range 60-80°C) of the eluent.

Ruthenium complex of thiophen-2-thiohydrazide Ru(thth)_2(Hthh) :-

The working solution containing RuCl₃ in dilute HCl (10.0 mL) (concentration ~18 mg ruthenium/10 mL) was evaporated nearly to dryness. The mass was re-dissolved in 6N HCl and added ~170 mg of thiophen-2-thiohydrazide ligand. A beautiful violet complex was obtained on digestion of the resulting mixture on water bath for about half an hour. It was collected and thoroughly washed with hot water to remove any excess ligand. To remove all adhered hydrochloric acid washing with cold distilled water was done thoroughly. The compound was dried in vacuum desiccator. Yield:- 85% based on starting ruthenium. TLC experiment was done to examine the purity of the compound.

TLC result of the prepared complexes showed a secondary spot along with the main spot. So the micro crystalline complex was further purified by column chromatography. Complex solution in chloroform was absorbed in silica gel supported column. The complex was eluted by ethyl acetate- petroleum ether (boiling range 60-80°C) mixture. A white band comprising oxidized product of the ligand was eluted first at 3:100 ethyl acetate: pet ether composition of the eluent. It was then followed by an intense violet
band of desired complex. The main complex eluted at 1:10 ethyl acetate- petroleum ether (boiling range 60-80°C) of the eluent.

Preparation of the broth:-
In 1L of distilled water 21.0 gm of the Mueller Hinton broth was suspended, heated to dissolve and sterilized by autoclaving at 15 psi pressure and at 121°C for 15 minutes.

Preparation of the agar media:-
In 1L of distilled water 38.0 gm of the MacConkey agar was suspended, mixed well and heated to boiling to dissolve it completely. It was then sterilized by autoclaving at 15 psi pressure and at 121°C for 15 minutes.

Preparation of the stock solution for study of antibacterial property:-
The stock solution for bactericidal study was prepared by dissolving 3 mg of ruthenium ortho-hydroxysthiobenzhydrazide complex (Ru(o-htbh)2(o-Hhtbh)) in 5 mL of distilled DMSO.

Methodology:-
Different volume of the stock solution (viz. 0.2 mL, 0.4 mL and 0.6 mL) were added to the broth previously inoculated with 0.2 mL of Escherichia coli (K12) grown in Muller-Hinton broth to get a final volume of 10 mL. Appropriate volume of distilled DMSO was added to each of the set so that total volume with respect to DMSO be 0.6 mL in
each set. Control was made using 0.6 mL of DMSO, 0.2 mL inoculum and 9.2 mL of broth. In control 0.6 mL DMSO was added to study whether DMSO has any bactericidal activity at this concentration. The culture tubes were then placed in a shaker incubator for 24 hrs. at 37°C. After 24 hours, 0.2 mL of the broth from each culture tube was drawn, diluted to 50mL with physiological saline. 0.2 mL of this suspension was further diluted to 50mL with physiological saline. 0.2 mL of this final suspension was added to 10 mL of Muller Hinton Agar previously melted and maintained at 50°C. It was then poured in a petri dish, immediately covered and incubated for 24 hrs. at 37°C. Number of the colonies was counted.

**Study of action on tumour cells :-**

**Thymidine Uptake:**

HELA cells (1x10^4/well) were cultured in 96 well flat bottom microtiter plates with varying concentrations of the drug for 48 hours in 0.2 mL medium in triplicate. Cell proliferation was quantified by incubation of cells with 0.5 mCi of [methyl-^3H] thymidine during last 18 hours of culture. The cells were harvested onto filters and radioactivity (mean cpm) was measured in a liquid scintillation counter (Perkin Elmer Life Sciences Inc., Boston, MA).
RESULTS AND DISCUSSION:

The properties of prepared ruthenium(II) complexes of thiohydrazide ligands are being discussed here.

Table-3.01.a- Analytical data of Ru(tbh)$_2$(Htbh) i.e. Ru(C$_7$H$_7$N$_2$S)$_2$(C$_7$H$_6$N$_2$S):-

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found(%)</td>
<td>44.82</td>
<td>3.67</td>
<td>14.87</td>
</tr>
<tr>
<td>Anal. Calculated(%)</td>
<td>44.97</td>
<td>3.93</td>
<td>14.99</td>
</tr>
</tbody>
</table>

The prepared micro crystalline compound is beautiful violet in colour, found to be soluble in chloroform, dichloromethane, carbon tetrachloride, ethanol, ethyl acetate, acetonitrile, benzene, dimethyl sulphoxide and in dimethyl formamide. Molar conductance in DMSO is nearly 2x10$^{-5}$ mho. Magnetic moment measurement shows the compound as diamagnetic in nature.

Elemental analyses of the prepared complex reveals a concurrence with calculated values indicating a 1:3 metal: ligand complex formation. Decomposition temperature is observed to be greater than 250°C.

Table-3.01.b: Analytical data of Ru(o-htbh)$_2$(o-Hhtbh) i.e.Ru(C$_7$H$_7$N$_2$OS)$_2$(C$_7$H$_6$N$_2$OS):-

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found(%)</td>
<td>42.20</td>
<td>3.38</td>
<td>13.32</td>
</tr>
<tr>
<td>Anal. Calculated(%)</td>
<td>41.79</td>
<td>3.65</td>
<td>13.93</td>
</tr>
</tbody>
</table>
The prepared micro crystalline compound is red-violet in colour, found to be soluble in chloroform, dichloromethane, carbon tetrachloride, ethanol, ethyl acetate, acetonitrile, benzene, dimethyl sulphoxide and in dimethyl formamide. Molar conductance in DMSO is nearly $5 \times 10^{-5}$ mho. Magnetic moment measurement shows the compound as diamagnetic in nature.

Elemental analyses of the prepared complex reveals a concurrence with calculated values indicating a 1:3 metal: ligand complex formation. Decomposition temperature is found to be greater than 250°C.

Table-3.01,c:-

Analytical data of Ru(fth)$_2$(Hfth) i.e. Ru(C$_4$H$_5$N$_2$OS)$_2$(C$_3$H$_6$N$_2$OS) :-

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found(%)</td>
<td>34.02</td>
<td>2.91</td>
<td>16.22</td>
</tr>
<tr>
<td>Anal.Calculated(%)</td>
<td>34.28</td>
<td>3.05</td>
<td>16.00</td>
</tr>
</tbody>
</table>

The prepared compound is red violet micro crystalline solid, soluble in chloroform, dichloromethane, carbon tetrachloride, ethanol, ethyl acetate, acetonitrile, benzene, dimethyl sulphoxide and in dimethyl formamide. Molar conductance in DMSO is nearly $1 \times 10^{-5}$ mho. Magnetic moment measurement shows the compound as diamagnetic in nature.
Elemental analyses of the prepared complex reveals a concurrence with calculated values indicating a 1:3 metal: ligand complex formation. Decomposition temperature is greater than 250°C.

**Table-3.01.d:-**

Analytical data of Ru(tht)₂(Htht) i.e. Ru(C₅H₂N₂S₂)₂(C₅H₆N₂S₂)₂:

<table>
<thead>
<tr>
<th></th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Found(%)</td>
<td>31.11</td>
<td>2.47</td>
<td>14.38</td>
</tr>
<tr>
<td>Anal.Calculated(%)</td>
<td>31.41</td>
<td>2.79</td>
<td>14.66</td>
</tr>
</tbody>
</table>

Colour of the prepared complex is violet, it is found to be soluble in chloroform, dichloromethane, carbon tetrachloride, ethanol, ethyl acetate, acetonitrile, benzene, dimethyl sulphoxide and in dimethyl formamide also. Molar conductance is nearly $3 \times 10^{-5}$ mho in DMSO. It is a diamagnetic compound as per magnetic moment measurement studies.

Elemental analyses of the prepared complex reveals a concurrence with calculated values indicating a 1:3 metal: ligand complex formation. Decomposition temperature is observed to be greater than 250°C.
3.2 ELECTRONIC SPECTRA:-

The electronic spectra of the prepared ruthenium(II) complexes of thiohydrazide ligands has been studied in different solvents viz. chloroform, dichloromethane, carbon tetrachloride, ethanol, ethyl acetate, acetonitrile and in dimethyl sulphoxide. For ethanolic solution spectra is taken in the range 210-800 nm whereas for other solvents lower limit is 250 nm due to high absorption of the solvents in lowest wave length limit. The solutions were prepared with concentration 1mg/10mL and were diluted accordingly as required. Assignment of transitions for the complexes have been made considering six coordinated geometry on the basis of [Ru(bipy)$_3$]$^{2+}$ [15] and other related complexes[16-19].
The simplified molecular orbital diagram for ruthenium(II) system[20] is given:-

\[
\begin{align*}
\pi_1^* & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad
\end{align*}
\]

On examination of molecular orbital diagram for the ruthenium(II) system it is evident that the highest occupied molecular orbitals are predominantly ruthenium d orbital. π-bonding orbital of ligand lie just below the occupied d orbitals. Where as the lowest unoccupied molecular orbital i.e. LUMO is ligand based π* orbital.
The thiohydrazide complexes of ruthenium(II) show three to four maxima in the region 210-800 nm. The bands are very intense \([E>1000 \text{ M}^{-1}\text{cm}^{-1}]\) and assigned as metal to ligand \([\text{M} \rightarrow \text{L}]\) or ligand to metal \([\text{L} \rightarrow \text{M}]\) charge transfer transitions or ligand centered transitions \([\text{L} \rightarrow \text{L}]\).

An attempt has been made to identify different transitions of the prepared ruthenium(II) complexes in different solvents along with a detailed look at the electronic spectra of such complexes.

**Ruthenium complex of thiobenzhydrazide \(\text{Ru(tbh)}_2(\text{Htbh})\):**

The ethanolic solution of ruthenium thiobenzhydrazide complex exhibits three peaks in the range of 200-800 nm. An intense peak responsible for violet colour of the complex is observed at 538 nm. This intense band has been assigned as metal to ligand charge transfer transition i.e. transition from ruthenium 4d \(\rightarrow\) \(\text{L(\pi^*)}\). Another weak absorption band was observed near 400 nm. This band appeared as shoulder on the left hand side of the previous band. This band may also be assigned as MLCT band.

A very intense and sharp band is observed at \(\sim\) 310 nm and this band is definitely due to ligand centered transition.

The electronic spectra of \(\text{Ru(tbh)}_2(\text{Htbh})\) in ethanol, acetone, acetonitrile, methanol, dimethyl sulphoxide and isoamyl acetate are reported in the figures 3.1a, 3.1b, 3.1c,
3.1d, 3.1e and 3.1f respectively. The table 3.02.a represents the electronic absorption data of the complex along with proposed assignment of transitions.

**Table 3.02.a:** Electronic absorption data of ruthenium-thiobenzhydrazide complex in different solvents.

<table>
<thead>
<tr>
<th></th>
<th>Ethanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>Methanol</th>
<th>DMSO</th>
<th>Isoamyl</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A&lt;sub&gt;max&lt;/sub&gt;/nm</td>
<td>538</td>
<td>540</td>
<td>530</td>
<td>537</td>
<td>549</td>
<td>531</td>
<td></td>
</tr>
<tr>
<td></td>
<td>410</td>
<td>420</td>
<td>419</td>
<td>402</td>
<td>410</td>
<td>420</td>
<td></td>
</tr>
<tr>
<td></td>
<td>313</td>
<td>327</td>
<td>287</td>
<td>286</td>
<td>320</td>
<td>318</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>240</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Assignment**
- MLCT
- L→L

Fig. 3.1a Electronic spectrum of Ru(tbh)<sub>2</sub>(Htbh) in ethanol (concentration 1.79 x 10<sup>-5</sup> M):
Fig 3.1.b Electronic spectrum of Ru(tbh)$_2$(Htbh) in acetone (concentration 1.79 x 10$^5$ M):

Fig 3.1c Electronic spectrum of Ru(tbh)$_2$(Htbh) in acetonitrile (concentration 1.79 x 10$^5$ M):
Fig 3.1d Electronic spectrum of Ru(tbbh)$_2$(Htbh) in methanol (concentration $1.79 \times 10^{-5}$ M):

Fig 3.1e Electronic spectrum of Ru(tbbh)$_2$(Htbh) in DMSO (concentration $1.79 \times 10^{-5}$ M):
Fig 3.1f (i) Electronic spectrum of Ru(tbh)$_2$(Htbh) in isoamyl acetate 
(concentration 1.79 x $10^{-5}$ M):

Fig 3.1f (ii) Electronic spectrum of Ru(tbh)$_2$(Htbh) in isoamyl acetate 
(concentration 1.79 x $10^{-6}$ M):
Ruthenium complex of *ortho-hydroxy thiobenzhydrazide* Ru(o-htbh)$_2$(o-Hhtbh):-

The ruthenium complex of ortho-hydroxy thiobenzhydrazide in ethanolic solution exhibits mainly four peaks in the 210-800 nm region. An intense peak at 544 nm is observed which is responsible for violet colour of the complex. This intense band has been assigned as metal to ligand charge transfer transition i.e. transition from ruthenium 4d to vacant anti bonding orbital L($\pi^*$). Another weak absorption band is observed as shoulder near 460 nm on the left hand side of the previous band. This band may also be assigned as MLCT band.

A very intense and sharp band is observed at ~ 317 nm and this band is definitely due to ligand centered transition (L$\rightarrow$L). The very intense band at 248 nm may be transition from ligand $\pi$ orbital to unoccupied $e$ orbital of the metal i.e. LMCT type of transition.

The electronic spectra of Ru(o-htbh)$_2$(o-Hhtbh) in ethanol, acetone, acetonitrile, methanol, dimethyl sulphoxide and isoamyl acetate are given in the figures 3.2a, 3.2b, 3.2c, 3.2d, 3.2e and 3.2f respectively. The table 3.02.b represents the electronic absorption data of the complex along with proposed assignment of transitions.

<table>
<thead>
<tr>
<th>$\lambda_{\text{max/\text{nm}}}$</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Acetone</td>
</tr>
<tr>
<td>544</td>
<td>545</td>
</tr>
<tr>
<td>460</td>
<td>465</td>
</tr>
<tr>
<td>317</td>
<td>326</td>
</tr>
<tr>
<td>248</td>
<td>247</td>
</tr>
</tbody>
</table>
Fig 3.2 a Electronic spectrum of Ru(o-hthb)$_2$(o-Hhtbh) in ethanol
(concentration $1.66 \times 10^{-5}$ M):

Fig 3.2 b Electronic spectrum of Ru(o-hthb)$_2$(o-Hhtbh) in acetone
(concentration $1.66 \times 10^{-5}$ M):
Fig 3.2 c Electronic spectrum of Ru(o-hibh)₂(o-Hhtbh) in acetonitrile (concentration $1.66 \times 10^5$ M):

Fig 3.2 d Electronic spectrum of Ru(o-hibh)₂(o-Hhtbh) in methanol (concentration $1.66 \times 10^5$ M):
Fig 3.2 e Electronic spectrum of Ru(o-htbh)$_2$(o-Hhtbh) in DMSO
(concentration 1.66 x 10$^{-5}$ M):

Fig 3.2 f Electronic spectrum of Ru(o-htbh)$_2$(o-Hhtbh) in isoamyl acetate
(concentration 1.66 x 10$^{-5}$ M):
Ruthenium complex of furan-2-thiohydrazide \( \text{Ru}(\text{fth})_2(\text{Hfth}) \):

The ruthenium complex of furan thiohydrazide in ethanolic solution exhibits four peaks in the 210-800 nm region. The transition responsible for the violet colour of the complex is observed as an intense peak at 555 nm. This intense band has been assigned as metal to ligand charge transfer transition i.e. transition from ruthenium 4d to vacant anti bonding orbital \( L(\pi^*) \). Another weak absorption band is observed as shoulder near 470 nm on the left hand side of the previous band. This band may also be assigned as MLCT band.

An intense and sharp ligand centered transition (\( L\rightarrow L \)) band is observed at \( \sim 313 \) nm.

The very intense band at 276 nm may be due to transition from ligand \( \pi \) orbital to unoccupied \( e \) orbital of the metal i.e. LMCT type of transition.

The electronic spectra of \( \text{Ru}(\text{fth})_2(\text{Hfth}) \) in ethanol, acetone, acetonitrile, methanol, dimethyl sulphoxide and isoamyl acetate are given in the figures 3.3a, 3.3b, 3.3c, 3.3d, 3.3e and 3.3f respectively. The table 3.02.c represents the electronic absorption data of the complex along with proposed assignment of transitions.

**Table 3.02.c:** Electronic absorption data of ruthenium-furan-2-thiohydrazide complex in different solvents.

<table>
<thead>
<tr>
<th>( \lambda_{\text{max/nm}} )</th>
<th>Ethanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>Methanol</th>
<th>DMSO</th>
<th>Isoamyl Acetate</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>554</td>
<td>556</td>
<td>553</td>
<td>565</td>
<td>555</td>
<td>MLCT</td>
<td></td>
</tr>
<tr>
<td>470</td>
<td>468</td>
<td>460</td>
<td>449</td>
<td>466</td>
<td>460</td>
<td>MLCT</td>
<td></td>
</tr>
<tr>
<td>313</td>
<td>327</td>
<td>322</td>
<td>323</td>
<td>327</td>
<td>319</td>
<td>L(\rightarrow)L</td>
<td></td>
</tr>
<tr>
<td>276</td>
<td>284</td>
<td>285</td>
<td>266</td>
<td>262</td>
<td>262</td>
<td>LMCT</td>
<td></td>
</tr>
</tbody>
</table>

95
Fig 3.3a Electronic spectrum of Ru(fth)$_2$(Hfth) in ethanol (concentration $1.9 \times 10^{-5}$ M):

Fig 3.3b Electronic spectrum of Ru(fth)$_2$(Hfth) in acetone (concentration $1.9 \times 10^{-5}$ M):
Fig 3.3 c Electronic spectrum of Ru(fth)$_2$(Hfth) in acetonitrile (concentration 1.9 x $10^5$ M):

Fig 3.3 d Electronic spectrum of Ru(fth)$_2$(Hfth) in methanol (concentration 1.9 x $10^5$ M):
Fig 3.3 e Electronic spectrum of Ru(fth)$_2$(Hfth) in DMSO (concentration 1.9 x $10^{-5}$ M):

Fig 3.3 f Electronic spectrum of Ru(fth)$_2$(Hfth) in isoamyl acetate (concentration 1.9 x $10^{-5}$ M):
The ruthenium complex of thiophen-2-thiohydrazide in ethanolic solution exhibits mainly four peaks in the 210-800 nm region. An intense peak near 556 nm is observed. This intense band has been assigned as metal to ligand charge transfer transition i.e. transition from ruthenium 4d to vacant anti bonding orbital L(\pi^*). The violet colour of the complex is due to this transition. Near 460 nm, on the left hand side of the previous band, another weak absorption band is observed as shoulder. This band may also be assigned as MLCT band.

A very intense and sharp band is observed at ~310 nm and this band is probably due to ligand centered transition (L \rightarrow L). The very intense band at 266 nm may be transition from ligand \pi orbital to unoccupied \epsilon orbital of the metal i.e. LMCT type of transition.

The electronic spectra of Ru(tth)_2(Htth) in ethanol, acetone, acetonitrile, methanol, dimethyl sulphoxide and isoamyl acetate are given in the figures 3.4a, 3.4b, 3.4c, 3.4d, 3.4e and 3.4f respectively. The table 3.02.d represents the electronic absorption data of the complex along with proposed assignment of transitions.

<table>
<thead>
<tr>
<th>( \lambda_{\text{max/\text{nm}}} )</th>
<th>Ethanol</th>
<th>Acetone</th>
<th>Acetonitrile</th>
<th>Methanol</th>
<th>DMSO</th>
<th>Isoamyl Acetate</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>556</td>
<td>560</td>
<td>561</td>
<td>556</td>
<td>564</td>
<td>558</td>
<td>MLCT</td>
<td></td>
</tr>
<tr>
<td>460</td>
<td>470</td>
<td>465</td>
<td>464</td>
<td>470</td>
<td>465</td>
<td>MLCT</td>
<td></td>
</tr>
<tr>
<td>310</td>
<td>325</td>
<td>312</td>
<td>337</td>
<td>324</td>
<td></td>
<td>L \rightarrow L</td>
<td></td>
</tr>
<tr>
<td>266</td>
<td>265</td>
<td>266</td>
<td>267</td>
<td>260</td>
<td></td>
<td>LMCT</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.02.d: Electronic absorption data of ruthenium-thiophen-2-thiohydrazide complex in different solvents.
Fig 3.4 a Electronic spectrum of Ru(tth)$_2$(Htth) in ethanol (concentration 1.75 x 10$^{-5}$ M):

Fig 3.4 b Electronic spectrum of Ru(tth)$_2$(Htth) in acetone (concentration 1.75 x 10$^{-5}$ M):
Fig 3.4 c Electronic spectrum of Ru(tth)$_2$(Htth) in acetonitrile (concentration 1.75 x 10$^{-5}$ M):

Fig 3.4 d Electronic spectrum of Ru(tth)$_2$(Htth) in methanol (concentration 1.75 x 10$^{-5}$ M):
Fig 3.4 e Electronic spectrum of Ru\((\text{th})_2(\text{Hth})\) in DMSO (concentration 1.75 x 10\(^{-5}\) M):-

Fig 3.4 f Electronic spectrum of Ru\((\text{th})_2(\text{Hth})\) in isoamyl acetate (concentration 1.75 x 10\(^{-5}\) M):-
From above discussion it is clear that almost all the prepared ruthenium complexes of thiohydrazide ligands exhibit four transition bands in the 210-800 nm region. Among them the band near ~550 nm region is sharp and high intense and responsible for beautiful violet colour of the complex. Comparison of position of this band with different solvents has been summarized in the table no.3.02.e.

Table 3.02 e: Electronic absorption data for MLCT band of the complexes in different solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Ru(tbh)$_2$(Htbh)</th>
<th>Ru(o-htbh)$_2$(o-Hhtbh)</th>
<th>Ru(fth)$_2$(Hfth)</th>
<th>Ru(tth)$_2$(Htth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>538</td>
<td>544</td>
<td>555</td>
<td>556</td>
</tr>
<tr>
<td>Methanol</td>
<td>537</td>
<td>543</td>
<td>554</td>
<td>556</td>
</tr>
<tr>
<td>Acetone</td>
<td>540</td>
<td>545</td>
<td>554</td>
<td>560</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>530</td>
<td>546</td>
<td>556</td>
<td>561</td>
</tr>
<tr>
<td>DMSO</td>
<td>549</td>
<td>549</td>
<td>565</td>
<td>564</td>
</tr>
<tr>
<td>Isoamyl acetate</td>
<td>531</td>
<td>546</td>
<td>555</td>
<td>558</td>
</tr>
</tbody>
</table>

It is clear from the above table that a bathochromic shift is observed in general with increasing polarity of the solvent. This shift in band position towards higher wavelength with increasing polarity of the solvent supports the assignment of the band as MLCT type of transition. Another interesting observation is that there is a progressive bathochromic shift of this band on moving from Ru(tbh)$_2$(Htbh) to Ru(tth)$_2$(Htth).
All the complexes exhibit another weak MLCT transition in the region ~460 nm and that band appears as shoulder at the left hand side of the aforesaid band. At still higher energy region bands with high intensity and sharpness is observed, which resembles with spectra of free ligands in ethanolic solvent and assigned as ligand centered transition band.

3.3 INFRARED SPECTROSCOPY:-

The infra red spectra of ruthenium complexes of thiohydrazide ligands have been studied in the region 4000-300 cm⁻¹. The characteristic absorption bands relevant to the elucidation of bonding are recorded in Table 3.03. Infrared spectra of the corresponding ligands were studied and analyzed in detail in chapter 2. The shift of frequency were noted for the following bands ν₅-H, ν₇-N₂, ν₁-C=O, ν₄-S-H. Other bands like aromatic C-C stretching, C-H bending and in plane and out of plane stretching, N-N stretching etc have not been considered as their value should not change on complex formation. The figures 3.5, 3.6(a&b), 3.7 and 3.8 represent the infrared spectra of ruthenium complexes of H(tbh), H(o-hbh), H(fth) and H(th) respectively.
Table-3.03: Characteristic frequencies (cm\(^{-1}\)) in the infrared spectra of thiophydrazone ligands and their ruthenium(II) complexes

<table>
<thead>
<tr>
<th></th>
<th>(\nu_{\text{NH}}) [21,22]</th>
<th>(\beta_{\text{NH}}) [23]</th>
<th>(\nu_{\text{C-S}}) [23,24]</th>
<th>(\nu_{\text{C-N}}[25])</th>
<th>(\nu_{\text{MN}}[26])</th>
<th>(\nu_{\text{MS}}[27,28])</th>
<th>(\nu_{\text{SH}}[29])</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(tbh)</td>
<td>3280, 3200</td>
<td>1595</td>
<td>1200, 1200, 1565, 1562, 1630</td>
<td>1450, 1450</td>
<td>508, 445</td>
<td>475</td>
<td>2515</td>
</tr>
<tr>
<td>Ru(tbh)(_2)(Htbh)</td>
<td>3124</td>
<td>1630</td>
<td>1200, 1165, 1070, 1000, 1454</td>
<td>1450</td>
<td>508, 445</td>
<td>475</td>
<td>2515</td>
</tr>
<tr>
<td>H(o-htbh)</td>
<td>3300, 3220</td>
<td>1608</td>
<td>1175, 1105, 1070, 1465</td>
<td>1465</td>
<td>508, 445</td>
<td>475</td>
<td>2515</td>
</tr>
<tr>
<td>Ru(o-htbh)(_2)</td>
<td>3100</td>
<td>1610</td>
<td>1165, 1125, 1040, 860, 1465</td>
<td>1500</td>
<td>508, 445</td>
<td>475</td>
<td>2515</td>
</tr>
<tr>
<td>H(fth)</td>
<td>3230, 3175</td>
<td>1590</td>
<td>1220, 1070, 1065, 1024, 825</td>
<td>1552, 1554, 1595, 550, 500</td>
<td>440</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ru(fth)(_2)(Hfth)</td>
<td>3120</td>
<td>1620</td>
<td>1215, 1168, 1065, 1024, 824</td>
<td>1554, 1595, 550, 500</td>
<td>440</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The bands due to N-H stretching of NH$_2$ and NH groups are observed near 3300-3200 cm$^{-1}$. On complexation these bands generally shifts towards lower frequency [30]. This was found to be true for the entire ruthenium complexes under study. The interesting feature observed here is broadening of the band in the prepared complexes. This may be due to overlapping of a number of bands in that region. Almost all the ruthenium complexes show similar phenomenon.

The number of bands in the complexes is greater than in the free ligands. The position of characteristic bands are similar to that observed in the complexes of ruthenium(II)-2,2′ bipyridyl with N-substituted thiosemicarbazides i.e. Ru(bipy)$_2$(L)(ClO$_4$)$_2$[1] and 1-(phenylacetyl and phenoxyacetyl)-4-phenyl-3-thiosemicarbazide complex of

<table>
<thead>
<tr>
<th></th>
<th>$\nu$NH$^{[21,22]}$</th>
<th>$\beta$NH$_2$$^{[23]}$</th>
<th>$\nu$C-S$^{[23,24]}$</th>
<th>$\nu$C-N$^{[25]}$</th>
<th>$\nu$MN$^{[26]}$</th>
<th>$\nu$MS$^{[27,28]}$</th>
<th>$\nu$SH$^{[29]}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(tth)</td>
<td>3290, 3225</td>
<td>1607</td>
<td>1295, 1157, 750</td>
<td>1505</td>
<td></td>
<td></td>
<td>2530</td>
</tr>
<tr>
<td>Ru(tth)$_2$(Hth)</td>
<td>3140</td>
<td>1605, 1290, 1270, 1150, 1080, 752, 690</td>
<td>1580</td>
<td>517, 500</td>
<td>455</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bands due to N-H stretching of NH$_2$ and NH groups are observed near 3300-3200 cm$^{-1}$. On complexation these bands generally shifts towards lower frequency [30]. This was found to be true for the entire ruthenium complexes under study. The interesting feature observed here is broadening of the band in the prepared complexes. This may be due to overlapping of a number of bands in that region. Almost all the ruthenium complexes show similar phenomenon.

The number of bands in the complexes is greater than in the free ligands. The position of characteristic bands are similar to that observed in the complexes of ruthenium(II)-2,2′ bipyridyl with N-substituted thiosemicarbazides i.e. Ru(bipy)$_2$(L)(ClO$_4$)$_2$[1] and 1-(phenylacetyl and phenoxyacetyl)-4-phenyl-3-thiosemicarbazide complex of
The interesting observation is that for almost every stretching two sets of band are observed in the prepared complexes, one set at more or less at the same position with that of pure ligand and another set at shifted position. This observation may be explained by the proposed composition of the complexes i.e. RuL₂(LH). That means two ligand molecules have been coordinated after deprotonation and the third one has been coordinated in neutral mode. This assumption has been found to be true for all the prepared ruthenium complexes. A careful study of the spectral data helps to assign such transition.

The C-N stretching frequencies provide further insight about this phenomenon. It is observed that $\nu_{C-N}$ band, which is known as Amide II band is situated near 1400-1600 cm$^{-1}$. The positions of this band in the prepared complexes are at two different frequencies. One band shifts towards higher frequency, indicating involvement of deprotonated ligand in complex formation while other relatively weaker band remains at the same position to that of the uncoordinated ligand.
The 1565 cm\(^{-1}\) band i.e. C-N stretching bands of H(tbh) undergo a shift to 1630 cm\(^{-1}\) in the corresponding ruthenium(II) complex while two other bands at 1560 and 1450 cm\(^{-1}\) are also present in the complex which have not undergone any shift. Similarly for H(o-highbh) the band at 1580 cm\(^{-1}\) found to be at 1580 and 1620 cm\(^{-1}\) in the Ru(o-highbh)\(_2\)(o-Hlhtbh) complex. The 1552 cm\(^{-1}\) band of Hfth goes to 1595 cm\(^{-1}\) in the complex along with a peak at 1554 cm\(^{-1}\) while for Ru(tth)\(_2\)(Hfth), the band is shifted by ~75 cm\(^{-1}\) to higher frequency in comparison to the free ligand.

The C-S stretching band, perhaps the most important band, is used to assign the mode of bonding in the prepared complexes. A detailed discussion about identification of such band has been given in chapter 2. Although the band is difficult to assign\(^{[23]}\), but comparison of spectral patterns in the region 1000-1200 cm\(^{-1}\) and 700-900 cm\(^{-1}\) for the free ligands and corresponding complexes enable a fair assignment of the band\(^{[25,32-} \)
In thioformamide, the C=S bond adjacent to C-N bond has a stretching frequency of 843 cm\(^{-1}\) and that band has almost pure C-S character\[24\]. This assumption along with the assumption that \(\nu_{c=c}/\nu_{c=s}\) should be nearly equal to 1.5\[33\] is of great importance to suggest the bands with most C-S character both in the ligands and in the prepared complexes. It has been observed that such bands suffer a shift towards lower frequency in the prepared complexes indicating thioenolisation before complexation for the deprotonated ligand while such shift is restricted to about 5-25 cm\(^{-1}\) in case of neutral ligands\[34\] coordinating through thiocarbonyl sulphur.

![Infrared spectrum of Ru(o-hthbh)\(_2\)(o-Hntbh) in KBr disk.](image)

**Fig. 3.6.a.** Infrared spectrum of Ru(o-hthbh)\(_2\)(o-Hntbh) in KBr disk.
The C-S stretching frequencies of H(fth) at 1220, 1070 and 825 cm\(^{-1}\) shifted to the position 1168, 1025, 755 cm\(^{-1}\) in the corresponding ruthenium complexes for the deprotonated ligands. A set of weak band with very small shift has also been observed as per prediction. This trend in shift in C-S stretching frequencies is also observed for other ligands and their corresponding complexes.

The most important stretching frequencies which proves the mode of coordination is the metal nitrogen (M-N) and metal sulphur (M-S) stretching frequency. Here also two set of band is expected for each of such stretching. It is obvious that metal sulphur bond strength for coordination with thioenolic and thiocarbonyl sulphur in two types of ligand would widely vary; similar is the case for metal nitrogen bond strength. The \(\nu_{M-N}\) is observed at 475-550 cm\(^{-1}\) with two set of signal for each frequency. That for \(\nu_{M-S}\) is observed \(\sim 440-460\) cm\(^{-1}\) for the prepared ruthenium complexes. The later band is observed in higher frequency region than expected. This may be due to the fact that, half of the coordination site of the metal ion is occupied by \(\pi\) acceptor sulphur moiety,
hence greater extent of π bond formation leads to the shift of M-S stretching frequency towards higher energy. Both the bands are absent in the free ligands.

Fig. 3.7 Infrared spectrum of Ru(fth)$_2$(H-fth) in KBr disk.

Fig. 3.8 Infrared spectrum of Ru(tth)$_2$(H-tth) in KBr disk.
The O-H stretching frequency for the ligand H(o-htbh) is observed near 3600 cm\(^{-1}\) [35].

This band remains unchanged on complexation in Ru(o-htbh)\(_2\)(o-Hhtbh), suggesting that the -O-H group does not take part in coordination.

### 3.4 PROTON NMR SPECTROSCOPY:

The chemical shift data for proton NMR spectroscopy of the free thiohydrazide ligands and their ruthenium complexes are tabulated in the table 3.04. The figures 3.9, 3.10, 3.11 and 3.12 represent the \(^1\)H nmr spectra of the complexes Ru(tbh)\(_2\)(Htbh), Ru(o-htbh)\(_2\)(o-Hhtbh), Ru(fth)\(_2\)(Hfth) and Ru(fth)\(_2\)(Hfth) respectively.

A proper picture about the change in environment of the protons due to complexation may be obtained from the analysis of the spectra. A detailed study of the \(^1\)H nmr spectra of the ligands has already been discussed in chapter 2. Signals due to aromatic protons obtained in the region 6.6 to 7.8 ppm [36], they undergo negligible shift on complexation in comparison to shift of the hydrazinic protons. Hence their shift has not been tabulated.
Table 3.04: Proton nmr data for the ligand and the thiohydrazide complexes of ruthenium(II). Chemical shifts (δ) are given in ppm, relative to TMS.

<table>
<thead>
<tr>
<th></th>
<th>NH</th>
<th>NH₂</th>
<th>OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>H(tbh)</td>
<td>12</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Ru(tbh)₂(Htbh)</td>
<td>10.27*</td>
<td>8.3, 8.0*</td>
<td></td>
</tr>
<tr>
<td>H(o-htbh)</td>
<td>11</td>
<td>5.71</td>
<td>11.26</td>
</tr>
<tr>
<td>Ru(o-htbh)₂(o-Hhtbh)</td>
<td>9.9*</td>
<td>12.47, 9.97*</td>
<td>11.46, 9.77*</td>
</tr>
<tr>
<td>H(fth)</td>
<td>10.4</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>Ru(fth)₂(Hfth)</td>
<td>9.91*</td>
<td>9.72, 8.26*</td>
<td></td>
</tr>
<tr>
<td>H(tth)</td>
<td>10.8</td>
<td>5.15</td>
<td></td>
</tr>
<tr>
<td>Ru(tth)₂(Htth)</td>
<td>9.6*</td>
<td>9.47, 8.13*</td>
<td></td>
</tr>
</tbody>
</table>

(* marked data represents shift for proton of neutral ligand LH)

The proton nmr spectra of the prepared ruthenium complexes are a little bit complicated with presence of five set of signals in the non aromatic region.

NMR spectra of the ligands in CDCl₃ have been taken followed by D₂O exchange study. Comparison of the spectra reveals that the signal near 10-12 ppm is due to δNH for amidic proton of the following group.

\[ \text{S} \]
\[ \text{C} \quad \text{NH} \quad \text{NH₂} \]
Disappearance of the signal in the complexes for amidic proton suggests the following thioenolisation before complexation:

\[
\begin{array}{c}
\text{S} \\
\text{C} \quad \text{N} \\
\text{H} \quad \text{NH}_2
\end{array}
\]

But in the prepared ruthenium complexes, unlike thiohydrazide complexes of nickel and palladium, the signal for amidic proton does not vanish.

Fig. 3.9 NMR spectrum of Ru(tbh)₂(Htbh) in DMSO d₆.
Fig. 3.10 NMR spectrum of Ru(o-hthb)$_2$(o-Hhtbh) in DMSO $d_6$.

Fig. 3.11 NMR spectrum of Ru(fth$_2$(Hfth) in DMSO $d_6$.
The position of $\delta$NH$_2$ i.e. hydrazinic proton suffers a down field shift as described in table no 3.04. This large down field shift suggests that the ligands are coordinated via the hydrazinic nitrogen atom with ruthenium(II) ions in the prepared complexes. Another set of signal due to hydrazinic proton also obtained with different down field shift. These signals have peak area nearly half to that due to NH$_2$ protons. Close inspection of the position and peak area of the two set of signals in the prepared ruthenium complexes conclusively prove the proposed composition of the complexes as RuL$_2$(LH) i.e. two ligand molecules have been coordinated after thioenolisation and ionization while the third one has been coordinated without deprotonation.
The signal for –OH proton appear ~ 11.3 ppm for the ligand o-Hhtbh and position of the signal remains essentially the same in Ru(o-htbh)\textsubscript{2}(o-htbh) complex. This suggests the phenolic –OH group does not take part in coordination. Here also two set of signals due to –OH proton obtained, supporting the previous assumption.

3.5 CYCLIC VOLTAMMETRY:-

Cyclic-voltammetry is one of the major tool for studying the nature of ruthenium complexes and this has been utilized to study and characterize different such complexes with various coordinating sites viz. Cl and PR\textsubscript{3} [37,38], N\textsubscript{6} [39,40], Carbonyl[41], Cl\textsubscript{2}N\textsubscript{4}[42,43], P\textsubscript{4}Cl\textsubscript{2}[44,45], NNS [46] etc.

The electrochemistry of the prepared ruthenium complexes have been studied in acetonitrile solution using glass carbon electrode and tetraethyl ammonium perchlorate as supporting electrolyte, with scan rate of 20 mV/s. The electrochemical data are given in the table 3.05 and the figures 3.13, 3.14, 3.15 and 3.16 represent cyclic voltammograms of ruthenium complexes of Htbh, o-Hhtbh, Hfth and Htth respectively.

<table>
<thead>
<tr>
<th>Complex</th>
<th>E\textsubscript{1/2, obs} (ΔEp/mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru(tbh)\textsubscript{2}(Htbh)</td>
<td>-0.694</td>
</tr>
<tr>
<td>Ru(o-htbh)\textsubscript{2}(o-Hhtbh)</td>
<td>-0.790</td>
</tr>
<tr>
<td>Ru(fth)\textsubscript{2}(Hfth)</td>
<td>-0.780</td>
</tr>
<tr>
<td>Ru(tth)\textsubscript{2}(Htth)</td>
<td>-0.787</td>
</tr>
</tbody>
</table>
The electrochemistry of the complexes are dominated by Ru(II)/Ru(III) oxidation. The separation between anodic and cathodic peaks \( E_{pa} - E_{pc} \) is nearly about \(-150\) mV and depends upon scan rate. As these peak separation values are much greater than the ideal Nernstian value of 59 mV and ratio of intensities of cathodic and anodic current is not equal to unity i.e. \( i_c/i_a \neq 1 \), we can conclude the electrochemical process as irreversible one.

In most of the cases the peaks are not well defined in the anodic portion of the cyclic-voltammograms. This may be due to oxidation of the ligands followed by change in environment of the complexes leading to irreversible process. Similar observation has been reported with ruthenium(II)-thiosemicarbazone derivative complexes with NNS donor sites [46].

All the four thiohydrazide ligands have the same donor sites so similar electrochemical behavior is expected for their ruthenium complexes. In actual practice the \( E_{obs} \) values of the ruthenium thiohydrazide complexes are similar. However a little bit lower value was obtained with the Ru(tbh)_2(Htbh) complex.

![Cyclic voltammogram of Ru(tbh)_2(Htbh)](118)

Fig. 3.13 Cyclic voltammogram of Ru(tbh)_2(Htbh) in acetonitrile solvent.
Fig. 3.14 Cyclic voltammogram of Ru(o-hthb)$_2$(o-Hhthb) in acetonitrile solvent.

Fig. 3.15 Cyclic voltammogram of Ru(fth)$_2$(Hfth) in acetonitrile solvent.

Fig. 3.16 Cyclic voltammogram of Ru(th)$_2$(Hth) in acetonitrile solvent.
Relation between electrochemical potentials and charge transfer energies for ruthenium complexes were also exploited. It is assumed that MLCT band in the UV-visible spectrum, arise from an electronic excitation. This electronic excitation is equivalent to the oxidation of the metal and reduction of the ligand. Thus the energy of the MLCT transition (in cm$^{-1}$) should be equal to the absolute difference in potential between corresponding oxidation and reduction process or be linearly dependent on them [46-51]. Such linear relationship is also valid for these prepared ruthenium complexes.

Table 3.06 represents $E_{obs}$ values of the prepared ruthenium complexes with corresponding metal to ligand charge transfer energies (in cm$^{-1}$) in acetonitrile solution.

<table>
<thead>
<tr>
<th></th>
<th>$E_{1/2 , obs}$ (ΔEp/mV)</th>
<th>$E_{MLCT}$ (cm$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ru(tbh)$_2$(Htbh)</td>
<td>-0.694</td>
<td>18868</td>
</tr>
<tr>
<td>Ru(o-htbh)$_2$(o-Hhtbh)</td>
<td>-0.790</td>
<td>18315</td>
</tr>
<tr>
<td>Ru(fth)$_2$(Hfth)</td>
<td>-0.780</td>
<td>17986</td>
</tr>
<tr>
<td>Ru(tth)$_2$(Htth)</td>
<td>-0.787</td>
<td>17806</td>
</tr>
</tbody>
</table>

Figure number 3.17 represents the correlation between $E_{obs}$ and $E_{MLCT}$ values. A nice linear correlation is obtained with equation $E_{MLCT} = 10928 \, E_{obs} + 26456$ and $R^2$ value has been found to be 0.9917. However the data for the complex Ru(o-htbh)$_2$(Ho-htbh) complex misfit the graph and has not been plotted.
Fig. 3.17 Plot of $E_{\text{MLCT}}$ (cm$^{-1}$) vs. $E_{\text{obs}}$ for ruthenium thiohydrazide complexes.

3.6 **ANTIBACTERIAL ACTIVITY :-**

The activity of the ruthenium-ortho-hydroxythiobenzhydrazide complex against *Escherichia coli* (K12) strain has been studied by plotting cell counts against volume of the stock solution to obtain the curve shown in figure 3.18. Extrapolation of the curve to zero percent growth gives the minimum volume of the stock solution, required for total inhibition of the growth of the micro organism. From the volume of the stock solution minimum inhibitory concentration (MIC) is calculated. The value is found to be 149 µM of Ru(o-htbh)$_2$(o-Hhtbh) complex *in vitro*.
% Growth = (Cell count at a particular concentration of the complex) / (Cell count in the control)

Concentration of the stock solution = 0.99 micro-moles/mL.

3.7 ACTION ON TUMOUR CELLS:

The activity of the ruthenium-thiohydrazide complexes against HELA cell line has been studied by plotting counts per minute (cpm) of $^3$H-thymidine against concentration of
the complexes to obtain the curve shown in figure 3.19. Relevant data are given in the table.

Table 3.07: Activity of the ruthenium(II) thiohydrazide complexes against HELA cell line.

<table>
<thead>
<tr>
<th>Concn. of the drug (nM)</th>
<th>Mean cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ru(htbh)$_2$(Htbhb)</td>
</tr>
<tr>
<td>0</td>
<td>8265</td>
</tr>
<tr>
<td>20</td>
<td>7164</td>
</tr>
<tr>
<td>40</td>
<td>5726</td>
</tr>
<tr>
<td>80</td>
<td>4235</td>
</tr>
<tr>
<td>150</td>
<td>3042</td>
</tr>
<tr>
<td>200</td>
<td>1950</td>
</tr>
<tr>
<td>250</td>
<td>1240</td>
</tr>
</tbody>
</table>

Plot of mean cpm vs. drug concn.

Hence 250 nM concentration of the drug inhibits HELA cell division in vitro.
CONCLUSION:

The ruthenium(II) complexes prepared with thiohydrazide ligands have the general composition Ru(L)_2LH, they are red-violet in colour, sufficiently thermal stable and diamagnetic in nature. Electronic spectra of the complexes represent transitions characteristic of hexacoordinating d^6 system. A charge transfer type of transition at ~550 nm is responsible for violet coloration of the complex both in solid and solution state. Infrared spectra of the complexes suggest an interesting insight about the composition of the complexes. The mode of coordination of the ligand to ruthenium(II) ion is both thiolato sulphur and thione sulphur along with hydrazinic nitrogen atom. NMR spectra supports the assumptions about coordination mode and finally cyclicvoltammetric study of the complexes suggest their electrochemistry, formal redox potentials and relation of electrode potentials with metal to ligand charge transfer energies. Several attempts to prepare single crystals suitable for X-ray crystallography by different methods were not successful. Therefore the structures could not be conclusively established with help of crystallographic data.

Antibacterial study against *Escherichia coli* (K12) strain shows that Ru(o-hthb)_2(o-Hhtbh) completely inhibits the growth of the micro organism at 149 µM concentration *in vitro*. On the other hand 250 nM concentrations of the ruthenium-thiohydrazide complexes inhibit HELA cell division *in vitro*. Further studies on animal model may prove the compounds as useful cancer inhibition drug.
REFERENCES:


125

20 G. A. Crosby, Accounts of Chem. Res. 8, 231, 1975

21 G. R. Burns, Inorganic Chemistry, 7, 277, 1968


36. ‘Studies on platinum thiohydrazide blues and thiosemicarbazide blues’ Sanjukta Ray, Ph.D. Dissertation, University of Calcutta, 2004


45. B.P. Sullivan and T.J. Meyer, Inorganic Chemistry, 21, 1037, 1982


