CHAPTER III

SYNTHESIS AND SPECTROSCOPIC STUDIES OF BRH_2

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[COPPER (II) COMPLEX OF 5 – PHENYLAZO – 3 – METHOXY SALICYLIDENE THIOSEMICARBAZONE]

Thiosemicarbazones and their metal compounds are well known for their wide spectrum of biological activity in general and antineoplastic activity in particular (Petering et al., 1964; Orlova et al., 1968; Saryan et al., 1979; Scovill et al., 1980). Their action also as antimicrobial and antiviral agents has been reviewed (Levinson, 1980). Several types of metal complexes have been shown to possess cytotoxic or antineoplastic properties. The behaviour of the ligands can be modified by the linkage to metallic ions such as Cu (II) and Fe (III) enhancing their biological activity (Antholine et al., 1976; Saryan et al., 1979) as was found for other thiosemicarbazone complexes (West et al., 1991).

2-formyl-pyridine thiosemicarbazone is known to have antileukaemic property through it is too toxic to be used clinically while 3- and 4-formyl isomers are inactive (Das, 1989). The 3-(m-aminobenzyloxy) derivative shows much less toxicity without any change of its antineoplastic potency while 2- Formyl-5-hydroxypyridine thiosemicarbazone is seen to have antineoplastic activity against leukaemia and many other types of cancers in mice when it is injected intraperitoneally (Das, 1989). 1-Formyl-isoquinoline

thiosemicarbazone and many of its 5- substituted derivatives are also found to have antineoplastic activity. 2—Formyl pyrazine thiosemicarbazone shows a more potential anticancer activity with a much higher LD_{50} value (Das, 1989).

As earlier, reported platinum (II) and palladium (II) complexes with 2, 2′ - bipyridine and amino acids, creates antineoplastic activity against L1210 lymphoid leaukemic, P388 lymphocytic leaukemic, sarcoma 180, and Ehrlich ascitic tumor cells (Puthraya et al., 1985; Kumar et al., 1985; Puthraya et al., 1986).

Though the anti cancer activity of different platinum metal complexes appears to be promising, but platinum metals being unnatural to the living body from their biological view point, the body does not possess any effective mechanism for their rejection, as for those metals (e.g., copper and iron) which occur naturally in body. Hence, it can rationally be expected that, if any, complex of anticancer activity of these biologically involved metal ions is available, its toxicity would be less. This is why, presently this area has been the most current topic to the bio-coordination chemists and this possibility of having anticancer properties among the complexes of biologically important metal ions is being vigorously explored all over the world (Das, 1989).

Diphthalate complexes of copper (II) have been found to be active against leukaemia. Administration of copper (II) dimethylglyoxime chelate, $[cu(dmgH)_2]$, where 'dmgH' represents the dimethylglyoximate anion, has been found to inhibit the tumor growth of the animals with Ehrlich Ascites Tumor and Sarcoma 180 (Das, 1989).

Different bis-(thiosemicarbazone) complexes of copper (II), such as copper pyruvaldehyde bis-(thiosemicarbazone), copper – 2 – Keto – 3 – ethoxybutyraldehyde bis – (thiosemicarbazone) [Fig. 3.1] have been found active against tumors.

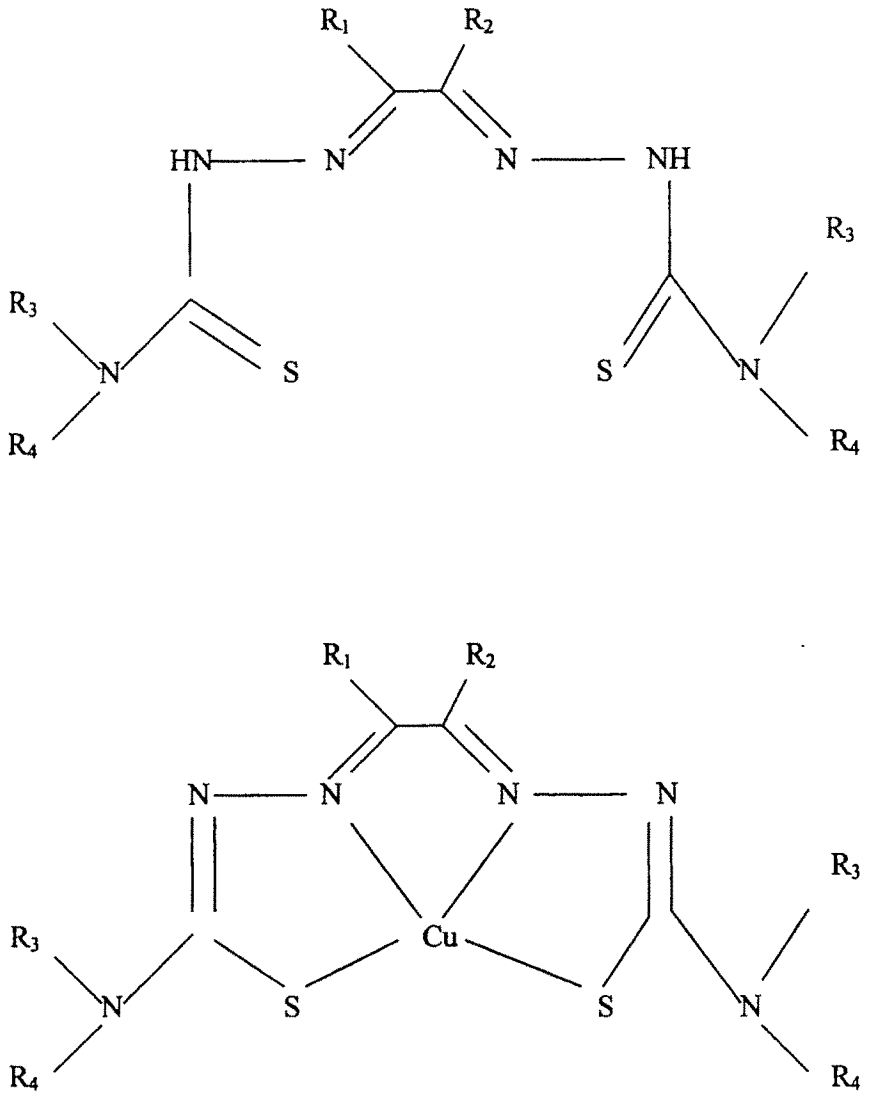


Fig. 3.1: Generalized structures of bis – (thiosemicarbazones) and their Copper (II) Complexes.

H2KTS (R1 = ethoxyethyl, R2 = R3 = R4 = H)

H2KTSM (R1 = ethoxyethyl, R2 = R4 = H, R3 = methyl)

H2KTSM2 (R1 = ethoxyethyl, R2 = H, R3 = R4 = methyl)

The last complex is generally represented as 'CuKTS' and the corresponding ligand as H_2KTS. H_2KTS is remarkably effective against different types of tumors such as, Walker 256, nitrogen mustard resistant carsinosarcomas, Sarcoma 180, spontaneous mammary tumors of DBA_2 mice; but it is not effective against leukemias. It has been established by definite experiments that the complex, not the ligand is responsible for the activity. On the other way it is observed that H_2KTS has no activity on W – 256 tumor growth in animals maintained on a copper deficient diet, but its activity is pronounced only when an excess Cu(II) salt is titrated back into the drinking water of the animals (Das, 1989).

Administration of CuKTS also shows antitumor activity, but it is toxic to the host. Certain members of ligands such as 1 – formylisoquinoline thiosemicarbazone and 5 – hydroxy – 2 – formylpyridine thiosemicarbazone have an excellent antitumor activity in some animals. Bleomycin, a family of glycopeptides are known to be antineoplastic by their own right. It has been established that in some cases, their copper (II) complex can increase their anticancer activities (Das, 1989).

It has been proved that some amino acid complexes of copper (II) show antiblastoma activity against the ascites form of Sarcoma 180 in vivo and against lympholeukaemia, L – 1210 in vitro. Mixed amino acid complexes of copper (II) have been found quite promising to inhibit the growth of tumors

of two strains – the mammary gland adenocarcinoma (AK – 755) and the cancer of the neck of the uterus (RShM – 5) [Das, 1989].

Salicylaldoxime and 2,4 – dihydroxybenzaldoxime copper (II) complexes have been found (Lumme et al., 1984) quite successful against L – 1210 and Ehrlich ascites carcinoma. The experiment with respect to L – 1210 shows that the activity arises due to the complex not due to the ligand or free metals i.e. Cu (II). Trans – bis – (salicylaldoximate) metal (II) chelates and specially that of copper (II) chelate to vitamin B_6 is, at least, partly responsible for the antineoplastic activity (Lumme et al., 1984). The cis – dicholoro diamine Pt (II) and bis – (thiosemicarbazonato) Cu (II) complexes, copper and iron complexes of α - N – hetrocyclic carboxy aldehyde formyl thiosemicarbazones and copper (II) complexes derived from thiophene – 2 – carbaldehyde thiosemicarbazone, have been studied extensively (Petering et al., 1974; Gale et al., 1975; Agarwal et al., Garcia –Tojal et al., 1999).

Various copper complexes have been found to possess cytotoxicity and antineoplastic properties (Petering et al., 1975; Agarwal et al., 1975; Antholine et al., 1976; Pickart et al., 1980; Saryan et al., 1981.). Therefore, an attempt has been made to synthesize a copper (II) complex of 5 – phenylazo – 3 – methoxy salicylidine thiosemicarbazone coded as BRH_2 (Patil et al., 1989) and its antineoplastic activity against Dalton's lymphoma.

THE PREPARATION AND CHARACTERIZATION OF BRH_2

Procedure for preparation of BRH_2 :

BRH_2 is a copper complex of 5 – phenylazo – 3 – methoxy salicylidene thiosemicarbazone.

Preparation of BRH_2 involves two steps –

A. **Preparation of ligand**

B. **Preparation of complex**

Materials required:

1. Aniline (Sigma)
2. Sodium Nitrite (Marck)
3. Hydrochloric acid (Himedia)
4. Orthovanillin (Aldrich)
5. Sodium hydroxide (Marck)
6. Thiosemicarbazide (Sigma)
7. Copper acetate (Marck)
8. Sodium acetate (Marck)

A. Preparation of ligand (5 – phenylazo – 3- methoxy salicylidene thiosemicarbazone):

Part – a : Preparation of 5 – phenylazo – 3- methoxy salicylaldehyde

Dissolving 4.5 gm of pure freshly distilled Aniline in 10 ml of concentrated hydrochloric acid in a beaker which is immersed in an ice bath. The solution is diluted with 20 ml of distilled water then diazotise aniline by drop wise addition of cold sodium nitrite solution (prepared by dissolving 4 gm of sodium nitrite in 20 ml of distilled water) maintaining temperature bellow 2 – 5^0C. The completion of the reaction is tested using starch iodide paper followed by addition of cold diazonium solution drop wise to O – Vanillin solution (7.5 g of O – Vanillin in 30 ml of aqueous sodium hydroxide) with constant stirring. The precipitate is allowed to stand overnight. The precipitate has been acidified with ice cold 0.05 N HCl solution. The resulting precipitate filtered and washed with ice cold HCl and with water. The precipitate is dried and recrystallised in ethanol.

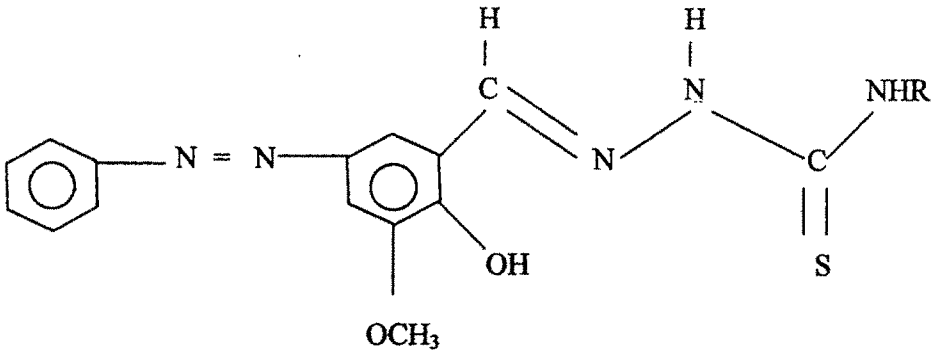
Part – b: ***Preparation of ligand***

The ethanolic solution of 0.01 m 5 – phenylazo – 3- methoxy salicylaldehyde and 0.01m thiosemicarbazide (1:1) was mixed in a round bottom flask and refluxed it for 10 minutes. The precipitate is filtered and washed with alcohol and water and then dried and stored.

B. Preparation of BRH_2:

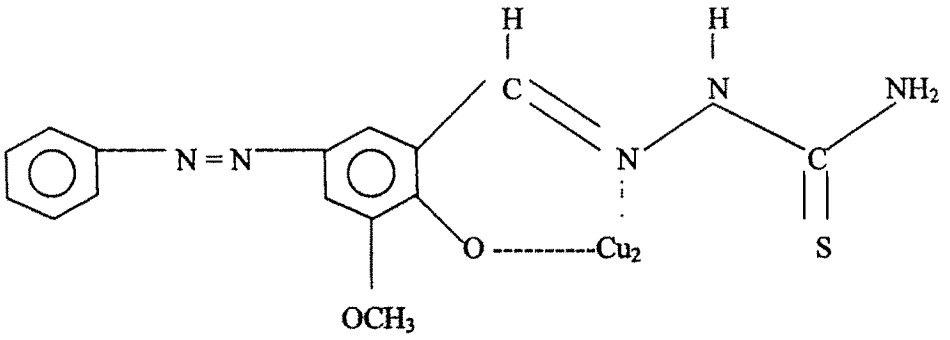
The copper acetate (0.01m) and the ligand (0.02m) [1:2] is taken in aqueous ethanolic medium and refluxed the mixture in presence of one gram of sodium acetate for about 4 hours. The complex was filtered and washed with alcohol and water. Finally dried it and this is BRH_2 [Cu (C_{15} H_{14} N_5 O_2 S)$_2$].

The characteristics of BRH_2 and the ligand were determined by Ultraviolet (UV) and Infrared spectra (IR).



Here, R = H, (HL^1)

BRH_2 Ligand



BRH_2 Complex

Fig. 3.2: Structure of BRH_2 Ligand and Complex

Determination of IR and UV spectra:

Infrared spectra of the ligands and complexes were recorded on *Perkin Elmer FT IR 1600 series Spectrophotometer*, and Ultra Violet Spectra of the ligands and complexes were recorded on *Hitachi U 3210 UV – Vis Spectrophotometer.*

Infrared spectra of ligands (HL^1) exhibit OH stretching frequency in the region 3,420 cm^{-1} (Ghosh et al., 1978). This disappears in the complexes, indicating that OH is deprotonated and oxygen is involved in the coordination. The band due to V (C=N) of the ligands that appears in the region1615 cm^{-1} (Biradar et al., 1976) shifts to lower frequency, indicating the involvement of nitrogen atoms of the azomethine group in the coordination. The band due to V (C = S) [Suzuki, 1962; Singh et al., 1969] observed in the region 780 cm^{-1} in the free ligands remains unaltered in intensity and position in the complexes, suggesting non-involvement of sulphur in the bonding. The involvement of Oxygen and nitrogen in the coordination is confirmed by appearance of new bands in the regions 530 cm^{-1} and 440 cm^{-1} (Lintvedt et al., 1978) which are assigned to V (M-N) and V (M-O) modes, respectively. These observations indicate the bidentate monobasic nature of the ligands.

The UV – Vis spectra shows complexes display two bands in the region 387.6 nm and 350.6 nm because of metal – ligand charge transfer

(Lintvedt et al., 1978; Folgado et al., 1988), and ligands display one band in the region 330.6 nm.

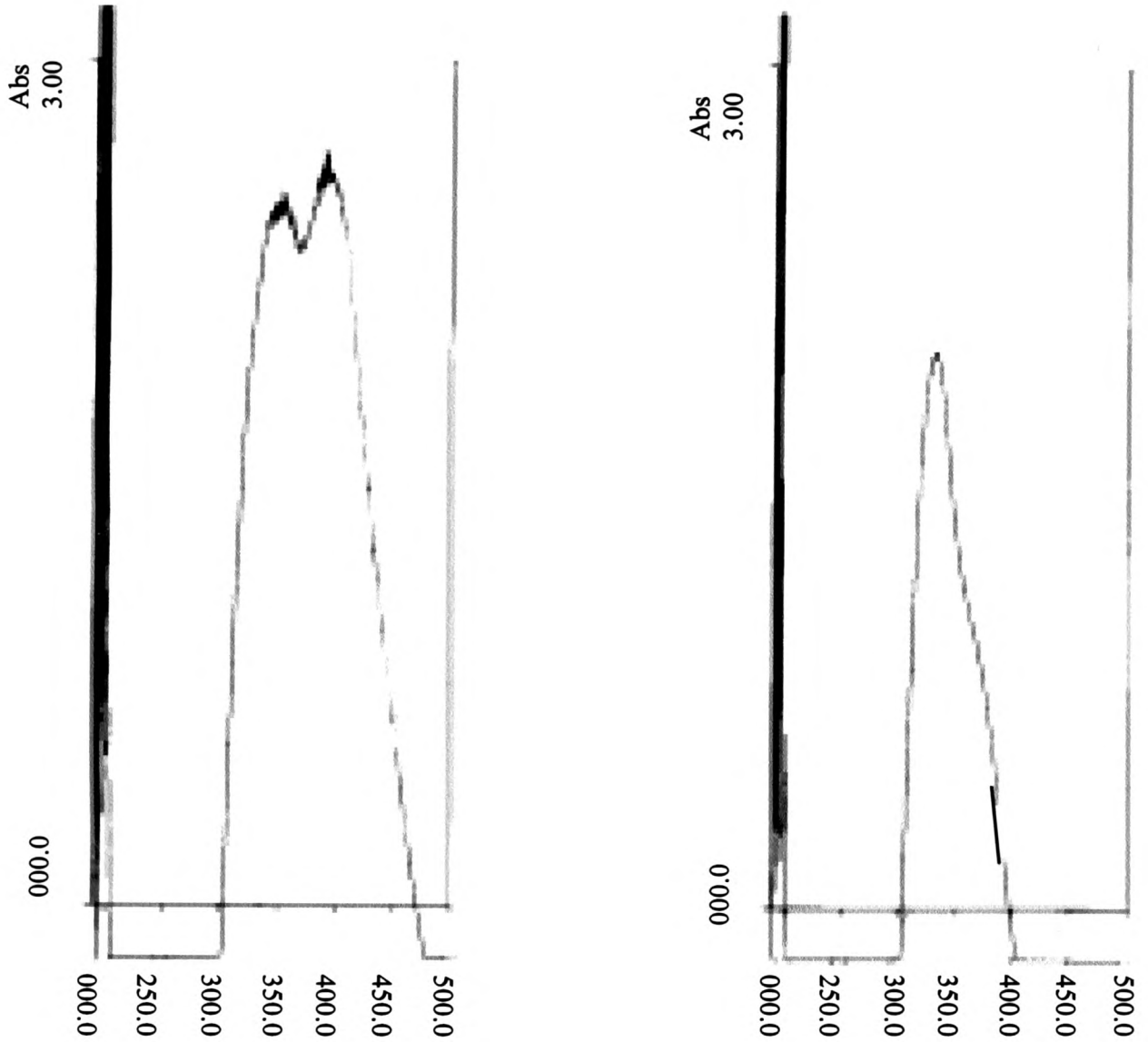
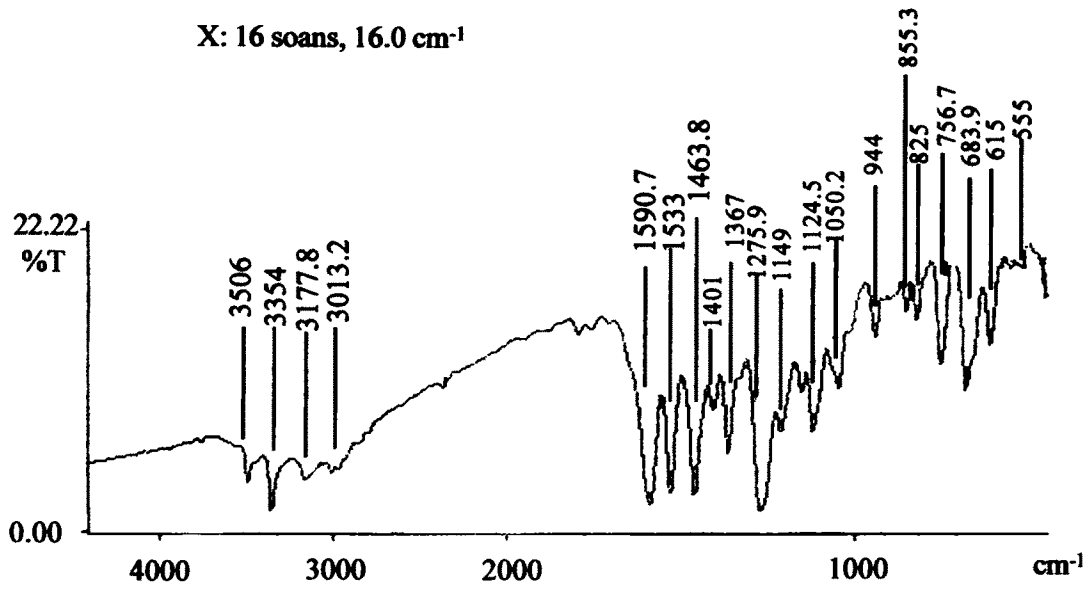
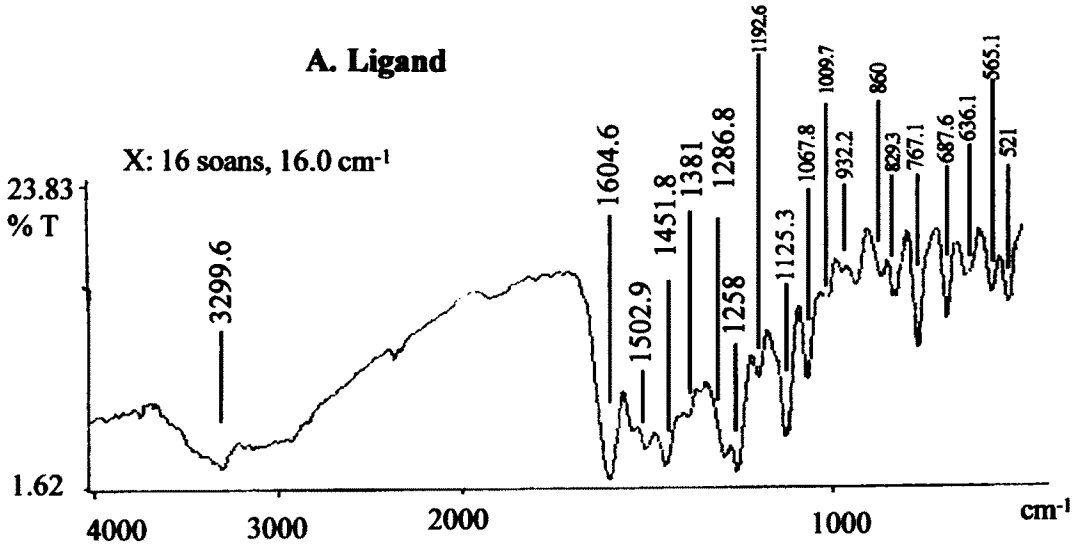


Fig. 3:3 UV-Vis Spectra of BRH_2 (in nm/min).



A. Ligand



B. Complex

Fig. 3:4 Infrared Bands of BRH_2 (in cm^{-1})