CHAPTER ONE
1.

Spectroscopy and structure of 3d metal complexes having the \([M_2N_2OS_2]\) coordination sphere

I. Exchange coupled dicopper(II) complexes of thiosemicarbazones

II. Structural diversity in dinickel(II) complexes of thiosemicarbazones

III. Coordination chemistry of Schiff-bases derived from mercapto-triazoles

IV Coordination chemistry of thiohydrazones

a. Furanthiocarboxyhydrazone

b. Phenyl thioacethydrazone

c. p-Methoxy thioacethydrazone

d. N-Methyl-indolyl-3-thiohydarzone

I.

Exchange coupled dicopper(II) complexes of thiosemicarbazones

A large section of coordination chemistry is now being dominated by organic thio derivatives of hydrazine, viz, thiosemicarbazide, thiosemicarbazones, thiohydrazide and thiohydrazones. As early as 1934 Domagk et al. [1] reported that thiosemicarbazones possessed anti-tubercular activity. Since then research on thiosemicarbazones and its metal complexes expanded dramatically. They have been found to be active against influenza [2], protozoa [3], smallpox [4], and certain kinds of tumor [5] and have been suggested as possible pesticides [6] and fungicides [7]. Their activity has frequently been thought to be due to their ability to chelate trace metals. Thus Liebermeister [8] showed that copper ions enhance the anti-tubercular activity of \(p\)-acetamidobenzaldehyde thiosemicarbazone.

Thiosemicarbazone derivatives are emerging as a new class of non-platinum experimental anticancer chemotherapeutic compounds [9,10] which not only show inhibitory activities against common cancers but which are also found to be potent inhibitors of a crucial enzyme, ribonucleotide diphosphate reductase (RDR), which is obligatory for DNA biosynthesis and cell division. The most active compounds of this class include copper complexes of 3-ethoxy-2-oxobutaraldehyde thiosemicarbazone [Fig.1], commonly known as CuKTS [11] and those of 4N-heterocycle thiosemicarbazone [12].
Motivated by the potential antitumour activity of Quinones, which are presently in use in clinical practices [13], Padhye et al. [14] synthesized napthoquinone-thiosemicarbazone hybrid molecules by combining structural features of both the groups with retention of their antitumor properties [Fig. 2]. Such 'hybrid' antitumor agents involving cisplatin and doxorubicin moieties have been found to possess lower therapeutic dosages, minimal cytotoxicities and reasonable kidney clearance [15].

Heterocyclic thiosemicarbazones also exert their therapeutic properties in mammalian cells by inhibiting ribonucletide reductase, a key enzyme in the synthesis of DNA precursors [16,17]. The interaction of thiosemicarbazones with various biochemical systems has been studied to understand the potential antitumour behaviour of this agent in vivo. For example the 1:1 copper (II) complex of 2-formyl pyridine thiosemicarbazone has been shown to inhibit the RNA dependent DNA polymerases [18].

Several copper(II) complexes with bis(thiosemicarbazones) have been studied to obtain a superoxide dismutase (SOD) like drug able to cross the cell membrane and reach intracellular superoxide generating sites [19]. Most thiosemicarbazones and their metal complexes are highly hydrophobic and their low solubility in water induces experimental limitations in biological studies [20]. Bis(thiosemicarbazones) act as a tetradeinate ligand and are excellent chelating agents, while mono(thiosemicarbazones)
form less stable complexes with metal ions [21]. The introduction of a hydrophilic group such as NH$_2$ in the heterocyclic ring systems should permit a soluble acid or sodium salt to be obtained with the goal of increasing the solubility in water [22]. Cu(II) complexes of 5-phenylazo-3-methoxy salicylaldehyde thiosemicarbazone and 4-$N$ substituted thiosemicarbazones were reported by Patil et al.[23]. One representative complex has been screened in vitro and in vivo against P388 lymphocytic leukemia cells sensitive and resistant to adriamycin (P388/S and P388/R) [Fig. 3].

Several $\alpha$-diketone-bis(thiosemicarbazone) copper(II) complexes have validated biological activity. Kethoxal-bis(thiosemicarbazone) copper(II), for example, has demonstrated carcinolytic and carcinostatic behavior against several established rat tumors [24]. Many attempts have been made [25, 26] to study their mechanism of action, although many aspects remain speculative. It is believed that the thiosemicarbazones may inhibit RNA dependent DNA polymerases and hence block the progress of diseases. There is also growing consensus on the involvement of toxic oxygen species, such as superoxide and hydroxyl radicals, in many of the disease states for which thiosemicarbazones have been shown to be effective. A recent study [27] has revealed the potential of using Cu(II) bis-thiosemicarbazones as superoxide dismutase (SOD) like drugs at intracellular sites.

EPR spectroscopy has been widely used in the study of complexes formed between metal ions and various ligands, both in frozen solution and liquid states, as it offers the potential to define local structure as well as provide information on the chemical reactions. Several studies [28, 29] have highlighted the dependence of the EPR spectra of copper(II) bis-thiosemicarbazones on pH, as a result of distinct species at low and neutral pH. The possible relationship between structure and function has been examined for several compounds and variations in the chemical arrangement at a molecular level have been proposed [30, 31]. In an attempt to explore further the origins of the difference in biological behaviour of mono- and bis-thiosemicarbazones,
particularly with regard to SOD-like activity, a series of mono and bis-thiosemicarbazones have been synthesized and their copper complexes were studied by EPR spectroscopy. Specifically copper(II) complexes of acetaldehyde thiosemicarbazone, pyruvic acid thiosemicarbazone and ribose bis-thiosemicarbazone have been prepared [32-34].

The copper complex [{CuL(MeC02)}2] (HL=2-formyl pyridine thiosemicarbazone) and related compounds have been shown to have marked antitumor activities, being more potent than the free ligands, against Ehrlich cells injected in to mice [35], Sarcoma 180 ascites tumours [36] and Chinese hamster ovary cells [37]. Until recently, when Ainscough [38] and others [39] reported the first systematic characterization of the complexes formed with HL, [Fig. 4] including the single-crystal X-ray crystallographic structures of [{CuL(MeC02)}2] and the protonated ligand complex [{Cu(HL)(SO4)}2], most reports had described solution or in vivo experiments.

![Fig. 4 (HL)](image)

The exact mechanism by which such copper complexes exert their antitumor activity is not clear due to the large number of potential sites of action within the cell and the difficulties associated with monitoring and unequivocally assigning a reaction to a particular step. One of the proposed mechanisms is the interaction of the copper(II) drug with the thiol-containing enzyme, ribonucleoside diphosphate reductase (RDR), which is required for the synthesis of DNA precursor [40], the CuL+ species or HL (released by the reduction of CuL+ with thiols) may displace the iron from the RDR metal binding site or CuL+ may bind to a thiol group of the enzyme.

Also it does appear that the drug can bind to intracellular thiols such as glutathione [N-(N-L-γ-glutamyl-L-cysteinyl)glycine] giving a thiolato complex with CuL+[41]. This in turn is able to promote redox reactions with other thiols and oxygen to produce the disulfide along with O2· and OH radicals which could be partly
responsible for the observed cytotoxicity. A second possible mechanism involves the binding of the copper(II) drug to the nitrogen bases of DNA or RNA, thus hindering or blocking base replication. Antholine et al. have studied the interaction of CuL\(^+\) with ethylenediamine in solution using ESR spectroscopy and deduced that adduct formation results [42].

In order to provide a firmer chemical basis for the proposed antitumour action of the CuL\(^+\) species, Ainscough et al. have examined its reaction with a range of N-donor ligands [viz. 2,2'-bipyridyl (bipy); 1,10-phenanthraline(phen) and 4-dimethylaminopyridine (dmop)] as well as few S, N and S,O donors ligands, and have shown that in fact stable ternary copper(II) complexes can be isolated and characterized even with thiolates. The single crystal X-ray structure of one of these adducts [CuL(bipy)]ClO\(_4\) is described [43].

Metal complexes of thiosemicarbazones can interfere in the complicated mechanisms that lead to a leukemic transformation [44]. The uncontrolled proliferation of the leukemic clone, the onset of maturation at the initial stage of hemopoiesis and the slowing down of cell turnover are the most important alterations that characterize the pathological manifestation of leukemia. Molecules that interfere with these mechanisms can contribute to the elimination of neoplastic cells by inhibiting replications; by inducing differentiation by triggering apoptotic processes and, in this way, act as potentially very important therapeutic substances. Ferrari et al. [45] carried out extensive investigation of 5-formyl uracil thiosemicarbazone complexes on the assays of proliferation inhibition on human leukemia cell lines K562 and CEM in vitro.

Comparative structural studies and approximate molecular orbital calculations have been developed by Tojal and Rojo [46] on Cu(II) complexes derived from pyridine-2-carbaldehyde thiosemicarbazone. The results allow to explain: (1) some significant structural differences between complexes containing the neutral ligand and those with the anionic one and (2) the formation of monomeric versus dimeric entities with the neutral ligand.

Although the investigations of metal complexes of a variety of thiosemicarbazides and thiosemicarbazones have been subject of intensive research, those of \(^4\)N substituted thiosemicarbazones and ligands that can bind two metal ions in close proximity have received little attention.

In continuation of their work on binucleating ligands Hoskin et al. [47] designed a novel phenoxo bridged binucleating thiosemicarbazone ligand and its Cu(II)
and Ni(II) complexes (Fig. 5) Also presented are some results of a single crystal X-ray study of one of the complexes, [Ni₂L(OC₂H₅)](DMF)₂.

![Chemical structure](image)

Literature records several reviews on coordination chemistry of thiosemicarbazones [48]. Recently Shetty et al. [49] reported binuclear molybdenum(V) and (VI) complexes of 2,6-diformyl-p-cresol bis[4-(X-phenyl thiosemicarbazonex)] (X= various substitution on phenyl ring) where the novel binucleating ligand is derived from the Schiff condensation of 2,6 diformyl-p-cresol and substituted thiosemicarbazides.

The ligands have few interesting features.

1. The ligands contain SNONS donor sequences possessing five potential coordinating sites.
2. The ligand can bind two metal ions leading to oxobridged binuclear complex keeping option for exogenous bridge.
3. The ligand can behave as monobasic, dibasic, or tribasic depending on reaction conditions and nature of metal ions.

In continuation of this work the we have attempted the synthesis and physico-chemical investigation of Cu(II), Ni(II) and Co(II) complexes using above ligands [chart 1.] and screened them for antimicrobial action. Exhaustive spectral, magnetic or redox studies of cobalt(II) complexes were not undertaken as we ended up with mononuclear complexes instead of expected binuclearity in the SNONS coordination sphere.

This work has been directed towards an understanding of the chemistry, electronic structures, spin-exchange coupling between metal centers and redox properties of these metal complexes.
Experimental

Preparation of 2, 6-diformyl-p-cresol

Preparation of 2,6-diformyl-p-cresol was carried out according to the method reported by Denton [50] with slight modification. Phosphorus pentoxide (75g) was mixed with preheated phosphoric acid (80ml). The reaction was carried out in a three-necked flask fitted with a stirrer, a guard tube and thermometer. The reaction mixture was stirred at 150°C until all phosphorus pentoxide dissolved into phosphoric acid. The resultant product, polyphosphoric acid was cooled to 50-60°C. Then, hexamine (30g) and p-cresol (12ml) were added slowly with stirring. The temperature was raised carefully up to 125°C. The yellow pasty mass was cooled and treated with water. The yellow precipitate was separated by filtration. The crude product was purified by steam distillation, which gave pale yellow needles. M.p 130-131°C. Yield 6g.

Synthesis of ligands

The synthesis of ligand involves two steps
1) Preparation of thiosemicarbazide and 2) Preparation of thiosemicarbazone

Aniline and substituted anilines and other chemicals used for the preparation were of reagent grade. Anilines were distilled or recrystallized prior to use.

Preparation of thiosemicarbazides [51]

Freshly distilled aniline (0.1mol) was dissolved in ammonia solution (20ml, d=0.88) and carbon disulphide (8.0ml) was added to it, gradually with stirring in ice bath. Ethanol (30ml) was added and stirring was continued till carbon disulphide completely dissolved. The reaction mixture was allowed to stand for 2-3 hours. An aqueous sodium chloroacetate (0.1mol) solution was added followed by hydrazine hydrate (10ml, 50%). The reaction mixture was stirred for 2-3 hours and allowed to stand overnight. The crystals separated were filtered and recrystallized from ethanol. Same method was followed for other substituted thiosemicarbazides using different substituted anilines. Yield 70-75%

Preparation of aryl thiosemicarbazone

0.021 Mole of aryl thiosemicarbazide in ethanol (100ml) was treated with 0.01 mole of 2,6-diformyl-p-cresol. The reaction mixture was refluxed for 3-4 hours. The yellow solid separated was filtered, washed with ethanol 2-3 times and dried. Yield 80-90%.

[Chart 1].
(a) structure of ligand (L¹-L¹⁰) (b) structure of complexes (C¹-C¹⁰)

R = -H
(L¹)
= -o, m, p -CH₃
(L², L³, L⁴)
= -o, m, p -OCH₃
(L⁵, L⁶, L⁷)
= -o, m, p -Cl
(L⁸, L⁹, L¹⁰)

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<th>Code</th>
<th>Ligand</th>
<th>M.p</th>
</tr>
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<tbody>
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<td>L¹</td>
<td>2,6-diformyl-p-cresol-bis(4-phenyl thiosemicarbazone)</td>
<td>&gt;280°C</td>
</tr>
<tr>
<td>L²</td>
<td>2,6-diformyl-p-cresol-bis(4-o-tolyl thiosemicarbazone)</td>
<td>&gt;270°C</td>
</tr>
<tr>
<td>L³</td>
<td>2,6-diformyl-p-cresol-bis(4-m-tolyl thiosemicarbazone)</td>
<td>&gt;266°C</td>
</tr>
<tr>
<td>L⁴</td>
<td>2,6-diformyl-p-cresol-bis(4-p-tolyl thiosemicarbazone)</td>
<td>&gt;282°C</td>
</tr>
<tr>
<td>L⁵</td>
<td>2,6-diformyl-p-cresol-bis(4-o-anisoly thiosemicarbazone)</td>
<td>&gt;270°C</td>
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<td>L⁶</td>
<td>2,6-diformyl-p-cresol-bis(4-m-anisoly thiosemicarbazone)</td>
<td>&gt;270°C</td>
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<td>L⁷</td>
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<td>&gt;264°C</td>
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<tr>
<td>L⁸</td>
<td>2,6-diformyl-p-cresol-bis(4-o-chlorophenyl thiosemicarbazone)</td>
<td>&gt;270°C</td>
</tr>
<tr>
<td>L⁹</td>
<td>2,6-diformyl-p-cresol-bis(4-m-chlorophenyl thiosemicarbazone)</td>
<td>&gt;270°C</td>
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<tr>
<td>L¹⁰</td>
<td>2,6-diformyl-p-cresol-bis(4-p-chlorophenyl thiosemicarbazone)</td>
<td>&gt;260°C</td>
</tr>
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</table>

*Chart 1.*
Preparation of the complexes

$[\text{Cu}_2(\mu-\text{Cl})\text{L}'\cdot\text{nH}_2\text{O}]$

A mixture of copper chloride (0.002 mol), appropriate thiosemicarbazone (0.001 mol) in EtOH were refluxed for 2 h. Separated complex was filtered, washed with EtOH and dried in air. Yield 80%, m.p. >250 °C.

Physical measurements

All the complexes were analyzed for their metal content by EDTA titration after decomposition with a mixture of HClO$_4$ and HCl followed by HCl. Sulfur was estimated as BaSO$_4$ [Details are given below]. Carbon, hydrogen and nitrogen were estimated on Thermoquest CHN analyzer. Magnetic susceptibility measurements were made at room temperature on a Gouy balance using Hg[Co(SCN)$_4$] as calibrant. Magnetic susceptibility on powdered samples of the complexes were measured in the temperature range 77-300 K using a PAR model 155 vibrating sample magnetometer and the instrument was calibrated with the use of metallic nickel. Results were converted into $X_m$ by the following equation,

$$X_m = \frac{[Y(\text{expt}) \cdot \text{Mol.wt} + \text{Wt. of complex} \cdot \text{field applied}]}{\text{diamagnetic corrections}}$$

Electronic spectra were recorded on Hitachi 2001 in DMF. I.r. spectra were recorded in the 4000-400 cm$^{-1}$ region (KBr disc) on a Nicolet 170 SX FT-IR. Far I.r. were recorded in the region 500-100 cm$^{-1}$ on Bruker IFS66V (Polyethylene discs). The $^1$H spectrum was obtained in d$_6$-DMSO on JEOL GSX 400 spectrometer using TMS as an internal reference. D$_2$O exchange is also recorded. $^{13}$C was recorded in DMSO on JEOL GSX 400 model. E.s.r. spectra of polycrystalline sample were recorded at room temperature on a Varian E-4 X-band spectrometer using TCNE/DPPH as $<g>$ marker. The $g_H$ and $g_L$ are compared with the resonance position of diphenyl picryl hydrazyl (DPPH) or tetracyanoethylene radical (TCNE). The $g_H$ and $g_L$ values are calculated using the following equations,

$$g_H = g_{\text{DPPH}} \cdot \text{H}_{\text{DPPH}}/\text{H}$$

$$g_{av} = 1/3(g_H + 2g_L)$$

Conductance measurements were done on ELICO-CM82 Conductivity Bridge, provided with a dip type conductivity cell fitted with platinum electrodes. The cell constant was determined by measuring the conductance of aqueous KCl solution of
known specific conductance. The value of cell constant was found to be 0.51. The molar conductance is calculated as follows.

\[ \Lambda_m = \frac{1000K \times \text{obs. Conductance (in mhos)/C}}{K \times \text{cell constant}} \]

\[ \Lambda_m = \text{molar conductance} \]

\[ K = \text{cell constant} \]

\[ C = \text{molar concentration (0.001M)} \]

T.g. studies were carried out in the 25-800 °C range using Rigaku TAS-100 Model thermal analyzer with a heating rate of 10 °C per min. in N\textsubscript{2} atmosphere. The FAB mass spectra were recorded on a JEOL EX 102/DA-6000 mass spectrometer using Argon as the FAB gas. m-Nitrobenzyl alcohol (NBA) was used as matrix. Electrochemical measurements were performed at room temperature in DMSO under O\textsubscript{2} free conditions using Optoprecision Potentiostat. A three-electrode assembly comprising a graphite-working electrode, a Pt auxiliary electrode and a saturated calomel reference electrode (SCE) were used. The supporting electrolyte was N\textsubscript{(Et)}\textsubscript{4}Cl with 0.1 M concentration and sample concentration was 0.001 M.

**Analysis of complexes**

The elemental analysis of complexes for metal, halogen and sulfur was carried out by the following standard methods [53].

**Estimation of Cobalt**

An accurately weighed (~ 0.100 g) complex was decomposed with a mixture of perchloric acid and concentrated hydrochloric acid (20 ml 1:1 v/v) on a sand bath. The solution was evaporated till the dense white fumes appeared and cooled to room temperature. The solution was diluted with distilled water (~ 50 ml) and transferred into conical flask. Three drops of xylene orange indicator were added followed by very dilute H\textsubscript{2}SO\textsubscript{4} until colour changes from red to yellow. Powdered hexamine was added with shaking until the deep-red colour is restored (pH-6). The solution was warmed to 60°C and titrated against standard EDTA. Colour change red to yellow-orange.

\[ \% \text{Co} = BR \times \text{Molarity of EDTA} \times 0.05894 \times 100 / \text{Wt of Complex} \]

**Estimation of Nickel**

An accurately weighed complex was decomposed and diluted as discussed in the estimation of cobalt. Freshly prepared murexide indicator (5-6 drops) was added followed by 10 ml of 1M ammonium chloride solutions. Concentrated ammonia solution was added drop wise until the pH of the solution reached to 7, which was indicated by the yellow colour of the solution. The solution was titrated against
standard EDTA until the end point is approached and the solution was made strongly alkali by addition of 10 ml of concentrated ammonia solution and then the titration was continued until the colour changes from yellow to bluish-violet.

% Ni = BR × Molarity of EDTA × 0.05871 × 100 / Wt of Complex

**Estimation of Copper**

An accurately weighed complex was decomposed and diluted as discussed in the estimation of cobalt. To the solution 5 ml of concentrated NH₃ and 5 drops of Fast-sulphon Black f indicator solution were added. The resulting solution was titrated against standard EDTA solution until the colour changed from blue to dark green.

% Cu = BR × Molarity of EDTA × 0.06354 × 100 / Wt of Complex

**Estimation of Chloride**

An accurately weighed (~ 0.150 g) complex was treated with 30 ml of dilute HNO₃ (1:1 v/v) on water bath for 1h. The solution was filtered through Whatman 40 filter paper to remove unwanted organic matter. Thus obtained solution was dilute to 100 ml and treated with AgNO₃ solution. The solution was heated nearly to boiling and allowed to stand for 2 h for complete coagulation. The process of precipitation and coagulation were performed in subdued light. The precipitate was filter through previously weighed sintered glass crucible (G-4) and washed with very dilute HNO₃ and dried at 130-140°C.

% Cl = Weight of AgCl x 0.2474 x 100/Weight of complex.

**Estimation of sulfur**

About 0.2g of the complex was fused with fusion mixture in a platinum crucible. The fused mass was treated with distilled water and quantitatively transferred into a beaker. This was heated to boiling and any undissolved particles were removed by filtration. The filtrate was acidified with HCl and then the solution was boiled and sulphate was precipitated with barium chloride. The precipitate was digested on a sand bath, filtered through Whatman No. 42 filter paper and washed with hot water until free from chloride. The precipitate was ignited in a previously weighed crucible. The residue was weighed as barium sulphate after cooling. The % of sulfur was computed as,

% of sulfur = [wt. of BaSO₄ * 0.1373 * 100 / wt. Of complex]

**Evaluation of antibacterial activity**

The antibacterial activity of the ligands and their metal complexes was assayed against two bacteria viz., *Escherichia coli* (gram negative) and *Bacillus cirrof*
lagellosus (gram positive) by cup-plate method [52]. Norfloxin and flucanozole nitrate were used as antibacterial and antifungal standards.

**Preparation of subcultures:**

One day prior to test, the cultures *Escherichia coli* and *Bacillus cirroflagellosus* were inoculated in nutrient broth (inoculation medium) and incubated at 37°C for overnight.

**Nutrient broth:**

Nutrient broth was prepared by dissolving peptone (0.5g), yeast extract (0.15 g), beef extract (0.15g), sodium chloride (0.35g), potassium hydrogen phosphate (0.36g) in distilled water (100 ml). The pH of the solution was adjusted to 7.2 by sodium hydroxide solution (4%) and then autoclaved for 20 minutes at 15 lbs pressure.

**Preparation of nutrient agar (base layer) medium:**

Nutrient agar was prepared by dissolving peptone (6 g), yeast extract (1.5 g) and agar (20 g) in distilled water (1000 ml). The pH of the solution was adjusted to 7.2 and autoclaved for 20 minutes at 15 lbs pressure.

**Preparation of seed layer medium:**

Peptone (0.6%), triptone (0.4%), yeast extract (0.3%), beef extract (0.15%), glucose (0.1%) and agar (2%) were dissolved in sterilized water.

**Preparation of drug solution:**

Drug solution was prepared by dissolving 5 mg of the compound in 5 ml of dimethyl formamide to give a drug concentration of 1000 μg/ml

**Method of testing:**

Nutrient agar, while hot, was poured into the sterilized petri dishes (20 ml in each dish) and allowed to attain room temperature. The seed layer medium was melted and cooled to 45-50°C with gentle shaking. Overnight grown liquid culture was added aseptically to the seed layer medium and mixed thoroughly to get the uniform distribution. Immediately, it was poured into petri dishes containing base layer (15 ml in each dish) and then allowed to attain room temperature. Thereafter, punching into the set agar with a sterile cork borer and scooping out the punched part, the cups were made. The diameter of each cup was 10mm. To these cups, 0.1 ml (100 μg) of drug solution added. These plates were allowed to cool for an hour to facilitate the diffusion. Then the plates were incubated at 37°C for 36 hours. The zone of inhibition was measured in millimeters.
Evaluation of antifungal activity

All the compounds prepared during the present investigation were tested for their antifungal activity, employing the fungi *Candida albicans* and *Aspergillus niger*.

Preparation of sub-culture

One day prior to the tests, inoculation of the above mentioned fungi was made in the inoculation medium and incubated at 37°C for 24 hours. The inoculation medium was prepared by dissolving yeast extract (0.6%), potassium dihydrogen phosphate (0.3%), peptone (1%), sodium chloride (0.5%) and agar (2%) in distilled water. The pH was adjusted to 7.0 and autoclaved for 30 minutes at the pressure of 15 lbs.

Preparation of nutrient agar (base layer) medium:

Nutrient agar (base layer) medium was prepared by the same method as explained under evaluation of antibacterial activity.

Preparation of fungal medium

The fungal medium was prepared by dissolving yeast (0.6%), potassium dihydrogen phosphate (0.3%), peptone (4%), sodium chloride (0.5%) and glucose (0.5%) in distilled water. The pH of the medium was adjusted to 7.0. The medium was sterilized at 15 lbs pressure for 20 minutes and cooled to 45-50°C with gentle shaking. Overnight grown culture was added aseptically to this medium and mixed thoroughly to get the uniform distribution.

Method of testing

The solutions of the compound in DMF were tested for antifungal activity in exactly the same manner as explained under evaluation of antibacterial activity. The zone of inhibition was measured in millimeters.

Results and discussion

All the complexes are non-hygroscopic. Complexes contain 2:1 metal-to-ligand ratio. They are formed by the loss of one, two or three proton(s). They are insoluble in water, EtOH, MeOH but soluble in DMF, DMSO and MeCN. Analytical data are presented in Table 1. Molar conductivities in DMF suggest that complexes are non-electrolyte (12-17 mho cm² mol⁻¹). Colour of the complexes ranges from pale black to dark brown. Melting points of all the complexes are above 250 °C.
Table 1. Analytical data of complexes.

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<th>Compound</th>
<th>Empirical formula</th>
<th>Found % (calc. %)</th>
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<tbody>
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<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>C1</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]</td>
<td>44.45(44.41)</td>
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<tr>
<td>C2</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]</td>
<td>46.20(46.18)</td>
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<td>C3</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]₂H₂O</td>
<td>43.70(43.76)</td>
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<tr>
<td>C4</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]</td>
<td>46.23(46.18)</td>
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<tr>
<td>C5</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]</td>
<td>44.10(44.02)</td>
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<td>C6</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]₂H₂O</td>
<td>41.90(41.81)</td>
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<td>C7</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]₂H₂O</td>
<td>41.85(41.81)</td>
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<td>38.09(38.00)</td>
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<td>C9</td>
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<td>39.83(39.98)</td>
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<td>C10</td>
<td>[Cu₂(C₂₃H₁₉N₆O₂S₂)Cl]</td>
<td>39.83(39.98)</td>
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</table>
Electronic spectral studies

Electronic spectral data are summarized in Table 2. The broad intense band around 260 nm in case of ligand is assigned to intra ligand π-π* transition. This band is almost unchanged in the spectra of complexes. The ligand also shows a broad band at 318 nm with a shoulder on low energy side, due to n→π* transition associated with azomethine linkage. This band in all complexes have shown red shift due to the donation of lone pair of electron to the metal and hence the coordination of azomethine. The shoulder centered around 380 nm in the ligand was assigned to n→π* of thioamide chromophore, which suffer blue shift in complex due to thioenolization. The moderately intense broad band for the complex in the region 403-410 nm is assigned to S→Cu(II) ligand to metal charge transfer transition (LMCT). The LMCT maxima for the phenolate complex show broadening, with a tail running into the visible part of the spectrum. This may result from a phenolate to Cu(II) LMCT band being superimposed on the low energy side of S→Cu(II) LMCT. The more intense charge transfer bands which extends deep into the visible region, prevents any analysis of d-d transition in the complex. Analysis of Table 2 reveals that the position of both the ligand bands and the LMCT bands is only slightly affected by the change in the substituents on the phenyl ring of thiosemicarbazides moiety in all complexes. Nevertheless, this small change cannot be related directly with the electron donor or acceptor ability of the substituents [54-56].

I.r spectral studies

Important I.r absorption bands are summarized in Table 3. The possibility of thione-thiol tautomerism (H-N-C=S ⇔ C=N-SH) in all the ligands has been ruled out, since there is no band around 2500-2600 cm⁻¹, which is characteristic of thiol group. The phenyl ν(NH) and hydrazine ν(NH) are observed around 3300 and 3100 cm⁻¹ respectively. Coupled vibration among thioamide bands I β(NH)+ν(CN), II ν(CN)+β(NH), III and IV are distributed around 1540, 1450, 1330 and 930 cm⁻¹.

The phenolic ν(OH) is found around 2900 cm⁻¹ as a weak broad band which disappears in all complexes indicating deprotonation and coordination to metal. This is further supported by shift of phenolic ν(C-O) at 1280 cm⁻¹ in ligand to higher frequency by about 50-60 cm⁻¹ in complex. Thioamide bands III and IV which have major contribution of ν(C=S) have undergone considerable reduction in intensity in all the complexes due to thioenolization and subsequent coordination to metal which is
Table 2. Electronic spectral and magnetic moment data

<table>
<thead>
<tr>
<th>Compound</th>
<th>$X_{\text{max}}$ (nm)</th>
<th>$\mu_{\text{eff}}$ (B.M)</th>
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</tr>
<tr>
<td>C2</td>
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<td>C3</td>
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<td>C4</td>
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</tr>
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<td>C10</td>
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Table 3. I.R spectral data of complexes

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<th>$\nu(\nu \nu)$</th>
<th>$\nu(\nu)$</th>
<th>Thioamide bands</th>
<th>$\nu(C-S)$</th>
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<td>$\nu(NH)$</td>
<td>$\nu(CN)$</td>
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<td>II</td>
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<td>-</td>
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</table>
supported by the appearance of a weak band around 610 cm\(^{-1}\) due to \(\nu(C-S)\) and disappearance of \(\nu(\text{N-H})\). The band due to \(\nu(\text{NH})\) persists in complexes. The broad band around 3400 cm\(^{-1}\) is due to \(\nu(\text{OH})\) of non coordinated water molecules. The presence of water molecules is confirmed by TG studies. The band around 1595-1603 cm\(^{-1}\) which is assigned to \(\nu(C=\text{N})\) has shifted to higher energy by 3-10 cm\(^{-1}\) supporting the coordination of azomethine nitrogen. Coordination of azomethine nitrogen has been proposed for the majority of thiosemicarbazone complexes with evidence based on shifting of \(\nu(C=\text{N})\). Interestingly, the shifting has been reported both to higher and lower energies. The shift of these bands depends on bond order of \(\nu(C=\text{N})\) on coordination which in turn depends on group attached to azomethine group. Thus the ligand act as tribasic pentadentate molecule. I.r spectra of ligand L\(^{10}\) and complex C10 are displayed in Spectrum 1 and 2.

The low frequency bands in the 500-470, 412-350 and 370-292 cm\(^{-1}\) regions are assigned to \(\nu(M-N)\), \(\nu(M-O)\) and \(\nu(M-S)\) respectively. The presence of a chloro bridge is evident from the IR bands in the 270-240 cm\(^{-1}\) region [57-61].

**Magnetochemistry**

*Orbital contribution in metal dimer complexes*

Transition metal complexes containing more than one metal atom with unpaired electrons can generally be categorized according to their magnetic behaviour into three main groups depending on the strength of the metal-metal interaction. In the non-interacting type the magnetic properties of the dimer are essentially unchanged from the paramagnetic monomer. In the strongly interaction type formation of relatively strong metal-metal bonds occurs, and the molecule will display simple diamagnetic behaviour (from even numbers of electrons).

Hoffmann et al [62] described the properties of weakly interacting metal ions. In such compounds this weak coupling between the electrons of the two metal ions leads to low-lying excited states of different spin, which can be populated at thermal energies (<1000 cm\(^{-1}\)). The resulting magnetic behaviour will be antiferromagnetic or ferromagnetic, depending on whether the low spin (spin paired) or high spin (spin parallel) state is the ground state, respectively. This interactions-often termed super exchange because of the large distance involved (3-5 Å) between the metal ions has been observed in a wide variety of compounds.
Spectrum 1. I.r spectrum of ligand L\textsuperscript{10}

Spectrum 2. I.r spectrum of complex C\textsubscript{10}
In experimental studies the magnetic interaction between spins $S_A$ and $S_B$ for atoms A and B is usually written in a form suggested originally by Heisenberg, Dirac, and Van Vleck

$$H = -2JS_A S_B \quad [1]$$

where the coupling constant $J$ is positive if the spins are parallel and negative if they are paired. If $|S_A| = |S_B| = S$ molecular states with total spin $S=0,1, \ldots, 2S$ are possible, and the energy difference between two states with spin $S$ and $S-1$ is given by

$$E(S) - E(S-1) = -2JS \quad [2]$$

In the most common case discussed is, $S=1/2$, and the triplet-singlet splitting, $E(1)-E(0)$, equals $-2J$.

Of all types of copper(II) dimers having an oxygen-atom bridge, the phenolate complexes are the most common and probably best studied. One reason for this is that phenolate was considered for many years as the most likely candidate for the endogenous bridging ligand in hemocyanin [63]. The apparent pKa of the protein bridging ligand, determined by Solomon several years after the initial proposal [64], is also consistent with that hypothesis. Another reason for the plethora of $\mu$-phenolate complexes is their easy synthesis and subsequent utility for the study of magnetic interactions between two Cu(II) ions.

Robson is generally credited with first synthesis of the classic Schiff base ligands used to prepare binuclear $\mu$-phenolate complexes [Fig. 6][65-67]. Subsequently, a large variety of complexes having different donors and geometries have been prepared and characterized [68-70], and representative complexes are shown in fig 7 and 8. with their magnetic data.

![Fig. 6](image)

Note that the chelate ring sizes of (4,6,6,4), (5,6,6,5), and (6,6,6,6) are all represented, although a direct correlation of structure and magnetic coupling is not readily made because the actual geometry of the copper atoms varies, as does the orbital overlap between the phenolate and copper ions.
In early 1980s, several research groups began to prepare ligands that could provide three nitrogen donors to each metal ion. Those ligands are not accessible by the Schiff-base route, and involve the coupling of two (N\textsubscript{3}) units to an appropriate aryl precursor.

A number of workers have treated bis(chloromethyl)-\textit{p}-cresol with bis(pyridylmethyl)amine or bis(benzimidazolylmethyl)amine to give ligands in which the N-N-N chelates are 5-membered rings\cite{71-73}.

The Cu(II) complexes prepared using these ligands show only modest magnetic coupling in most cases, and the copper ion geometry is sometimes trigonal bipyramidal.

With complexes of these ligands having two, single atom bridges; there is apparently some associated strain, which can be relieved in solution, generating a complex having a single phenolate bridge \cite{74}. The bis(aquo) complex prepared by Stephen \cite{Fig. 9} is the only structurally characterized Cu(II) dimer having a phenolate bridge without another bridging group. Having bound water molecules makes this complex potentially a good model for metHc(aquo), except that the phenolate ion is equatorially bound to one copper ion and axially coordinated to the other. As a result, the magnetic interaction is actually slightly ferromagnetic rather than antiferromagnetic.
During same period, Sorrell’s and Karlins’s group independently synthesized ligands providing three nitrogen donors to each copper ion. For these ligands, all of the chelate rings are 6-membered, which allows the copper(II) ions to attain a tetragonal geometry without strain. Sorrell and co-workers were successful in preparing a diamagnetic exogenously azido-bridged complex by incorporating 5-membered chelates into the ligand backbone so that Cu-Cu separation would be increased and the azide would be forced to bridge in a 1,3-fashion [Fig. 10]. This is highly magnetically coupled system with $2J > 1800\text{ cm}^{-1}$ [75-79].

We find it appropriate to compare the structure, geometry around metal ions and a physical parameter i.e, coupling constant value of above reported systems with one developed by us, although sulfur ligation is totally ruled out.

All the complexes prepared in the present investigation have low room temperature magnetic moments falling in the range $\mu_{\text{eff}} = 0.89 - 1.10\ \mu_B$ indicating strong antiferromagnetic exchange interaction between metal centers.

Variable temperature magnetic susceptibility measurement was performed on powdered sample of copper complex (C2) in the temperature range 77-300 K. The best fit of the data to the Bleany-Bowers equation (1) [80] (using isotropic (Heisenberg)
exchange Hamiltonian, $H = -2J \hat{S}_1 \cdot \hat{S}_2; S=1/2$) for exchange-coupled pairs of copper (II) ions was determined with two variable non-linear regression analyses.

\[
\chi_m = \frac{N\beta^2 g^2}{3kT} \left[ 1 + \frac{1}{3} \exp\left( \frac{-2J}{kT} \right) \right]^{-1} \left( 1 - \rho \right) + \left[ \frac{N\beta^2 g^2}{4kT} \right] \rho + N\alpha \quad (1)
\]

In this expression $-2J$ is the singlet-triplet splitting or exchange integral and the other terms have their usual meaning. $\rho$ represents the fraction of a possible magnetically dilute monomeric Cu(II) impurity. The temperature independent paramagnetism $N\alpha$ was taken as $60 \times 10^{-6}$ cgs units/mol for copper, and $\rho$ was treated as a floating parameter. A typical experimental variable temperature susceptibility data and magnetic moment including the best fit theoretical line is shown in Graph 1. for $-2J = 625 \text{ cm}^{-1}$ and $g=2.09$. This $\chi_m T$ vs $T$ curve is typical of a strongly antiferromagnetically coupled system, as expected for a phenoxy-bridged Cu$_2$ dimer. The magnetic moment decreases noticeably as the temperature decreases. Because of close proximity of the two metal centers in the SNONS compartment, we describe this magnetic behavior in large part to an intramolecular antiferromagnetic exchange interaction. This interaction is likely to be propagated by the endogenous bridging oxygen atom and to a lesser extent by exogenous bridging chloride but not via a direct metal-metal interaction. The discrepancy factor $\sigma = \left[ \sum (\chi_{obs} - \chi_{calc})^2 / \sum \chi_{obs} \right]^{1/2}$ in the least squares fits was $2.1 \times 10^3$. In line with their magnetic behavior, all the complexes are E.p.r silent [81-83].

Although sulfur ligation is ruled out in hemocyanin *biomimic*, there are number of copper proteins where presence of sulfur coordination is confirmed. As we find it now, these magnetically coupled systems could be added to the list of the previously reported pharmacologically important thiosemicarbazone metal chelate systems.
Enzymes containing dinuclear Cu(II) centers play important roles in nature and, consequently, characterization of their structure and function is a problem of outstanding importance. A fundamental and, as yet, largely unexplored issue is the determination of the structural and magnetic properties of binuclear copper(II) centers using NMR spectroscopy. $^1$H NMR is a natural technique to probe these systems because only protons proximate to the paramagnetic centers are affected [84].

During the past decade, the isotropic NMR shift phenomenon has gained increasing importance as a tool for investigating molecular properties. The term "isotropic shift" is meant to include nuclear resonance shifts arising from Fermi hyperfine contact interactions or from electron-nuclear dipolar (pseudocontact) interactions in paramagnetic substances.

A large portion of the work involving isotropic shifts has been carried out by chemists interested in the bonding and stereochemistry of paramagnetic transition metal complexes. In these systems the hyperfine contact interaction is the result of an imbalance of electron spin on the ligand due to spin transfer between metal and ligand. The spin imbalance may reach ligand nuclei by means of s or p molecular orbitals, causing changes in local magnetic fields and resulting in binuclear resonance shifts. The dipolar interaction is most simply described as a through-space coupling between ligand nuclei and unpaired electrons on the metal ion. The dipolar NMR shift for a nucleus depends on its orientation with respect to the ligand-field axis of the complex.
and upon its distance from the metal ions. Dipolar shifts occur only for complexes having g-value anisotropy. In many transition metal complexes, both types of interaction contribute to the observed NMR shifts [85].

Use of isotropic shifts is by no means restricted to transition metal complexes. Of interest to biochemists is the use of the isotropic shift phenomenon for elucidating structures of large molecules such as ferredoxin, hemoglobin, cytochromes c and others. At present the general utility of the isotropic shift phenomenon is severely restricted because most paramagnetic substances either fail to give detectable nuclear resonances or give very broad, poorly resolved signals as a result of rapid nuclear spin relaxation induced by the paramagnetism. Those paramagnetic transition metal complexes upon which the most successful NMR studies have been carried out have very short electron relaxation times. Reasonably narrow NMR signals may be obtained for these complexes and, coupled with isotropic shifts, the overall resolution may be quite good. Unfortunately, only few transition metal ions appear to have short enough relaxation times to give the required resolution. A few years ago Eaton, [86] made systematic study of paramagnetic acetylacetonate complexes in order to determine which metal ions have the very short electron relaxation times necessary for observation of NMR signals of ligand protons. A short electron relaxation time precludes observation of EPR signals, and thus those ions giving the best resolved NMR spectra proved to be those not especially amenable to EPR studies.

$^1$H N.m.r spectroscopy is not generally viewed as a viable solution characterization technique for a paramagnetic copper(II) complexes, because slow electronic relaxation leads to broad resonance. However a few reports have described copper(II) complexes that produce narrow $^1$H N.m.r resonance. The copper complexes, which produce such $^1$H N.m.r spectra, involve two paramagnetic centers, which produce effective relaxation mechanisms. In most cases these are binuclear copper(II) complexes with moderate antiferromagnetic coupling (2J values ranging from -156 to -546 cm$^{-1}$). Balch and Satcher [87] reported $^1$H N.m.r spectra of binuclear complexes in which variation of a bridging ligand causes a marked change in the magnetic coupling. Further they reported a quantitative relationship, between the chemical shifts and magnetic coupling, over a wide range (2J= +26 to -1100 cm$^{-1}$) of coupling constants.

$^1$H N.m.r study of few nonsymmetrical binuclear complexes were reported by Lubben et al.[88] in the copper centers which are weakly antiferromagnetically coupled
with \(2J=-15 \text{ cm}^{-1}\). The weak antiferromagnetic coupling is reflected in the large isotropic shifts for the ligand protons in the \(^1\text{H}\) N.m.r spectrum. The resonance is well resolved and covers a range of 180 ppm. NMR assignments were deduced from peak integration, selective substitution, 2D-COSY experiments. Lesowski [89] reported \(^1\text{H}\) N.m.r study of heteronuclear macrocyclic complexes. In one of the complexes [NiCoL](ClO\(_4\))\(_2\).2H\(_2\)O [L=macrocyclic ligand] the imino proton has been identified at 403 ppm.

Maekawa and his coworkers [90], who made an exhaustive investigation of \(^1\text{H}\) N.m.r of dicopper(II) complexes with binucleating ligands containing imidazoles, reported \(2J\) values ranging from 156 to 545 cm\(^{-1}\) for the complexes. \(^1\text{H}\) N.m.r of their complexes cover a moderately short range of 0 to 100 ppm.

Usually mononuclear copper (II) complexes provide quite broad \(^1\text{H}\) NMR signals. On the other hand, when two copper (II) ions are close to each other so that superexchange interactions occurs effectively, the narrowing of NMR signals as well as the decrease in isotropic shift occurs. An equation of the isotropic shift [90] for the antiferromagnetic coupling systems (\(H=2JS_iS_j; S=\frac{1}{2}\)) has been given by the explicit use of the \(J\) value,

\[
\Delta\delta_{iso} = \frac{\gamma^2 \beta A}{(2\pi)kT}[(\exp(-2J/kT)+3)^{-1}]
\]

where \(A\) is the hyperfine coupling constant of a nucleus with an electronic spin moment in the \(S=1\) level in the magnetically coupled copper homodimer, \(\gamma\) is gyromagnetic ratio of an observed nucleus, and \(k\) is Boltzmann constant. The isotropic shifts are governed by population of distributions in the \(S=0\) and \(S=1\) states of dimeric copper (II) complexes, thus being decreased by a factor of \([(\exp(-2J/kT)+3)^{-1}\]. The antiferromagnetic interactions in complexes significantly influence both isotropic shifts and line widths with the propensity to reduce them. We find it interesting to investigate any relationship between \(J\) and/or \(\Delta\delta_{iso}\) and \(\Delta V_{1/2}\). We have selected few complexes (C4-C7) for the \(^1\text{H}\) N.m.r studies. \(^1\text{H}\) N.m.r spectra for ligand L4 and complex C4 are presented in Spectrum 3 and 4.
Spectrum 3. N.m.r spectrum of ligand L⁴

Spectrum 4. N.m.r spectrum of complex C4
Relationship between NMR Parameters and Magnetic Properties of Binuclear copper (II) Complexes [90]

In order to observe the relationship between NMR parameters and magnetism, the magnetic moments of binuclear copper (II) complexes in solution are required. The magnetic moments obtained in the solid-state do not, necessarily agree with those in solution because the coordination of solvent molecules and/or the dissociation of coordinated ligands may occur. We can estimate the magnetism from a magnetic susceptibility study of the solution by N.m.r spectroscopy [91]. The magnetic susceptibility was measured in d6-DMSO. From these $\chi_m$, the effective magnetic moment ($\mu_{eff}$) are readily obtained according to the standard equation, $\mu_{eff}=2.83(\chi_m T)^{1/2}$, and presented in Table 4 together with the $\mu_{eff}$ values calculated from 2J values in the solid state. Results reveal that the $\mu_{eff}$ (solution) values are comparable with those in the solid state.

Graph 2 Plots of observed $^1$H N.m.r chemical shifts ($\Delta$obs) vs magnetic moment ($\mu_{eff}$) (—) and observed line width at half-height vs $\mu_{eff}$ (-----) 

Ligand dissociation is unlikely in these chelating systems because no evidence of the dissociation was obtained by NMR spectra. The structures predicted in solution state are similar to those characterized in the solid state.

The magnetic susceptibility of a Cu(II) dimer is modeled by

$$\chi_{dimer} = 2Ng^2\beta^2/3kT[1+(1/3)\exp(-2J/kT)]^{1} \ldots \ldots [3]$$

We used the magnetic susceptibility of half of $\chi_{dimer}$ in order to obtain the magnetic susceptibility per copper, $\chi_{Cu}=\chi_{dimer}/2$.

The 2J values in the solid state have been determined much more accurately
than those in solution because the measurements of magnetic susceptibility can be 
made over a wide temperature range in the solid state. The experimental temperature 
range in the case of solution is quite restricted giving rise to an ambiguity in the 2J 
value.

Both the observed shifts [with respect to azomethine signal] and the magnetic 
moment increase in the order C7, C6, C5. This relationship is well illustrated in 
Graph.2. These results support the relationship between the isotropic shift and the 
magnetic moments as in equation 2. The similar relation is also found in the line width 
at half-height, being presented in the same Graph.2. Although the Solomon-
Bloembergen equation has been available for the line widths of paramagnetic systems, 
there are no relations for the line widths of copper (II) complexes, which give relatively 
narrow NMR signals as well as small isotropic shifts.

Table 4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \mu_{\text{eff}} )</th>
<th>( 2J )</th>
<th>( \Delta \delta_{\text{obs}} )</th>
<th>( \Delta V_{1/2} )</th>
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<td>Solid(^b)</td>
<td>(ppm)(^d)</td>
<td>(Hz)</td>
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<td>0.80</td>
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\(^a\)obtained at room temperature from 3mM solution sample by the Evan’s method; error 
limit 0.1 \( \mu_{\text{eff}} \)

\(^b\)room temperature magnetic moment of solid sample by Gouy method with 
diamagnetic corrections.

\(^d\)from cryomagnetic investigation

\(^d\)relative in ppm to solvent resonance

*The determination of the paramagnetic susceptibility of metal complexes in solution 
by nuclear magnetic resonance*

The position of a line in the proton resonance spectrum of a molecule depends on bulk 
susceptibility of the medium in which the molecule is situated. For an inert substance 
(dioxan) in aqueous solution, the shifts caused by paramagnetic ions are given by the 
thoretical expression.

\[
\Delta H/H = (2\pi/3) \Delta k------[4]
\]

where \( \Delta k \) is the change in volume susceptibility.

For aqueous solution of paramagnetic substances about 2% of t-butyl alcohol in
water also placed in the nuclear magnetic resonance tube which is spun during the measurements. The change in the susceptibility of the dissolved compound caused by the t-butyl alcohol will normally be completely negligible. Two resonance lines will normally be obtained from the methyl protons of the t-butyl alcohol in the two solutions owing to the difference in their volume susceptibilities; with the line from the more paramagnetic, $\chi$, of the dissolved substance is then given by the expression

$$\chi = \frac{3\Delta f}{2\pi fm} + \chi_0 + \frac{\chi_0(d_0-d_s)}{m} \tag{5}$$

where

$\Delta f$ = frequency separation between the two lines in cycles/sec

$f$ = frequency at which the proton resonance are being studied, in cycles/sec

$m$ = mass of the substance in 1 ml of the solution

$\chi_0$ = mass susceptibility of the solvent

d$_0$ is the density of the solvent and d$_s$ that of the solution. For highly paramagnetic substances the last term can be often be neglected without serious error. Acetone or dioxan can also be used in place of t-butyl alcohol or, for non-aqueous solutions, cyclohexane or tetramethyl silane. Alternatively a resonance line of the organic solvent itself can be used as a reference, provided care should be taken that the concentration of solute is not too high, otherwise the line from the solution will be too broad. From these $X_m$, the effective magnetic moment are readily calculated [Table 4.] according to the standard equation

$$\mu_{\text{eff}} = 2.83(X_m T)^{1/2}$$

**Electrochemistry**

The potential for ligand reduction/oxidation was confirmed by investigating the ligand and analogous binuclear zinc complex, which exhibits no redox, waves in the working range [-0.2 to +0.65V]. The cyclic voltammogram of complex C2 in DMSO (graphite electrode) in the positive potential range exhibits [Graph 3.] one metal-centered reversible one-electron oxidation step.

$E_{\text{ox}} = 0.37 \text{ V}, \ E_{\text{red}} = 0.30 \text{ V}, \ E_{1/2} = 0.34 \text{ V} \ with \ \Delta E_p = 70 \text{ mV}$

$[E_{1/2} = 0.5(E_{\text{ox}}+E_{\text{red}}), \ \Delta E_p = E_{\text{ox}}-E_{\text{red}}].$

It is not surprising that only one metal center of the SNONS cavity has undergone oxidation. It is reasonable to assume that bulkier substitution on the phenyl ring of the thiosemicarbazide limb probably has squeezed the SNONS cavity to the extent that the ligand frame work is unable to expand to accommodate the bigger Cu$^+$,
if the metal ion gets reduced. But is liberal enough to hold smaller Cu$^{III}$ ion as revealed by a well-defined anodic peak in the +ve potential. The ratio $i_{pa}/i_{pc}$ remained practically constant when the scan rate is varied between 0.015 to 0.15 Vs$^{-1}$ and the redox couple is practically same even after repeated scanning rules out any electrode reaction or geometrical change after oxidation [92-97].

*Graph 3. Cyclic voltammogram of C2 in DMSO at a scan rate of 0.015 Vs$^{-1}$*
Thermal studies and FAB mass spectral studies

Thermal studies for the complexes C3, C7 and C8 were carried out in the temperature range 25 to 800 °C. Initially there is gradual loss in weight up to 140 °C which corresponds to the loss of two non-coordinated water molecules. In the first step of dehydrated complexes, the weight loss corresponds to combined loss of a thiosemicarbazide limb plus chloride. Partially decomposed, rearranged, intermediate complex continues to lose weight up to 590 °C. The plateau is obtained beyond 590 °C indicating formation of CuO. Weight losses from the TG, agrees well with theoretical calculations. It should be pointed out that definite horizontal weight levels were not obtained in the curve. Thus, the composition of the intermediate complexes may be fortuitous although the weight losses indicated the above stoichiometries.

FAB mass spectroscopic data (C1 and C6) clearly suggested that binuclear complexes have been formed in each case. For C1 a molecular ion was observed (m/z 659) corresponding to the mass of the entire complex including bridging chloride, while it corresponds to the dehydrated complex in C6 (m/z 716).

Biological activity

The result of the inhibitory activity of the complexes, on few species of bacteria and fungi, suggest that none of the complexes are active against the species used.
References


