STUDIES ON METAL COMPLEXES WITH NITROGEN, OXYGEN AND SULPHUR DONOR LIGANDS

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By

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CERTIFICATE

This is to certify that the thesis entitled, **"Studies On Metal Complexes With Nitrogen, Oxygen And Sulphur Donor Ligands"** is an authentic record of the research work carried out by **Mrs. Priya. N.P,** under my supervision in partial fulfilment of the requirements for the Degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

Dr. K.K. Aravindakshan
(Supervising Teacher)

This thesis is dedicated to my husband M.J. Manoranjan, Retd. Associate Professor in Chemistry for his wholehearted co-operation, encouragement and suggestions during the entire course of this work

DECLARATION

I, Priya. N.P., hereby declare that this thesis entitled, **"Studies on Metal complexes with Nitrogen, Oxygen and Sulphur Donor Ligands"**, submitted to the University of Calicut in partial fulfilment of the requirements for the award of Doctor of Philosophy in Chemistry, is a bonafide research work done by me under the supervision and guidance of Dr. K.K. Aravindakshan.

I further declare that this thesis has not previously formed the basis for the award of any other degree, diploma or other similar title.

Calicut University
Date : **PRIYA. N.P**

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PREFACE

Coordination chemistry has been enriched due to the synthesis and characterization of several transition metal complexes in which the metal is coordinated by nitrogen, oxygen and sulphur donor ligands. Thiosemicarbazones and their metal complexes have been the subject of great interest due to their wide range of applications that stretch from their use in analytical chemistry through pharmacology to nuclear medicine. Literature survey revealed the fascinating ligational behaviour of C=N–NH–C=S group and the complexation behaviour of thiosemicarbazones. It was noticed that condensation of N(4)-substituted thiosemicarbazide with β-diketo derivatives leads to the formation of new class of N(4)- substituted thiosemicarbazones with significant and interesting structural properties. Coordination of N(4)-substituted thiosemicarbazones with transition metals leads to the formation of new metal complexes with novel structures. These metal complexes of substituted thiosemicarbazones have attracted much attention due to their antibacterial-, antifungal- and antitumour properties. They were also found to be useful inhibitors of metal corrosion.

Earlier reports on the synthesis, characterization and antimicrobial studies of some transition metal complexes of thiosemicarbazones are encouraging and useful for further research. Hence it was considered to be worthwhile and interesting to synthesize N(4)-methyl(phenyl)thiosemi-carbazones of 1,3 diketo derivatives and several complexes of these ligands.

In the present programme of research work, five different ligands i.e., acetylacetone N(4)-methyl(phenyl)thiosemicarbazone, benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone, acetoacetanilide N(4)-methyl-(phenyl)thiosemicarbazone, ω–bromoacetoacetanilide N(4)-methyl-(phenyl)thiosemicarbazone and N-ethylacetoacetanalide N(4)-methyl(phenyl)

thiosemicarbazone were synthesised and four of them were characterized. Their complexing behaviour towards several typical transition metal ions like Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were studied and new solid complexes were prepared. These complexes were characterized using various analytical techniques. Based on these analysis, tentative structures for these complexes were suggested.

The matter in this thesis is divided in to two Parts consisting of 9 Chapters. Chapter I of Part I, 'Synthesis and Characterization', gives a brief description of the history, importance and applications of coordination complexes. Brief review on the complexes of thiosemicarbazones and the significance and scope of the present investigation are also included in this chapter.

The materials used, methods adopted for the preparation of the ligands and the physico-chemical techniques employed for the characterization of complexes are given in Chapter II.

The synthesis and characterization of the ligands, acetylacetone N(4)-methyl(phenyl)thiosemicarbazone, benzoylacetone N(4)-methyl(phenyl)-thiosemicarbazone, ω–bromoacetoacetanilide N(4)-methyl(phenyl)thiosemi-carbazone and N-ethylacetoacetanalide N(4)-methyl(phenyl)thiosemicar-bazone and their transition metal complexes are described in Chapters III, IV, V and VI, respectively. These six chapters are included in Part I of the thesis.

In addition to the synthesis and structural studies of the compounds, their Electrochemical- and Biotoxic evaluations have been carried out and the results are presented in Part II. The corrosion inhibition effect of acetylacetone N(4)-methyl(phenyl)thiosemicarbazone and ω–bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone in 1M HCl

medium on mild steel has been investigated at room temperature. This study is included in Section A of Part II in the thesis.

Apart from this, three ligands, acetylacetone N(4)-methyl(phenyl)-thiosemicarbazone, ω–bromoacetoacetanilide N(4)-methyl(phenyl)thiosemi-carbazone and N(4)-ethylacetoacetanalide N(4)-methyl(phenyl)thiosemicar-bazone and their metal complexes were screened for antibacterial- and antifungal activities against selected microbial species. The details of these studies are presented in Section B of Part II in the thesis.

The cytotoxic - and antitumour studies of acetoacetanalide N(4)-methyl(phenyl)thiosemicarbazone and its metal complexes were conducted. Copper complex of acetoacetanalide N(4)- methyl(phenyl)thiosemicarbazone was selected for *in vivo* antitumour studies. The details of these investigations are presented in Section C of Part II in the thesis.

The references cited in the text are arranged in serial order at the end of each chapter /section.

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CHAPTER I
INTRODUCTION

Coordination compounds are those in which the central metal atom is linked to a number of ions or neutral molecules called ligands by coordinate bonds, (i.e. by donation of lone pair of electrons by these ions or neutral molecules to the central metal atom). For e.g. nickel tetra carbonyl, $[Ni(CO)_4]$ in which carbonyl molecules are linked to the central nickel atom by coordinate bonds. Coordination compounds are also called as complexes. The complex ion with positive charge is called cationic complex, eg. $[Cu(NH_3)_6]^{3+}$ and the complex ion with negative charge is called anionic complex, eg. $[Fe(CN)_6]^{4-}$. If the complex carries no net charge it is called a neutral complex or simply complex, eg. $[CoCl_3(NH_3)_3]$.

1. History of coordination compounds

The earliest known coordination compound used in India is the bright red alizarin-dye which is a calcium aluminium chelate of hydroxy anthraquinone. The first scientifically recorded observation about complexes was made by the German Alchemist, Andreas Libavius in 1597. He explained that the blue colour formed, when lime water containing ammonium chloride (NH_4Cl) comes in to contact with brass is due to the formation of a typical complex ion, $[Cu(NH_3)_4]^{2+}$. The coordination

compound, Prussian blue, ferriferrocyanide, had been used from the beginning of 18th century and it was known as Artist's Pigment. In 1760, the coordination compound, potassium hexachloroplatinate(II), $K_2[(PtCl_6)]$, was prepared to refine platinum. The systematic developments of modern coordination chemistry have begun with the discovery of a compound formed from ammonia solution of cobalt chloride in 1798 by the French Chemist, B.M. Tassaert. Even though he failed to follow up his discovery, his attempts in this area persuaded other scientists and they isolated an independent, stable new chemical compound, $[Co(NH_3)_6]Cl_3$ from cobalt chloride and ammonia. Its properties were found to be different from the constituent compounds. A series of coordination compounds of cobalt, iridium and platinum were prepared in the next fifty years. In 1862, the Swedish Chemist, Christian Wilhelm Blomstrand, suggested different structures for $Co(NH_3)_6Cl_3$ based on the amount of AgCl precipitated upon the addition of silver ions and he suggested that there must be two different types of chlorides in these complexes. However, some of the structures that he proposed were incorrect. In 19th century, more complexes were discovered and different theories were proposed to account their formation and properties. The most successful and widely accepted theory among them was the chain theory suggested by Blomstrand in 1869. This theory was modified by the Danish Chemist, Sophus Med Jorgensen. He prepared a lot of complexes and this provided experimental foundation for the coordination theory suggested by the German

scientist, Alfred Werner in 1893. Werner's theory was based on accurate conductivity measurements. Werner explained the spatial arrangement of ligands around the metal ions of complexes by suggesting two types of valencies, primary- and secondary valencies. Werner's ideas became popular and he prepared first optically active octahedral complex that does not contain carbon. In 1913, Werner was awarded Nobel Prize for his work and he is universally recognized as the founder of Coordination Chemistry and Structural Inorganic Chemistry.

Even though Werner's theory described the structures of many coordination compounds, it was unable to explain the nature of bonding within the coordination sphere and also it failed to account for the preference of 4- and 6 coordination numbers among complexes. A number of attempts have been made to extend the existing different theories to explain the bonding of coordination compounds.

Sidgwick introduced an electronic concept of coordination to explain the bond between the ligand and the metal ion. According to him, the ligands donate electron pairs to the central metal ion to form coordination compounds. In this concept, ligands are electron donors and central metal ions are acceptors and the bonds are dative- or coordinate bonds. The Effective Atomic Number rule (EAN), also known as the 18-electron rule was suggested by Sidgwick. This rule states that the total number of electrons

around the central metal ion including those gained through coordination by the ligands is EAN and in many cases this number is equal to the atomic number of the next higher inert gas. Most of the coordination compounds obey EAN rule.

There are other structural theories based on modern principles of bonding such as Valence Bond Theory (VBT), Crystal Field Theory (CFT), Ligand Field Theory (LFT) and the Molecular Orbital Theory (MOT). The concept of donation of electron pairs to metal ions forming coordinate bonds was applied to coordination compounds by Linus Pauling and Slater. This theory is concerned with the shapes and magnetic behaviours of metal complexes, which is called the Paulings theory of complexes or Valence Bond Theory (VBT). This theory was very useful in predicting both physical- and chemical properties of the complexes. But more satisfactory explanation for the bonding and the properties of the complexes was developed by Hans Bethe and John Vleek and is called Crystal Field Theory. This theory was very effective in describing the metal-ligand interaction and visible absorption spectra of metal complexes. According to this theory, metal ion is placed in an electrostatic field formed by the ligand molecules or ions. This electric field changes the energies of the d electrons in the case of transition metal ions and the properties of the complexes are related to these energy changes. As these interactions are similar to those between the ions in a crystal, it is called Crystal Field Theory. Valance Bond Theory considers the metal-ligand

bond to be covalent, but Crystal Field Theory considers the bond to be ionic. In the mid 1960's coordination chemists depended on another theory called Molecular Orbital Theory (MOT) which accounts both ionic- and covalent nature in the bonding interactions.

2. Importance and applications of coordination compounds

Coordination compounds are widely present in the minerals, plants and animals and are known to play many important functions in the area of analytical chemistry, metallurgy, biological systems, industry and medicine. Hardness of water is estimated based on the principle that the Ca^{2+} and Mg^{2+} ions form stable complexes with EDTA. Certain metals like silver and gold are extracted by the complex formation. Similarly, purification of metals can be carried out through the formation of coordination compounds. Certain articles can be electroplated with silver and gold much more smoothly and evenly from solutions of their complexes. Coordination compounds are used as catalysts for many industrial processes. If the complex ions formed are of metal chelate type, they can be widely used in industry and laboratory for the softening of hard water, in the separation of lanthanides and actinides, in the detection of metals in qualitative analysis and in the quantitative estimation of nickel, magnesium and copper ions. The interaction of transition metal ions with biological molecules provide one of the most fascinating areas of coordination chemistry. Many metal complexes are used as drugs and

chemotherapeutic agents. The pigment responsible for photosynthesis, chlorophyll is a coordination compound of magnesium. Hemoglobin, the red pigment of blood which acts as oxygen carrier is a coordination compound of iron. Vitamin B_{12} is a coordination compound of cobalt. Thus the chemistry of coordination compounds is an important and challenging area of modern Inorganic Chemistry.

3. Transition metal ions and their complexes

Transition elements with incomplete 3d-, 4d- and 5d subshells are also called‘d’ block elements. Except mercury, all transition elements have typical metallic structure and metallic properties. For the d block elements, the partly filled 3d-, 4d- and 5d orbitals project well out the periphery of the atoms and ions, so that the electrons occupying them are strongly influenced by the surroundings and in turn, are also able to influence the environment significantly. This is the reason why d block metals are very prominent and important in coordination chemistry. A large number of complex compounds are formed by transition metal ions with very useful properties like antifungal-, antibacterial-, antitumour-, antituberculosis- and good catalytic properties. The role of these complexes in chemotherapy and chelation therapy has opened a new path in coordination chemistry.

Transition elements form complexes easily due to their small size, large effective nuclear charge, large charge/size ratio and the presence of

vacant d orbitals to accept the lone pair of electrons donated by the ligands. The capacity to form complex compounds vary from element to element. The oxidation state and coordination number of the metal ion determine the physical- and chemical properties of the complexes. The coordination numbers 4- and 6 are the most prominent ones.

4. Schiff bases as ligands

Schiff bases, named after Hugo Schiff are the condensation products of primary amines and carbonyl compounds with a general formula $RCH = NR^1$, where R and R^1 represent alkyl or aryl substituents. The N-substituted imines called azomethines contain carbon-nitrogen double bond with nitrogen atom connected to an aryl or alkyl group[1] with a general formula $R^1R^2C = NR^3$, where R's are organic side chains. The chain on the nitrogen makes the Schiff base a stable imine. The N-substituted imines form an important class of compounds with various applications,[2] especially in medicinal- and pharmaceutical fields. They show biological activities including antibacterial-[3,4], antifungal-[5,6] and antitumour[7] activities.

Hydrazones are azomethines characterized by the presence of triatomic group, > C=N-N < as shown in Figure 1.

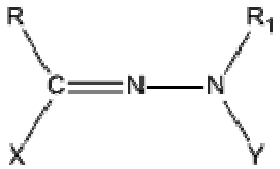

Figure 1.

where R, R_1, X and Y are different groups. In the Figure 1, if R_1 = NH_2CO the compound is semicarbazone and if R_1 = NH_2 CS it is thiosemicarbazone.

5. Thiosemicarbazone and their metal complexes - a brief review

Thiosemicarbazone and related compounds show keto-enol tautomerism as in Figure 2.

Figure 2.

where X = O, S or Se

It is observed that in most of the metal complexes, thiosemicarbazone coordinates in the keto form. The coordination to the metal atoms takes place in the *cis*- configuration, as in Figure 3, bonding through thiol sulphur- and hydrazine nitrogen atoms in a bidentate manner.

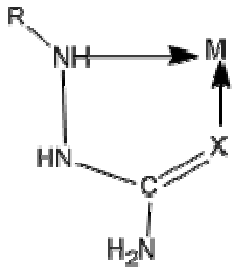

Figure 3.

If the thiosemicarbazone molecule is in the *trans*- configuration, bonding takes place only through the sulphur atom as in Figure 4.

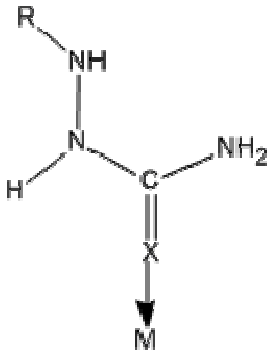

Figure 4.

If an additional coordinating atom is present in the proximity of S,N donating centres, thiosemicarbazone will act as tridentate ligand as in Figure 5.

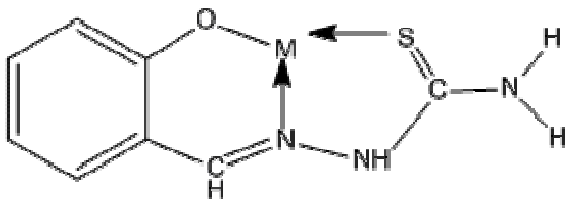

Figure 5.

Due to the high polarizability of electrons on the sulphur atom and the unoccupied 3d orbitals available for back bonding, a large number of metal

ions combine with sulphur containing ligands giving a variety of metal complexes. The structural- and stereochemical aspects of transition metal complexes of thiosemicarbazones and semicarbazones were reviewed by Campbell[8] and George Kauffmma[9]. Earlier works in this area have been carried out by Livingstone[10] and later by Akbar Ali and Livingstone[11]. The chemistry of thiosemicarbazones has attracted many scientists due to their structural diversity, variable bonding modes, promising biological activities and ion sensing ability[12-14]. Thus thiosemicarbazones are now considered as the main class of sulphur donor ligands particularly for transition metal ions. The review of the literature reveals the importance and applications of thiosemicarbazones as ligands in the field of coordination chemistry.

Schiff base compounds, including thiosemicarbazones, can react with metal ions giving a number of complexes and chelate compounds with industrially- and biologically important properties[15]. Studies on thiosemicarbazones of β-diketones by Samus *et al*[16] showed that they are good tridentate O, N, S ligands forming complexes with Cu(II), Ni(II) and Co(II) ions.

Douglas X West *et al*[17] studied the properties of iron(II) complex of thiosemicarbazone derived from 2-acetylpyridine and reported that this complex inhibited the growth of various bacteria[18]. The physico- chemical- and structural characteristics of dioxo-molybdenum complexes of

thiosemicarbazones were studied by Neeta Kanoongo *et al*[19] and they confirmed the significant antimicrobial activity of these compounds. The utility of transition metal complexes of substituted cuminaldehyde thiosemicarbazone in DNA binding and nuclear activity was studied by Panchangam Murali Krishna *et al*[20]. Considerable work was done on the thiosemicarbazone derived from salicylaldehyde and 2-hydroxy acetophenone, particularly those with substitution at the N(3) position of the thiosemicarbazone moiety[21]. The research programme related to the biological activity of Ni(II) and Cu(II) complexes of benzyloxy benzaldehyde-4-phenyl-3-thiosemicarbazone were conducted by Prathima *etal*[22]. They evaluated the *in vitro* antibacterial and antioxidant activities of these compounds. Synthesis, characterization and biological activities of some transition metal complexes of thiosemicarbazone derived from 4[1-4-(methylphenyl sulfonyl)-1H-indol-3-yl] but-en-2-one were studied by J. Q. Qu *et al*[23] and they proposed tentative coordination structures for these complexes.

Monomeric oxovanadium(IV) complexes of cyclohexanone semicarbazone and thiosemicarbazone of the type $[VOL_2X]$ X, where L is the ligand and X = Cl^-, Br^- or 0.5 SO_4^{2-}, have been prepared and studied by Chandra and Pandeya[24]. These complexes have tetragonal structures with anions interacting *trans*- to the oxygen atoms. This proved that geometries other than octahedral and square planar are possible for these complexes. Jain *et al*[25] have described dimeric vanadyl(IV) complexes of α-pyridyl

thiosemicarbazone of the type $[LVO(\mu\text{-}x)_2VOL]X_2$, where L= the ligand, x=OH, Cl or CN and X = H_2O, Cl or CN. The vanadyl ions have square-pyramidal geometry with the oxygen at the apex, and with the bidentate ligand coordinating *via* the pyridine nitrogen and thione sulphur. Iron(III) complex of substituted salicylaldehyde thiosemicarbazone was synthesized and its structural- and magnetic features were studied by Ryabova *et al*[26]. The antitumour studies of Co(II) and Cu(II) thiosemicarbazones of methyl- or ethylacetoacetates were conducted by Jayasree[27]. Pradhan and Rao reported octahedral complexes of manganese(II) with acetone thiosemi- carbazone[28]. The ligands like pyruvic acid thiosemicarbazone[29], 4-nicotinamide thiosemicarbazone[30] and thiosemicarbazones of benzaldehyde-, salicylaldehyde- and acetophenone[31,32] were selected for this study. Paramagnetic octahedral complexes of the type $[CrL_3]$, where L is the *para* substituted benzaldehydethiosemicarbazone were prepared and studied[33]. The use of iron(III) complex of α-(N)-heterocyclic carboxal-dehydethiosemicarbazone and 2-acetylpyridine carboxaldehydethiosemi-carbazone in chelation therapy was found out by Spingarn *et al*[34]. M.C. Jain[35] showed that 4-benzylamidothiosemicarbazone and 1-(α)-furyl-4-benzylamidothiosemicarbazone formed tetragonally distorted octahedral complexes with Cu(II) ions. Their tetragonal symmetry was confirmed by ESR spectra. The activities of Zn(II) and Cd(II) chelates of vanillin

thiosemicarbazone against pathogenic fungi was reported by Thimmaiah *et al*[36].

The Pt(II) complex of 4-phenyl-1-benzaldehydethiosemicarbazone was prepared and characterized by elemental analysis. Its antitumour activity was also studied [37]. According to this report, the complex had a tetra nuclear geometry and it showed antitumour activity. Ying-Ying Liu e*t al*[38] synthesized a new ligand by condensing p-amino acetophenone with thiosemicarbazide and acetylacetone. The Zn(II) and Ni(II) complexes of this new ligand, 4N-(acetylacetone amine) acetophenonethiosemicarb- azone were subjected to X-ray diffraction analysis and found to be of distorted tetrahedral configuration. The studies on salicylaldehyde N(4)-cyclohexylthiosemicarbazone copper(II) complex by Bindu and Kurup[39] revealed the distorted square pyramidal structure of the complex. Previous work on thiosemicarbazones derived from 2-acetylpyridine and related ketones showed that they were capable of inhibiting the growth of bacteria[40]. Dilworth *et al.*[41] synthesized and characterized Cu(I) and Cu(II) complexes of pyridylhydrazinethiosemicarbazone. The trigonal- bipyramidal geometry of Cu(I) and Cu(II) complexes of 1-salicylaldehydethiosemicarbazone was reported by El-Shazly *et al*[42].

Ruthenium(II) complexes of NSO donor ligands such as ring-substituted 4-phenylthiosemicarbazones of salicylaldehyde and o-hydroxy-

acetophenone were described by Parbati *et al*[43]. Ni(II) complexes of ONS chelating thiosemicarbazones with triphenylphosphine co-ligands were studied by Şükriye *et al*[44]. *Douglas et al*[45] studied iron(III) complexes of thiosemicarbazones derived from 2-acetyl- pyridine. An interesting series of sulphur-bonded unusual bicoordinated thallium(I) complexes of the type TlL, where L is the anion of a monobasic bidentate thiosemicarbazone have been prepared and studied by Bhardwaj[46]. Kiran *et al*[47] reported ^{1}H- and ^{13}C-NMR spectral studies of several new complexes of Sb(III) and Bi(III) with thiosemicarbazones of furfuraldehyde and thiophene-2-carbaldehyde. Cobalt(II) complexes of 4-acetamidobenzaldehyde N(4)-substituted thiosemicarbazones have been prepared in ethanolic solution and their magnetic susceptibilities and molar conductivity measurements were reported by Douglas *et al*[48].

A latest paper dealing with the synthesis and antityrosinase mechanism of p-hydroxybenzaldehydethiosemicarbazone and p- methoxy benzaldehydethiosemicarbazone was published by Liang *et al*[49]. In these studies, both compounds were evaluated for their inhibition activity on mushroom by tyrosinase and this research supplied the basis for the development of new food preservatives and cosmetic additives. A novel di-2-pyridylthiosemicarbazone was synthesized and studied for its antiproliferative effect by Jun Yuan *et al*[50]. Transition metal complexes of furan-2-aldehydethiosemicarbazone was reported by Kurup *et al*[51] and found

that furan oxygen was not involved in the complex formation. The platinum complexes of salicylaldehydethiosemicarbazone with variable coordination mode were studied by Indranipal *et al*[52] and observed that in these complexes the ligand coordinated to the metal as a bidentate S,N-donor, forming a four member chelate ring. Anticonvulsant activity of aryl- and heteroaryl semicarbazone and thiosemicarbazone was reported by Yogeswari *et al*[53]. The partial transformation of thioamide group into nitrile in pyridine-2-carbaldehydethiosemicarbazonatocopper(II) entities in aqueous- basic medium was reported by Saize *et al*[54].

The preparation and spectral properties of nickel(II) mixed-ligands complexes derived from 2-acetylpyridinethiosemicarbazones and some nitrogen/sulphur monodentate ligands such as thiophene, ammonia, picoline, pyridine and aniline were described by Iniama *et al* [55]. These complexes had been characterized on the basis of ^{1}H- and ^{13}C-NMR, IR- and electronic spectra. These data revealed that the primary ligand was a tridentate thiosemicarbazone that coordinated in a square planar conformation to nickel(II) *via* azomethine N, pyridyl N, thiolate S atoms and a monodentate donor molecule. Unusual coordination modes of thiosemicarbazone ligands were found out by Falguni *et al*[56] in benzaldehydethiosemicarbazone. They observed that the benzaldehyde thiosemicarbazone coordinated to the metals through the hydrazinic nitrogen and sulphur with a bite angle of ~67^{0}, forming

a four-membered chelate ring. But acetaldehydethiosemicarbazone coordinated through the imine nitrogen and sulfur, forming a five-membered chelate ring with a bite angle of ~81^0. The studies on copper(II) complexes of hybrid hydroxyquinolinethiosemicarbazone ligands were conducted by Hickey *et al*[57] and they observed that the ligands formed stable complexes with Cu(II), where the copper ion was four coordinate and essentially square planar as characterised by single crystal X-ray crystallography. The preparation and spectroscopic study of a series of ten heterobimetallic compounds containing Fe and Sn, in which ferrocene derived semi-carbazones and thiosemicarbazones reacted with organotin acceptors of $RSnCl_3$ and R_2SnCl_2 types were carried out by Eugenio *et al*[58]. The reactivity of the phosphinothiosemicarbazone ligand, 2-[2-(diphenyl-phosphino)benzylidne]-*N*-ethylthiosemicarbazone, (HLPEt) toward M(I) halides (M = Cu, Ag, Au) was studied [59].

Even though the metal complexes of thiosemicarbazones[60] and oximes[61] were studied separately, there are only a few work on the metal complex containing both thiosemicarbazone and oxime as ligands. Structural studies of two N(4)-substituted thiosemicarbazones prepared from 1–phenyl-1,2-propanedione-2-oxime and their binuclear nickel(II) complexes were conducted by Kaminsky *et al*[62]. Both oximes and thiosemicarbazones can function as neutral or anionic ligands and both are capable of acting as

bridging ligands between metal centers in binuclear Cu(II) and Ni(II) complexes[63]. Complex formation between the organotin (IV) moiety and the anions of 2,3-dihydroxybenzaldehyde thiosemicarbazone and 2,3-dihydroxybenzaldehyde 4-methylthiosemi- carbazone occurred. These complexes were proposed to have tetrahedral geometry. The studies by Abdurazag *et al*[64] revealed the above facts. Maia *et al*[65]. synthesized Pd(II) complexes from 2-acetylpyridinethio- semicarbazone, 2-acetylpyridine *N*(4)-methylthiosemicarbazone and 2-acetylpyridine *N*(4) phenylthiosemicarbazone. It was observed that the monoanionic thiosemicarbazonate ligands acted in a tridentate mode, binding to the metal through the pyridine nitrogen, the azomethine nitrogen and the sulfur atoms. The cytotoxic activity against the breast cancer cell line, MDA-MB231 and *Mycobacterium tuberculosis*, H_{37}Rv ATCC 27294 were evaluated for these compounds. Amal *et al*[66] prepared and studied the crystal and molecular structure of 2-amino- acetophenone *N*(3)-dimethylthiosemicarbazone.

5.1. Conclusion

Thiosemicarbazones have been the subject of great interest of several researchers for a number of years due to the diversity in chemical- and structural properties shown by them. Besides, these compounds are blessed with wide spectrum of biological activities which include antibacterial-, antifungal-, antimalarial-, antiviral-, antineoplastic- and antileprotic

activities[67,68]. The reason behind this is that thiosemicarbazones {$R^1R^2C=N^3$-N^2H-(C=S)-N^1R^3R} posses several donor atoms like N^2, S and N^3 which can binds with biologically important metal ions like Fe, Cu and Ni forming different chelate complexes[69-72].

Our literature survey revealed that there are only a few reports on the synthesis, characterization and application of thiosemicarbazones from β-diketones. Therefore, in the present programme of research, we have decided to discuss the synthesis, characterization and the applications of metal complexes of N(4)-methyl(phenyl)thiosemicarbazones of β-diketo derivatives.

6. Significance and scope of the present investigation

Thiosemicarbazones and their metal complexes are important owing to their significant antifungal-, antibacterial- and anticancer activities. From our literature survey, it is observed that the systematic investigations on the coordination behaviour of thiosemicarbazones of β-diketo derivatives may provide many useful informations. If the hydrogen atom of the active methylene group or imino group or phenyl group of diketo derivative is substituted by halogen or alkyl group, changes in the ligational behaviour of the resulting compounds are anticipated. By virtue of the presence of active thiosemicarbazone moiety, we hope that their complexes will be potentially useful chemotherapeutic agents.

In the present investigation, we have synthesized N(4)-methyl (phenyl)thiosemicarbazones of 1,3-diketo derivatives and several complexes of these ligands. The main ligands that we have prepared during our studies and their abbreviations are given below :

1. Acetylacetone N(4)-methyl(phenyl)thiosemicarbazone (Aac MPTCH_2)
2. Benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone (Bac MPTCH_2)
3. Acetoacetanilide N(4)- methyl(phenyl)thiosemicarbazone (A acd MPTCH_2)
4. ω-Bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (ω-BAacd MPTCH_2)
5. N-Ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (EAacd MPTCH_2)

We have prepared the complexes of these ligands with Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions. These metal complexes were characterized by various physico-chemical methods like elemental analysis, electrical conductance, magnetic susceptibility, IR-, UV-Vis and ^{1}H-NMR techniques. Tentative structures of the compounds have been suggested. The synthesis and characterization of the ligands and the complexes are included in Part I of the thesis.

Besides the structural studies of the complexes, we have conducted corrosion inhibition studies of Acetylacetone N(4)-methyl-(phenyl)thiosemicarbazone(AacMPTCH_2) and ω-Bromoacetoacetanilide N(4)- methyl(phenyl)thiosemicarbazone (ω-BAacd MPTCH_2) using mild steel in 1M hydrochloric acid. Inhibition effects were investigated by weight loss- and electrochemical methods, including potentiodynamic polarization, electrochemical impedance [EIS] spectroscopy, adsorption- and surface morphological studies. The details of these studies are presented in Part II of the thesis (Section A).

We have also carried out investigations on antibacterial and antifungal behaviour of selected ligands and the complexes. The results are included in Part II of the thesis (Section B).

Literature survey, revealing the antitumour activities of thiosemi-carbazones, persuaded us to conduct the cytotoxic- and antitumour studies of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (Aacd MPTCH_2) and its complexes. Copper(II) complex of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone was selected for conducting the *in vivo* antitumour- and for the long term cytotoxicity (MTT assay) studies. The results are included in Part II of the thesis (Section C).

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CHAPTER II

REAGENTS, APPARATUS AND PROCEDURAL DETAILS

A brief account of the general reagents used, experimental details and procedure adopted for the analysis of the ligands and the complexes are given in the following pages. The different types of instruments, with their operational characteristics and reference materials, used for the physico-chemical studies of the compounds are also given. However, all specific synthetic procedures and experimental set up are explained in the relevant chapters.

1. Metal salts

Mainly acetates of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were used for the synthesis of complexes. The metal salts used were of BDH AnalaR quality.

2. Solvents

The solvents used for the synthesis, extraction and recrystallisation of the ligands and the complexes were ethanol, methanol, diethyl ether, petroleum ether, etc. Commercially available solvents like ethanol and methanol were purified by standard methods. Others were *E. Merck* reagents

and used as such. The solvents, such as methanol, dimethylsulfoxide and dimethyl formamide, used for spectral and conductivity measurements were of spectroscopic grade.

3. Ligands

The ligands were synthesized from three different β- ketoanilides[1]; acetoacetanilide, ω-bromoacetoacetanilides and N-ethyl acetoacetanilide and two different β-diketones, acetylacetone and benzoylacetone, by condensing them with N(4)-methyl(phenyl)- thiosemicarbazide. Acetylacetone, acetoacetanilide, benzoylacetone, etc. were purchased from Merck. Carbondisulphide, N-methylaniline, hydrazinehydrate, sodiumhydroxide pellets, sodium chloroacetate, conc. HCl, etc. were of AnalaR grade and were used as such. Details of the synthesis of the ligands will be given in the respective chapters. Substituted acetoacetanilides were prepared and purified according to the reported procedures[2] . N(4)-methyl(phenyl)thiosemicarbazide and the substituted β-ketoanilides were prepared as follows.

3.1. Synthesis of N(4)-methyl(phenyl) thiosemicarbazide

3.1.1. Preparation of carboxymethyl N-methyl(phenyl)dithiocarbamate

A mixture of 12.0 ml (15.2 g, 0.20 mol) of carbon disulphide and 21.6 ml (21.2 g, 0.20 mol) of N-methylaniline was treated with a solution of 8.4 g of (0.21 mol in 250 ml) NaOH . It was stirred at room temperature for

about 2 h, when a pale orange coloured homogeneous solution resulted. To this solution was then added in small portions about 23.2 g (0.20 mol) of sodium chloroacetate with stirring. The solution was allowed to stand for 6-7 h. To the resulting pale golden yellow solution was added 25 ml of conc. HCl and the resulting solid was collected and dried. (Yield: 82%; M.P. = 197-198^{o}C).

3.1.2 Preparation of N(4)-methyl(phenyl)thiosemicarbazide

A solution of 17.7 g of (0.0733 mol) carboxymethyl N-(4)methyl-(phenyl)dithiocarbamate in 20 ml of 98% hydrazine hydrate and 10 ml of water was heated on a water bath for about 10 minutes when colourless crystals began to appear. The heating was continued for another 5 minutes, filtered washed with water, and dried under IR lamp. The crude product was recrystallised from a mixture of ethanol and water to get colourless , triclinic crystals. (Yield: 81%; M.P. = 125^{o}C).

3.2. Preparation of substituted acetoacetanilide

3.2.1 ω-Bromoacetoacetanilide

To a magnetically stirred solution of acetoacetanilide (0.1 mol) in glacial acetic acid (150 ml) containing traces of iodine, a solution of bromine (5.2 ml, 0.1 mol) in glacial acetic acid (80 ml) was added slowly. The mixture was stirred for 1 h and kept overnight at room temperature. On

pouring the reaction mixture in to water (4 litre), white crystalline product was obtained, which was filtered, washed successively with cold water, aqueous ethanol and petroleum ether and dried. The product obtained was recrystalized from benzene. (Yield : 70%; M.P. = 129^{o}C).

3.2.2 N-Ethylacetoacetanilide

N-ethylacetoacetanilide was obtained as its sodium derivative. N-ethylaniline (12.6 ml, 0.1 mol) and ethylacetoacetate (11.4 ml, 0.1 mol) were heated on a sand bath at boiling temperature for 15 minutes. The oily product was cooled and treated with 2N NaOH (200 ml). The white product that formed was filtered, washed successively with diethyl ether and petroleum ether. The product, sodium-N-ethylacetoacetanilide was recrystallised from ethanol. (Yield: 66%; M.P.=172^{o}C). This sodium derivative was used for the preparation of the ligand.

4. Analytical methods

Semi-micro analysis were carried out by standard methods to check the purity of the compounds. Carbon, hydrogen and nitrogen were estimated by microanalysis using Hitachi CHN-O rapid analyser at Sophisticated Test and Instrumentation Centre, Kochi. For the estimation of metals and sulphur, a known amount of the complex was digested with a mixture of conc.HNO_3 (10 ml) and 2 or 3 drops of perchloric acid. The digestion process was repeated for 3 or 4 times by adding fresh amount of conc.HNO_3. Finally, the process

was carried out with conc. HCl. The digested mass was extracted with distilled water and used for estimations. Copper present in the solution was estimated iodometrically, using standard sodium thiosulphate solution. Cobalt, zinc and cadmium were determined by EDTA titrimetric methods. Manganese, iron, nickel and sulphur were estimated gravimetrically. Sulphur was estimated as barium sulphate and bromine was estimated as silverbromide[3].

5. Physico-chemical methods

The physico-chemical analysis of the ligands and the complexes were accomplished using magnetic-, IR-, ^{1}H-NMR- and electronic spectral studies.

5.1. Magnetic measurements

The magnetic susceptibility measurements of the complexes were carried out on a Gouy-type magnetic balance. All measurements were done at room temperature, using $Hg[Co(NCS)_4]$ as calibrant and Magway MSBMK1 Magnetic Susceptibility Balance. Diamagnetic corrections[4] using Pascal's constants were applied by adding the diamagnetic contributions of various atoms and structural units. The effective magnetic moments in Bohr Magneton (B.M.) were calculated from the corrected molar susceptibilities.

5.2. Electronic spectra

UV-visible spectra of the soluble complexes were recorded using their solutions (10^{-3}M) in ethanol, methanol, dimethyl sulphoxide and dimethylformamide on UV-1601 Shimadzu spectrophotometer, with a scanning range of 10000 – 50000 cm^{-1}.

5.3. Infrared spectra

The infrared spectra of the ligands and the complexes, in the range 4000-400 cm^{-1}, were recorded using KBr pellets on FTIR DR – 810 1A Shimadzu spectrometer.

5.4 ^{1}H-NMR spectra

The ^{1}H-NMR spectra of ligands and the diamagnetic Zn(II) complexes were recorded in $CDCl_3$ or DMSO-d_6 by using 300 MHz, Bruker Advanced DPX spectrometer.

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CHAPTER III

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF ACETYLACETONE N(4)-METHYL (PHENYL)THIOSEMICARBAZONE

1. Introduction

The chemistry of transition metal complexes of thiosemicarbazones has played an important role in the development of coordination chemistry as a whole. Thiosemicarbazones are considered as very good ligands because they can be easily attached to the metal ions forming stable coordination compounds. The earlier studies revealed that chelating thiosemicarbazones with S, N and O donor atoms form metal complexes with interesting physico-chemical properties and pronounced biological activities[1, 2]. Besides, several thiosemicarbazone complexes act as very good catalysts[3, 4]. Thiosemicarbazones and their transition metal complexes are used as anticancer-[5], antitubercular-[6] and antibacterial[7] reagents. This may be due to the presence of lone pair of electrons in either a π. or sp^2 hybridized orbital or trigonally hybridized nitrogen in the >C=N-group.

Literature survey revealed that comparatively less work has been done on transition metal complexes of ligands derived from acetylacetone and substituted thiosemicarbazone. Therefore, in the present work, a new ligand formed by the condensation of acetylacetone and N(4)-methyl(phenyl)

thiosemicarbazide, acetylacetone N(4)-methyl(phenyl)thiosemicarbazone, ($AacMPTCH_2$) (L2H) has been reported and used as a chelating ligand for the preparation of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes. The ligand and the complexes were characterized and tested for different uses. The structure of the ligand is given in Figure 1.

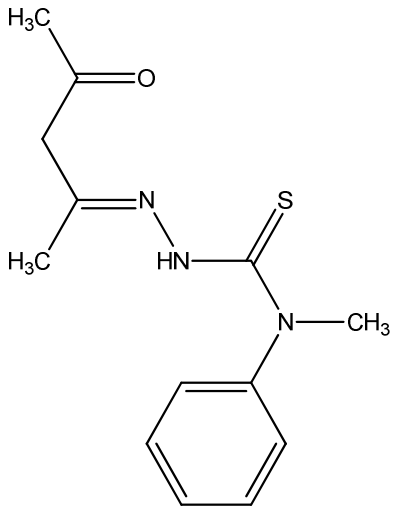

Figure 1.

Acetylacetone N(4)-methyl(phenyl)thiosemicarbazone ($AacMPTCH_2$) (L2H)*

2. Experimental

2.1. Materials and methods

Details regarding the chemicals used and the methods adopted for the characterization of the compounds are described in Chapter II.

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*IUPAC Name:
(E)-N-methyl-2-(4-oxopentan-2-ylidene)-N-phenylhydrazinecarbothioamide

2.2. Preparation of the ligand, (AacMPTCH$_2$) (L2H)

A mixture of N(4)-methyl(phenyl)thiosemicarbazide (0.005 mol) in methanol (30 ml) and acetylacetone (0.005 mol) was refluxed for 2h on a water bath. The reaction mixture was allowed to stand for one day. The pale yellow coloured solid separated was washed with petroleum ether and dried in a desiccator. The product was recrystallised from ethanol (Yield: 80%, M.P = 92°C).

2.3. Preparation of the complexes

A methanolic solution of metal acetate (0.025 mol in 20 ml) was added to a methanolic solution of AacMPTCH$_2$ (0.05 mol in 40 ml) and the mixture was refluxed for 4h. The reaction mixture was evaporated and cooled. The solid complex that formed was filtered off, washed several times with methanol. It was dried in a desiccator. All the complexes were prepared using a reaction mixture containing metal acetate and ligand in 1:2 molar ratio. Yield and M.P. of the complexes were noted.

3. Results and discussion

The data obtained from the analytical and physico-chemical studies have been correlated to explain the properties, structure and bonding of the compounds.

3.1. Characterization of the ligand

The ligand, pale yellow coloured crystals, was soluble in methanol, ethanol, etc. TLC technique was adopted for testing the homogeneity and purity of the ligand. The ligand was characterized by elemental analysis (Table 3.1.1), electronic- (Table 3.1.2), IR- (Table 3.1.3 and Figure 2) and ^{1}H NMR- (Table 3.1.4 and Figure 3) spectral studies.

3.1.1. Analytical data

The ligand was subjected to elemental analysis on a CHN-O instrument. The experimentally found out- and calculated percentages of C, H, N and S were in good agreement confirming the molecular formula of the ligand as $C_{13}H_{17}N_3OS$.

Table 3.1.1. Physico-chemical and analytical data of the ligand

Compound	**Colour**	**Yield (%)**	**M.P (^{o}C)**	**Elemental analysis (%) found (calculated)**			
				C	**H**	**N**	**S**
Ligand	Pale yellow	80	92	60.53 (59.00)	7.10 (6.46)	16.20 (15.96)	12.06 (12.16)

3.1.2. Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 260 nm (38461cm^{-1}) which can be attributed to $\pi \rightarrow \pi^*$ transition. Similarly a

band at 390 nm (25641cm^{-1}) in the ligand spectrum may be attributed to n→ π* transition[8]. The data are given in Table 3.1.2.

Table 3.1.2. Electronic spectral bands of the ligand

Spectral bands		**Assignments**
(nm)	**(cm^{-1})**	
260	38461	π→ π*
390	25641	n→ π*

3.1.3. IR spectrum

IR spectral data of the ligand with the probable assignments are given in the Table 3.1.3 and Figure 2. There is a possibility of thione-thiol tautomerism in the ligand due to the presence of –NH-C (=S)-NR_2 group. But the absence of a band ~ 2500 cm^{-1}, characteristic of thiol form, ruled out this possibility in the ligand[9]. The band at 3434 cm^{-1} in the ligand spectrum account for ν_{N-H} stretching vibration[10]. The enolic –OH stretching band around 3500 cm^{-1} was absent in the ligand spectrum. The bands at 2921 and 2852 cm^{-1} represent the asymmetric-and symmetric stretching vibrations of –CH group[11]. The band at 3066 cm^{-1} may be due to ν_{C-H} (aromatic) stretching vibration. The band due to $\nu_{C=O}$ appears at 1638 cm^{-1}. The band at 1594 cm^{-1} represents the azomethine stretching vibration (due to $\nu_{C=N}$ moiety)[12]. The bending vibration of C=S is found at 791cm^{-1} while the band due to

stretching vibration is seen at 1125 cm^{-1} [13]. The band at 1021cm^{-1} may be assigned to ν_{N-N}[14].

Table 3.1.3. Significant IR spectral bands of the ligand

Bands(cm^{-1})	**Assignments**
3434	ν_{N-H} (stretching)
3066	ν_{C-H} (aromatic)
2921	ν_{C-H} (asymmetric stretching)
2852	ν_{C-H} (symmetric stretching)
1638	$\nu_{C=O}$
1594	$\nu_{C=N}$
1125	$\nu_{C=S}$ (stretching)
1021	ν_{N-N}
791	$\delta_{C=S}$ (bending)

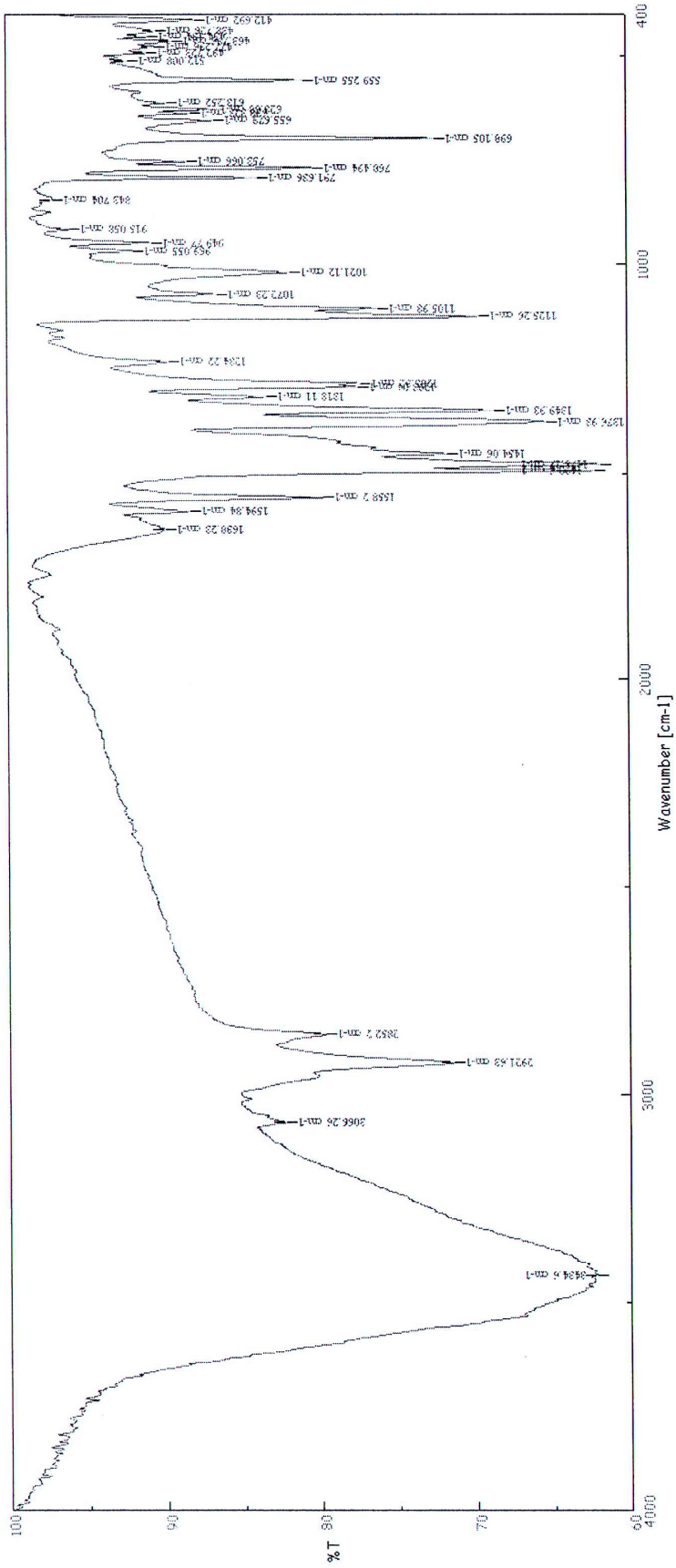

Figure 2.
IR spectrum of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone ($AacMPTCH_2$)(L2H)

3.1.4. ^{1}H NMR Spectrum

The ^{1}H NMR spectrum of the ligand was recorded in DMSO-d_6 and the assignments are given in Table 3.1.4 and Figure 3. The multiplet observed between 7.49-7.12 ppm has been assigned to C_6H_5 protons[15]. A sharp singlet at 5.80 ppm in the spectrum of the ligand is assignable to -NH proton and since this is a low value, the compound may be in the E form[16,17]. A singlet at 1.86 ppm is due to CH_3 proton adjacent to the C=N group. A singlet at 3.9 ppm is due to CH_3 proton of acetyl group. The signals appeared at 3.30 ppm are due to the active methylene protons. The CH_3 proton adjacent to the phenyl group was observed as a singlet at 2.30 ppm.

Table 3.1.4. ^{1}H NMR assignments of the ligand.

δppm	**Proton**
7.49 – 7.12	Aromatic
5.80	-NH
1.86	-CH_3 (adjacent to C=N)
3.90	-CH_3 (acetyl group)
3.30	-CH_2 (active methylene group)
2.30	-CH_3 (adjacent to phenyl group)

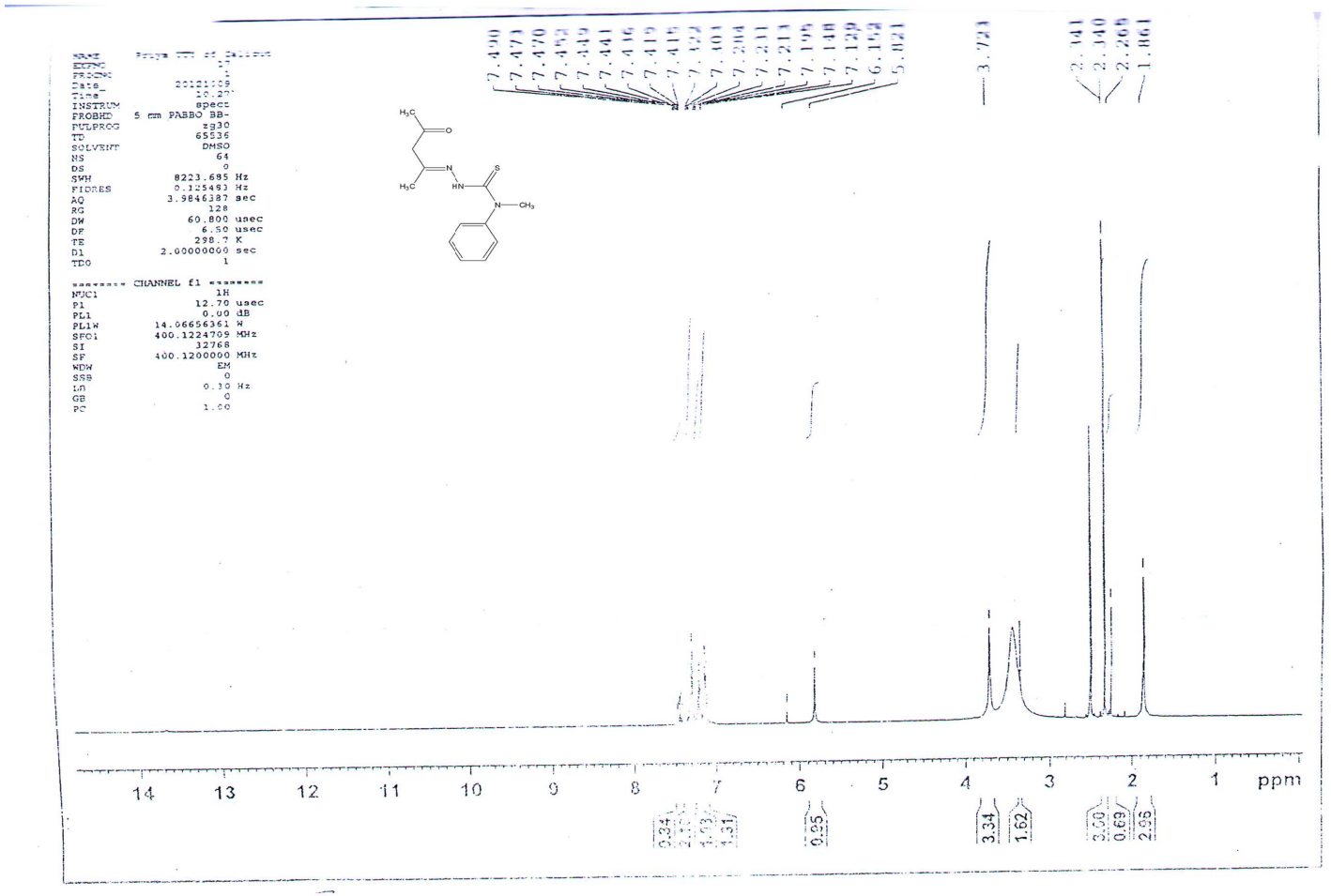

Figure 3.
^{1}H NMR spectrum of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone ($AacMPTCH_2$)(L2H)

3.2. Characterization of the complexes

The elemental analytical data of the complexes were obtained from the CHN-O rapid analyzer. Most of the complexes were non-hygroscopic, air- and photo stable. Generally, they were soluble in ethanol, methanol and DMSO. The electrical conductance measured in DMSO and the calculated molar conductance values indicated that they were non-electrolytes[18].

3.2.1. Elemental analysis

The analytical data and physical properties of the ligand ($AacMPTCH_2$)(L2H) and its complexes are listed in Table 3.2.1. The complexes were found to have a general formula $[M(L2H)_2X_2]$, where M=Co(II), Ni(II), Zn(II) or Cd(II) and X=CH_3 COO^- and $[Cu(L2H)(OH)_2]$.

Table 3.2.1. Physico-chemical and analytical data of the ligand and its complexes

Compounds	Colour	Yield (%)	MP (^oC)	Elemental analysis (%) found (calculated)				
				C	H	N	S	Metal
Ligand	Pale Yellow	80	92	60.53 (59.00)	7.10 (6.46)	16.20 (15.96)	12.06 (12.16)	---
$[Co(L2H)_2X_2]$	Brownish Black	72	285	50.01 (51.20)	5.30 (5.60)	11.10 (11.95)	8.79 (9.10)	8.29 (8.55)
$[Ni(L2H)_2X_2]$	Greenish Yellow	74	289	50.19 (51.20)	5.48 (5.60)	11.10 (11.96)	8.90 (9.10)	8.40 (8.48)
$[Cu(L2H)(OH)_2]$	Dark Green	70	290	42.14 (43.00)	4.68 (4.72)	11.23 (11.66)	8.10 (8.88)	18.20 (18.44)
$[Zn(L2H)_2X_2]$	White	80	266	50.08 (50.21)	5.14 (5.60)	11.34 (11.84)	8.90 (9.02)	9.98 (10.01)
$[Cd(L2H)_2X_2]$	Pale Yellow	70	278	46.31 (47.59)	5.14 (5.28)	10.98 (11.10)	8.00 (8.46)	14.16 (14.88)

X= CH_3COO^-

3.2.2. Magnetic behaviour

The magnetic moment values of the complexes calculated from the corrected magnetic susceptibilities are given in Table 3.2.2.

Table 3.2.2. Magnetic moments of the complexes

Compound	**μ_{eff} (B.M)**
$[Co(L2H)_2X_2]$	4.75
$[Ni(L2H)_2X_2]$	2.64
$[Cu(L2H)(OH)_2]$	1.97

T= 304K μ_{eff} = Effective magnetic moment

X = CH_3COO^- B.M. = Bohr Magneton

Octahedral- and tetrahedral Co(II) complexes differ in their magnetic properties[19]. The intrinsic orbital angular momentum in the high-spin octahedral ground state ($^4T_{1g}$) results in a considerable orbital contribution and leads to magnetic moment values between 4.70 and 5.20 B.M at room temperature. Low-spin octahedral Co(II) complex has a ground term, ^2E_g and the observed magnetic moment is close to the spin-only value for one unpaired electron (1.72 B.M).

Tetrahedral Co(II) complex, with ground term ($^4A_2$), has no orbital contribution and the expected value of magnetic moment is 3.89 B.M. However, if there is mixing up of the ($^4T_2$) state by spin-orbit coupling

perturbation, the magnetic moment value, observed will be in the range, 4.40-4.70 B.M. It is generally assumed that high-spin 4-coordinate complexes are tetrahedral.

Square planar Co(II) complexes are of low-spin type and show magnetic moments between 2.20 to 2.90 B.M.

The Co(II) complex reported in this chapter, $[Co(L2H)_2X_2]$ registered a magnetic moment value of 4.75 B.M. This value together with colour of complex clearly indicates octahedral geometry around the Co(II) ion.

Tetrahedral Ni(II) complexes show high magnetic moments in the range 3.60 to 4.00 B.M. which is due to the orbital contribution of T ground state towards the spin-only value of 2.83 B.M, corresponding to two unpaired electrons. Large distortions in the field of the coordinated ligands are found to produce magnetic moments with small orbital contribution and the observed values are as low as 3.20 B.M[20]. Square planar Ni(II) complexes have a spin - singlet ground state and hence are diamagnetic.

Octahedral Ni(II) complexes should register magnetic moments nearly equal to the spin-only value, as the ground state 'A' lacks orbital contribution. However, observed magnetic moment values fall in the range 2.60 to 3.30 B.M due to spin-orbit coupling or higher state mixing with the ground state. The Ni(II) complex, $[Ni(L2H)_2X_2]$ investigated here registered a magnetic moment value 2.64 B.M indicating its octahedral geometry.

Magnetic moment values of Cu(II) complexes are rather insensitive to stereochemistry. A regular octahedral Cu(II) complex shows a magnetic moment which ranges from 1.80 to 2.10 B.M. Slightly higher than the spin-only value is due to the fact that spin-orbit coupling in the ion can mix the ground state, representing no orbital momentum, with higher level of identical multiplicity. For regular tetrahedral Cu(II) complex, a room temperature moment value of about 2.20 B.M is predicted. However, the observed moment fall in the range 1.95 to 2.00 B.M [21]. The copper complex obtained here showed a magnetic moment of 1.97 B.M indicating its square planar structure.

3.2.3 Electronic spectra

The important electronic spectral bands of the ligand, Co(II), Ni(II) and Cu(II) complexes and their assignments are given in the Table 3.2.3.

Table 3.2.3. Electronic spectral bands of the ligand and complexes

Compound	Bands	
	(nm)	**(cm^{-1})**
Ligand	260 390	38461 25641
$[Co(L2H)_2X_2]$	405 620 1045w	24691 16129 9569
$[Ni(L2H)_2X_2]$	410 630 br 1020 w	24390 15873 9803
$[Cu(L2H)(OH)_2]$	415 569	24096 17574

br = broad, w = weak

For octahedral Co(II) complexes, the following transitions are generally observed

$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$

and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$

Among these, $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ corresponds to highest energy transition. In octahedral system, since the $^4A_{2g}(F)$ level and $^4T_{1g}(P)$ level are very close, the transitions to these two levels are also close together. In octahedral complexes, the spin allowed transition $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ takes place generally in the near infrared region.

The known transitions for tetrahedral Co(II) complexes are :

$^4A_2(F) \rightarrow {}^4T_1(F)$ ~ 1580 nm

$^4A_2(F) \rightarrow {}^4T_2(F)$ ~ 660 nm

$^4A_2(F) \rightarrow {}^4T_1(P)$ ~ 510 nm

The visible spectrum of tetrahedral of Co(II) complex is dominated by the highest energy transition, $^4A_2(F) \rightarrow {}^4T_1(P)$. The transition, $^4A_2(F) \rightarrow {}^4T_1(F)$ occurs in the infrared region. In the case of Co(II) complexes, the dark colour together with some weak and broad bands present in their spectra in the region 1000-1200 nm are characteristic of square planar geometry[22].

In the present investigation, $[Co(L2H)_2X_2]$ registered three bands at 405, 620 and 1045 nm which may be due to, respectively,

$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$

and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transitions of an octahedral complexes.

For octahedral Ni(II) complexes, the known transitions are :

$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ~ 1160 nm

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ ~ 740 nm

$^3A_{2g}(F) \rightarrow {}^1E_g(D)$ ~ 640 nm

$^3A_{2g}(F) \rightarrow {}^1A_{1g}(G)$ ~ 540 nm

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ ~ 390 nm

The spectra usually consist of bands in the infrared region at ~ 1160 nm, a close pair of bands ~ 640 nm followed by a weaker band ~ 540 nm and a stronger band in the blue end ~ 390 nm. A large number of octahedral Ni(II) complexes have been examined and the assignments of transitions have been made conclusively[23].

The spectrum of tetrahedral Ni(II) complex is expected to consist of three spin- allowed transitions,

$^3T_1(F) \rightarrow {}^3T_{2g}(F)$ ν_1 3300 ~ 2000 nm

$^3T_1(F) \rightarrow {}^3A_{2g}(F)$ ν_2 1540 ~ 1000 nm

$^3T_1(F) \rightarrow {}^3T_1(P)$ ν_3 840 ~ 590 nm

The ν_1 band is often marked by the absorption of either organic part of the molecule or the solvent. The ν_2 band has an appreciable molar absorptivity (ε15 to 50) and ν_3 band is found to be in the visible region and shows and intense absorption (ε100 to 200). The planar Ni(II) complexes are generally red, yellow or brown, due to the presence of an absorption band of medium intensity in the 450-600 nm region. However, other colours do occur when additional absorption bands are present[24].

In the present case, $[Ni(L2H)_2X_2]$ registered an intense band ~ 410 nm and two broad bands at about 630 and 1020 nm. They may be due to the following transitions of a typical octahedral Ni(II) complex:

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$

$^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$

Generally, all Cu(II) complexes are blue or green in colour. This colour is due to the presence of absorption bands in the 600-900 nm region of the spectrum. The single d-d transition that occurs is assigned as $E \rightarrow T_2$. The energy level diagram for a Cu(II) complex having D_4h symmetry predicts three vibronically induced transitions *viz*, $^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_g$.

In tetrahedral- or pseudo-tetrahedral Cu(II) complexes, the d-d transitions occur in the range 1430 – 1000 nm. If the region 1000-500 nm, where the square planar or distorted octahedral Cu(II) complexes normally absorb, is blank, it is reasonable to infer that the complexes have tetrahedral geometry. The energy of the bands of tetrahedral Cu(II) complexes are low compared to those of the square planar or tetragonal complexes. In the present investigation, the 4–coordinate $[Cu(L2H)(OH)_2]$ registered two bands at 415 and 569 nm, which may be respectively, due to $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2A_{1g}$ transitions of square planar Cu(II) complex.

3.2.4. Infrared spectra

The significant IR spectral bands of the ligand (Figure 2) and the complexes (Figures 4-8) along with their probable assignments are given in Table 3.2.4. These assignments are made by the comparison of the IR spectra of acetylacetone, thiosemicarbazide, thiosemicarbazones of similar β-dicarbonyl compounds and their metal complexes[25] .

The ligand and all the metal complexes except that of copper(II) exhibited a broad region with a few characteristic bands around 3500 cm^{-1} in their IR spectra. This may be assigned as the stretching frequency of –NH group[26]. But in the case of the copper(II) complex, the band due to -NH group is found to at 2958 cm^{-1}. This change may be due to the presence of the OH group in the copper(II) complex.

The IR spectrum of the ligand shows characteristic bands in the 2800-2900 cm^{-1} region, which may be assigned to the symmetric- and asymmetric stretchings of –CH group[11]. In the spectra of all the complexes these bands are retained in the same region.

The ligand spectrum shows a band at 1638 cm^{-1}. This may be assigned to $\nu_{C=O}$[27]. In the spectra of the complexes, there is not much change in the position of this band, indicating the non-participation of this group C=O in

coordination. The small change in the frequency supports the hydrogen bond formation[28] with the –NH group in the thiosemicarbazone moiety.

A strong band at 1594 cm^{-1} in the ligand spectrum may be assigned to $\nu_{C=N}$ [12]. In the spectra of all the complexes this band is found to be shifted to a lower frequency region by a few cm^{-1} indicating the participation of azomethine nitrogen in coordination[29].

The band at 1021 cm^{-1} in the spectrum of the ligand may be assigned to ν_{N-N}[13]. In the spectra of the complexes this band is found to be shifted to a higher frequency region, indicating the participation of azomethine nitrogen in coordination.

Medium bands around 450 cm^{-1} in the spectra of all the complexes may be assigned to M-N bond[30].

The bending vibrations due to C=S , found at 791 cm^{-1} and the stretching vibrations at 1125 cm^{-1} in the ligand spectrum, are shifted to lower frequency by 20-30 cm^{-1} in the spectra of the complexes . This may be due to the coordination through the sulphur atom of –NH-C=S group of the ligand (without enolisation) to the metal ions[13].

The broad feature of medium intensity due to ν_{O-H} around 3550 cm^{-1} in the IR spectrum of the copper(II) complex[31].

The band at ~ 1610 cm^{-1} is assigned to $\nu_{asym\ COO^-}$ and another one at ~ 1420 cm^{-1} to $\nu_{sym\ COO^-}$. The presence of these bands in all the complexes, except that of copper(II), indicates the coordination of the acetate anion to the metal ions in a unidentate manner[32].

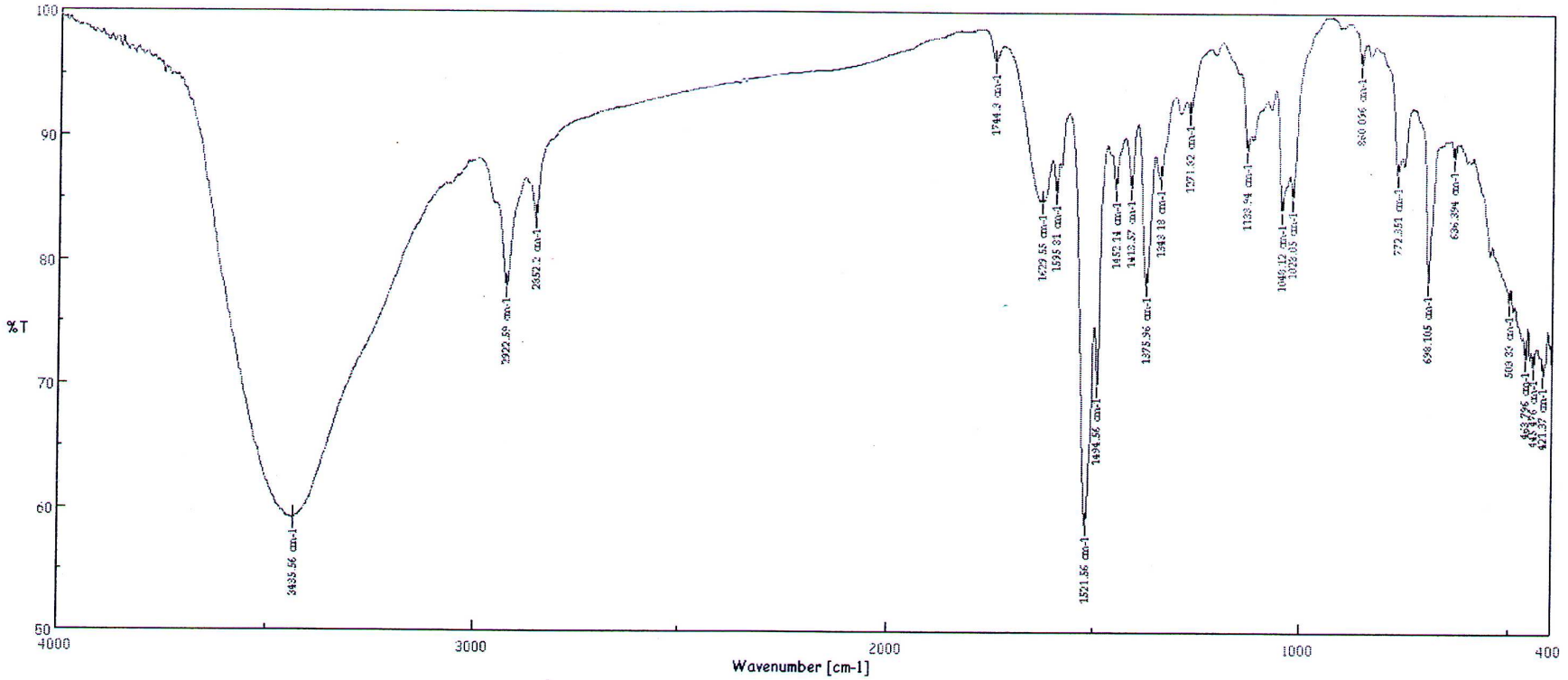

Figure 4.
IR spectrum of Co(II) complex of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone
$[Co(L2H)_2X_2]$

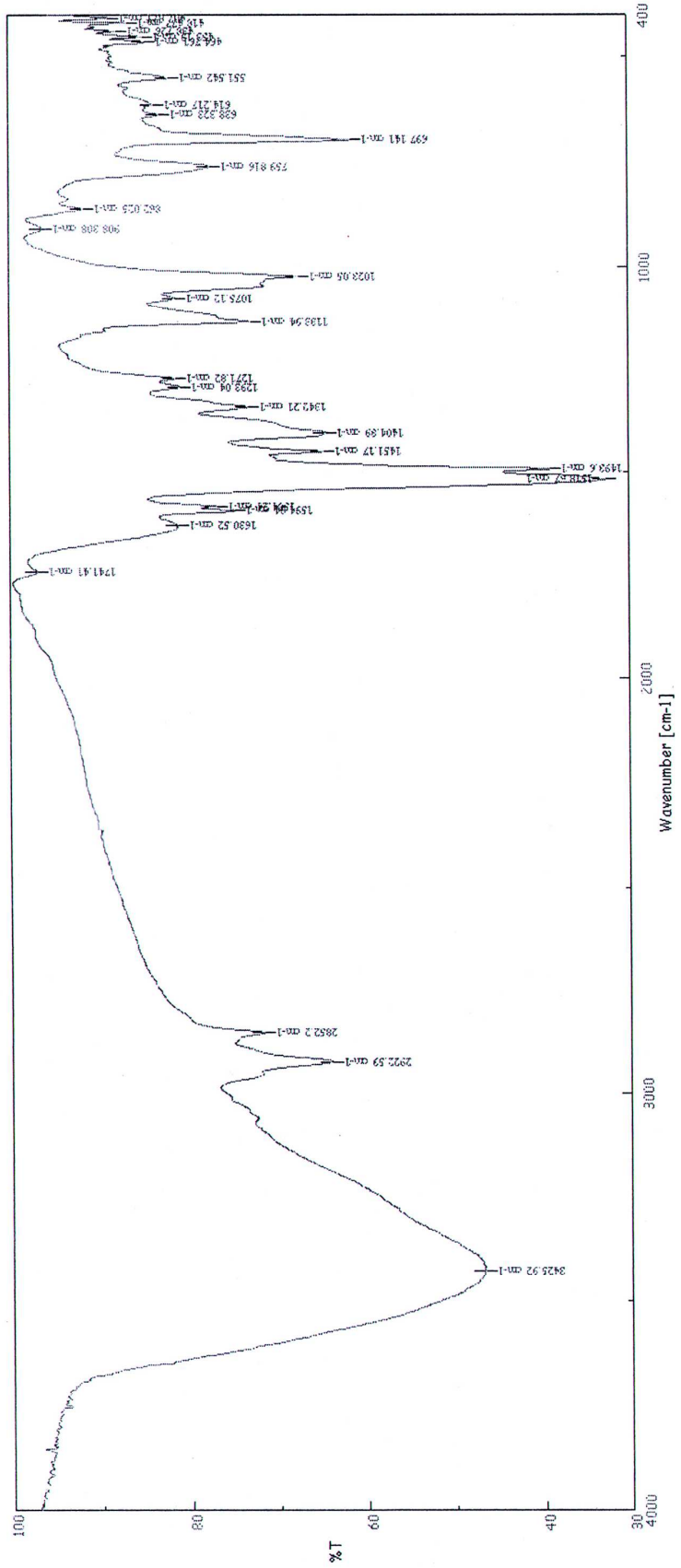

Figure 5.
IR spectrum of Ni(II) complex of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone $[Ni(L2H)_2X_2]$

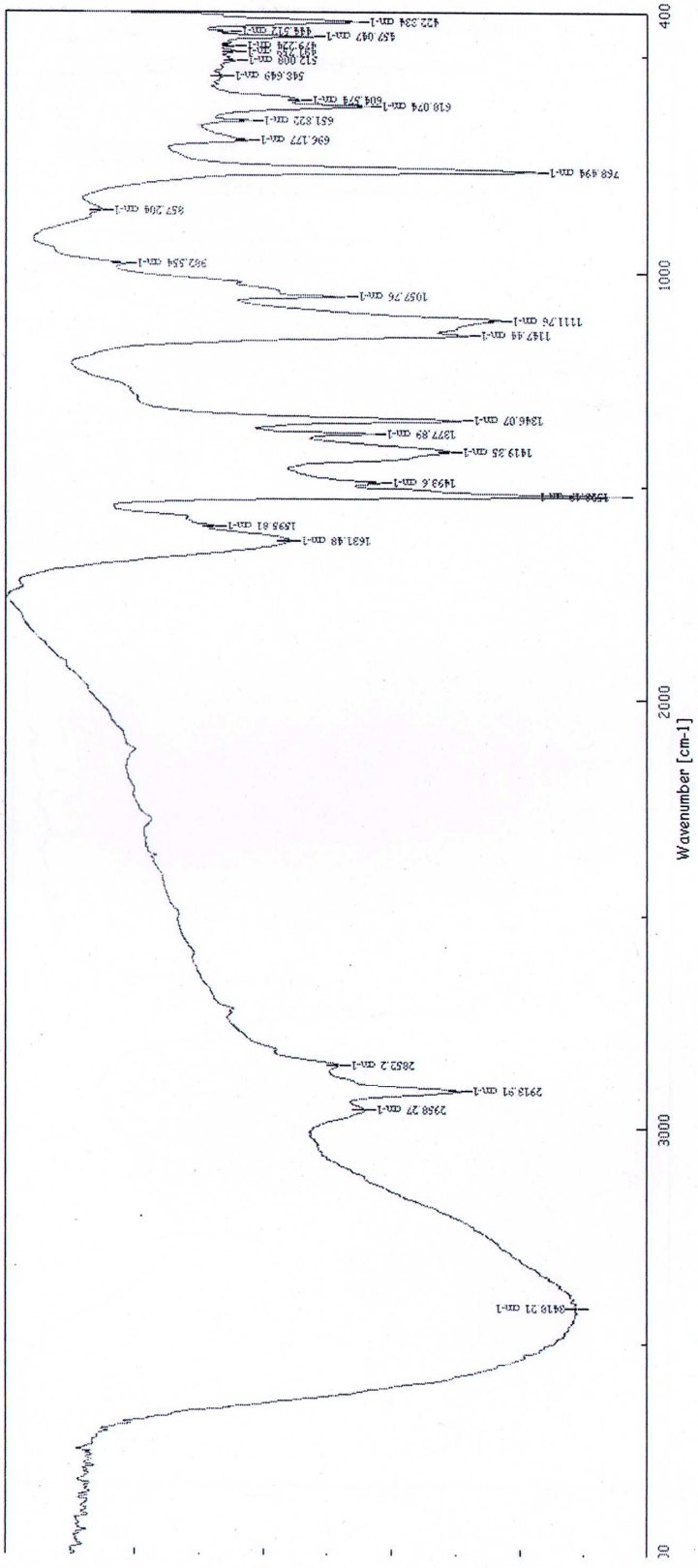

Figure 6.
IR spectrum of Cu(II) complex of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone $[Cu(L2H)(OH)_2]$

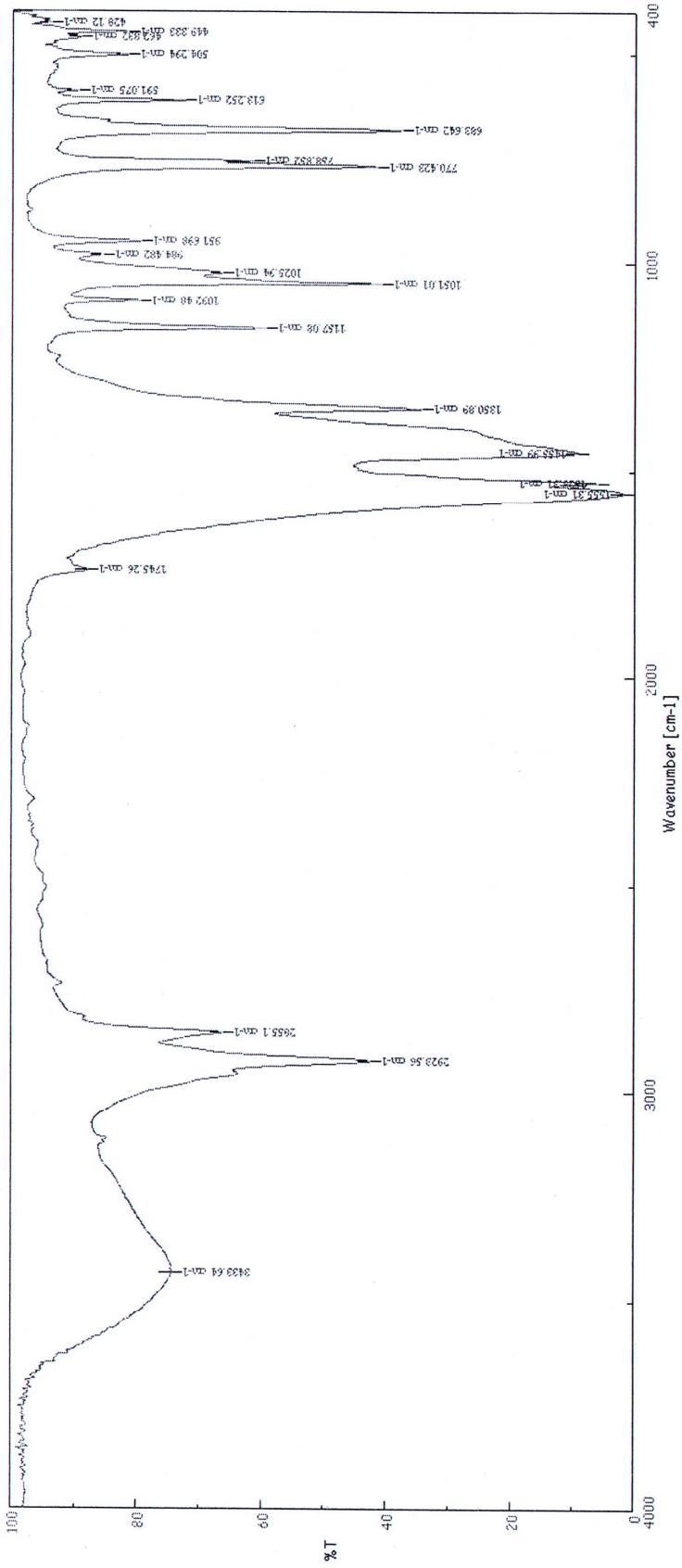

Figure 7.
IR spectrum of Zn(II) complex of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone $[Zn(L2H)X_2]$

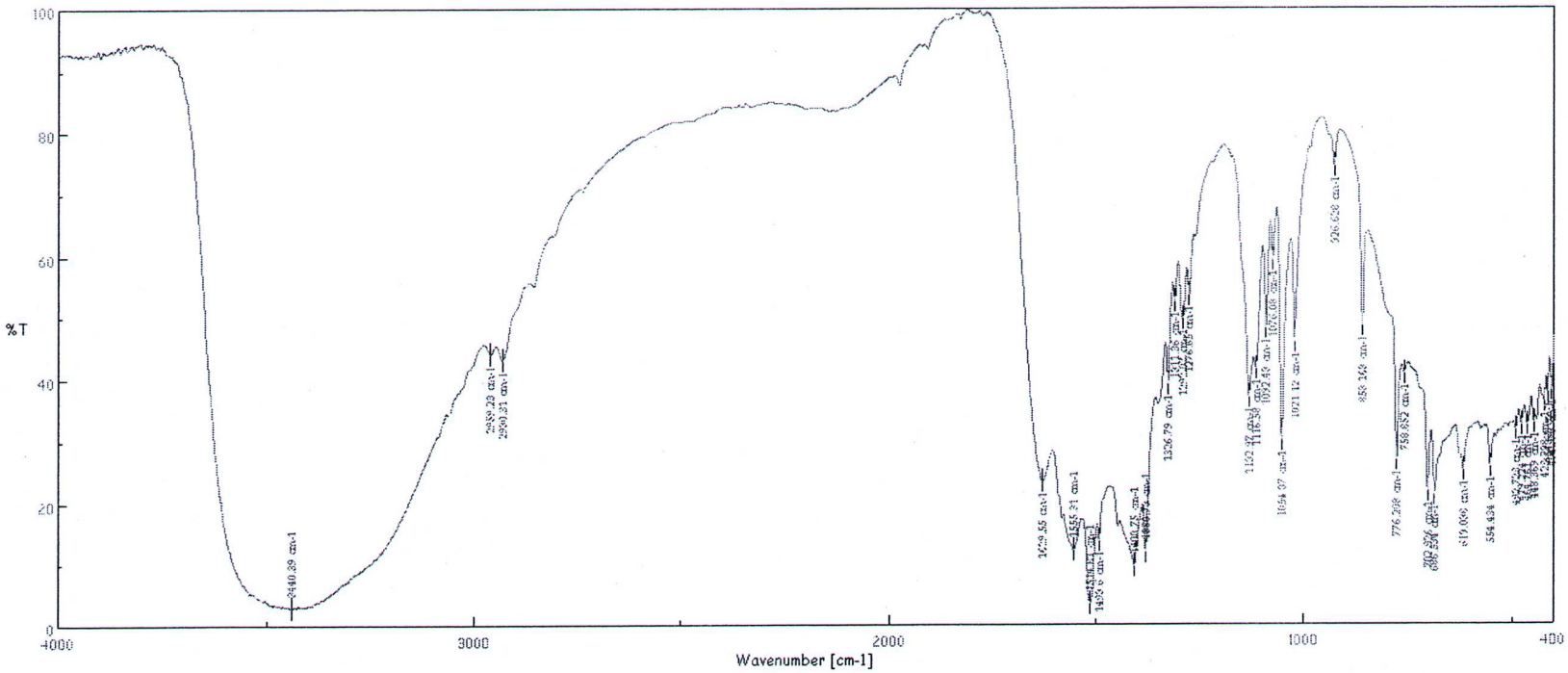

Figure 8.
IR spectrum of Cd(II) complex of acetylacetone N(4)-methyl(phenyl) thiosemicarbazone
$[Cd(L2H)_2X_2]$

Table 3.2.4. Significant infrared spectral bands (cm^{-1}) of the ligand and its metal complexes

Ligand	$[Co(L2H)_2X_2]$	$[Ni(L2H)_2X_2]$	$[Cu(L2H)(OH)_2]$	$[Zn(L2H)_2X_2]$	$[Cd(L2H)_2X_2]$	Assign-ments
- 3434	- 3435	- 3425	3419 2958	- 3433	- 3440	$\nu_{(O-H)}$ $\nu_{(N-H)}$
2921	2922	2922	2913`	2923	2923	$\nu_{(C-H)}$ asy.str
2852	2852	2852	2852	2855	2959	$\nu_{(C-H)}$ sym.str
1638	1629	1630	1631	1610	1629	$\nu_{(C=O)}$
1594	1521	1518	1520	1556	1555	$\nu_{(C=N)}$
-	1494	1493	-	1433	1493	$\nu_{(COO^-)}$
1125	1048	1023	1111	1051	1054	$\nu_{(C=S)}$ stretching
791	772	760	768	770	776	$\delta_{C=S}$ bending
-	445	464	422	449	424	$\nu_{(M-N)}$

$X=CH_3COO^-$

4. Conclusion

Coordination compounds of Co(II), Ni(II), Cu(II), Zn(II), and Cd(II) with a multidentate ligand, acetylacetone N(4)-methyl(phenyl)thiosemicar-bazone were prepared and their physico-chemical properties have been studied. The complexes have the general formulae $[M(L2H)_2X_2]$, where M=Co(II), Ni(II), Zn(II) or Cd(II) and X = CH_3COO^- The structure of the complex is given in Figure 9. Based on magnetic measurements and various

spectral studies, geometries have been assigned to the complexes. Except the Cu(II) complex, all the others were found to be octahedral. The Cu(II) complex, $[Cu(L2H)(OH)_2]$ was found to be 4-coordinate with square planar geometry. The suggested structure is given in Figure 10.

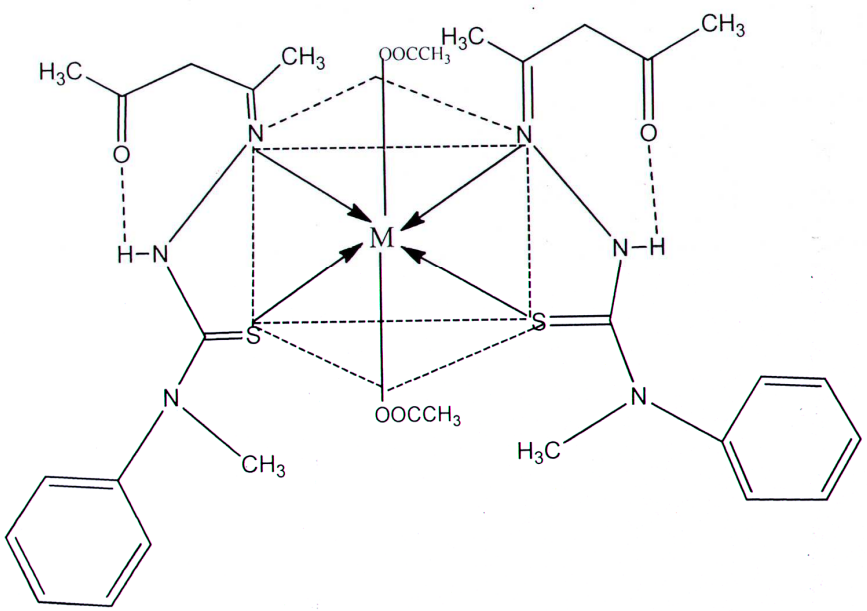

Figure 9.

Suggested structure of the complexes of the formula $[M(L2H)_2X_2]$, where M = Co(II), Ni(II), Zn(II) or Cd(II)

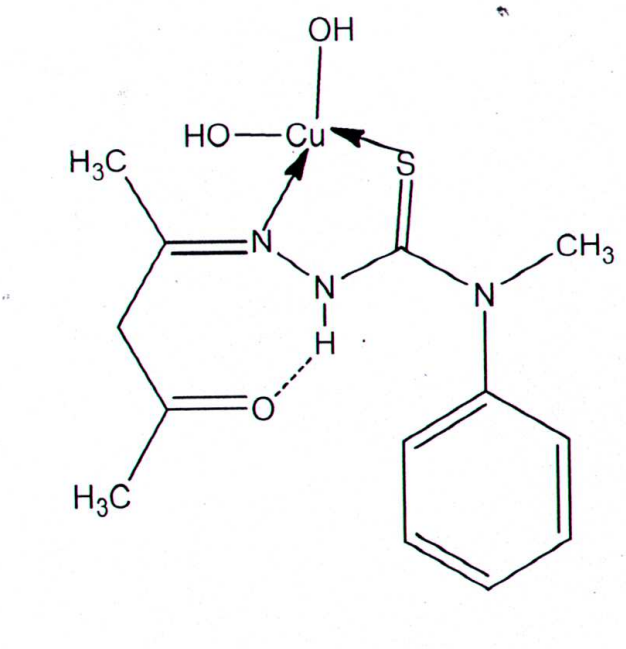

Figure 10.

Suggested structure of $[Cu(L2H)(OH)_2]$

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CHAPTER IV

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF BENZOYLACETONE N(4)-METHYL-(PHENYL)THIOSEMICARBAZONE

1. Introduction

Literature survey revealed that 1,3-diketones and related com- pounds received considerable attention on ligands[1-2]. Schiff bases of acetylacetone, benzoylacetone, dibenzoylmethane, and thienoyltrifluoro- acetone with ethanolamine and 2-aminophenol were synthesized and characterized by various analytical and spectral studies[3]. Ramadan *et al*[4] synthesized and characterized benzoylacetone isonicotinoyl hydrazone complexes of divalent transition metals. Investigations on the synthesis and toxic effects of Ni(II) and Cu(II) complexes of benzoylacetone S-methylisothiocarbazone on *Paramecium caudetum* was conducted by Kurasova *et al*[5]. Later, structural investigations on Ni(II) and Fe(III) complexes of the same ligand were also conducted[6,7]. Recently, influence of mononuclear Cu(II) and Ni(II) complexes of this ligand on the activity of enzymes synthesized by the fungal stain, *Aspergillus niger* was investigated by Maria *et al*[8]. However, there are no reports on the coordi- nation behavior of N-substituted thiosemicarbazone of benzoylacetone. Hence it appeared worthwhile and interesting to prepare benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone, ($BacMPTCH_2$)

(L2H) and to study its reactions with several typical transition metal ions so as to isolate these solid complexes. The structure of the ligand is given in Figure 1.

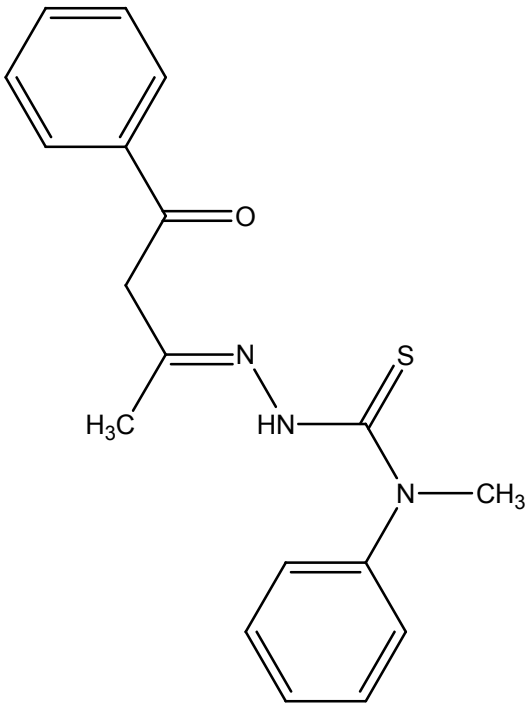

Figure 1.

Benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone

(BacMPTCH_2)(L2H)*

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*IUPAC Name :

(E)-N-methyl-2-(4-oxo-4-phenylbutan-2-ylidene)-N-phenylhydrazinecarbo-thioamide

2. Experimental

2.1. Materials and methods

Details regarding the chemicals used and the methods adopted for the characterization of the compounds are described in Chapter II.

2.2. Preparation of the ligand, ($BacMPTCH_2$) (L2H)

Benzoyl acetone in 30 ml methanol (0.05 mol) was added to N(4)-methyl(phenyl)thiosemicarbazide in 30 ml methanol (0.005 mol) and refluxed on a water bath for 2h. The product was washed with petroleum benzene for several times and dried in vaccum. (Yield: 60%, M.P = 92ºC)

2.3. Preparation of the complexes

A methanolic solution of metal acetate (0.025 mol in 30 ml) was added to a methanolic solution of $BacMPTCH_2$ (0.05 mol in 40 ml) and the mixture was refluxed for 4h. The product was filtered and washed with methanol for several times and dried. Yield and the melting points of the complexes were noted.

3. Results and discussion

The data obtained from the analytical and physico-chemical studies have been correlated to explain the properties, structure and bonding of the compounds.

3.1. Characterization of the ligand

The ligand, benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone ($BacMPTCH_2$) (L2H) obtained was a colourless solid, soluble in methanol, ethanol, etc. This ligand was characterized by elemental analysis (Table 3.1.1.), electronic- (Table 3.1.2), IR- (Table 3.1.3 and Figure 2) and ^1H NMR- (Table 3.1.4 and Figure 3) spectral studies.

3.1.1. Analytical data

The ligand was subjected to elemental analysis on a CHN-O instrument. The experimentally found out- and calculated percentages of C, H, N and S were in good agreement confirming the molecular formula, $C_{18}H_{19}N_3OS$.

Table 3.1.1 Physico-chemical and analytical data of the ligand

Compound	**Colour**	**Yield (%)**	**M.P (^oC)**	**Elemental analysis (%) found (calculated)**			
				C	H	N	S
Ligand	Colour less	60	92	65.10 (66.40)	5.12 (5.84)	12.70 (12.92)	9.34 (9.84)

3.1.2. Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 280 nm (37037 cm^{-1}) due to $\pi \rightarrow \pi^*$ transition. The band at 350 nm (28571

cm^{-1}) in the ligand spectrum may be attributed to $n \rightarrow \pi^*$ transition[9]. The data are given below.

Table 3.1.2 Electronic spectral bands of the ligand

Spectral bands		**Assignments**
(nm)	(cm^{-1})	
280	37037	$\pi \rightarrow \pi^*$
350	28571	$n \rightarrow \pi^*$

3.1.3. IR spectrum

IR spectrum of the ligand with the probable assignments are given in the Table 3.1.3. and Figure 2. The spectrum showed bands of medium intensities at 3377 and 3045 cm^{-1} due to ν_{N-H} and ν_{C-H} (aromatic) respectively. The bands at 2923 and 2853 cm^{-1} are due to asymmetric- and symmetric stretching vibrations of –CH group [10]. The band at 1614 cm^{-1} is due to $\nu_{C=O}$. This shows that ligand exists in the keto form without enolisation[11]. Ligand shows a band at 1594 cm^{-1} which is the characteristic of azomethine group[12]. The bands at 1117 and 804 cm^{-1} are assigned to stretching and bending vibrations of C=S, respectively. This indicates that the enolisation of –NH–C(=S) group to –N=(C–SH) has not taken place[13]. There is a band at 1059 cm^{-1} due to ν_{N-N}[14]

Table 3.1.3 Significant IR spectral bands of the ligand

Bands(cm^{-1})	**Assignments**
3377	ν_{N-H} (stretching)
3045	ν_{C-H} (aromatic)
2923	ν_{C-H} (asymmetric stretching)
2853	ν_{C-H} (symmetric stretching)
1614	$\nu_{C=O}$
1594	$\nu_{C=N}$
1117	$\nu_{C=S}$ (stretching)
1059	ν_{N-N}
804	$\delta_{C=S}$ (bending)

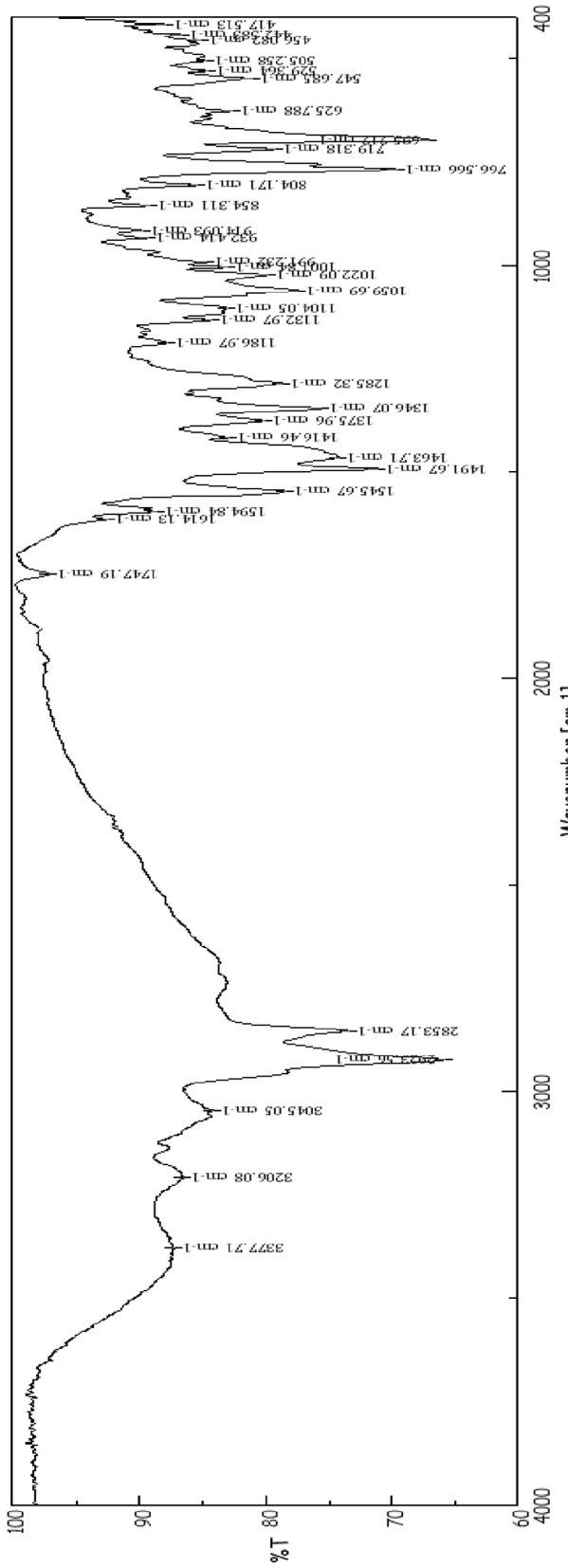

Figure 2.
IR spectrum of benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone
$BAcMPTCH_2(L2H)$

3.1.4. ^{1}H NMR spectrum

The deshielding multiplets at 7.48–7.40 and 7.35–7.16 ppm in the spectrum of the ligand are due to the aromatic protons[15] of thiosemicarbazone part and benzoylacetone moiety, respectively. A high deshielding singlet observed at 13.60 ppm is due to –NH proton[16]. A singlet at 2.20 ppm is due to $–CH_3$ protons, adjacent to the C=N group. A deshielding singlet at 3.30 ppm is due to $–CH_2$ group. CH_3 protons adjacent to the phenyl group of thiosemicarbazone moiety are observed as a singlet at 2.80 ppm[17]. The assignments are given in Table 3.1.4. and Figure 3.

Table 3.1.4 ^{1}H NMR assignments of the ligand

δ ppm	**Proton**
7.48 – 7.40	Aromatic (from thiosemicarbazone part)
7.35-7.16	Aromatic (from benzoylacetone)
13.60	-NH
2.20	$-CH_3$ (adjacent to C=N group)
2.80	$-CH_3$ (adjacent to thiosemicarbazone)
3.30	$-CH_2$

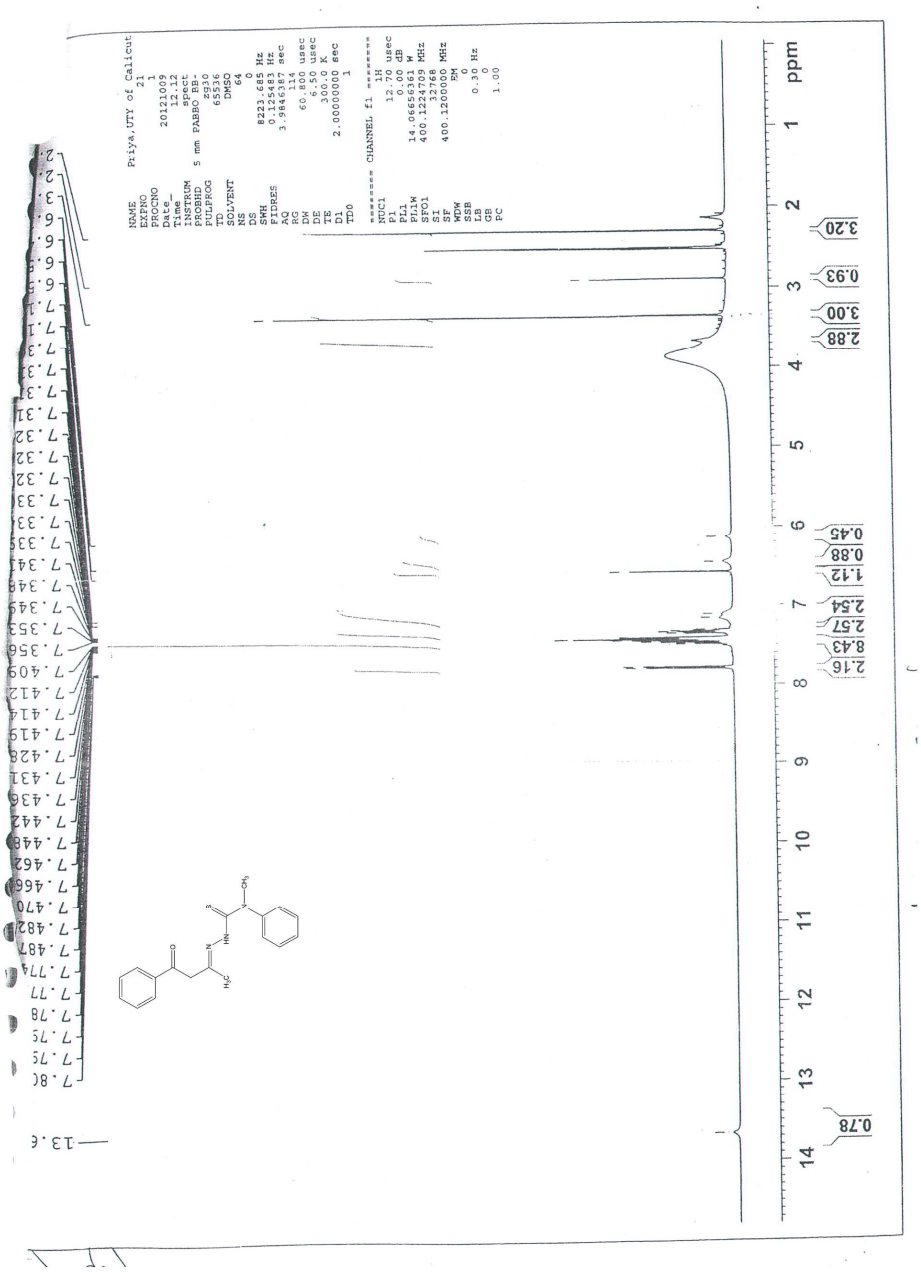

Figure 3.
^{1}H NMR spectrum of benzoylacetone N(4)-methyl(phenyl) thiosemicarbazone
BacMPTCH$_2$(L2H)

3.2. Characterization of the complexes

Benzoylacetone N(4)-methyl(phenyl)thiosemicarbazone formed stable complexes with various transition metal ions, which are soluble in DMSO, DMF, etc. These complexes were characterized as follows :

3.2.1. Elemental analysis

The analytical data and physical properties of the ligand, $BacMPTCH_2$ and its complexes are listed in Table 3.2.1. The complexes were found to have a general formula $[M(LH)_2]$, where LH is the monovalent anion of the ligand (L2H).

Table 3.2.1 Physico-chemical analytical data of the ligand and its complexes

Compound	**Colour**	**Yield (%)**	**MP (^oC)**	**Elemental Analysis (%) found (calculated)**				
				C	H	N	S	Metal
Ligand	Colour less	60	92	65.10 (66.40)	5.12 (5.84)	12.70 (12.92)	9.34 (9.84)	--
$[Co(LH)_2]$	Dark brown	68	279	60.00 (60.90)	4.98 (5.07)	11.20 (11.80)	9.30 (9.02)	8.10 (8.30)
$[(Ni(LH)_2]$	Light green	66	280	61.20 (60.90)	5.10 (5.00)	12.20 (11.80)	4.90 (4.15)	9.00 (8.28)
$[Cu(LH)_2]$	Dark blue	64	281	60.40 (60.50)	4.90 (5.04)	11.92 (11.77)	4.91 (4.48)	9.16 (8.89)
$[Zn(LH)_2]$	Colourless	69	280	60.20 (60.38)	5.13 (5.03)	11.43 (11.74)	4.13 (4.47)	9.12 (9.14)
$[Cd(LH)_2]$	Pale yellow	62	283	58.10 (56.60)	5.20 (4.72)	12.20 (11.00)	5.20 (4.19)	15.30 (14.74)

3.2.2. Magnetic behaviour

The calculated magnetic moment values are given in the Table 3.2.2.

Table 3.2.2 Magnetic moments of the complexes

Compound	**μ_{eff} (B.M)**
$[Co(LH)_2]$	4.91
$[Ni(LH)_2]$	3.20
$[Cu(LH)_2]$	1.91

T= 304K μ_{eff} = Effective magnetic moment

B.M = Bohr Magneton

The octahedral- and tetrahedral Co(II) complexes differ in their magnetic properties. The intrinsic orbital angular momentum in the high- spin octahedral ground state ($^4T_{1g}$) results in a considerable orbital contribution and leads to magnetic moment values between 4.70 and 5.20 B.M at room temperature[18]. In the present case, the Co(II) complex showed a magnetic moment value of 4.91 B.M. This clearly indicates the octahedral geometry of the Co(II) complex.

Octahedral Ni(II) complexes should register magnetic moments nearly equal to the spin-only value, as the ground state 'A' lacks orbital contribution[19]. However, the observed magnetic moment values fall in the range 2.60 to 3.30 B.M due to spin-orbit coupling or higher state mixing with

the ground state. The Ni(II) complex investigated here registered a magnetic moment value 3.20 B.M indicating its octahedral geometry.

For regular octahedral Cu(II) complexes, fall the magnetic moments in the range from 1.80 to 2.10 B.M. This is higher than the spin-only value and is due to spin-orbit coupling[20]. For a regular tetrahedral Cu(II) complex, a room temperature moment value of about 2.20 B.M is predicted. However, the observed moments fall in the range 1.95-2.00 B.M. The copper complex obtained in this investigation, showed a magnetic moment of 1.91 B.M indicating its octahedral structure.

3.2.3 Electronic spectra

The important electronic spectral bands of the ligand and Co(II), Ni(II) and Cu(II) complexes are given in the Table 3.2.3.

Table 3.2.3. Electronic spectral bands of the ligand and complexes

Compound	**Bands**	
	(nm)	**(cm^{-1})**
Ligand	280 350	37037 28571
$[Co(LH)_2]$	410 615 1030	24390 16260 9708
$[Ni(LH)_2]$	400 625	25000 16000
$[Cu(LH)_2]$	650	15384

In the spectrum of the Co(II) complex, three bands are observed at 24390, 16260, 9708 cm^{-1} which may be due to the following transitions :

$^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ ν_1 24390 cm^{-1}

$^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ ν_2 16260 cm^{-1}

$^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ ν_3 9708 cm^{-1}

The bands may be assigned to the transitions of an octahedrally coordinated Co(II) ion[21].

The nickel complex has two absorption bands at 25000 and 16000 cm^{-1} and are due to the following transitions :

$^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ ν_1 25000 cm^{-1}

$^3A_{1g}(F) \rightarrow {}^3T_{1g}(F)$ ν_2 16000 cm^{-1}

These observations supported an octahedral structure for the complex[9]. In the electronic spectrum of $[Cu(LH)_2]$, a peak observed at 15384 cm^{-1}, due to the transition, $E_g \rightarrow T_{2g}$ indicated an octahedral structure for the complex[22]. Based on the electronic spectra, it has been assumed that all these complexes have octahedral geometry.

3.2.4. Infrared spectra

The significant IR spectral bands of the ligand (Figure 2) and the complexes (Figures 4 – 6) along with their probable assignments are given in the Table 3.2.4.

In the IR spectra of the complexes, the bands due to ν_{N-H} , and ν_{C-H} around 3300 and 3000cm^{-1}, respectively are retained. A sharp band observed at 1614 cm^{-1} in the ligand, due to $\nu_{(C=O)}$ has undergone a shift to lower frequency region which demonstrates the involvement of the carbonyl group in complex formation with the metal ions. The observed band in the metal complexes around 1050 cm^{-1} may be assigned to ν_{C-O} formed as a result of enolization of C=O during coordination[23]. The strong band at 804 cm^{-1} in the spectrum of ligand, due to $\delta_{C=S}$ is found to be shifted to lower frequency region, due to the participation of sulphur atom in coordination. Absence of the characteristic band of ν_{C-S} in spectra of the complexes, rule out the possibility of enolization of C=S group. This observation can be correlated to the coordination through sulphur atom without enolization and deprotonation in all the complexes[24].

A band of medium intensity at 1594 cm^{-1} in the spectrum of the ligand may be assigned to $\nu_{C=N}$. However, in the spectra of the complexes, this band

is found to be shifted to a lower frequency region by a few cm^{-1} indicating the participation of azomethyl nitrogen in coordination[25].

The mode of coordination of the ligand through O, N, and S is further supported by the ν_{M-O} and ν_{M-N} at ~ 420 and 520 cm^{-1}, respectively, in the spectra of complexes[26].

Table 3.2.4. Significant infrared spectral bands (cm^{-1}) of the ligand and its metal complexes

Ligand	**$[Co(LH)_2]$**	**$[Ni(LH_2)]$**	**$[Cu(LH)_2]$**	**$[Cd(LH)_2]$**	**$[Zn(LH)_2]$**	**Assign-ments**
3377	3425	3433	3428	3414	3429	ν_{N-H}
2923	2923	2923	2923	2923	2923	$\nu_{C-H\ asy.}$
2853	2853	2853	2853	2853	2853	$\nu_{C-H\ sym..}$
1614	--	--	--	--	-	$\nu_{C=O}$
1594	1520	1520	1516	1514	1521	$\nu_{C=N}$
--	1049	1024	1026	1054	1023	ν_{C-O}
804	774	764	761	776	773	$\delta_{C=S}$
--	510	496	508	512	523	ν_{M-N}
--	417	414	419	412	418	ν_{M-O}

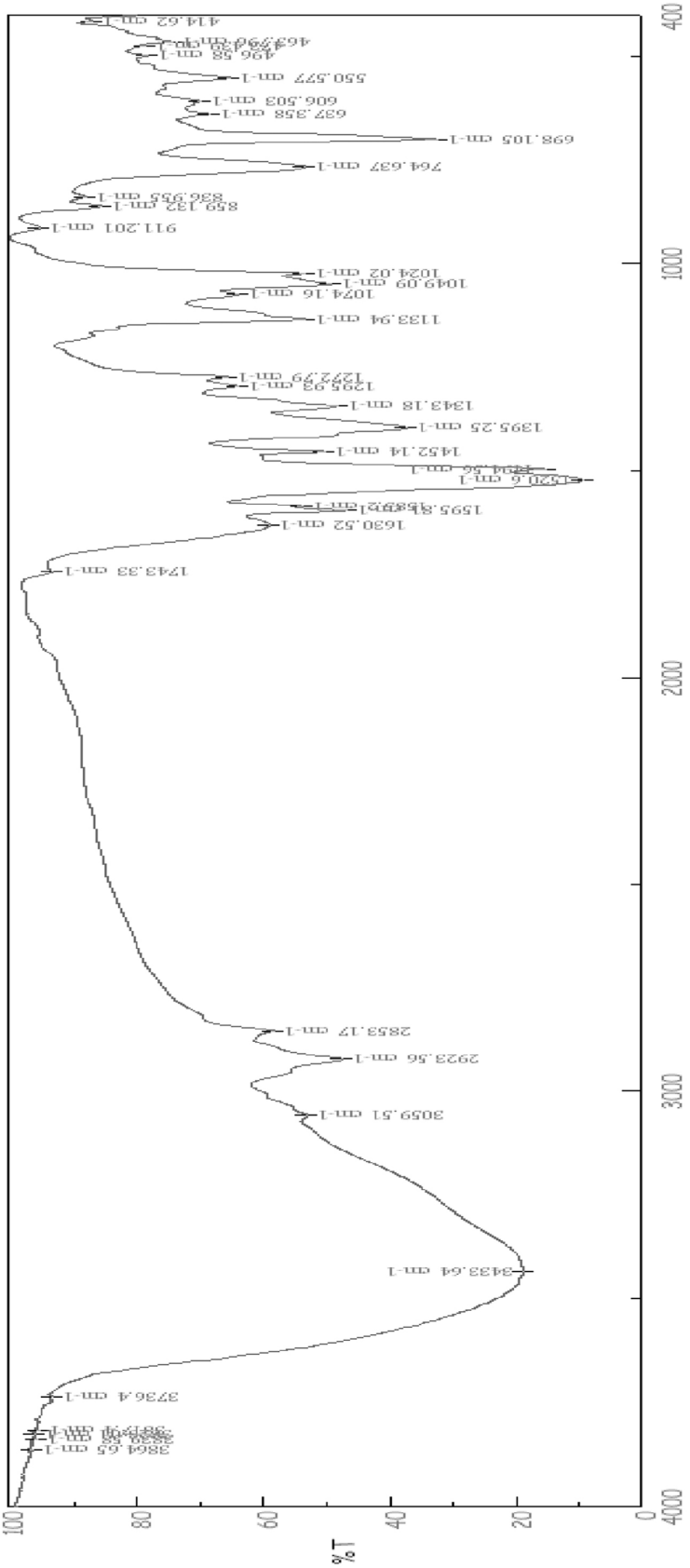

Figure 4.
IR spectrum of Ni(II) complex of benzoylacetone N(4)-methyl(phenyl) thiosemicarbazone $[Ni(LH)_2]$

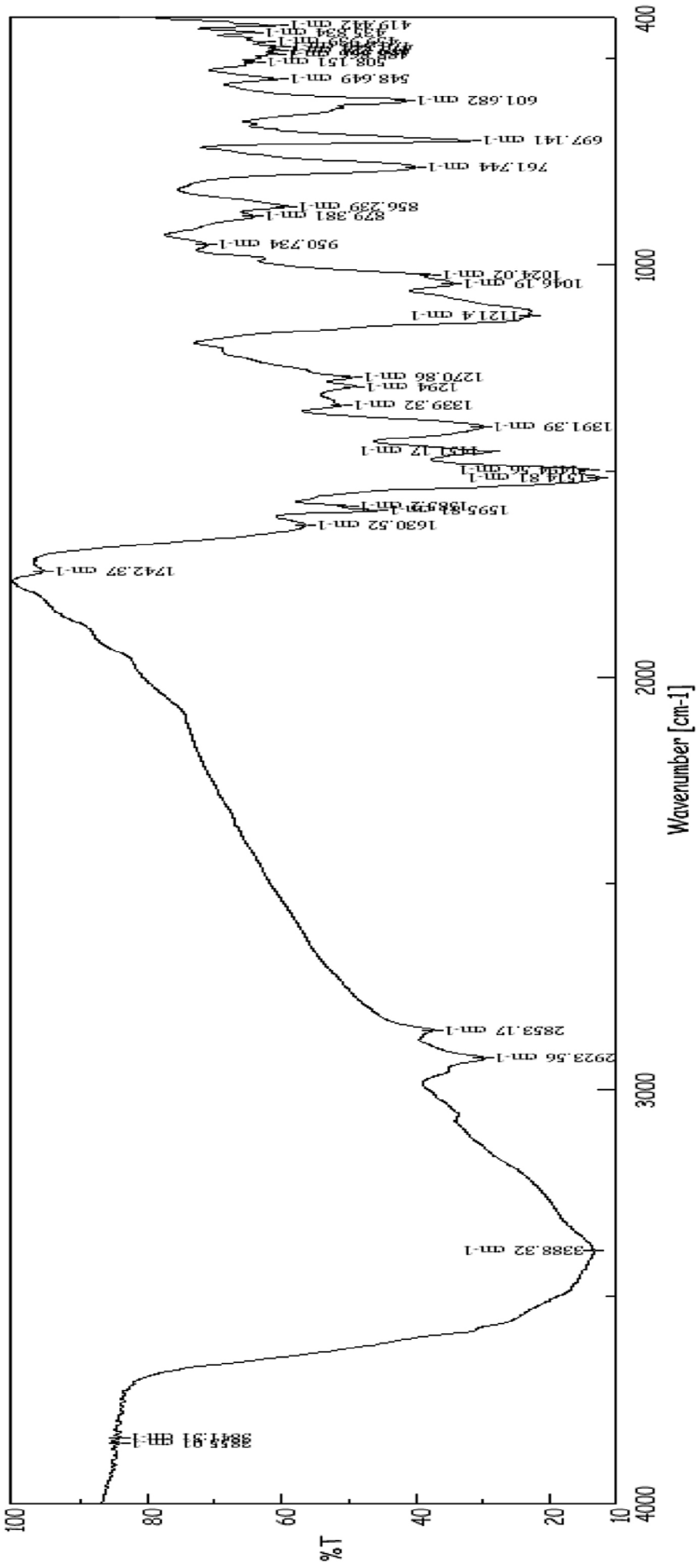

Figure 5.
IR spectrum of Cu(II) complex of benzoylacetone N(4)-methyl(phenyl) thiosemicarbazone, BAcMPTCH$_2$ [Cu(LH)$_2$]

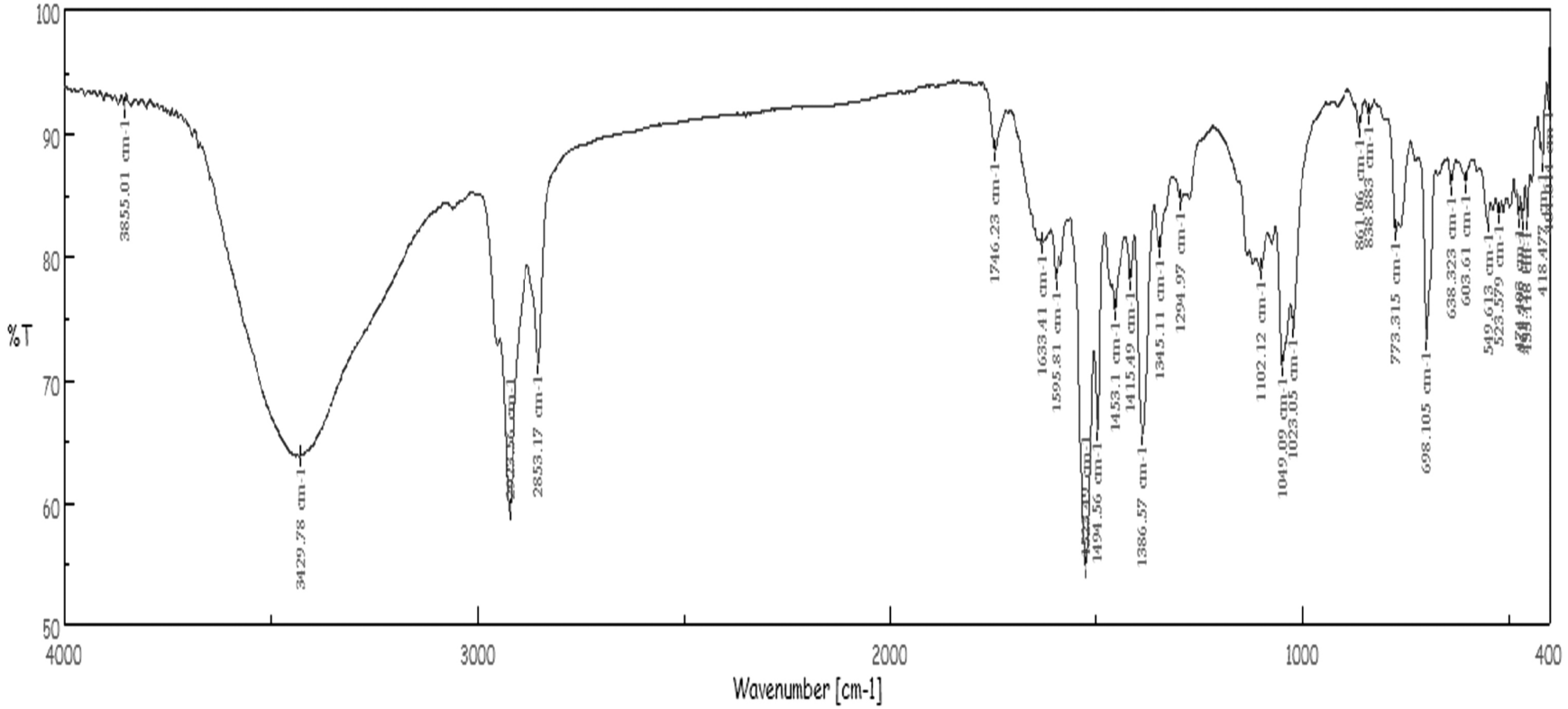

Figure 6
IR spectrum of Zn(II) complex of benzoylacetone N(4)-methyl(phenyl) thiosemicarbazone $[Zn(LH)_2]$

4. Conclusion

The Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of benzoyl-acetone N(4)-methyl(phenyl)thiosemicarbazone(BacMPTCH_2)(L2H) were synthesized. Based on the magnetic and spectral studies, these complexes were assigned octahedral geometries. The structure of the complexes is given in Figure 7.

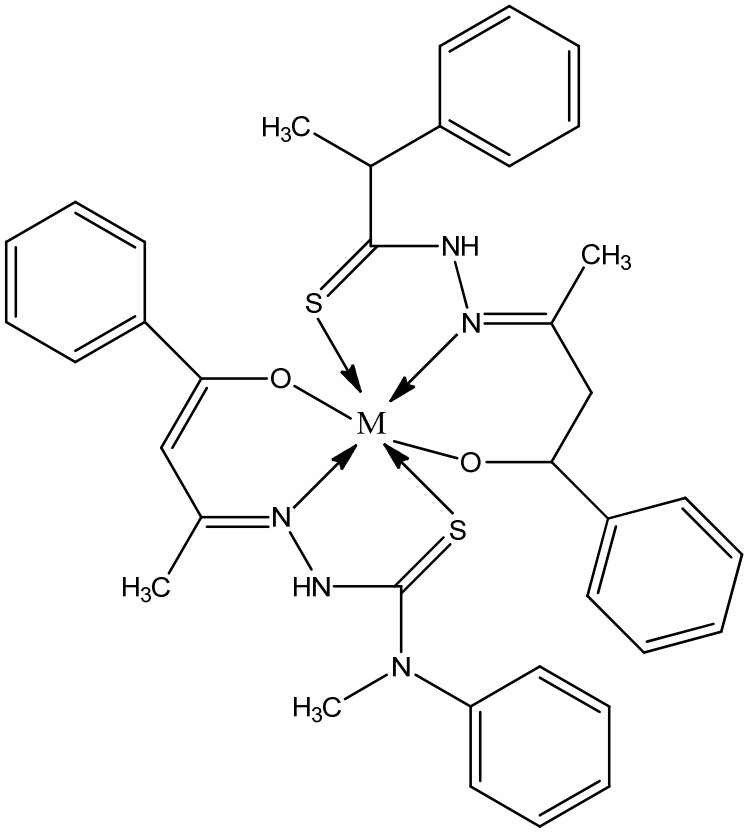

Figure 7.

Suggested structure of the complex of the formula [M(L$H)_2$],

where M = Co(II), Ni(II), Cu(II), Zn(II) or Cd(II)

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CHAPTER V

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF ω-BROMOACETOACETANILIDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE

1. Introduction

Detailed investigations on the coordination chemistry of amides and anilides are fascinating in view of their similarities to peptides and proteins, which are essential constituents of life. As ligands, thiosemicarbazones of β-keto anilides haven't received the kind of attention that they really deserve. Earlier reports on acetoacetanilidesemicarbazones- and thiosemicarbazones showed that they possess antitumour activity[1]. Recently, it has been shown that substitution on the 4th nitrogen of thiosemicarbazone can enhance its biological activity[2]. We have also observed the effective substitution of acetoacetanilide changes considerably the chemical properties and ligational behavior of compounds derived from it[3-5]. With this view, a detailed investigation on the synthesis and characterization of ω-bromoacetoacetanilide N(4)- methyl(phenyl)thiosemicarbazone, (ω-BAacd-MPTCH$_2$) (L2H) and its transition metal complexes is presented in this chapter. The structure of the ligand is given in Figure 1.

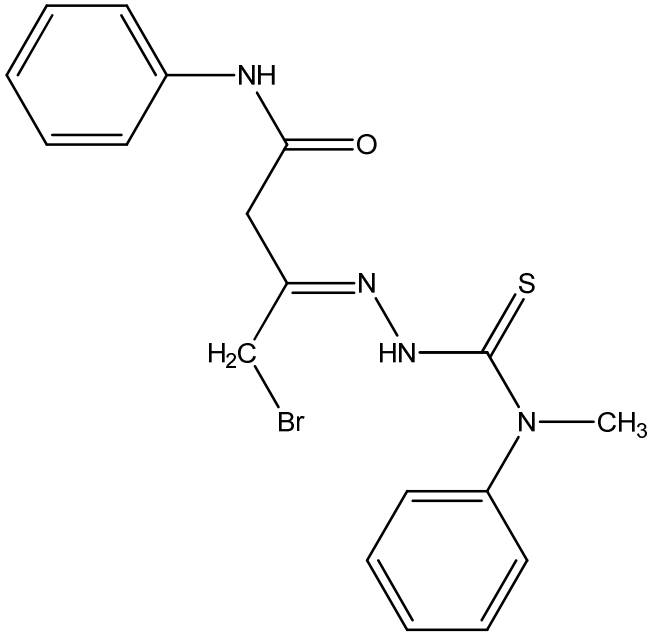

Figure 1.

ω-Bromoacetanilide N(4)-methyl(phenyl)thiosemicarbazone
(ω-BAacd MPTCH_2)(L2H)*

2. Experimental

2.1. Materials and methods

Details of the chemicals used for the synthesis and characterization of the ligand and the complexes are given in Chapter II.

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*IUPAC Name :

(Z)-4-bromo-3-{2-[methyl(phenyl)carbamothioyl]hydrazono}-N-phenylbutanamide

2.2. Preparation of the ligand, (ω-BAacd $MPTCH_2$)(L2H)

A solution of N(4)- methyl(phenyl)thiosemicarbazide in ethanol (0.1 mol, 250 ml) was added to ω-bromoacetoacetanilide in ethanol (0.1 mol, 250 ml) and refluxed for 3 h. The solution obtained was evaporated, cooled and stirred several times with petroleum ether. The solid product that separated was filtered and kept in a desiccator under reduced pressure over anhydrous calcium chloride. The product was recrystallised from acetone (Yield : 40% , M.P = 110ºC).

2.3. Preparation of the complexes

To the ethanolic solution of the ligand (0.005 mol in 50 ml) added a methanolic solution of metal acetate (0.0025 mol in 25 ml) and stirred for 1 h. The solid complex formed was filtered, washed with water and finally with methanol. The products were dried over anhydrous calcium chloride. Yield and M.P. of the complexes were noted.

3. Results and discussion

Using the data obtained from the analytical and physico- chemical studies, the structure and geometries of the complexes were discussed.

3.1. Characterization of the ligand

The ligand, ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemi-carbazone (ωBAacdMPTCH_2)(L2H) was soluble in methanol, ethanol, etc. It was characterized by elemental analysis (Table 3.1.1), electronic- (Table 3.1.2), IR- (Table 3.1.3 and Figure 2.) and ^{1}H NMR (Table 3.1.4 and Figure 3) spectral studies.

3.1.1. Analytical data

The ligand was subjected to elemental analysis on a CHN-O instrument .The experimentally found out- and calculated percentages of C, H, N and S were in good agreement confirming its molecular formula as $C_{18}H_{19}N_4OSBr$.

Table 3.1.1 Physico-chemical and analytical data of the ligand

Compound	**Colour**	**Yield (%)**	**M.P (^{o}C)**	**Elemental analysis (%) found (calculated)**			
				C	**H**	**N**	**S**
Ligand	Colourless	40	110	48.99 (51.60)	4.22 (4.55)	12.53 (13.39)	8.01 (7.65)

3.1.2. Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 268 nm (37313 cm^{-1}) which can be attributed to $\pi \rightarrow \pi^*$ transition. Similarly a

band at 412 nm (24271 cm^{-1}) in the ligand spectrum may be attributed to $n \rightarrow \pi^*$ transition. The data are given in Table 3.1.2.

Table 3.1.2 Electronic spectral bands of the ligand

Spectral bands		**Assignments**
nm	cm^{-1}	
268	37313	$\pi \rightarrow \pi^*$
412	24271	$n \rightarrow \pi^*$

3.1.3. IR spectrum

IR spectral data of the ligand (Figure 2) with the probable assignments are given in the Table 3.1.3. The assignments are made in comparison with the spectra of similar type of compounds[6].

The broad bands in the spectrum of the ligand in the higher frequency region may be due to ν_{N-H} (3500-3300 cm^{-1}). A medium band at 1676 cm^{-1} in the spectrum may be assigned to $\nu_{C=O(anilide)}$ and a band at 1598 cm^{-1} may be assigned to $\nu_{C=N}$. The ν_{N-N} band in the ligand spectrum appears at 1020 cm^{-1}. The strong band at 503 cm^{-1} in the spectrum of the ligand may be assigned to ν_{C-Br}. The strong band at 1075 cm^{-1} in the ligand spectrum may be assigned to $\nu_{C=S}$.

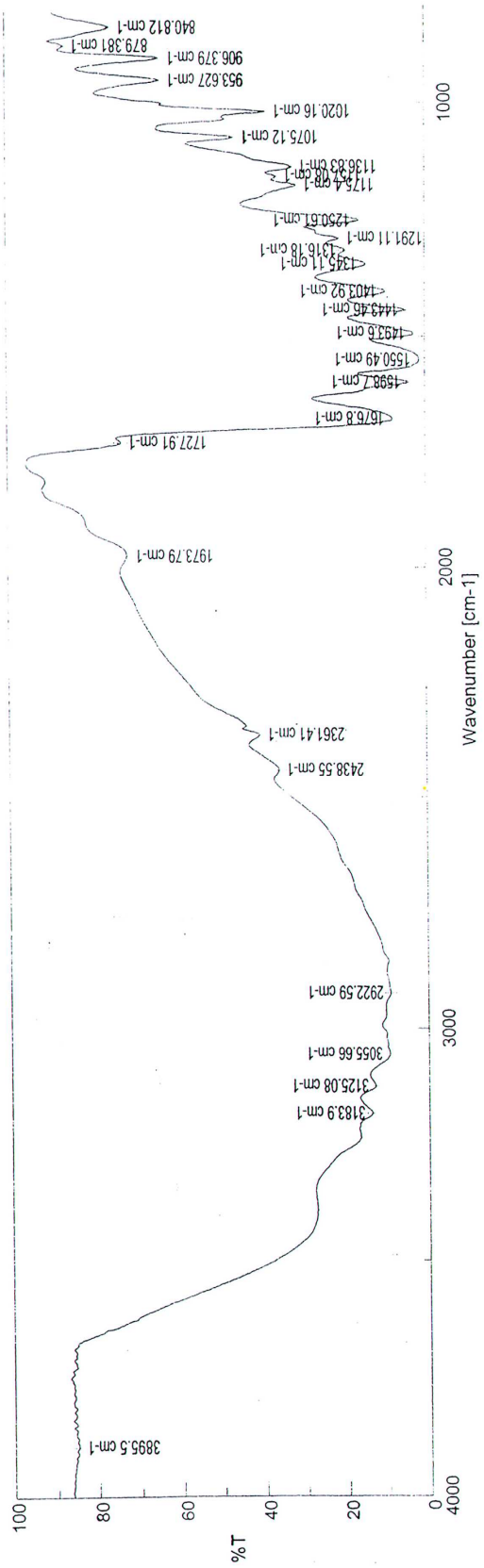

Figure 2.
IR spectrum of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone
(ω-BAacdMPTCH2)(L2H)

Table 3.1.3. Significant IR spectral bands of the ligand

Bands (cm^{-1})	**Assignments**
3500 3000	ν_{N-H} and ν_{C-H} (aromatic)
1676	$\nu_{C=O\ (anilide)}$
1598	$\nu_{C=N}$
1075	$\nu_{C=S}$
1020	ν_{N-N}
503	ν_{C-Br}

3.1.4. ^{1}H NMR spectrum

The spectrum of the ligand shows two singlets at 10.23 and 6.8 ppm which may be assigned to NH protons of anilide ring and thiosemicarbazide moieties, respectively. The multiplets observed at 7.42-7.30 ppm and 7.20-7.10 ppm may be assigned to aromatic ring of anilide part and the phenyl ring of the substituted thiosemicarbazide moiety, respectively. The singlets observed at 3.64 and 3.42 ppm may be assigned to α-CH_2 and ω-CH_2 protons, respectively. The singlet observed at 2.72 ppm may be assigned to CH_3 group of the substituted thiosemicarbazide. The assignments are given in Table 3.1.4. and Figure 3.

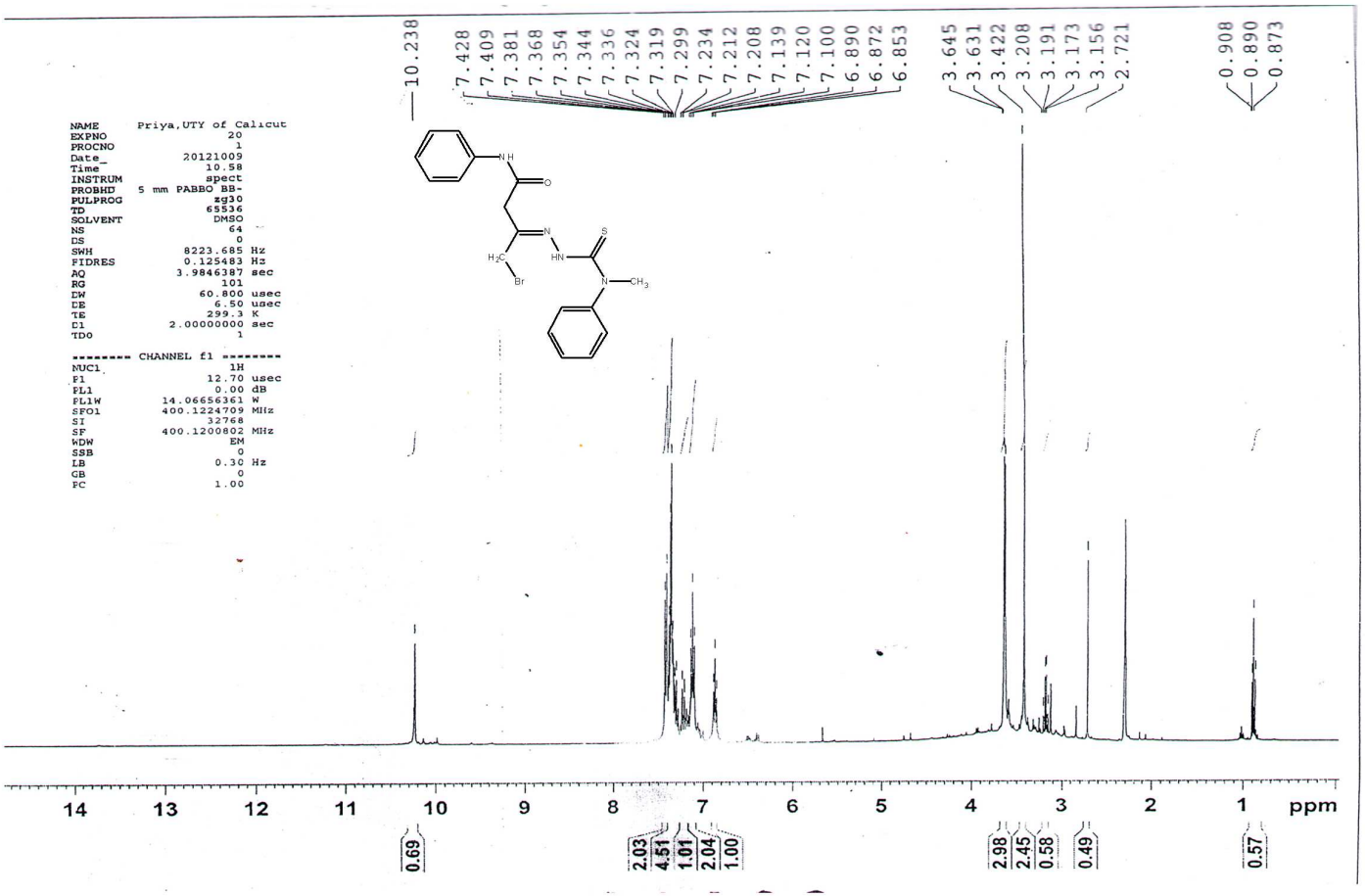

Figure 3.

^{1}H NMR spectrum of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (ω-BAacdMPTCH2)(L2H)

Table 3.1.4. ^1H NMR assignments of the ligand

δ(ppm)	Proton
10.23	-NH(anilide)
7.42-7.30	Aromatic (anilide)
7.20-7.10	Aromatic (substituted thiosemicarbazide)
6.80	-NH (thiosemicarbazide)
3.64	α-CH_2
3.42	ω-CH_2
2.72	-CH_3 (substituted thiosemicarbazide)

3.2. Characterization of the complexes

The ligand, (ω-BAacdMPTCH_2)(L2H) formed stable complexes with transition metal ions. The complexes were soluble in DMSO, DMF, etc. They were characterized as follows :

3.2.1. Elemental analysis

The analytical data and physical properties of the ligand (ω-BAacd MPTCH_2) (L2H) and its complexes are listed in Table 3.2.1. The complexes were found to have general formulae [Co$(LH)_2$], [NiL$(H_2O)_3$] and [ML(H_2O)], where M=Cu(II), Zn(II) or Cd(II) and LH^- is the monovalent anion- and L^{--} is the bivalent anion of L2H.

Table 3.2.1. Physico-chemical and analytical data of the ligand and its complexes

Compound	Colour	Yield (%)	MP (^oC)	Elemental Analysis (%) found (calculated)				
				C	H	N	S	Metal
Ligand	Colour less	40	110	48.99 (51.60)	4.22 (4.55)	12.53 (13.39)	8.01 (7.65)	--
$[Co(LH)_2]$	Dark brown	65	282	49.20 (48.20)	3.10 (3.79)	13.60 (12.50)	7.91 (7.15)	6.89 (6.58)
$[NiL(H_2O)_3]$	Dark green	64	274	40.70 (42.09)	4.70 (4.18)	10.55 (11.32)	12.05 (12.60)	6.03 (6.82)
$[CuL(H_2O)]$	Dark green	65	278	44.60 (43.20)	4.60 (4.20)	12.04 (11.20)	6.10 (6.40)	12.90 (12.70)
$[ZnL(H_2O)]$	Off white	68	272	44.11 (43.08)	5.02 (4.18)	12.21 (11.16)	7.18 (6.38)	13.90 (13.04)
$[CdL(H_2O)]$	Pale yellow	70	275	40.14 (39.38)	4.12 (3.82)	10.88 (10.21)	6.12 (5.83)	21.46 (20.49)

3.2.2. Magnetic behaviour

The calculated magnetic moments of the complexes are given in the Table 3.2.2.

Table 3.2.2 Magnetic moments of the complexes

Compound	μ_{eff}(B.M)
$[Co(LH)_2]$	4.64
$[NiL(H_2O)_{3]}$	2.79
$[CuL(H_2O)]$	1.90

T=304K μ_{eff} = Effective magnetic moment

B.M = Bohr Magneton

The Co(II) complex registered a magnetic moment value of 4.64 B.M. Based on this value, we can assign octahedral geometry for this complex. The Ni(II) complex registered a magnetic moment value of 2.79 B.M. which is in agreement with octahedral geometry. The magnetic moment value of the copper(II) complex was 1.90 B.M. and therefore, it may be assigned a square planar structure[7]. Both the Zn(II) and Cd(II) complexes were found to be diamagnetic.

3.2.3. Electronic spectra

The important electronic spectral bands and the assignments of the ligand and the Co(II), Ni(II) and Cu(II) complexes are given in the Table 3.2.3.

Table 3.2.3. Electronic spectral bands of the ligand and complexes

Compound	**Bands**	
	(nm)	**(cm^{-1})**
Ligand	268 412	37313 24271
$[Co(LH)_2]$	448 616 1025	22321 16233 9756
$[NiL(H_2O)_3]$	390 666 1018	25641 15015 9823
$[CuL(H_2O)]$	440 525	22727 19047

The bands shown by the Co(II) complex at 448, 616 and 1025 nm were assigned to $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, respectively. These bands are characteristic of octahedral geometry of the Co(II) complex.

The Ni(II) complex registered all the three bands characteristic for 6-coordinate octahedral Ni(II) ion at 390, 666, and 1018 nm. They may be assigned respectively to $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ transitions.

The Cu(II) complex, showed two weak bands corresponding to the transitions of a square planar Cu(II) complex, $^2B_{1g} \rightarrow {}^2E_g$ and $^2B_{1g} \rightarrow {}^2B_{2g}$ at 440 and 525 nm respectively.

3.2.4 Infrared spectra

The significant IR spectral bands of the ligand (Figure 2) and the complexes along with their probable assignments are given in the Table 3.2.4 and Figures 4-8. The assignments were made based in comparison with the spectra of similar type of compounds[8].

As we have mentioned earlier, a broad band in the higher frequency region, 3500-3000 cm^{-1} is present in the spectra of the complexes of Ni(II), Cu(II), Zn(II) and Cd(II). This region is found to be broad due to the presence of water coordination. The anilide carbonyl stretching frequency at 1676 cm^{-1}

in the ligand is found to be lowered in the spectrum of the Co(II) complex, indicating the participation of carbonyl oxygen in coordination. But in the spectra of complexes of Ni(II), Cu(II), Zn(II) and Cd(II) this band disappears and a new band around 1150 cm^{-1} is observed due to the enolisation of $–CH_2$-C=O to –CH=C-OH followed by the participation of deprotonated oxygen in coordination. The stretching frequency of azomethine nitrogen is observed at 1598 cm^{-1} in the ligand spectrum. This band is found to be lowered in the complexes due to the participation of azomethine nitrogen in coordination. A strong band at 1075 cm^{-1} in the ligand spectrum, due to $\nu_{(C=S)}$ disappears and new bands around 750-850 cm^{-1} due to ν_{C-S} appears in the spectra of all the complexes. This is due to the enolization of =N-NH-C=S to =N-N=C-SH, followed by the coordination through deprotonated sulphur.

A medium band around 1020 cm^{-1} is observed both in the case of ligand and complexes, is due to the presence of ν_{N-N}. A strong band around 505-510 cm^{-1} in the spectra of the ligand and the complexes may be assigned to ν_{C-Br}. The other bands in the spectra of the complexes around 550-450 cm^{-1} may be assigned to $\nu_{M-N,}$ and ν_{M-O}.

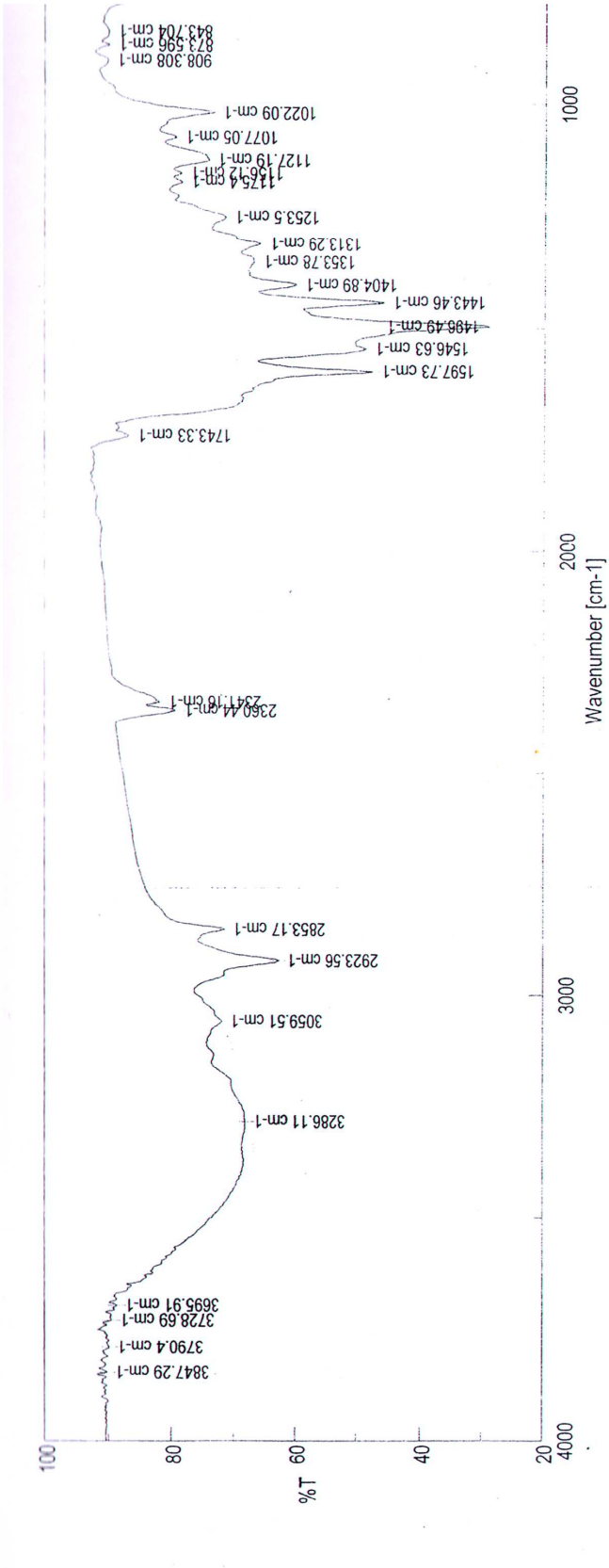

Figure 4.

IR spectrum of Co(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone $[Co(LH)_2]$

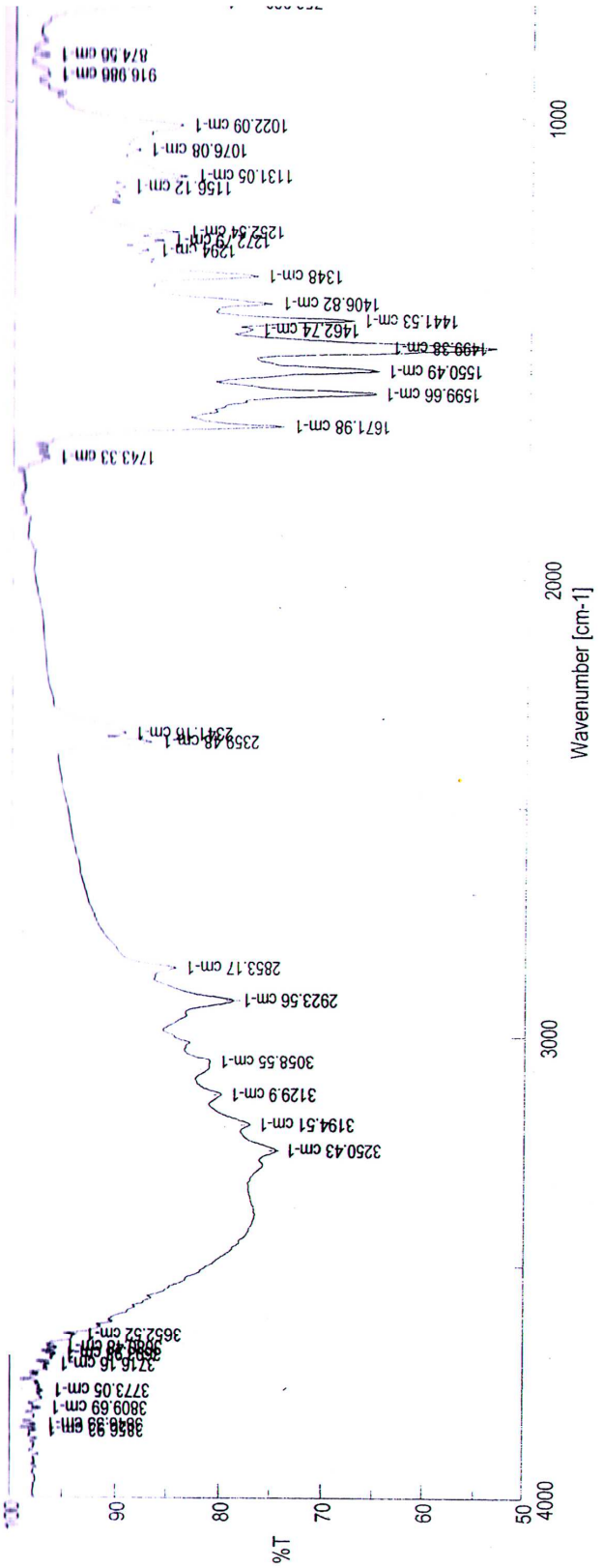

Figure 5.

IR spectrum of Ni(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone $[NiL(H_2O)_3]$

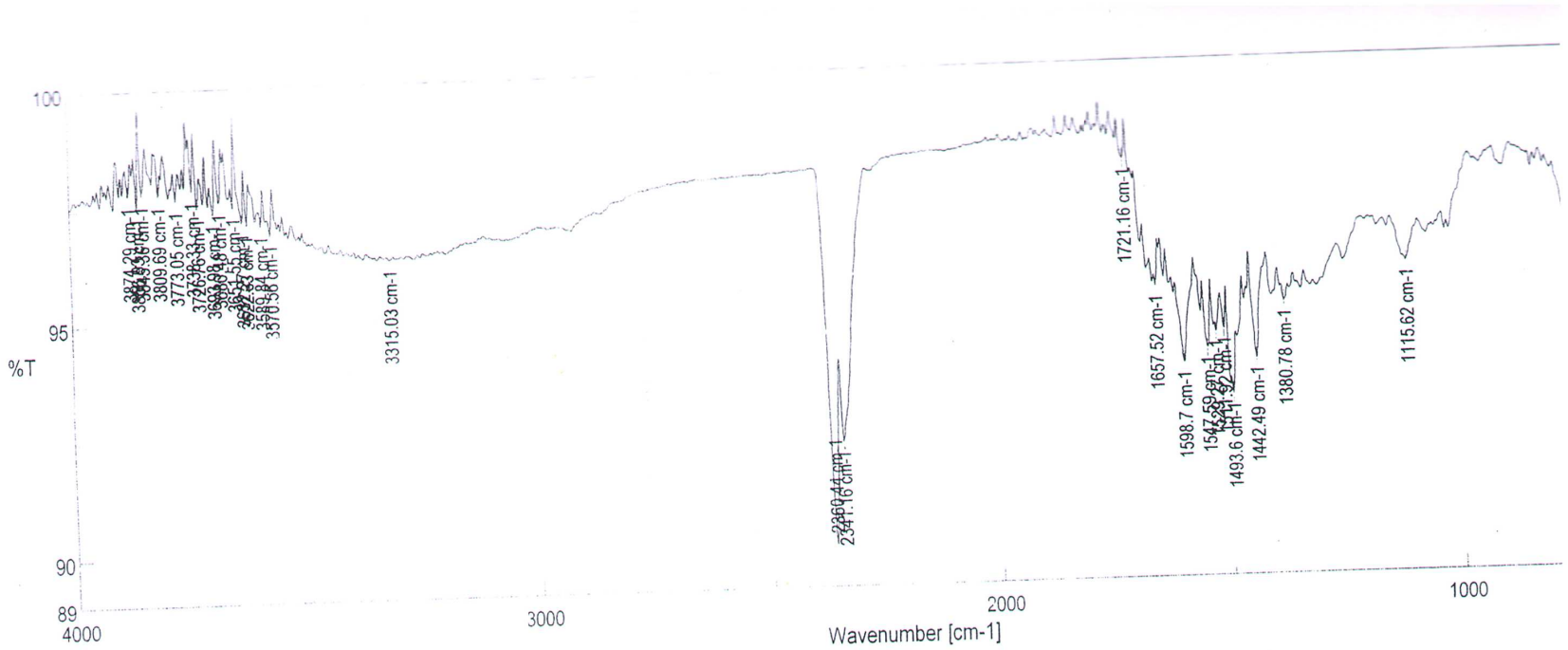

Figure 6.

IR spectrum of Cu(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone $[CuL(H_2O)]$

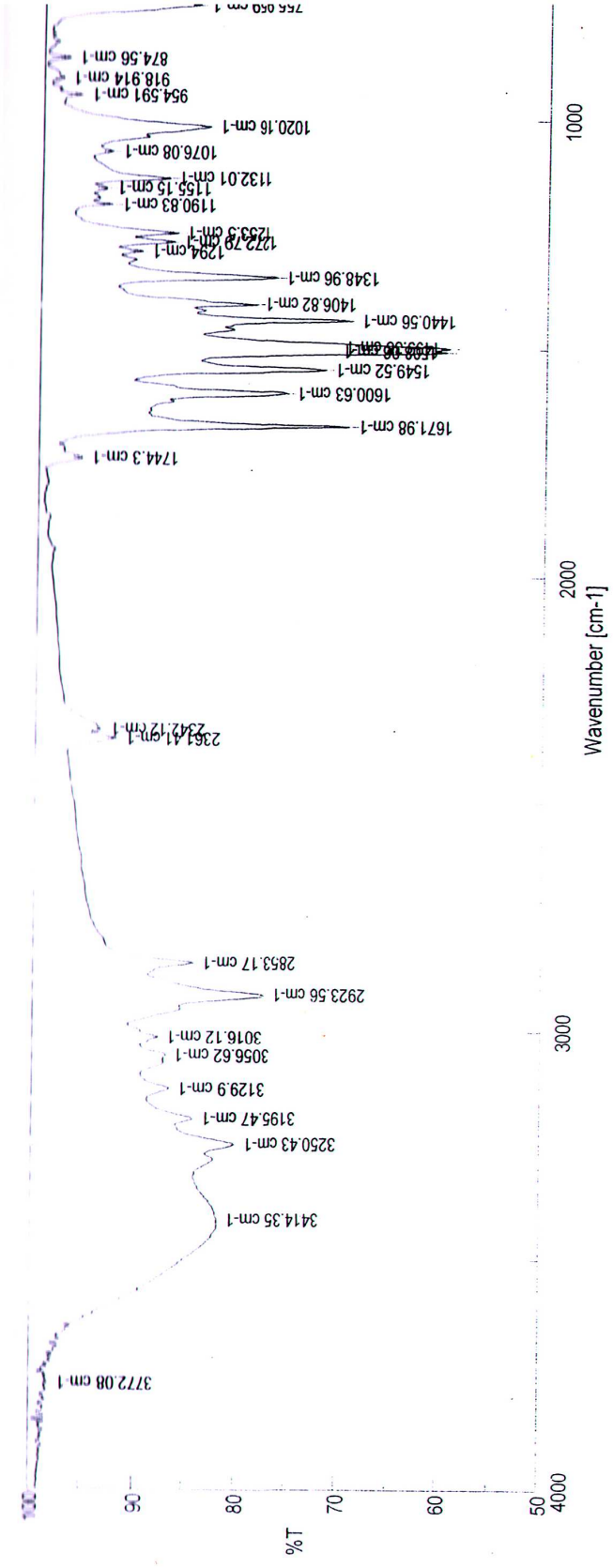

Figure 7.

IR spectrum of Zn(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone [$ZnL(H_2O)$]

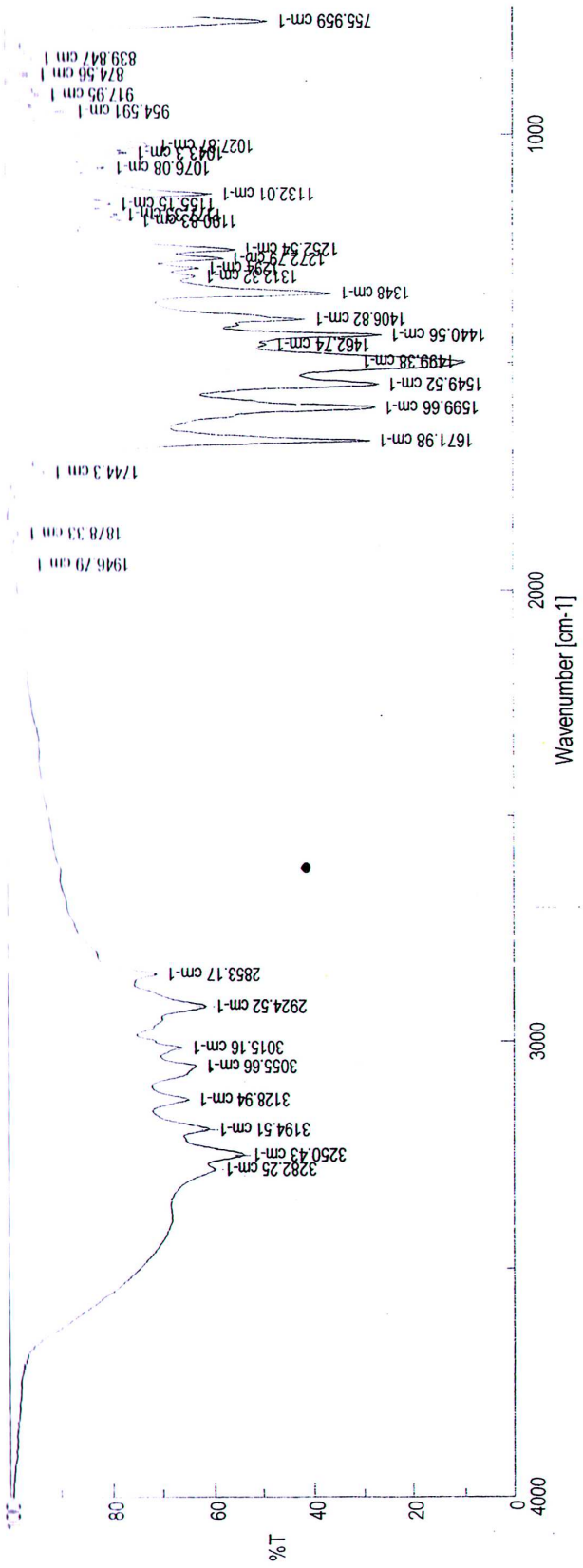

Figure 8.

IR spectrum of Cd(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone [$CdL(H_2O)$]

Table 3.2.4. Significant infrared spectral bands (cm^{-1}) of the ligand and its metal complexes

Ligand	$[Co(LH)_2]$	$[NiL(H_2O)_3]$	$[CuL(H_2O)]$	$[ZnL(H_2O)]$	$[CdL(H_2O)]$	Assign-ments
3500 3300	3695 3286	3652 3250	3570 3315	3600 3282	3414 3195	$\nu_{(N-H)}$, $\nu_{(O-H)}$ $\nu_{(C-H)}$ aromatic
1676	1597	--	--	--	--	$\nu_{C=O}$
		1156	1155	1155	1155	$\nu_{(C-O)}$ anilide
1598	1546	1550	1598	1599	1549	$\nu_{(C=N)}$
1075	--	--	--	--	--	$\nu_{(C=S)}$
1020	1022	1022	1115	1027	1020	$\nu_{(N-N)}$
--	843	874	756	839	874	$\nu_{(C-S)}$ + $\nu_{(C-N)}$
503	507	502	503	507	507	$\nu_{(C-Br)}$
--	542	532	541	530	530	$\nu_{(M-N)}$
--	455	455	457	435	460	$\nu_{(M-O)}$

3.2.5. ^1H NMR spectrum of Zn(II) complex

Figure 9 shows the significant NMR spectral lines of the Zn(II) complex. The signals due to -NH proton of anilide group, aromatic ring of anilide group and the substituted aromatic ring are observed at 10.19, 7.32-7.26 and 7.05-6.90 ppm, respectively. The -NH proton signal which is present at 6.80 ppm in the ligand spectrum disappears due to the enolisation of =N-NH-C=S to =N-N=C-SH and subsequent deprotanation during complex formation. The α-CH_2 proton which appears at 3.64 ppm in the

ligand spectrum is not observed in the spectrum of the complex. But another signal is observed at 4.60 ppm due to α-CH proton formed by the enolization of $–CH_2$-C=O to –CH=C-OH during complex formation. Two other signals are observed at 3.60 ppm and 2.50 ppm due to ω-CH_2 and -CH_3 (substituted), respectively.

Table 3.2.5. Significant ^{1}H NMR spectral assignments of Zinc(II) complex

δppm	**Proton**
10.19	-NH(anilide)
7.32-7.26	Aromatic (anilide)
7.05-6.90	Aromatic (Substituted thiosemicarbazide)
6.94	-NH
4.60	α-CH
3.60	ω-CH_2
2.50	-CH_3 (Substituted thiosemicarbazide)

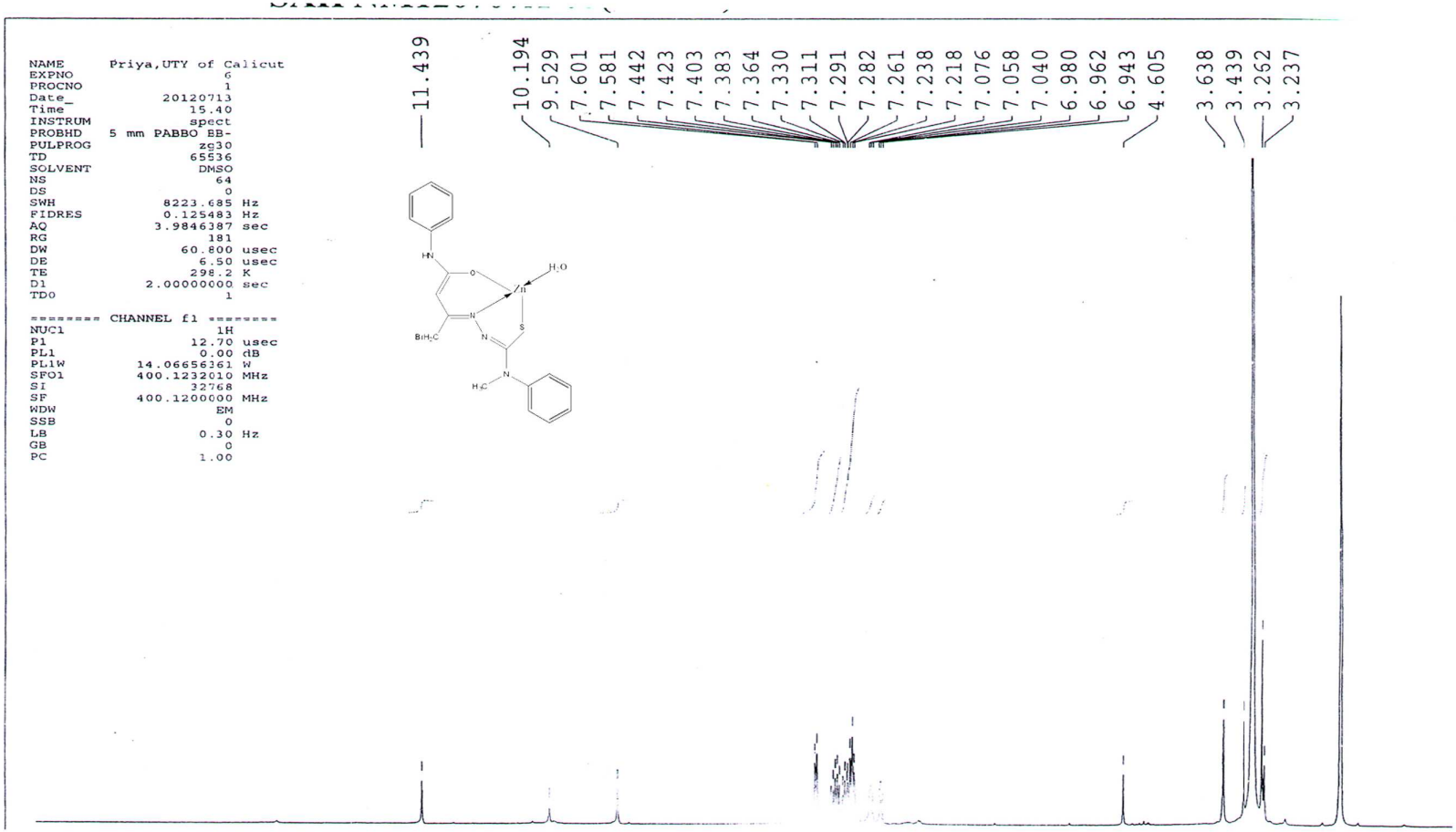

Figure 9.

^{1}H NMR spectrum of Zn(II) complex of ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone $[ZnL(H_2O)]$

4. Conclusion

The Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of ω-BAacd-MPTCH$_2$ (L2H) were synthesized. Based on the magnetic and spectral studies, Co(II) and Ni(II) complexes were assigned octahedral structures and Cu(II), Zn(II) and Cd(II) were assigned tetrahedral geometry (Figures 10, 11 and 12). The presence of water in some of the complexes was established by IR studies.

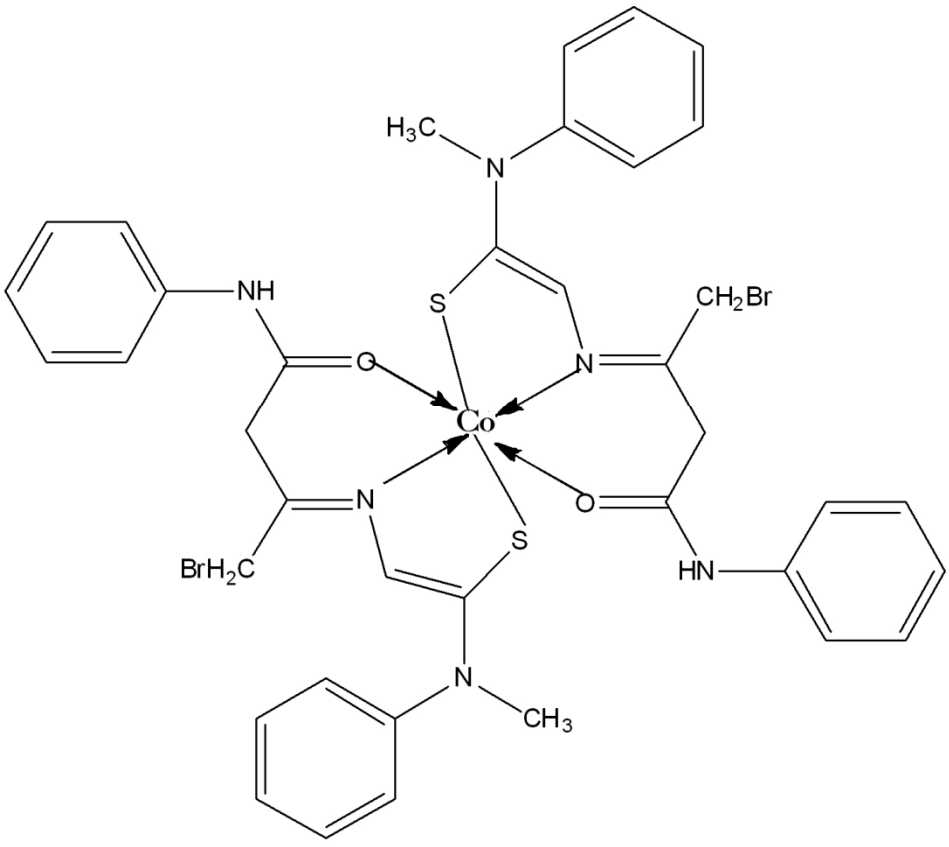

Figure 10.

Suggested structure of [Co(LH)$_2$]

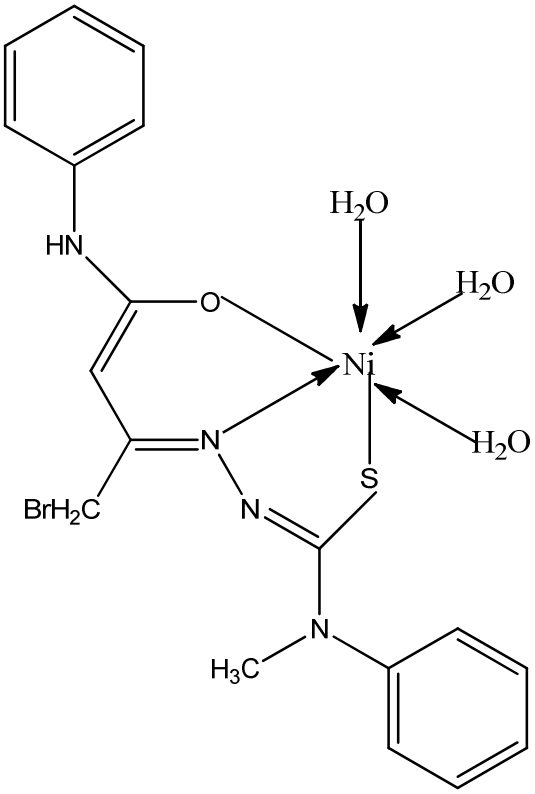

Figure 11.

suggested structure of $[NiL(H_2O)_3]$

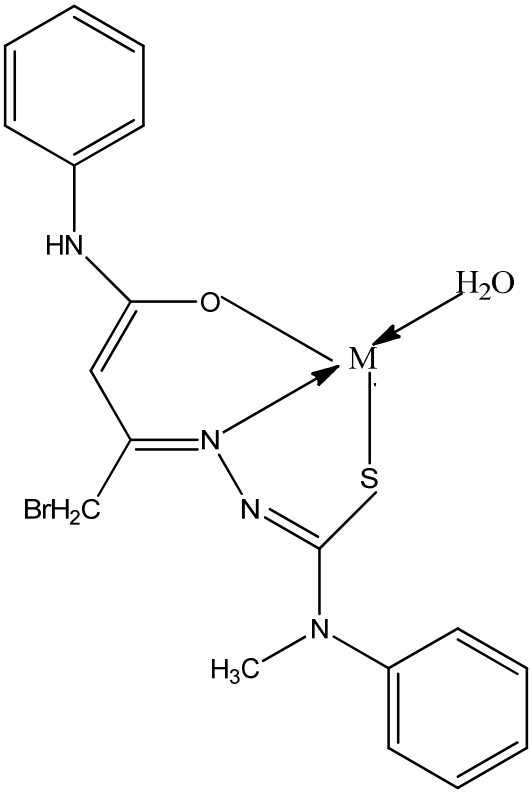

Figure 12.

Suggested structure of the complex of the formula [ML(H_2O)],

where M = Cu(II) , Zn(II) or Cd(II)

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CHAPTER VI

SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES OF N-ETHYLACETOACETANILIDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE

1. Introduction

The study of the coordination behaviour of thiosemicarbazones towards transition metal ions showed that the resulting complexes possess diverse solubility, stability and beneficial biological activities[1]. It has been reported that replacing the hydrogen atom of the -NH group of acetoacetanilide by straight chain alkyl groups enhances their solubility and thereby improves their chelating ability[2]. In continuation of our investigations on thiosemicarbazones of β 1,3-diketo derivatives, it was found to be worthwhile and interesting to synthesize and characterize 4-N-substituted thiosemicarbazone of N-substituted acetoacetanilide and their metal complexes. Therefore, in this chapter, we present our studies on the synthesis and characterization of N-ethylacetoacetanilide N(4)-methyl (phenyl)thiosemicarbazone (EAacdMPTCH_2) (L2H) and its complexes with a few typical transition metal ions. The structure of the ligand is given in Figure 1.

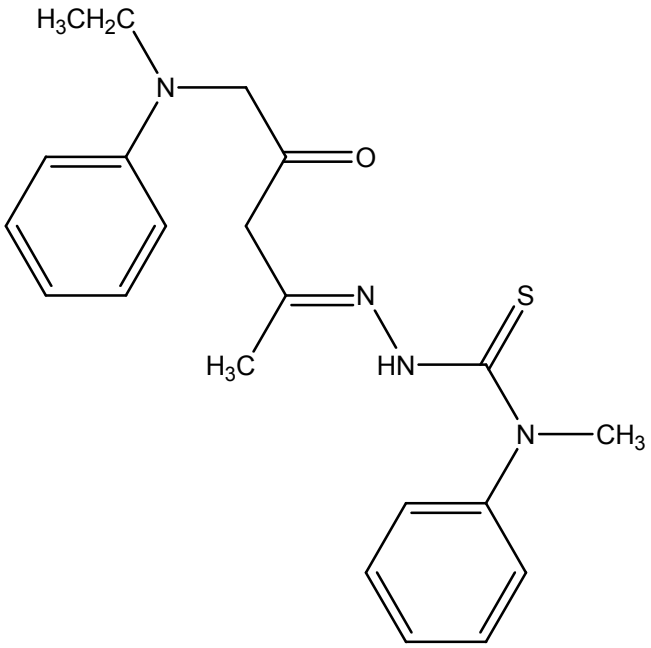


Figure 1.

N-Ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone ($EAacdMPTCH_2$) (L2H)*

2. Experimental

2.1. Materials and methods

Details regarding the chemicals used and the methods adopted for the characterization of the compounds are described in Chapter II.

---

*IUPAC Name :

(E)-2-(5-(ethyl(phenyl)amino)-4-oxopentan-2-ylidene)-N-methyl-N-phenylhydrazinecarbothioamide

2.2. Preparation of the ligand, (EAacd MPTCH_2) (L2H)

N(4)-methyl(phenyl)thiosemicarbazide in 150 ml ethanol (0.05 mol) was added to sodium N-ethylacetoacetanilide in 150 ml ethanol-water (4:1) mixture (0.05 mol) and refluxed on a water bath for 2h. The resulting solution was evaporated to reduce the volume and stirred for 2h. The pale-yellow solid which formed was filtered off, washed several times with water and dried in a desiccator over anhydrous calcium chloride. The product was recrystallised from methanol (Yield : 45% , M.P = 160ºC).

2.3. Preparation of the complexes

To a magnetically stirred hot methanolic solution of the ligand (0.005 mol in 20 ml), added slowly a methanolic solution of metal acetate (0.005 mol in 15 ml). The mixture was stirred for 1h. The solid complex that formed was filtered off, washed with water and finally with methanol. It was dried in a desiccator over anhydrous calcium chloride. Yields and melting points of the complexes were noted.

3. Results and discussion

The data obtained from the analytical and physico-chemical studies have been correlated to explain the properties, structure and bonding of the compounds.

3.1. Characterization of the ligand

The ligand, N-ethylacetoacetanilide N(4)-methyl(phenyl) thiosemi-carbazone (EAacdMPTCH_2) (L2H) is a colourless solid soluble in methanol, ethanol, etc. From the elemental analysis, (Table 3.1.1.) the following empirical formula, $C_{20}H_{24}N_4OS$ was assigned to the ligand. It was further characterized by electronic-(Table 3.1.2) and IR (Table 3.1.3 and Figure 2) spectral studies.

3.1.1. Analytical data

The ligand was subjected to elemental analysis on a CHN-O instrument. The experimentally found out and calculated percentages of C, H, N and S were in good agreement confirming with the molecular formula, $C_{20}H_{24}N_4OS$.

Table 3.1.1 Physico-chemical and analytical data of the ligand

Compound	**Colour**	**Yield (%)**	**M.P (^oC)**	**Elemental analysis (%) found (calculated)**			
				C	**H**	**N**	**S**
Ligand	Colourless	45	160	66.40 (65.21)	7.10 (6.52)	15.90 (15.21)	9.01 (8.69)

3.1.2. Electronic spectrum

The electronic spectrum of the ligand showed an intense band at 282 nm (35460 cm^{-1}) due to $\pi \rightarrow \pi^*$ transition. A band at 345 nm

(28985 cm^{-1}) in the ligand spectrum may be attributed to $n \rightarrow \pi^*$ transition. The data are given in Table 3.1.2.

Table 3.1.2 Electronic spectral bands of the ligand

Spectral bands		**Assignments**
nm	cm^{-1}	
282	35460	$\pi \rightarrow \pi^*$
345	28985	$n \rightarrow \pi^*$

3.1.3. IR spectrum

IR spectrum (Figure 2) of the ligand with the probable assignments are given in the Table 3.1.3. The IR spectrum showed bands at 3573 and 3417 cm^{-1} due to ν_{C-H} and $\nu_{N-H,}$ respectively[3]. Bands at 1607 and 1556 cm^{-1} corresponded to $\nu_{C=O}$ and $\nu_{C=N,}$ respectively. The band at 1051 cm^{-1} may be assigned to $\nu_{C=S}$. There was a band at 1023 cm^{-1} due to ν_{N-N}.

Table 3.1.3. Significant IR spectral bands of the ligand

Bands (cm^{-1})	**Assignments**
3573 3417	$\nu_{C-H,} \nu_{N-H}$
1607	$\nu_{C=O}$
1556	$\nu_{C=N}$
1051	$\nu_{C=S}$
1023	ν_{N-N}

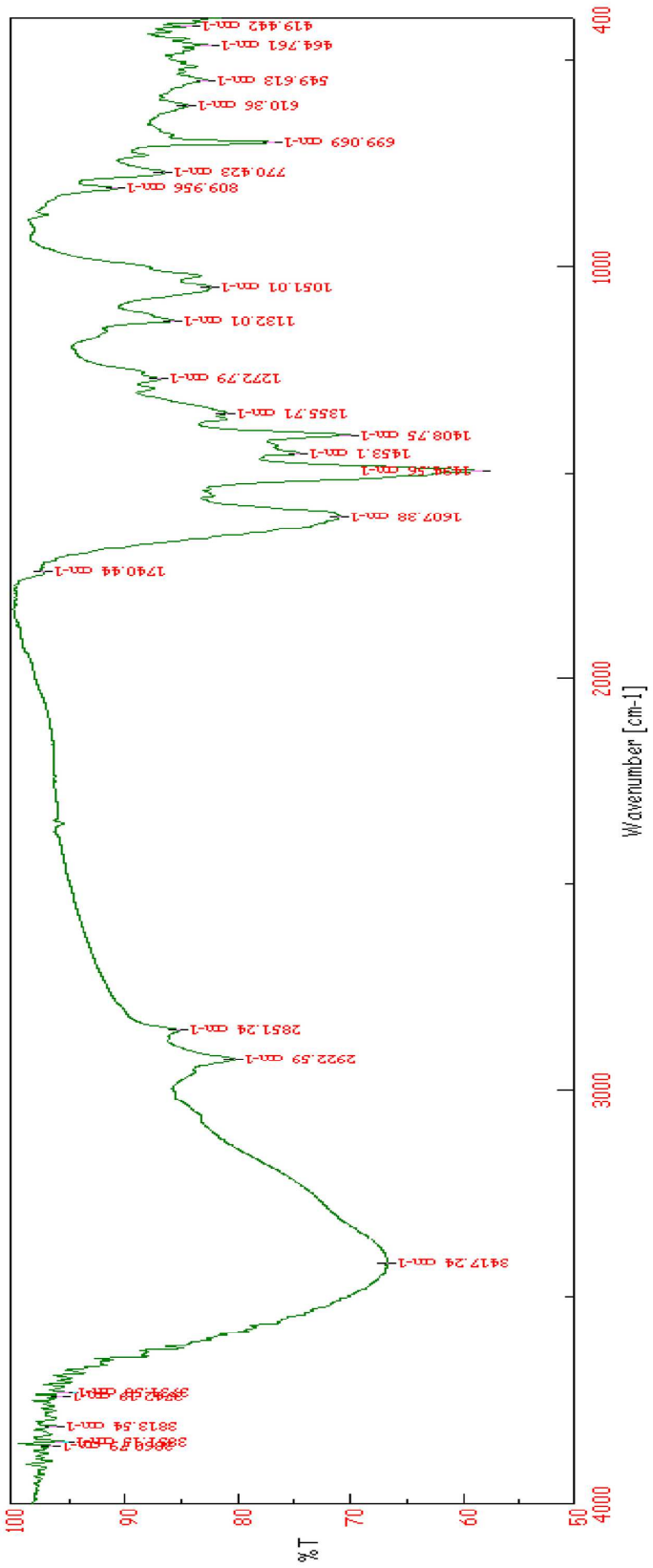


Figure 2.
IR spectrum of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone
($EAacdMPTCH_2$)(L2H)

3.2. Characterization of the complexes

N-ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone formed stable complexes with general transition metal ions, which were soluble in DMSO, DMF, etc. The complexes were characterized as follows :

3.2.1. Elemental analysis

The analytical data and physical properties of the ligand, (EAacd $MPTCH_2$) (L2H) and its complexes are listed in Table 3.2.1. The complexes were found to have a general formula $[ML(H_2O)_3]$, where L^{-2} is the divalent anion of the ligand, L2H.

Table 3.2.1 Physico-chemical and analytical data of the ligand and its complexes

Compound	**Colour**	**Yield (%)**	**MP (^oC)**	**Elemental Analysis (%) found (calculated)**				
				C	**H**	**N**	**S**	**Metal**
Ligand	Colour-less	45	160	66.40 (65.21)	7.10 (6.52)	15.90 (15.21)	9.01 (8.69)	--
$[CoL(H_2O)_3]$	Dark brown	55	282	50.34 (49.90)	7.80 (6.23)	12.10 (11.60)	6.98 (6.65)	14.40 (12.24)
$[NiL(H_2O)_3]$	Light green	60	280	50.70 (49.92)	7.40 (6.64)	12.20 (11.23)	7.10 (6.65)	13.30 (12.21)
$[CuL(H_2O)_3]$	Dark blue	58	278	52.40 (49.43)	6.98 (6.17)	11.94 (11.53)	6.98 (6.59)	14.14 (13.07)
$[ZnL(H_2O)_3]$	Colourless	60	282	56.10 (55.37)	6.12 (5.55)	13.23 (12.92)	8.01 (7.38)	16.30 (15.08)
$[CdL(H_2O)_3]$	Pale yellow	55	280	46.30 (44.90)	5.92 (5.61)	11.30 (10.40)	6.71 (5.98)	22.30 (21.00)

3.2.2. Magnetic behaviour

The calculated magnetic moment values are given in the Table 3.2.2.

Table 3.2.2 Magnetic moments of the complexes

Compound	**μ_{eff} (B.M)**
$[CoL(H_2O)_3]$	4.85
$[NiL(H_2O)_3]$	3.11
$[CuL(H_2O)_3]$	2.10

T= 304 K μ_{eff} = Effective magnetic moment

B.M = Bohr Magneton

The Co(II) complex registered a magnetic moment of 4.85 B.M., indicating its high-spin octahedral structure. The Ni(II) complex registered a magnetic moment of 3.11 B.M. This value indicates the octahedral geometry around Ni(II) ion. The magnetic moment value of $[CuL(H_2O)_3]$ was observed to be 2.10 B.M. which is the characteristic of a 6- coordinated Cu(II) complex[4]. The complexes of Zn(II) and Cd(II) were found to be diamagnetic[5].

3.2.3. Electronic spectra

The important electronic spectral bands of the ligands, Co(II), Ni(II) and Cu(II) complexes and their assignments are given in the Table 3.2.3.

Table 3.2.3 Electronic spectral bands of the ligand and complexes

Compound	Bands	
	(nm)	**(cm^{-1})**
Ligand	282 345	35460 28985
$[CoL(H_2O)_3]$	465 555 br 1045 w	21505 18018 9569
$[NiL(H_2O)_3]$	409 620 1010 w	24449 16129 9900
$[CuL(H_2O)_3]$	639	15649

br =broad, w=weak

In the spectrum of the Co(II) complex, three bands are observed at 21505, 18018 and 9569 cm^{-1} may be due to the following transitions, respectively,

$^4T_{1\,g}(F) \rightarrow {}^4T_{1g}(P)$

$^4T_{1\,g}(F) \rightarrow {}^4A_{2g}(F)$

$^4T_{1\,g}(F) \rightarrow {}^4T_{2g}(F)$

The Ni(II) complex had three absorption bands at 24449, 16129, and 9900 cm^{-1} which may be due to the following transitions, respectively,

$^3A_{2\,g}(F) \rightarrow {}^3T_{1g}(P)$

$^3A_{2\,g}(F) \rightarrow {}^3T_{1\,g}(F)$

$^3A_{2\,g}(F) \rightarrow {}^3T_{2\,g}(F)$

In the UV-visible spectrum of $[CuL(H_2O)_3]$ we observed a peak at 15649 cm^{-1}, which indicated the electronic transition, $E_g \rightarrow T_{2g}$. The above observations are consistant with octahedral geometry for all the complexes.

3.2.4. Infrared spectra

The significant IR spectral bands of the ligand (Figure 2) and the complexes (Figures 3 - 7) along with their probable assignments are given in Table 3.2.4.

Table 3.2.4 . Significant infrared spectral bands (cm^{-1}) of the ligand and its metal complexes

Ligand	**$[CoL(H_2O)_3]$**	**$[NiL(H_2O)_3]$**	**$[CuL(H_2O)_3]$**	**$[CdL(H_2O)_3]$**	**$[ZnL(H_2O)_3]$**	**Assign-ments**
-- 3417 3573	3397 2923 --	3408 2955 --	3426 3059 --	3426 2923 --	3407 3060 --	ν_{O-H} ν_{C-H} ν_{N-H}
1607	--	--	--	--	--	$\nu_{C=O}$
1556	1494	1494	1498	1493	1494	$\nu_{C=N}$
--	1117	1118	1116	1116	1118	ν_{C-O} (new)
1051	--	--	--	--	--	$\nu_{C=S}$
1023	1022	1023	1023	1023	1024	ν_{N-N}
--	865	863	873	873	872	ν_{C-S} + ν_{C-N}
--	548	551	551	551	553	ν_{M-N}
--	440	457	465	451	494	ν_{M-O}

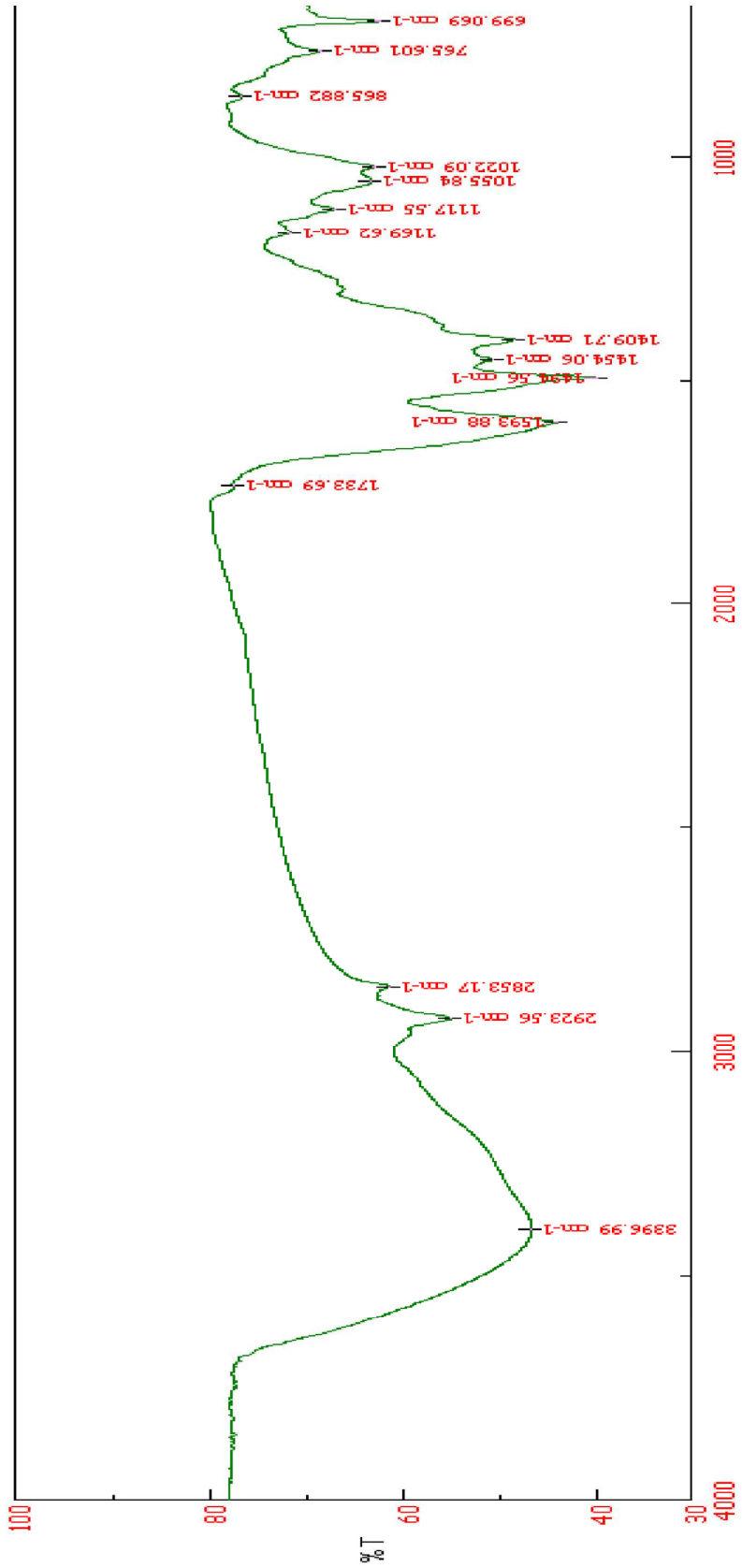


Figure 3.

IR spectrum of Co(II) complex of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone $[CoL(H_2O)_3]$

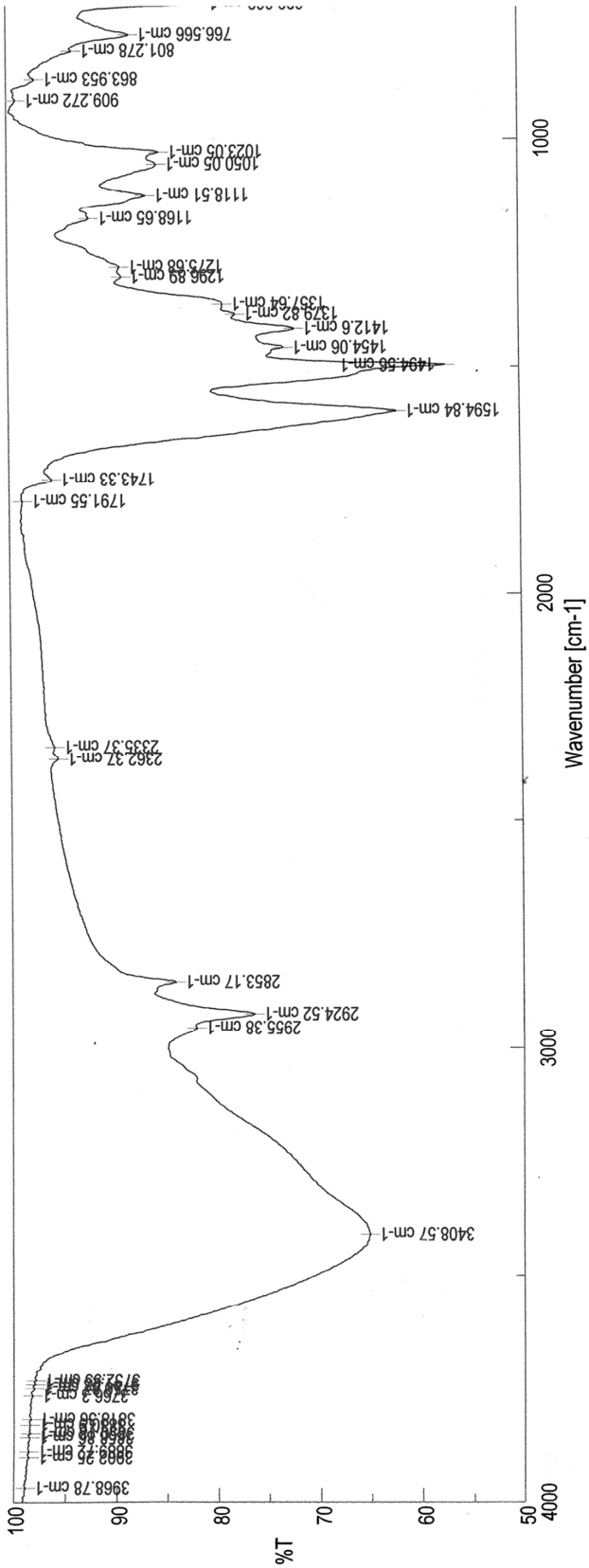


Figure 4.

IR spectrum of Ni(II) complex of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone $[NiL(H_2O)_3]$

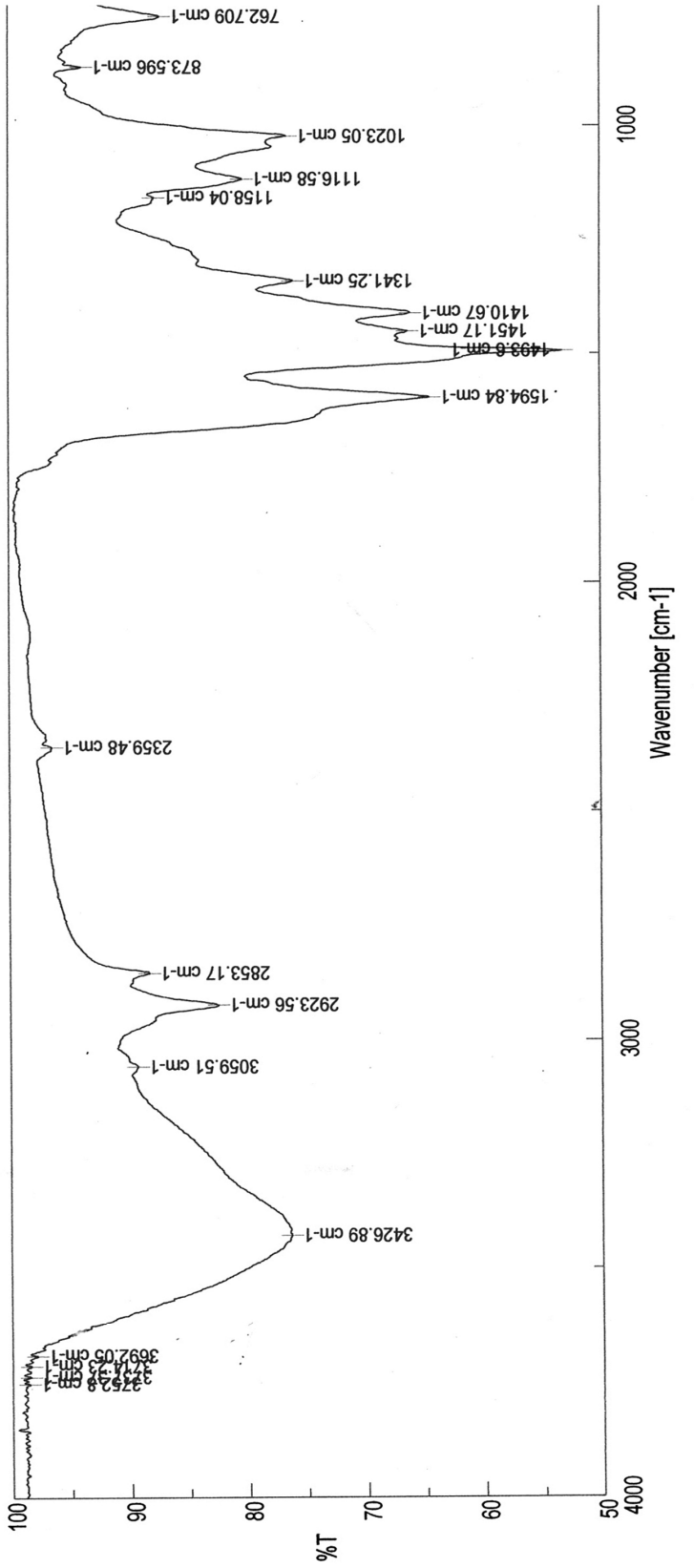


Figure 5.

IR spectrum of Cu(II) complex of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone $[CuL(H_2O)_3]$

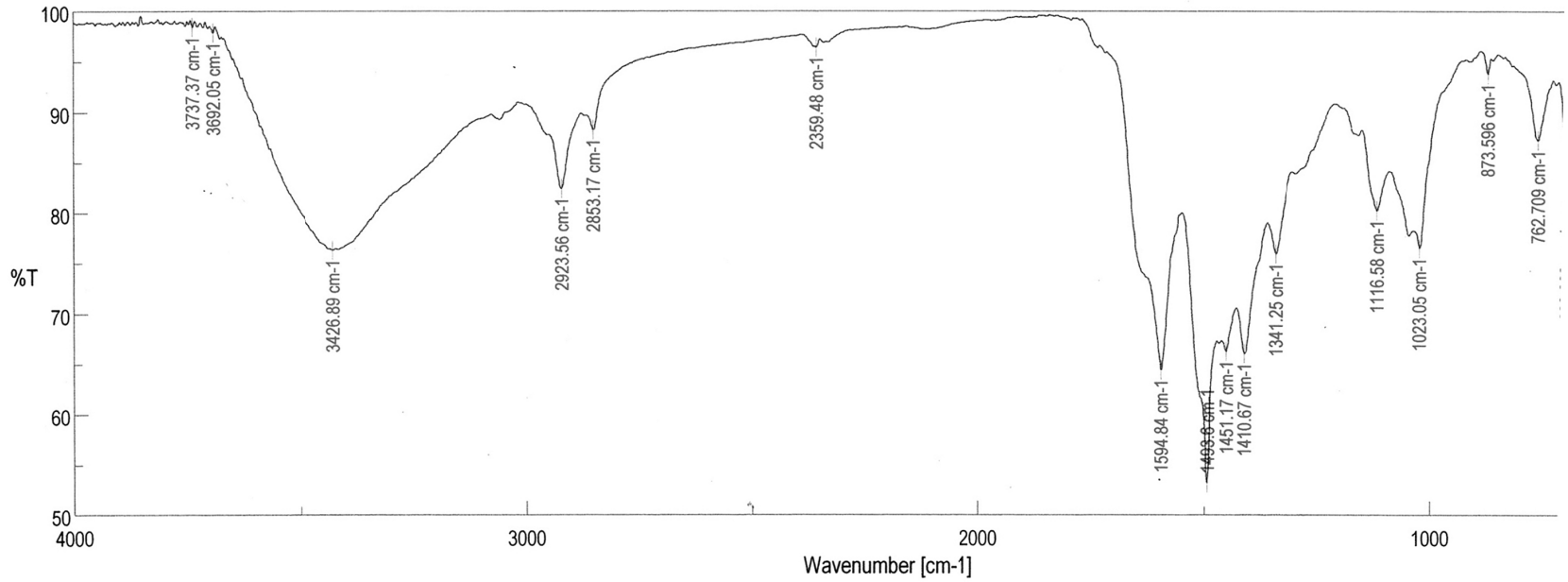


Figure 6.

IR spectrum of Cd(II) complex of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone $[CdL(H_2O)_3]$

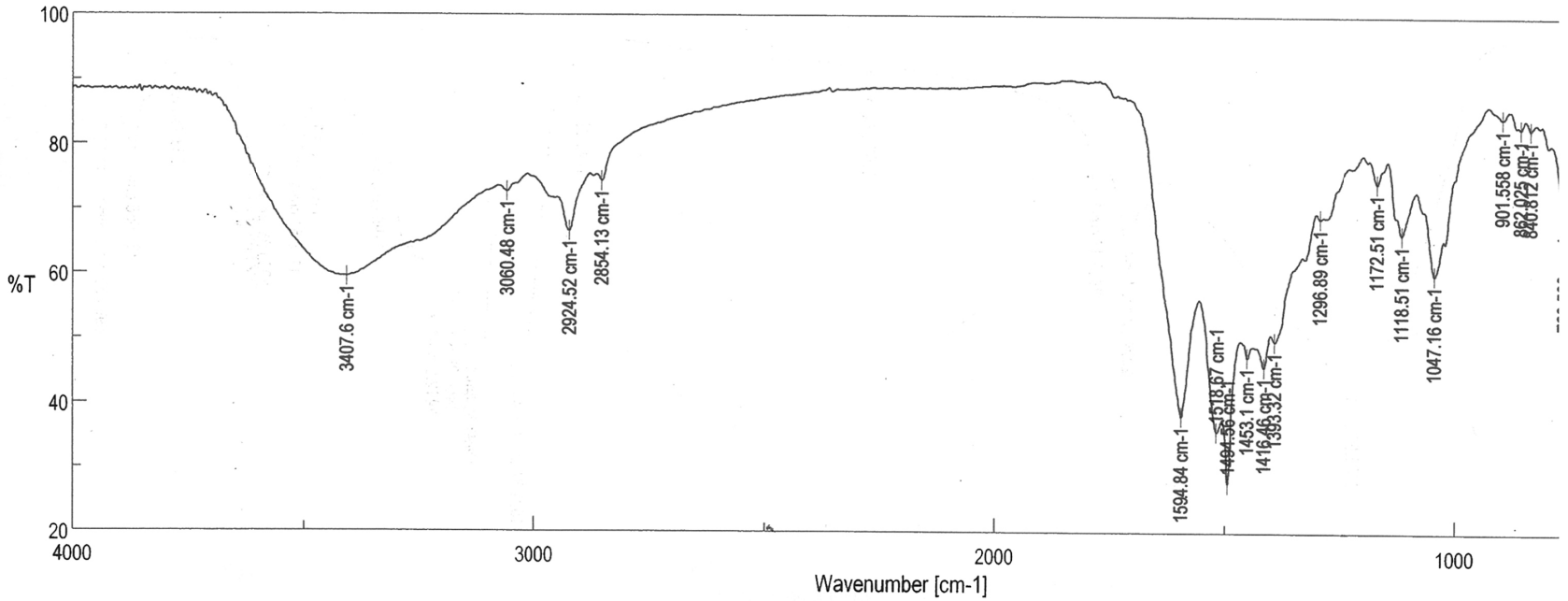


Figure 7.

IR spectrum of Zn(II) complex of N-ethylacetoacetanilideN(4)-methyl(phenyl)thiosemicarbazone $[ZnL(H_2O)_3]$

The strong band at 1607 cm^{-1} in the ligand spectrum may be assigned to $\nu_{(C=O)}$ anilide[6]. In the spectra of all the complexes, this band disappeared and a new band appeared around 1116 cm^{-1}. This may be due to enolization of $–CH_2$-C=O to –CH=C-OH and subsequent coordination through the deprotonated oxygen. In the spectrum of ligand, absence of a band at 2353 cm^{-1} indicated its thioketo nature in the solid state. A strong band at 1051 cm^{-1} in the ligand spectrum may be attributed to $\nu_{(C=S)}$. This band suffered considerable changes during complex formation. In the spectra of all the complexes, this band disappeared and a new band appeared at about 865 cm^{-1} due to $\nu_{(C-S)}$ with some contribution from $\nu_{(C-N)}$. The enolization of =N-NH-C=S to =N-N=C-SH and subsequent coordination through deprotonated sulphur atom might have taken place here.

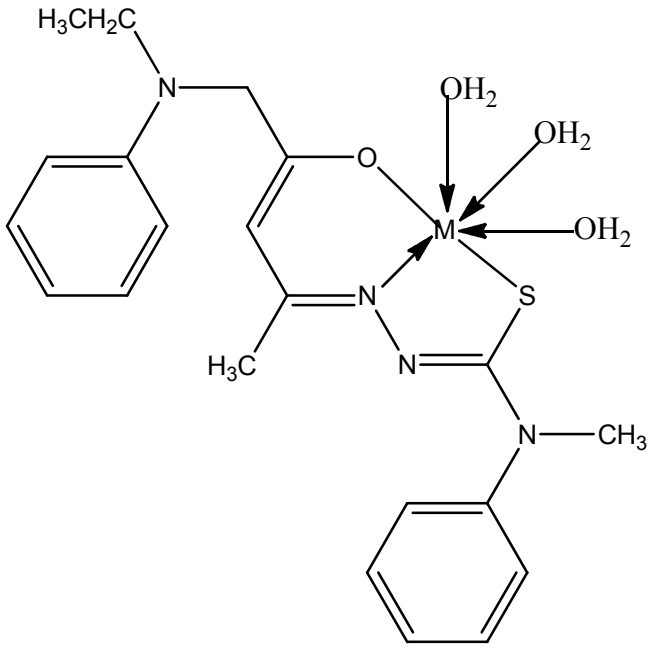
The strong band at 1556 cm^{-1} in the spectrum of ligand may be assigned to $\nu_{(C=N)}$. In the spectra of all the complexes, this band shifted to lower frequency region by a few cm^{-1}, indicating the participation of azomethine nitrogen in coordination.

The band at 1023 cm^{-1} in the spectrum of the ligand may be assigned to $\nu_{(N-N)}$[7]. A shift of this band to a higher frequency region was noticed in the spectra of all the complexes, indicating the participation of azomethine nitrogen in coordination.

In the spectra of all the complexes, broad bands around 3500 cm^{-1} due to ν_{O-H} together with strong bands around 660 cm^{-1} suggest the presence of coordination of water molecules. The other bands in the spectra of the complexes around 550-450 cm^{-1} may be due to ν_{M-N} and ν_{M-O}.

4. Conclusion

Complexes of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) with N-ethyl acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (EAacdMPTCH_2) (L2H) were synthesized and their physico-chemical properties have been studied. On the basis of magnetic moments, electronic – and other spectral data, all the complexes were assigned octahedral geometry with a general molecular formula [ML $(H_2O)_3$], where M = Co(II), Ni(II), Cu(II), Zn(II) or Cd(II), which is given in Figure 8.



Suggested structure of the complex of the formula [ML $(H_2O)_3$],

where M= Co(II), Ni(II), Cu(II), Zn(II) or Cd(II)

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CHAPTER I
CORROSION

1. Introduction

Any process of deterioration and consequent loss of a solid metallic material through an unwanted chemical or electrochemical attack by its environment, starting at its surface is called corrosion. In simple words, corrosion can be defined as the destruction of metals, or alloys by the surrounding, or the environment through chemical or electrochemical changes. The father of corrosion science Ulick R. Evans, the British scientist described corrosion as an electrochemical process, in which the destruction by chemical or electrochemical agencies takes place[1]. Fontana, a famous scientist defined corrosion as the destructions or deterioration of a material because of reaction with its environment[2]. The word corrosion includes not only the destruction of metals alone, but the destruction of non-metallic materials also. The deterioration of paint and rubber by sunlight or chemicals will also come under corrosion in this sense. Corrosion can be fast or slow. Polythionic acid will attack the sensitized steel body in hours. However,the railway track will resist corrosion for a long period.

Corrosion is a spontaneous slow chemical interaction of metal or alloy with its environment, resulting in the formation of one of its compounds such as oxide, hydrated oxide, carbonate, sulphide, sulphate, etc. Extraction

of metals from their ores is an endothermic process. Pure metals, being highly energetic have natural tendency to revert back to their combined metal ores.

$$\text{Metal Ores} \underset{\text{Corrosion}}{\overset{\text{Extraction}}{\rightleftharpoons}} \text{Metal}$$

Thus in metals, corrosion is a process of reverse of extraction. The most familiar examples of corrosion are rusting of iron when exposed to the atmospheric conditions and the formation of green film of basic carbonate on the surface of copper when exposed to moist air containing carbondioxide.

The phenomenon of corrosion is often complicated by the simultaneous occurrence of two or more forms of corrosion. Chemical- or electro chemical reaction, physical factors, such as surface forces often play an important role in corrosion[3].

1.1. The mechanism of corrosion

The mechanism of corrosion involves 3 steps.

i. Metal undergoes oxidation releasing electrons.

$Fe \rightarrow Fe^{+2} + 2e^-$ at the anode region.

ii. At the cathodic region, reduction of O_2 in presence of water to hydroxyl ions takes place

$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$

iii. The metal ions formed at the anode combine with hydroxyl ions formed at the cathode to produce metallic hydroxide , $Fe(OH)_2$, which on further oxidation forms hydrated ferric oxide (rust).

$2Fe^{2+} + 4OH^- \rightarrow 2Fe(OH)_2$

$2Fe(OH)_2 + O_2 + (X-2)H_2O \rightarrow Fe_2O_3.X.H_2O$

1.2. Gravity of corrosion problem

The process of corrosion is slow and occurs only at surface of metals, but the losses incurred, due to corrosion are enormous waste which includes destruction of machines, equipments and different types of metallic products. Losses occurring due to corrosion cannot be measured in terms of the cost of metals alone, but the high cost of fabrication in to equipment, machine tool or structures should also be considered. The severity of the problem may be made clear by the approximate loss of metal due to corrosion, as 2 to 2.5 billion dollars per annum all-over the world. Obviously, we must understand the mechanism of corrosion, if its effects are to be minimized and then only we can avoid corrosion conditions and provide protection against corrosion[4]. Corrosion is one of the most significant problems faced by advanced industrial societies. It is estimated that about 30-40% of iron and steel produced annually is used just to replace the rusted iron materials. Corrosion costs of automobiles, fuel systems, radiators, exhaust systems are in billions. Corrosion affects all appliances used inside and outside home, on the road, in

the sea, in power plants and in aerospace vehicles. It is very surprising to see that the total annual cost of floods, hurricanes, fires, lightning and earthquakes are less than the cost incurred due to corrosion.

1.3. Types of electrochemical corrosion

1.3.1. Galvanic (Differential metal) corrosion

When two dissimilar metals (eg. zinc and copper) are in contact with each other, in a corrosive environment, the metal with low reduction potential (anode) undergoes oxidation (corrosion) and metal with high reduction potential (cathode), undergoes reduction. This type of corrosion is called galvanic corrosion. As the difference between the electrode potentials increases, the corrosion rate increases.

1.3.2. Concentration cell (Differencial aeration) corrosion

This type of corrosion occurs when a metal is exposed to differential air concentrations or oxygen concentrations.

1.3.3. Waterline corrosion

In this type of corrosion, the metal portion just below the waterline is more anodic and undergoes corrosion compared to the portion above the waterline. Ocean going ships, water storage steel tanks and water lifting pipes suffer this type of corrosion.

1.3.4. Crevice corrosion

Crevice corrosion generally occurs in the area with less oxygen. Since oxygen cannot penetrate a crevice (eg. joints, bolts, nuts, washers, etc.) corrosion takes place rapidly.

1.3.5. Pitting corrosion

This type of corrosion arises when a metallic surface is defective (with cracks) or occupied by dust, sand or water drop. This type of corrosion is usually seen in chloride medium.

1.3.6. Stress corrosion (Stress cracking)

The internal stress of metal due to mechanical operations such as pressing, hammering, bending, welding, etc, bring stress corrosion. The metal stressed part becomes more reactive, acts as anode and undergoes corrosion under specific corrosion environments. Cracking of brass and caustic (seasonal cracking) embrittlement of steel boilers are the two examples of stress corrosion.

1.3.7. Intergranular corrosion (Grainboundary corrosion)

This type of corrosion involves the attack of corrosion products on the grains boundaries of a metal, generally observed in alloys. Such corrosion can be observed in stainless steel (18% Cr, 8% Ni) containing more than 0.1% carbon when it is heated to very high temperature and cooled slowly.

1.3.8. Microbiological corrosion

This type of corrosion involves the degradation of materials by fungi, moulds, bacteria or their bye products, bascillus, algae diatoms, etc , as a result of the consumption of oxygen by the bacteria, which results in corrosion.

1.3.9. Soil corrosion

Soil contains moisture, ionic species like chlorides, micro organisms like bacteria which enhance the corrosion, and this comes under soil corrosion.

1.3.10.Erosion corrosion

The corrosion on the surface of a metal due to mechanical wear and tear leads to the relative motion of the corrosive liquid over the metal surface and leads to erosion corrosion. It is commonly observed on copper tubes.

1.4. Factors affecting corrosion

The rate of corrosion is influenced by two factors. Primary factors related to the metal and secondary factors related to the environment.

1.4.1. Primary factors

i. Nature of the metal :- The tendency of a metal to corrode depends on its nature. The metals with low reduction potential will corrode easily

eg. Li, Na, Mg and Zn. The noble metals with high reduction potential will resist corrosion.

ii. Electrode potential difference: - When two metals are in contact, greater is the difference in electrode potentials between the cathode and anode, the faster is the rate of corrosion of the anode.

iii. Surface state of the metal: - Corrosion at the surface of the metal is accelerated in the presence of water droplets or dust particles. If the metal has a tendency to form a passive protective stable film (usually metal oxide), the further corrosion of the metal will be prevented.

1.4.2. Secondary factors

i. pH :- In general, lower the pH of the corrosion medium, higher is the corrosion rate, i.e., acidic media (pH < 7) are more corrosive than alkaline and neutral media. But it is observed that metals like A1, Zn and Pb undergo fast corrosion in highly alkaline medium.

ii. Temperature :- With the increase of temperature, corrosion rate is enhanced. This is because, increases in temperature, increases the conductance of the medium, reduces passivity of the metal and increases the rate of corrosion.

iii. Humidity :- The rate of corrosion increases with the increase in humidity up to a certain value called critical humidity.

iv. Relative areas of anode and cathode parts :- If the ratio of anode to cathode region is small, i.e., smaller anode region and larger cathode region, the overall corrosion will be more effective. The corrosion will be less intense if the anodic area is larger compared to cathodic region.

1.5. Protection against corrosion

Corrosion control is more realistic than corrosion prevention. Some of the corrosion control methods are mentioned here.

1.5.1. Design and material selection

The materials with greater number of angles, corners, edges, etc., should be avoided since it will be difficult for efficient surface treatment. Metal corrosion rate can be reduced by the use of a single metal than the dissimilar metals. If two dissimilar metals are in contact, the area of the anode should be larger than the area of the cathode.

1.5.2. Protective coatings

Protecting the surface of an object by the application of coating is the oldest of the common procedures for corrosion prevention. Protective coatings include metallic coatings, chemical conversion coatings and organic coatings.

1.5.3. Cathodic protection

The principle involved in this method is that the elimination of anode site and conversion of the entire metal into cathodic site by providing electrons from the external source, so that the specimen always remains cathode.

1.5.4. Anodic protection

Certain metals like, Ti, Ni and Cr will become passive, by the application of suitable anodic current. Few metals and alloys like steel exhibits passivity by forming their oxide layers. Thus the prevention of corrosion by impressed anodic current is called anodic protection.

1.6. Corrosion inhibitors

There are the substances which minimize the corrosion by retarding either anodic or cathodic reactions. Thus a corrosion inhibitor can be defined as a substances which when added in small quantities to the aqueous corrosive environment, decreases the corrosion rate of the metal. There are different type of inhibitors.

i. Anodic inhibitors :- Chromates, phosphates, tungstates and other anions with high oxygen content will form sparingly soluble compounds with newly formed metal ions at the anode, and will get

adsorbed on the metal surface forming a protective layer and reduces the corrosion rate.

ii. Cathodic inhibitors :- In this, the corrosion is reduced by slowing down the diffusion of H^+ ions to the cathode by the organic inhibitors like amines, heterocyclic nitrogen compounds, substituted thiourea, etc., which are capable of being adsorbed at the metal surface. The adherent deposit film at the cathode increases the hydrogen over voltage and there by decreases the corrosion.

iii. Mixed inhibitors :- Substances which affect both cathodic and anodic reaction are called mixed inhibitors. They are typically film forming compounds that cause the formation of precipitate on the surface, blocking both cathodic and anodic sites indirectly. The most common inhibitors of this category are the silicates and phosphates.

iv. Green inhibitors :- They are a special class of inhibitors important due to their non-toxic and environment friendly nature. They do not contain heavy metals. The extract of the leaves of Aquilaria crassna is a very good green inhibitor.

1.6.1. Limitations of inhibitors

Eventhough inhibitors reduces the corrosion of metals in many environments, there are some limitations for this type of corrosion prevention.

i. The addition of inhibitor to the corrosive system may contaminate the environment.

ii. Some inhibitors are toxic and one cannot use it properly (Arsenic salts)

iii. Certain inhibitors will lose their ability as the concentration and the temperature of the environment increases.

1.7. Schiff bases as corrosion inhibitors

Among the organic compounds[5,6], Schiff bases, the condensation products of amines and ketones or aldehydes have attracted immense attention in the field of corrosion inhibition science[7-12]. This is because, they can easily be synthesized from inexpensive starting materials. Their ecofriendly properties also contribute to this. Schiff bases posses many coordinating centres, which enhance their inhibitory properties[13]. Due to their synergistic effect, they act like a bridge between the inhibitor cation and the metal surface in acid solutions[14,15]. The solubility of Schiff bases in acids is a main factor for their selection as industrially suitable inhibitors. The presence of –N=CH (imine) group in the Schiff bases are very helpful in studying the mechanism of the reactions in chemical systems. The main point behind the corrosion inhibition nature of Schiff base is the presence of π electrons in triple bonds or conjugated double bonds and the electronegative functional groups. The literature review mentioned here will reveal the importance of Schiff bases as effective corrosion inhibitors.

Sorkhabi et al[16] determined the inhibition efficiency of pyramidine Schiff bases towards mild steel in 1M HCl medium by weightloss- and polarization methods. These studies showed that the compounds are good corrosion inhibitors even at low concentrations. The adsorption followed Langmuir isotherm. The corrosion inhibition studies of Schiff base compounds (derived from diamines and o-hydroxy- and o-methoxy aromatic aldehydes) with stainless steel in tap water and HCl showed that they possess 93% inhibition efficiency. The adsorption obeyed Langmuir isotherm[17]. Emregal and Atakol[18] observed the inhibition property of N-(2-hydroxyphenyl) salicylaldimine on iron in 1M HCl using weight-loss, polarization and EIS technique and they obtained positive results. The corrosive nature of zinc in HCl solution containing glutaraldehyde and methionine showed 92.5% inhibition efficiency. It was a cathodic inhibitor which followed Tempkim adsorption isotherm[19].

The capacity of Schiff base compounds to decrease the corrosion rate was confirmed by the studies of Li and co-workers[20]. Using polarization and EIS techniques, they showed that N,N^{I}-o-phenylen-bis (3-methoxysalicylidenimine) is a good corrosion inhibitor on copper in 1M HCl. Conversion of an amine to Schiff base will improve the inhibitory action. This was confirmed by Desai *et al*[21], based on their studies of Schiff bases derived from benzaldehyde and aromatic- and aliphatic primary amines on aluminium alloy steel in HCl. The presence of electronegative atoms like chlorine and

bromine as para substituents on phenol ring will increase the inhibition capacity. The studies of Schiff base, like 5-bromosalicylaldehyde-etansulphonylhydrazone and 5-chlorosalicylaldehydeetansulphonylhydrazine on AA 3102 Aluminium in 1M HCl by EIS technique and hydrogen evolution test, conducted by Aytac *et al*[22] proved this. Bansiwal *et al*[23] carried out weight loss and thermometric studies to evaluate the inhibition effects of different Schiff bases such as 2-anisalidine- pyridine, 2-anisalidenepyrimidine on the corrosion of aluminium in HCl solution and showed that they are efficient corrosion inhibitors.

1.8. Thiosemicarbazones as corrosion inhibitors

The corrosion inhibition property of thiosemicarbazones and their derivatives was studied by many scientists[24-30]. They are considered as efficient inhibitors of corrosion, because they contain good π-electron conjugation increasing its coordination and an abundance of heteroatoms increasing their adsorption on to the surface of the metal[31]. These compounds will get adsorbed on the metal surface through the lone pair of electrons on nitrogen and sulphur atoms and also through the π-electrons in the molecules[32-35.]

Thiosemicarbazones of pyrazole carboxaldehyde derivatives were prepared and their corrosion inhibition efficiencies were studied using carbon steel in HCl and observed that they were mixed type inhibitors. Their

inhibition efficiencies increases with increasing inhibitor concentration and temperature[36]. The corrosion inhibition of furoin thiosemicarbazone towards mild steel in l M HCl solution was studied using weight loss, Tafel polarization and electrochemical impedance spectroscopy techniques and observed that it is an efficient corrosion inhibitor even at low concentration[37]. U.J. Ekpe *et al*[38] showed that 2-acetylpyridine 4-phenylthiosemicarbazone and 2-acetylpyridine 4-methylthiosemicarbazone exhibited maximum inhibition efficiency on the corrosion of mild steel in HCl. Inhibition effect of 3-pyridinecarboxaldehydethiosemicarbazone on the corrosion of mild steel in l M HCl was studied using weight loss, potentiodynamic polarization and electrochemical impedance spectroscopy. The results showed that it is an effective inhibitor for mild steel. It followed Langmuir adsorption isotherm and acted as a mixed type of inhibitor.

2. Scope of the present investigation

Mild steel is an important raw material used in petroleum - and power generation. Hydrochloric acid solution is commonly used for pickling, acid cleaning and for other applications. The systems, instruments or vessels will get damaged due to the corrosive nature of hydrochloric acid. Mild steel is used in the gas - and oil industries because of its resistance against corrosion. But unfortunately, it will easily undergo pitting corrosion in acid media, especially in HCl. Thus, it has become a challenge to find out methods to

provide longer life time to the metallic instruments. The use of corrosion inhibitors is considered as the most effective method for the protection of mild steel in HCl. It is observed that the adsorption of inhibitors takes place through hetero atom such as nitrogen, oxygen, phosphorus, sulphur[39], multiple bonds or aromatic rings. The order of inhibition efficiency is O < N < S < P. Sulphur compounds showed great affinity for transition metal and will get coordinated to the metal surface easily[40]. The aim of this work is to evaluate the inhibitory action of two thiosemicarbazones (i.e., acetylacetone N(4)-methyl(phenyl)thiosemicarbazone and ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone on mild steel in lM HCl at room temperature by weight loss measurements, electrochemical analysis, adsorption studies and surface morphological studies.

CHAPTER II

MATERIALS, METHODS AND INSTRUMENTS

1. Introduction

In the present work the corrosion inhibition efficiency of thiosemicarbazones on mild steel in l M HCl was evaluated by four methods. They are, weight loss measurements, electrochemical analysis, adsorption studies and surface morphological studies. The electrochemical methods include potentiodynamic polarization- and electrochemical impedance spectroscopic techniques. The weight loss coupon method is considered as a classical corrosion test method, which involves the determination of weight loss of metal coupons exposed to the corrosive environment using laboratory immersion corrosion testing method. This is a traditional and most widely used corrosion inhibition testing method. The potentiodynamic polarization method is based on the idea that the electrochemical corrosion is a activation controlled process. In such process, an activation controlled reaction is involved for which the rate of reaction is controlled by the rate of electrochemical charge transfer process on the metal surface. This type of analysis is also referred as Tafel slope analysis. The percentage of inhibition efficiency is calculated using the corrosion current densities. Electrochemical independence spectroscopy is a powerful technique for the characterization of

electrochemical systems and provides a wealth of kinetic- and mechanistic informations. Adsorption studies and surface morphological studies are considered as the important methods for studying the metal - inhibitor interactions.

2. Materials

2.1. Mild steel

Mild steel is an iron-carbon alloy containing less than 0.25 percent carbon and is comparatively mild. Due to its ferromagnetic properties, a large number of electrical devices are manufactured using this. Mild steel having 99.22% Fe, 0.019% Mn, 0.28% Ni and 0.30% carbon as determined by the chemical analysis (by X-ray fluorescene spectrophotometer) with size 48 mm × 19 mm × 1 mm were used for the weight loss measurements. The same pieces with 1 cm^2 area were used in electrochemical studies. The specimen pieces were polished with different grade emery papers to mirror polish. These coupons were accurately weighed using electronic balance after washing with methanol, acetone and distilled water and drying. ASTM standard G1- 72 procedures, were followed. The observations were recorded at the room temperature[41-43].

2.2. Inhibitor solution

Thiosemicarbazones were prepared by the methods described in the Part 1. Solutions of different concentration of inhibitors were prepared by dissolving the required amount in l M HCl at room temperature. 80 ml of l M HCl without inhibitor was used as blank test solution.

2.3. Medium

The medium for the study was prepared from reagent grade HCl (E. Merck) and doubly distilled water. The inhibitor was dissolved in l M HCl and the experiments were conducted under standard conditions and at room temperature.

3. Methods

The following methods were adopted for ascertaining the corrosion inhibition efficiency.

3.1. Weight loss method

This is a conventional and widely used method of determination of corrosion inhibition. This involves the calculation of weight loss of metal specimens exposed to corrosive environment using laboratory immersion corrosion testing method for *in situ* corrosion monitoring[44,45]. The basic principle of this method is that from the weight loss of metal coupons, measured at regular time intervals exposed in acid solution, we can calculate

the corrosion rate and the corrosion inhibition efficiency. The calculation of weight loss and corrosion rate of mild steel specimens in HCl is based on the standard procedure for laboratory immersion corrosion testing published in 1990 by American Society for Testing Materials (ASTM) G 3-72 [46,47].

For the weight loss measurements, mild steel specimen coupons with a very a small hole at one end for the passage of thread is used. After weighing accurately, the specimens were immersed in 100 ml beaker which contained 80 ml l M HCl with- and without the addition of different concentrations of inhibitors at room temperature (30°C). All the acid solutions were kept open to air. They were exposed for a period of 96 h and measurements were taken at an interval of 24 h. After 24 h, the specimens were taken out, washed with acetone and water, dried and weighed accurately using an analytical balance. The weight losses of the specimens were recorded up to a period of 96 h at an interval of 24 h.

The percentage corrosion inhibition efficiency, η% was calculated using the relation,

$$\eta\% = \frac{W_u - W_i}{W_u} \times 100$$

where W_u and W_i are the weight loss of metal in the uninhibited solution and the inhibited solution , respectively.

The average corrosion rates of the coupons in various environments were determined by using the relation[48-50],

$$\text{Corrosion rate (mpy)} = \frac{534W}{DAT}$$

where W is the weight loss is milligram, D is the density of the metal in mg/cm^3, A is the area of coupon in inches, T is the exposure time in hours and mpy is milliinches of corrosion per year. The proportionality constant, 534 varies depending on the units required for the corrosion rate. Surface coverage of the inhibitor can also be obtained from weight loss method. The surface covered by the inhibitor, θ is obtained by the relation,

$$\theta = \frac{\eta_o - \eta_i}{\eta_o}$$

were η_o and η_i are the corrosion rates of coupons without- and with inhibitor, respectively.

3.2. Electrochemical studies

The corrosion is an electrochemical phenomenon. So the mechanism of corrosion can be studied by employing the electro chemical methods which are fast and accurate. They are used for the evaluation of corrosion inhibitor efficiency[51]. There are various methods in the electrochemical analysis, like,

potentiodynamic polarization, galvanodynamic- and potentiokinetic measurements and cyclic polarization. For the present investigation, the common electrochemical techniques, potentiodynamic polarization and electrochemical impedance spectroscopy were used. The instrument is shown in Figure 1. Electrochemical tests were carried out in a three electrode cell with metal specimen as the working electrode, platinum sheet (1 cm^2 surface area) as auxiliary electrode and the saturated calomel electrode as the reference electrode. At first the working electrode is kept in the test solution. Then a steady state open circuit potential will be developed. A Gill AC computer controlled electrochemical work station (model No. 1475, ACM, UK) was used for the measurements. Potentiodynamic polarization curves (Tafet plots) were obtained in the potential range of -250 mV to +250 mV at a sweep rate of 1 mV/s. The electrochemical impedance spectroscopic (EIS) measurements were carried out with an amplitude of 10 mV alternating current.

3.2.1. Electrochemical impedance spectroscopy (EIS)

The electrochemical Impedance spectroscopy involves the measurement of a circuit's tendency to resist (or impede) the flow of an alternating electric current. The real - and imaginary components of the impedance response of the system represent the resistance and capacitance. In this method, we are applying a sinusoidal alternating potential signal to the

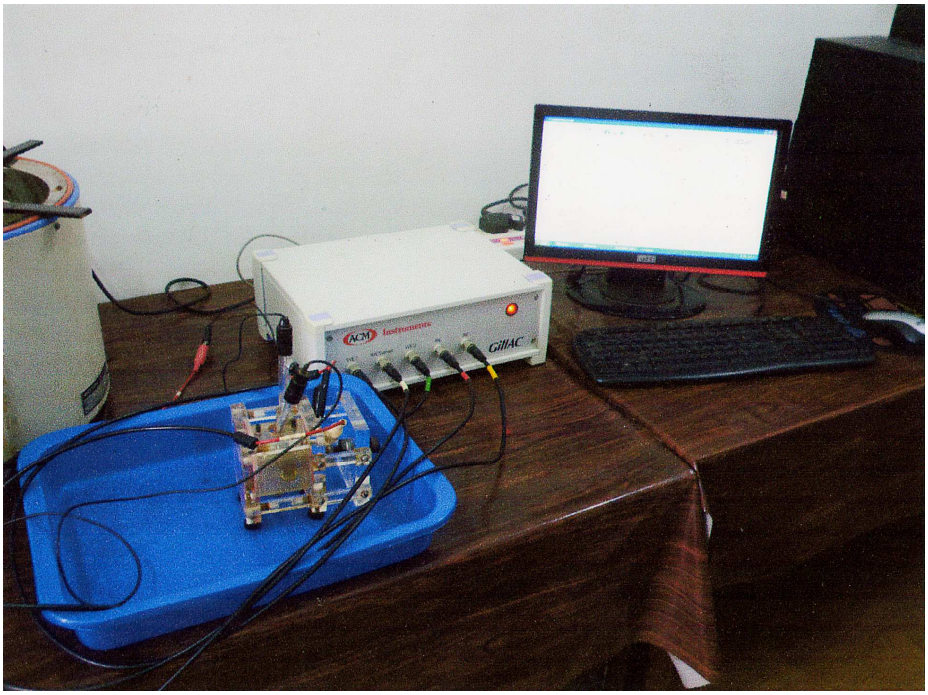
test system with a range of frequency and a frequency dependent impedance Z (ω) will be obtained [52]as ,

$$Z(\omega) = \frac{V(t)}{I(t)} = \frac{V_0 \sin \omega t}{I_0 \sin(\omega t+\theta)}$$

where ω is the frequency; t is the time; $V_{(t)} = V_o \sin \omega t$ sinusoidal alternating potential signal, $I(t) = I_o \sin(\omega t + \theta)$ is the time dependent current response and θ is the phase angle between $V_{(t)}$ and $I_{(t)}$. Impedance is given by a complex number with a real component $Z^1(\omega)$ and an imaginary component $Z^{11}(\omega)$ as given in the equation,

$$Z(\omega) = Z^1(\omega) + jZ^{11}(\omega)$$

where $j = \sqrt{-1}$.



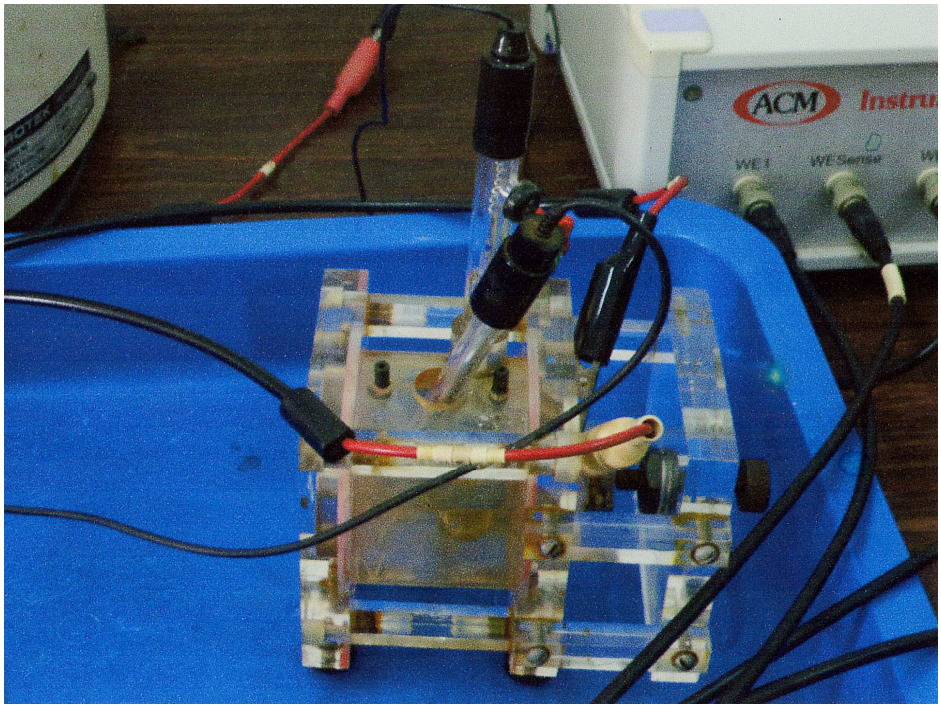


Figure 1.

Gill A.C., computer controlled electro chemical work station

(Model No. 1475, ACM, UK)

The experimental arrangements for the impedance measurement consist of an electrochemical cell (the system under study), a potentiostat and a frequency response analyzer. A very small voltage (5 to 10 mV) is applied between the specimen and a reference electrode. The impedance of the system is determined by the analysis of sine wave by the analyzer. The plot with Z^{1} (ω) against Z^{11} (ω), called Nyquist plot (cole-cole plot) is obtained by applying different frequencies. The plot is obtained as a semi circle in the complex plane, which intercepts the real axis twice. In real case , Nyquist plots are depressed semicircles due to the roughness and other non - homogeneities of the metal surface (dispersing effect). (R_Ω is the uncompensated resistance between the working electrode and reference electrode (solution resistance), R_p is the polarization resistance at the metal-solution interface. At high frequency, the impedance of the system is due to ohmic resistance, R_Ω and at maximum frequency; the semicircle touches left most end of the semicircle searching the X axis. The right end of the semicircle corresponds to the lower frequency). The corrosion inhibition efficiency is calculated by the relation,

$$IE\ \% = \frac{R_P^{1} - R_p}{R_p^{1}} \times 100$$

where R_p and R_p^{1} denote polarization resistance of electrode with and without inhibitor, respectively. Shapes of impedance plots are same for both the inhibited and uninhibited electrodes, even though the presence of inhibitor

increases the impedance. This study shows depressed capacitive loop at high frequency range (HF) whose diameter increases in inhibitor concentration followed by an inductive loop that is observed at low frequency (LF) region. The depressed capacitive loop with centre below the real axis may be due to the contribution from surface roughness, distribution of active sites, adsorption of inhibitior and formation of porous layers. The HF capacitive loop can be attributed to charge transfer reaction. By analyzing Nyquist plot, the transfer resistance, R_{ct} and double layer capacitance, C_{dl} were determined. The inhibition efficiency at different concentrations can be calculated from the charge transfer resistance using the following equation,

$$IE\% = \left(\frac{R_{ct}^{*} - R_{ct}}{R_{ct}^{*}} \right) \times 100$$

where R_{ct}^{*} and R_{ct} are the charge transfer resistance in the presence and absence of inhibitor. The values of R_{ct} increase with the increase in inhibitor concentration and this indicates that charge transfer process mainly controls the corrosion process. The value of C_{dl} decreases due to adsorption of inhibitor molecules, which displaces water molecules originally adsorbed on the metal surface and decreases the active surface area. The decrease in the values of double layer capacitance with increase in inhibitor concentration indicates that inhibitor molecules act by adsorption at the metal-solution interface, leading to the formation of a protective film on the metal surface

and decreasing the extent of dissolution reaction. This is also helpful in the study of rate inhibitor performance, and passive layer characteristics[53-59].

3.2.2. Potentiodynamic polarization method

In potentiodynamic polarization measurements, the current- potential curves were recorded by polarizing the specimen. The mixed potential theory provides the basis for the determination of corrosion rate by Tafel extrapolation. It starts at a potential of about -250 mV cathodically and +250 mV anodically with respect to open circuit potential at a scan rate of 1mV/s. The electrode potential is plotted against the logarithm of current density and this is called Tafel plot. The line describing the behavior in the Tafel region can be extrapolated to the corrosion potential to determine the corrosion rate E_{corr}. The extrapolation of these regions to the reversible potential of oxidation and reduction reactions provides a measure of the exchange current density of the reaction. In this method, when the metal is kept in an acid solution, the cathodic and anodic processes takes place, which depends on the rate of electron transfer reaction at the metal surface. The rate of oxidation-reduction reaction which is responsible for E_{corr} is expressed in terms of their electron flow per unit time or current. For a system at E_{corr}, the oxidation reaction occurs at a rate equal to that of the reduction reaction, i.e., if $i_{ox} = i_{red}$, the current flowing through the specimen will be zero. But in order to measure the current for getting the corrosion rate, a potential other than E_{corr} is

applied which leads to the polarization of metal - solution interface. As a result, either oxidation or reduction reaction will be accelerated which will increase the current flow. The value of i_{ox} or i_{red} is extrapolated to get the corrosion current, i_{corr}. If E_{corr} is accelerating the oxidation reaction, it is called anodic current and if E_{corr} is accelerating the reduction reaction it is called cathodic current.

The percentage inhibition efficiency is calculated using the corrosion current densities[60,61.]

$$I\,E\,\% = \frac{I_{corr}^{*} - I_{corr}}{I_{corr}^{*}} \times 100$$

where I_{corr} and I_{corr}^{*} are uninhibited and inhibited corrosion densities, respectively. Tafel equation[62,63] represents the anodic and cathodic polarization of metal surface.

$$\eta = \beta \log \frac{i}{i_{corr}}$$

where η is the over voltage (difference between the potential of the metal and its corrosion potential), β is Tafel constant, i_{corr} is the corrosion current and i is the current at over voltage η. On rearranging the equation we get,

$$\eta = \beta (\log i - \log i_{corr})$$

A plot of η against log I will give a straight line with a slope β, the Tafel constant. β_a and β_c represent the anodic- and cathodic portions of the Tafel plot, respectively. On extrapolation, these linear portions will intersect at a point. The current at this intersection point, i_{corr} (current density) is proportional to the corrosion rate. The slopes of anodic - or cathodic polarization curves decide whether the inhibitor is anodic, cathodic or both (mixed).

3.3. Adsorption studies

The mechanism of effective corrosion inhibition can be explained by the adsorption of corrosion inhibitors on metal surface which is determined by their chemical structure, nature and surface charge of the metal and nature of the media[64,65]. It is believed that the inhibitors form a protective film on the metal surface through the adsorption and separate the metal surface from the corrosive medium. The adsorption process can be considered as the substitution process between the organic inhibitor in the aqueous solution (Inh_{sol}) and water adsorbed at the metal surface (H_2O_{ads})[66].

$$Inh_{sol} + X\ H_2O_{(ads)} \longleftrightarrow Inh_{(ads)} + H_2O_{(sol)}$$

where X represents the number of water molecules replaced by one molecule of adsorbed inhibitor. The relationship between the amount of substance adsorbed in a liquid or gas phase and the concentration at constant

temperature is known as adsorption isotherm. The interaction between the inhibitor and surface of the metal can be explained by the adsorption isotherm. There are several types of adsorption isotherms, but most commonly used ones in connection with the metal inhibitor interaction are Langmuir-, Freundlich- and Tempkin isotherms. Tempkin adsorption isotherm is regarded as the best one for providing information regarding the behavior of Schiff's bases[67]. The relationship connecting the surface coverage and concentration of inhibitor is given by the following equations.

For the Langmuir isotherm,

$$\theta = \frac{K_{ads}C}{1 + K_{ads}C}$$

ie, $C / \theta = 1/ K_{ads} + C$

where θ is the degree of surface coverage, C is the molar inhibitor concentration in the bulk solution and K is the equilibrium constant of the adsorption process

For the Freundlich,

$$\theta = K_{ads}\, C^{n}$$

where n is a positive integer.

For Temkin,

$$KC = e^{-2a\theta}$$

Where ‘a’ is molecular interaction parameter.

The plot for Langmmir isotherm is log $\frac{\theta}{1-\theta}$ versus log C and that for Tempkin isotherm is θ versus log C. The values are plotted and the equation which gives a straight line will be the suitable isotherm for the particular inhibitor. Using the percentage inhibition efficiency obtained from weight loss method, θ can be calculated using the following relation.

$$\theta = \frac{\text{Percentage inhibition efficiency}}{100}$$

The thermodynamic parameter can be calculated as follows:

The K_{ads} is given by the relation,

$$K_{ads} \quad \frac{\theta}{(1-\theta)C_{inh}}$$

where θ is the degree of coverage on the metal surface and C_{inh} the concentration of inhibitor in mol L^{-1}. We can calculate the standard free energy of adsorption using the relation,

$$\Delta G°_{ads} = RTln(55.5K_{ads})$$

where R is the universal gas constant, T is the temperature in absolute scale, K is the equilibrium constant for adsorption process and 55.5 is the concentration of water in the solution expressed in Moles.

3.4. Surface morphological studies (SEM)

The surface morphology of the samples under study in the absence - and presence of inhibitors was investigated using the instrument, JEOL JSM – 5600 LV, Scanning Electron microscope. All micrographs of the specimens were taken at a magnification of 50 μm.

CHAPTER III

CORROSION INHIBITION STUDIES OF ACETYLACETONE N(4)-METHYL(PHENYL)-THIOSEMICARBAZONE ON MILD STEEL IN 1M HYDROCHLORIC ACID

1. Introduction

Steel is the most important engineering and construction material in the world. Acid solutions are widely used in industry, especially hydrochloric acid. There are a number of situations in which steel comes in contact with hydrochloric acid and the corrosive attack of the acid on steel causes a lot of problems. Therefore, the development of corrosion inhibitors of steel in acid solution has become a subject of great interest. Literature survey revealed that Schiff bases and thiosemicarbazones are very good corrosion inhibitors for steel in acid, particularly in hydrochloric acid. With this view, attempt has been made to synthesize new thiosemicarbazones which can be used as corrosion inhibitors. The present work describes the study of corrosion inhibition action of acetylacetone N(4)-methyl(phenyl)thiosemicarbazone ($AacMPTCH_2$) on mild steel in 1 M HCl solutions by different methods like weight loss, potentiodynamic polarization, electrochemical impedance spectroscopy (EIS), adsorption and scanning electron microscopy (SEM). The selection of the compound is based on molecular structure considerations. Existence of N, S and O atoms, aromatic rings and pi-electrons[68,69] on

AacMPTCH$_2$ is expected to enhances its interaction on the mild steel surface. The structure (Figure 1) and name of the compound are given below.

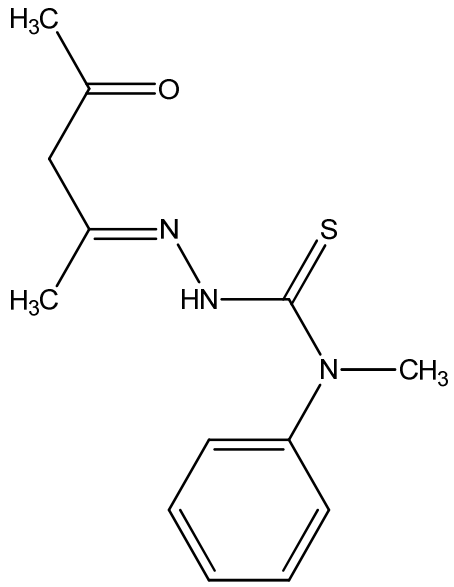


Figure 1.

Acetylacetone N(4)-methyl(phenyl)thiosemicarbazone (AacMPTCH$_2$)(L2H)

2. Experiments

The results obtained from weight loss experiments, electrochemical analysis and SEM analysis were used to find the corrosion inhibition efficiencies of AacMPTCH$_2$. The results are presented in this chapter.

3. Results and discussion

3.1. Weight loss measurements

The inhibition efficiency, the surface coverage and the corrosion rate (mpy) under different intervals of time (24, 48, 72, 96 h) in the absence- and

presence of different concentrations (50 ppm, 100 ppm and 150 ppm) of AacMPTCH$_2$ as inhibitor are given in Table 3.1. The results showed that the inhibition efficiency is high in a time interval 24 h. Corrosion inhibition efficiency of AacMPTCH$_2$ increases with inhibitor concentration at different time intervals. Here an efficiency of 96.5% was obtained at lower concentrations of 50 ppm and a maximum efficiency of 97.9% was obtained at a concentration of 150 ppm after 24 h. It was also observed that the inhibition efficiency of AacMPTCH$_2$ did not further increase after 150 ppm. i.e. 150 ppm is optimum concentration for AacMPTCH$_2$. The surface coverage θ was increasing with increasing inhibitor concentration. But as the immersion time increases θ values decreases. The corrosion rate was found to decrease with increase in the concentration of inhibitor molecule added. Table 3.1 clearly indicates these trends. Figures 2, 3 and 4 show the variation of corrosion rate with concentration of ligand, variation of inhibition efficiency with concentration of ligand and the variation of inhibition efficiency with time, respectively.

Table 3.1. Variation of inhibition efficiency and surface coverage of AacMPTCH$_2$ and corrosion rate of mild steel with concentration of inhibitor added and time of immersion.

AacMPTCH$_2$	**Conc. (ppm)**	**Time in hours**			
		24	**48**	**72**	**96**
Inhibition efficiency(η%)	50	96.50	92.40	87.70	85.00
	100	97.10	93.10	91.30	89.90
	150	97.90	96.00	93.90	91.90
Corro. rate (mpy)	0	1058.32	539.80	191.80	280.80
	50	36.70	40.09	44.96	41.97
	100	30.60	36.80	31.49	28.21
	150	22.00	20.98	21.99	22.49
Surface coverage (θ)	50	0.965	0.924	0.877	0.850
	100	0.971	0.931	0.913	0.899
	150	0.979	0.960	0.939	0.919

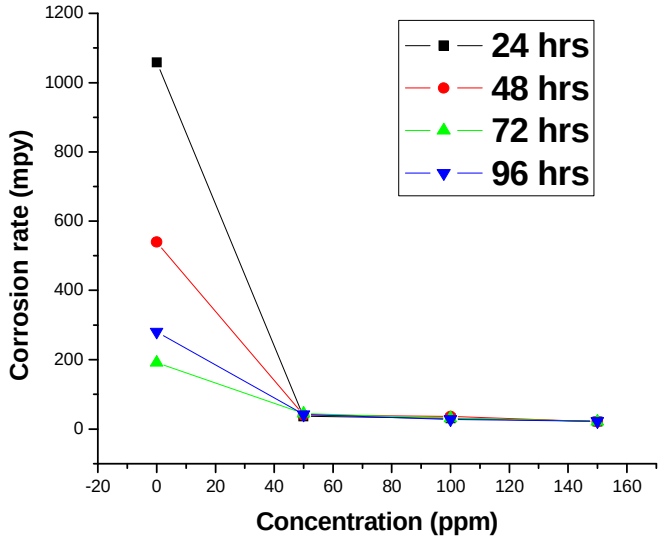


Figure 2.

Variation of corrosion rate with concentration of ligand AacMPTCH$_2$

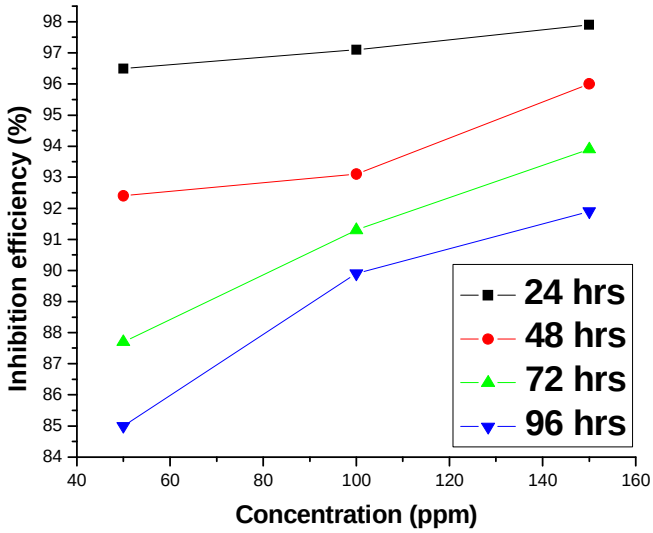


Figure 3.

Variation of inhibition efficiency with concentration of ligand AacMPTCH$_2$

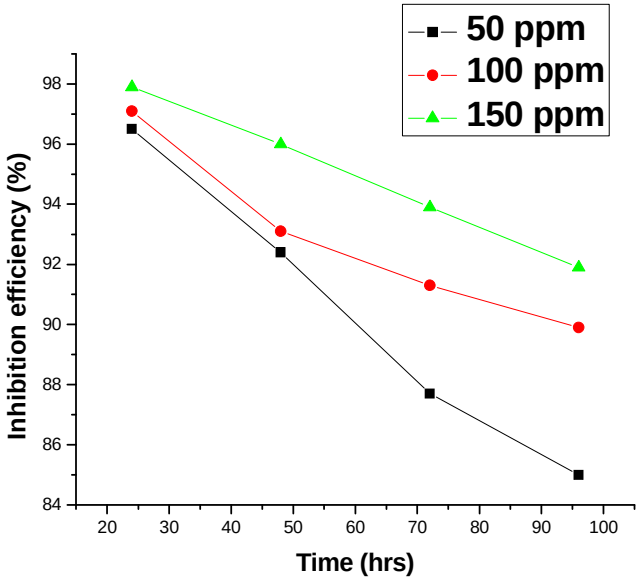


Figure 4.

Variation of inhibition efficiency with time of immersion for ligand AacMPTCH$_2$

3.2. Electrochemical analysis

3.2.1. Electrochemical impedance spectroscopic method

Nyquist plot and the impedance parameters derived from it in the presence of AacMPTCH$_2$ are given in Figure. 5 and Table 3.2.1. .

The results of electrochemical studies indicate that the values of charge- transfer resistance (R_{ct}) increases and corrosion rate decreases by increasing the inhibitor concentration. The values of corrosion rate in electrochemical studies are in good agreement with those obtained in weight loss method. But the values vary in different methods due to the difference in the immersion time in each method. However, there is consistency in the trends.

Table 3.2.1. The R_{ctt} (Ohm cm^2), C_{dl} (F), I_{corr}(mA/cm^2) and corrosion rate (mm/year) for the mild steel in 1M HCl without and with different concentration of AacMPTCH$_2$

Conc. (ppm)	**Rct (Ohm cm^2)**	**C_{dl}(F)**	**I_{corr} (mA/cm^2)**	**Corr. rate (mm/year)**	**η%**
0	11.28	1.454×10^{-4}	2.313	26.800	--
50	1427	1.766×10^{-5}	0.01828	0.2119	99.2
100	2266	3.451×10^{-5}	0.01151	0.1334	99.5
150	3296	1.878×10^{-5}	0.007915	0.09173	99.7

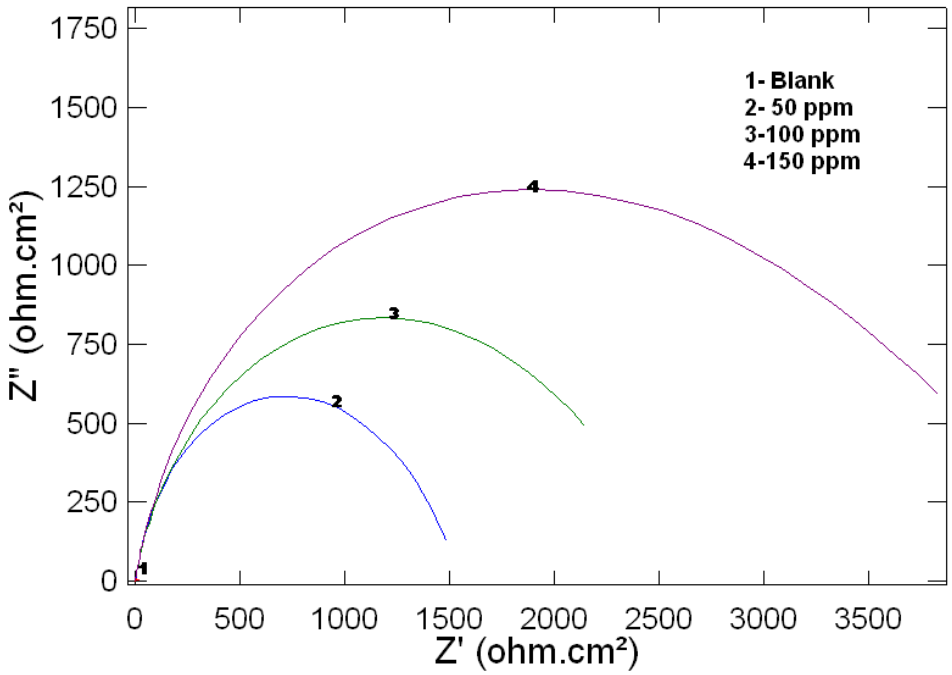


Figure 5.

EIS plot for ligand AacMPTCH$_2$

3.2.2. Potentiodynamic polarization method

Polarization studies on mild steel have been made for the AacMPTCH$_2$ in l M HCl. Cathodic-and anodic curves were recorded potentiodynamically with a sweep rate of 1000 mV/min and in a potential range from -250 to +250mV. Cathodic- and anodic potentiodynamic polarization curves obtained with selected concentrations (50 ppm, 100 ppm and 150 ppm) of AacMPTCH$_2$ are shown in Figure 6. Electrochemical parameters, such as corrosion potential E_{corr}, corrosion current I_{corr}, anodic- and cathodic Tafel slopes (βa and βc) were obtained from this experiment and are given in Table 3.2.2.

Table 3.2.2. Parameters from Tafel plot

AacMPTCH$_2$ Conc. (ppm)	**E_{corr} (mV)**	**β_a (mV dec^{-1})**	**β_c (mV dec^{-1})**	**I_{corr} (mA/cm^2)**	**CR (mm/year)**	**η%**
Blank	-453	97.381	126.200	0.8916	10.333	--
50 ppm	-499	45.398	107.530	0.0105	0.1216	98.8
100 ppm	-493	44.815	99.924	0.0089	0.1029	99.0
150 ppm	-444	32.545	136.270	0.0045	0.0517	99.5

Form the Tafel plot and based on E_{corr}, β_a and β_c values it is observed that the compound may be acting as mixed type of inhibitor.

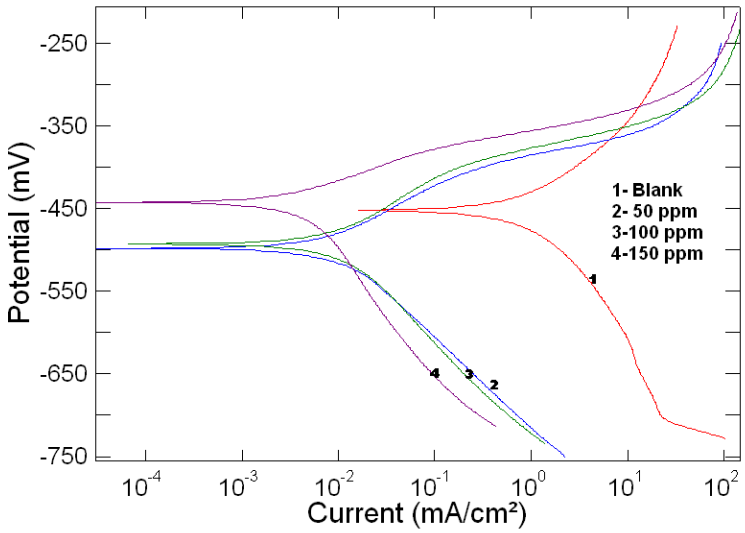


Figure 6.

Tafel plot for ligand AacMPTCH$_2$.

3.3. Adsorption studies

The mechanism of corrosion inhibition by AacMPTCH$_2$ on the mild steel surface was studied on the basis of adsorption isotherm. The values of surface coverage (θ) for AacMPTCH$_2$ are taken for these studies and are shown in Table 3.3(a).

Table 3.3(a). Surface coverage θ values

Conc. (ppm)	**Surface coverage (θ)**
50	0.965
100	0.971
150	0.979

Attempts were made by fitting the surface coverage values to various isotherms like Langmuir, Frumkin and Tempkin isotherms for which it is suitable[70]. It was observed that Langmuir isotherm was found to provide best description of the adsorption behavior of AacMPTCH$_2$ i.e., the plot of concentration on X axis verses C/θ on Y axis gave a straight line, which confirmed that the corrosion inhibition mechanism follows Langmuir adsorption isotherm as shown in Figure 7.

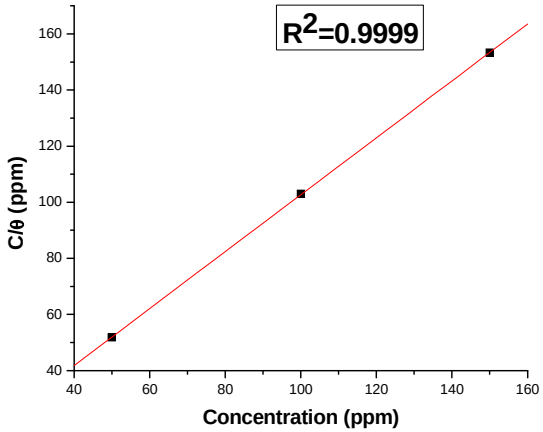


Figure 7.

Langmuir adsorption isotherm for ligand AacMPTCH$_2$.

Using the θ values and concentration in moles, K_{ads} was calculated using the relation,

$$K_{ads} = \frac{\theta}{1-\theta} \times \frac{1}{C}$$

The thermodynamic parameter, $\Delta G°_{ads}$ was calculated knowing the value of K_{ads}, using the relation, $\Delta G°_{ads} = -RT \ln (55.5\ K_{ads})$. Negative value of $\Delta G°_{ads}$ indicates the spontaneity of the adsorption process and since the value of ΔG° is lower, the process may be physisorption. These values are shown in Table 3.3(b).

Table 3.3(b) Parameters from Langmuir adsorption isotherm

Conc. ppm	**24 hours**	
	K_{ads}	**$\Delta G°_{ads}$(kjmol^{-1})**
50	145036.44	-40.87
100	88066.17	-38.30
150	81744.77	-38.30

3.4. Scanning electron microscopy (SEM) analysis

SEM photographs of the metal pieces were taken using solution of 200 ppm inhibitor concentration. Figure 8 represents the plain metal piece. Figure 9 represents the metal piece dipped in 1M HCl without the inhibitor. This SEM photograph reveals the presence of more pits. Figure 10 exhibits the formation of monolayer adsorption of the inhibitor, AacMPTCH$_2$ on the metal surface. Due to the presence of this film, corrosion of metal is prevented and its surface seems to be smoother. The images were taken after an immersion time of 24 h and at a magnification of 50 μm.

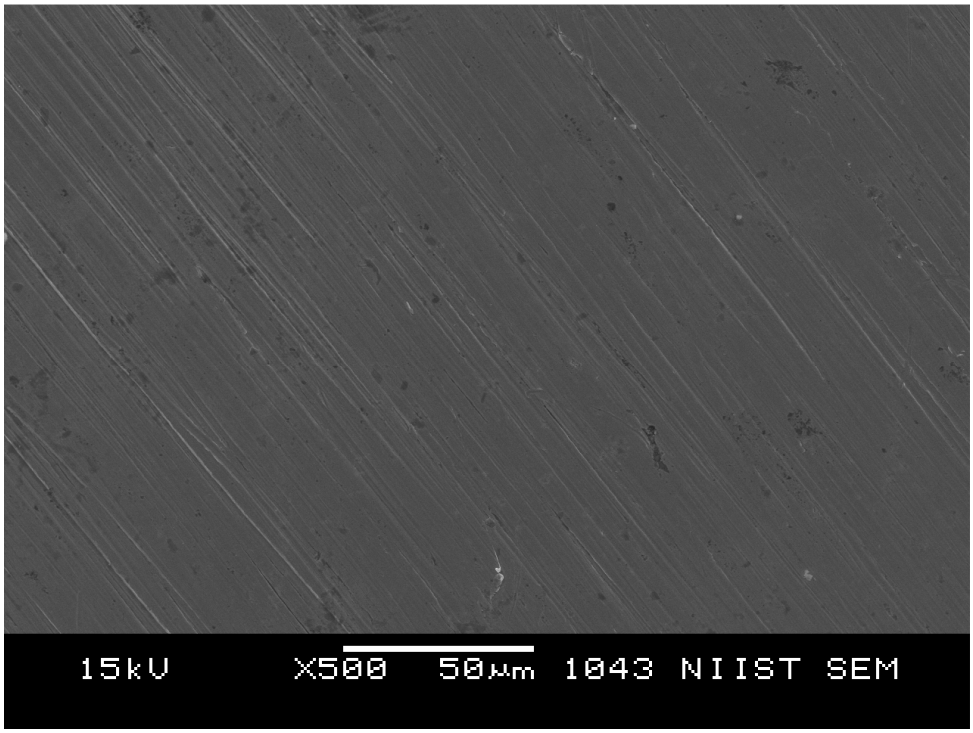


Figure 8.
Plain metal piece

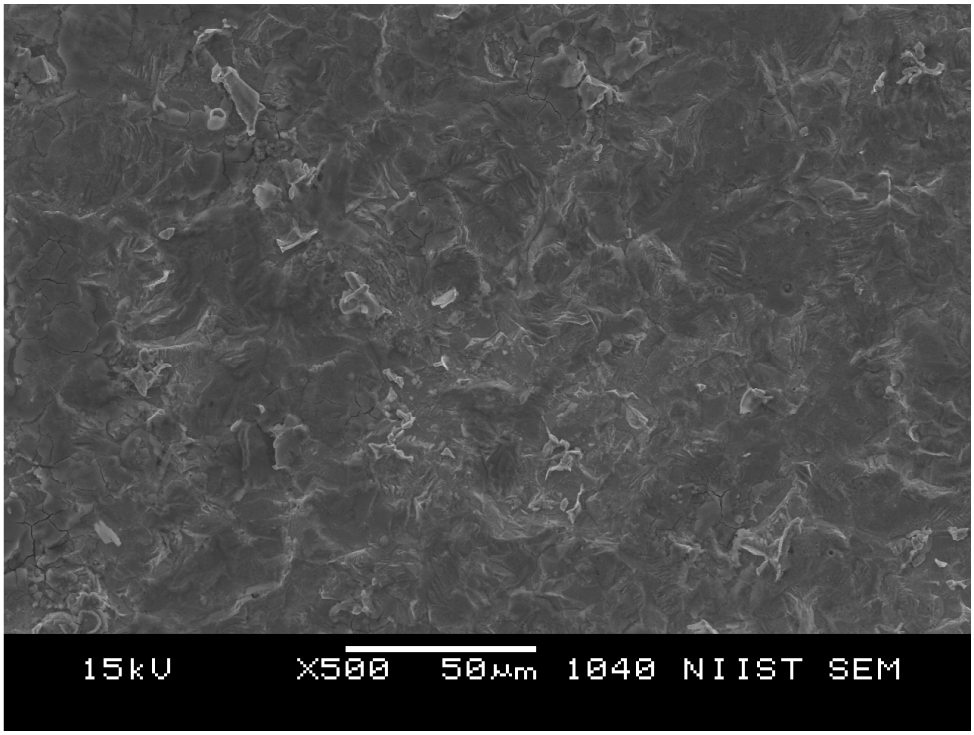


Figure 9.
Plain metal piece in 1M HCl

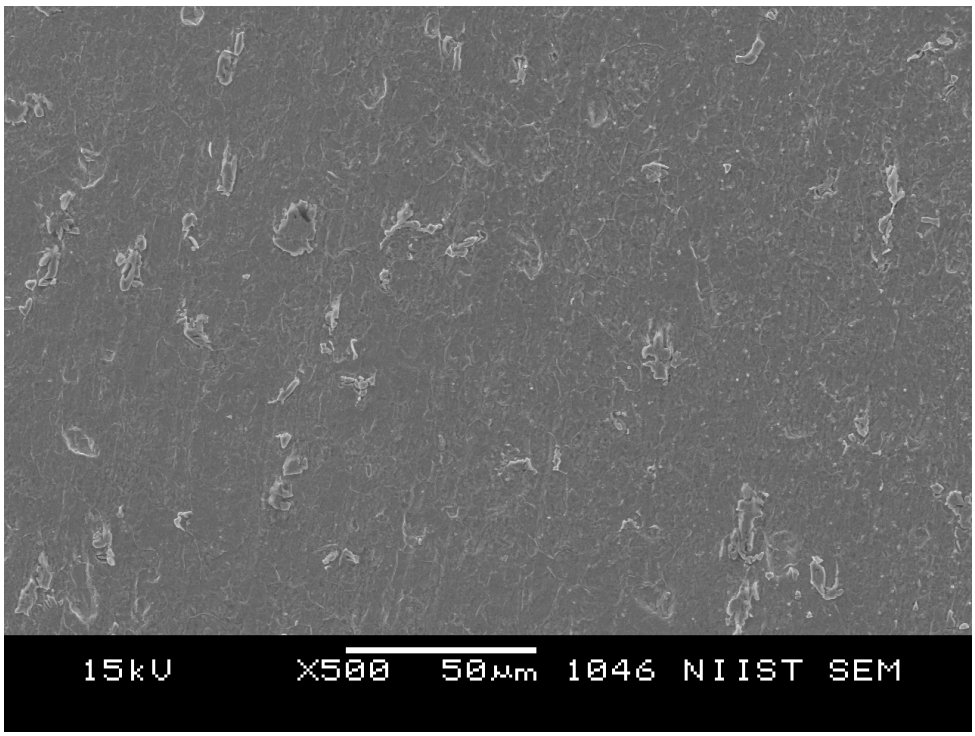


Figure 10.

Plain metal piece in 1M HCl with inhibitor

3.5. Conclusion

The results of the interaction of AacMPTCH$_2$ with mild steel in l M HCl have been studied and the final conclusions about the behavior of AacMPTCH$_2$ in the acid medium and its efficiency as corrosion inhibitor are given below.

i. AacMPTCH$_2$ is a very good inhibitor of corrosion of mild steel in l M HCl. The inhibition efficiency increases with the increase in concentration of the inhibitor. This may be due to its structural

characteristics, i.e., with hetero atoms, aromatic rings, multiple bonds, etc.

ii. The potentiodynamic polarization curves (Tafel plots) indicate that AacMPTCH$_2$ is a mixed type of inhibitor.

iii. The values of corrosion rate are consistent in different methods.

iv. The adsorption of inhibitor molecule on the metal surface is indicated by the decrease of C_{dl} values.

v. The mechanism of adsorption process follow Langmuir adsorption isotherm.

vi. The negative value of ΔG^{o}_{ads} indicates that the process is spontaneous.

CHAPTER IV

CORROSION INHIBITION STUDIES OF ω–BROMOACETOACETANILIDE N(4)-ETHYL(PHENYL)THIOSEMICARBAZONE ON MILD STEEL IN 1M HYDROCHLORIC ACID

1. Introduction

Corrosion is an important, serious problem in association with the usage of metals. The damage caused by corrosion is very high and that need a lot of money for the maintenance. Mild steel is used under different conditions for various purposes in acid media and this will lead to corrosion of the metals. One of the best ways of protecting metals from corrosion is to use corrosion inhibitors. The review of literature revealed that the structural characteristics of Schiff bases have considerable inhibition efficiency than the amine and aldehyde from which they are prepared. As per the earlier studies, this is due the presence of unoccupied π-orbital in the Schiff base, which enables the back donation from the transition metal d-orbitals, stabilizing the metal inhibitor bond, which is not possible in amines. The aim of the present work is to study the corrosion protection characteristics of ω-bromoacetoacetnilide N(4)-methyl(phenyl)thiosemicarbazone (ω-BAacd $MPTCH_2$) using mild steel in 1M hydrochloric acid. The presence of the features for a good corrosion inhibitor in this ligand persuaded us to undertake this work. The structure (Figure 1) and name of the compound are given in below.

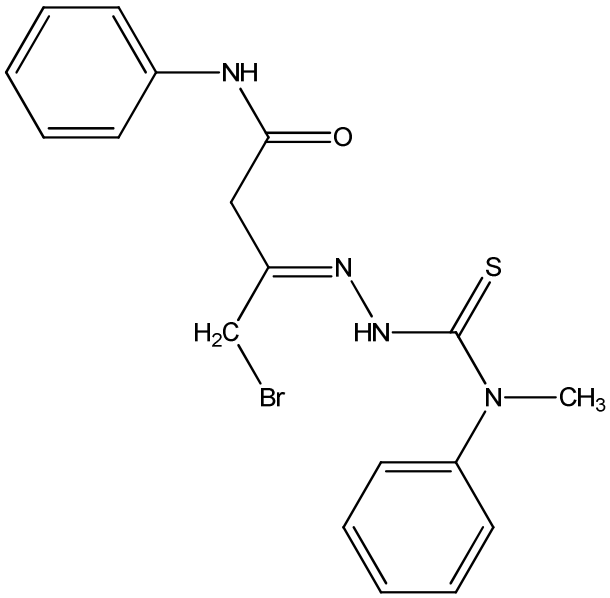


Figure 1.

ω-Bromoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (ω-BAacd MPTCH_2)(L2H)

2. Experiments

Different concentrations (50 ppm, 150 ppm and 200 ppm) of the ligand, ω-BAacdMPTCH_2 in 1M HC1 was prepared. The corrosion inhibition efficiency and the related characteristic properties of this ligand using mild steel in 1M HC1 was conducted by weight loss measurements, electrochemical analysis (Electrochemical impedance spectroscopy and Potentiodynamic polarization method) and SEM analysis.

3. Results and discussion

3.1. Weight loss measurements

From the study of weight loss measurements, it is clear that in the case of this ligand its corrosion inhibition efficiency is high and it increases with increasing concentration of the ligand. But as the time of immersion in HCl increases the inhibition efficiency decreases. These variations are shown in Table 3.1. Surface coverage, θ values increase with the increase of concentration of the inhibitor, while the corrosion rate decreases with increase in the concentration of the inhibitor. Figures 2, 3 and 4 show the variation of corrosion rate with concentration of the ligand, variation of inhibition efficiency with concentration of ligand and the variation of inhibition efficiency with time for the ligand, respectively.

Table 3.1. Variation of inhibition efficiency and surface coverage of ω-BAacdMPTCH$_2$ and corrosion rate of mild steel with concentration of inhibitor added and time of immersion.

ω-B Aacd MPTCH$_2$	**Conc. (ppm)**	**Time in hours**			
		24	**48**	**72**	**96**
Inhibition efficiency (η%)	50	97.40	94.70	92.70	90.60
	100	98.50	96.60	94.20	92.10
	150	98.60	96.70	94.90	92.50
	200	98.80	97.60	96.30	94.00
Corro. rate (mpy)	0	1028	559	379.30	288.35
	50	27.13	30.26	27.78	27.08
	100	15.62	18.64	21.93	22.74
	150	14.25	18.54	19.45	21.57
	200	11.32	13.27	14.18	17.37
Surface coverage (θ)	50	0.974	0.947	0.927	0.906
	100	0.985	0.966	0.942	0.921
	150	0.986	0.967	0.949	0.925
	200	0.988	0.976	0.963	0.940

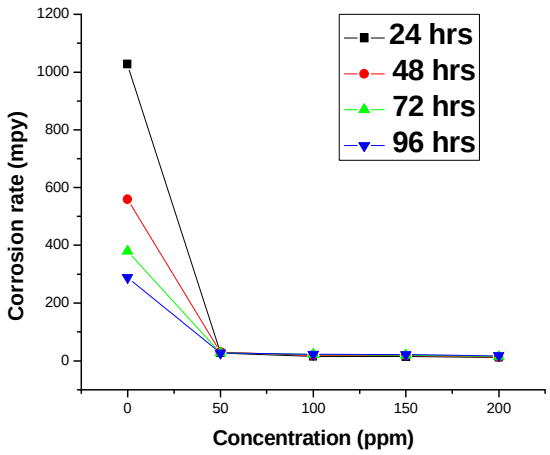


Figure 2.

Variation of corrosion rate with concentration of ligand ω-BAacd $MPTCH_2$

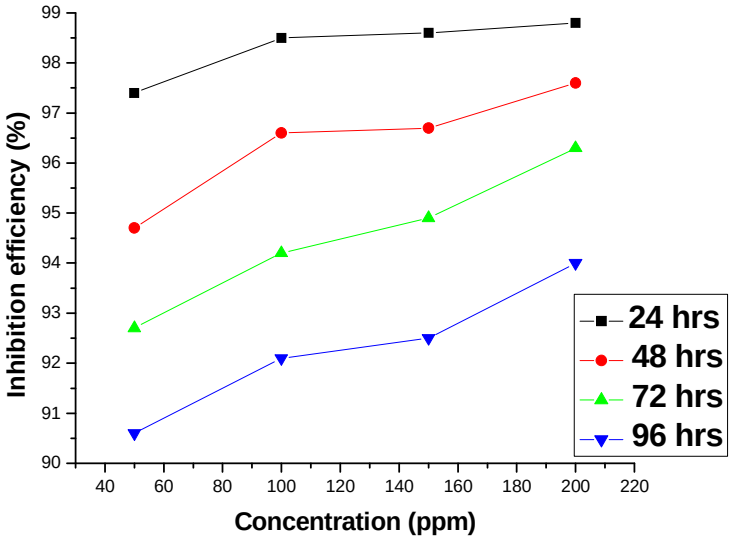


Figure 3.
Variation of inhibition efficiency with concentration of ligand ω-BAacd $MPTCH_2$

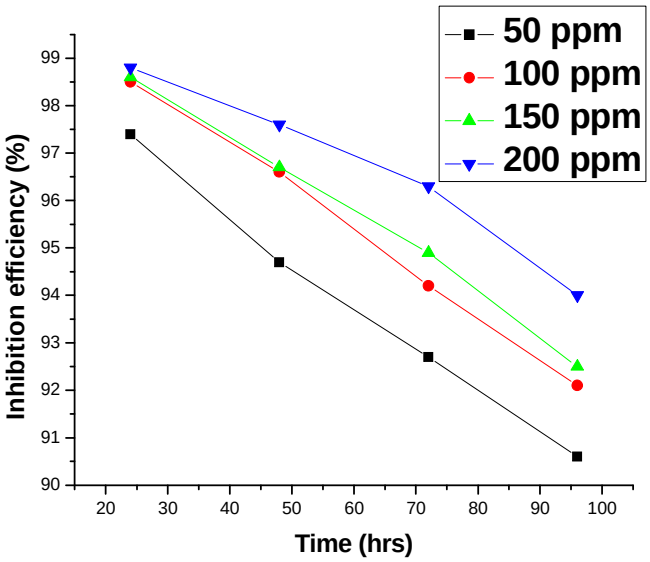


Figure 4.

Variation of inhibition efficiency with time of immersion for ligand ω-BAacd MPTCH$_2$

3.2. Electrochemical analysis

3.2.1. Electrochemical impedance spectroscopy (EIS)

This is a rapid and convenient method to measure the inhibition efficiency of a compound. In this method, the measurements were carried out with an amplitude of 10mV ac sine wave at a frequency range of 10 KHz to 10 Hz with reference to standard calomel electrode (SCE). The Nyquist plots for the mild steel after 1 h exposure in uninhibited and inhibited 1M HCl are given in Figure 5. The semicircular appearance of the impedance diagram shows that a charge-transfer process mainly controls the corrosion of mild

steel[71]. The important parameters obtained from the impedance spectra, R_{ct} and C_{dl} values, have opposite trends throughout the analysis. These are shown in Table 3.2.1. The decrease in electrical double layer capacitance with increase in inhibitor concentration may be attributed to the formation of a protective layer on the electrode surface and thus reducing corrosion rate. The result obtained from the electrochemical measurements supported the data obtained from the weight loss measurements.

Table 3.2.1. The R_{ctt} (Ohm cm^2), C_{dl} (F), I_{corr}(mA/cm^2) and corrosion rate (mm/year) for the mild steel in 1M HCl without and with different concentration of ω-BAacdMPTCH$_2$

Conc. (ppm)	**R_{ct} (Ohm cm^2)**	**C_{dl} (F)**	**I_{corr} (mA/cm^2)**	**Corr. Rate (mm/year)**	**η%**
0	11.28	1.454×10^{-4}	2.3130	26.800	--
50	1298	1.606×10^{-5}	0.0201	0.2329	99.1
100	1925	1.543×10^{-5}	0.01355	0.1571	99.4
150	2317	1.361×10^{-5}	0.01126	0.1305	99.5
200	2658	1.175×10^{-5}	0.009815	0.1138	99.6

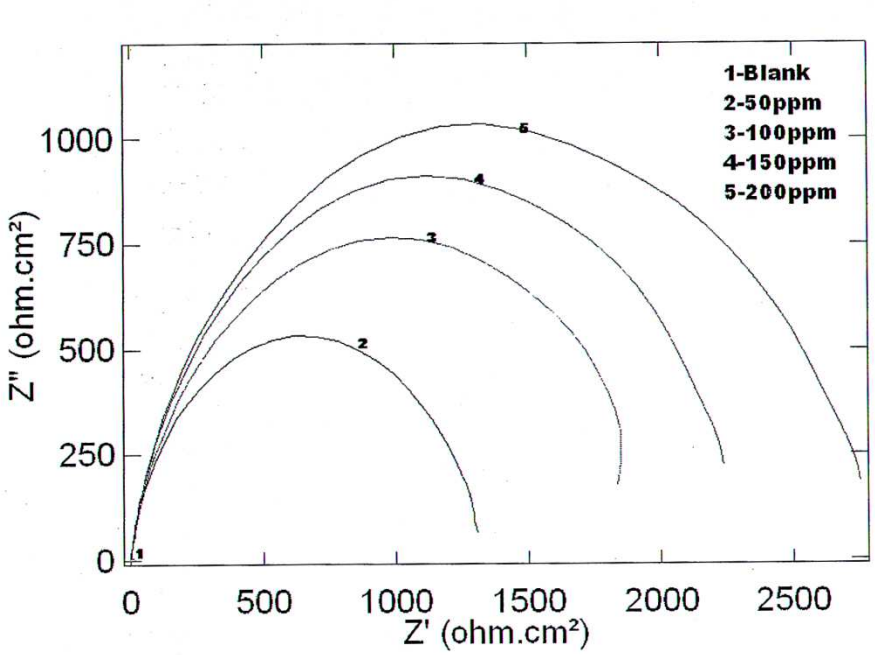


Figure 5.

EIS plot for ligand ω-BAacdMPTCH$_2$

3.2.2. Potentiodynamic polarization studies

Potentiodynamic polarization studies of mild steel in 1M HCl in the absence and presence of inhibitors were carried out in a potential range from -250mV to +250mV and with a sweep rate of 1000 mV/minutes. The cathodic-and anodic polarization curves obtained for mild steel in 1M HCl with various inhibitor concentrations are given in Figure 6. The electrochemical parameters like, corrosion potential (E_{corr}), cathodic- and anodic Tafel slopes (β_c and β_a) and corrosion current density (I_{corr}) obtained from Tafel extrapolation of polarization curve are given in Tabel 3.2.2. These results suggest that ω-B AacdMPTCH$_2$ acts as mixed type corrosion

inhibitor[72]. It was noted that when the concentration of inhibitor is increased, the corrosion current density is decreased.

Table 3.2.2. Parameters from Tafel Plot

ω-BAacdMPTCH$_2$ Conc. (ppm)	E_{corr} (mV)	β_a (mV dec^{-1})	β_c (mV dec^{-1})	I_{corr} (mA/cm^2)	CR (mm/year)	η%
Blank	-453	97.381	126.200	0.8916	10.3330	--
50 ppm	-489	52.766	111.180	0.0110	0.7278	98.8
100 ppm	-509	70.411	104.920	0.0097	0.1119	98.9
150 ppm	-490	63.663	112.900	0.0076	0.0876	99.2
200 ppm	-488	60.907	88.427	0.0046	0.0537	99.5

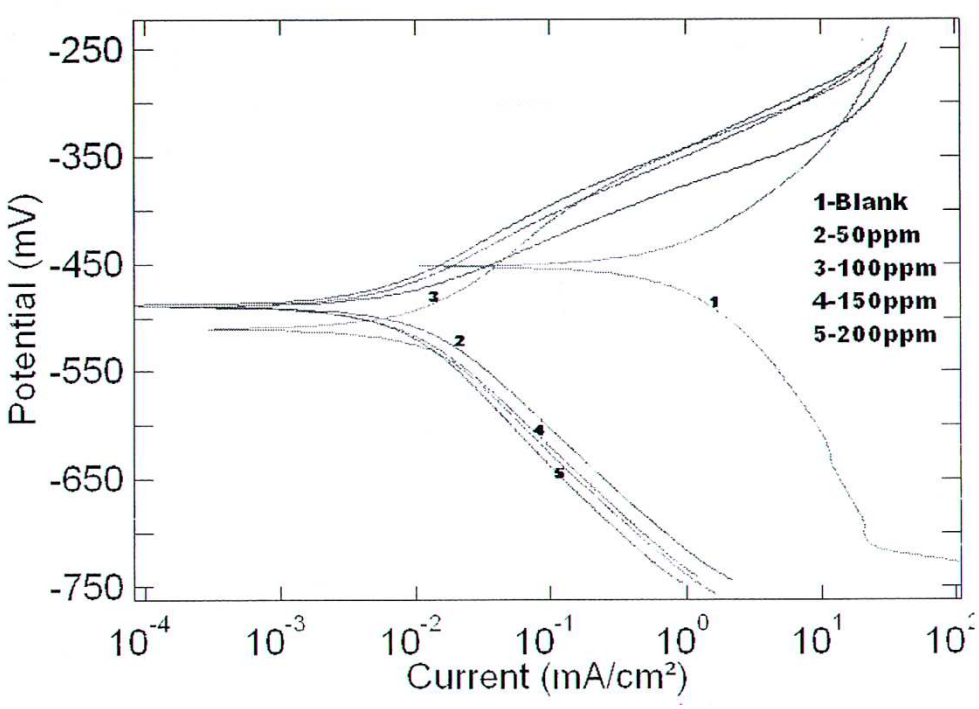


Figure 6.

Tafel plot for ligand ω-BAacdMPTCH$_2$

3.3. Adsorption studies

The mechanism of corrosion inhibition by ω-BAacd $MPTCH_2$ on the mild steel was studied on the basis of adsorption isotherm studies. The values of surface coverage (θ) for ω-BAacd$MPTCH_2$ are taken for these studies and are shown in Table 3.3(a).

Table 3.3(a). surface coverage θ values

Conc. (ppm)	Surface coverage (θ)
50	0.974
100	0.985
150	0.986
200	0.988

These studies were conducted on the basis of adsorption isotherm which provides the basic information on the interaction of inhibitor with the metal surface [73,74]. Adsorption isotherm was obtained by the surface coverage values (θ) for different concentrations of inhibitor in 1M HCl. Using these data, different graphs have been constructed to find out the most suitable adsorption isotherm. A plot of log θ/1-θ against log C gave a straight line, indicating that the adsorption of ω-BAacd$MPTCH_2$ on mild steel surface obeys Langmuir adsorption isotherm as shown in Figure 7.

Using θ values and concentration in moles, K_{ads} was calculated using the relation,

$$K = \frac{\theta}{1-\theta} \times \frac{1}{C}$$

The thermodynamic parameter, $\Delta G°_{ads}$ was calculated knowing the value of K_{ads} and using the relation, $\Delta G°_{ads} = -RT\ 1n\ (55.5\ K_{ads})$. Negative value of $\Delta G°_{ads}$ indicates the spontaneity of the adsorption process and since the value of ΔG° is lower, the process may be physisorption. These values are shown in Table 3.3(b).

Table 3.3(b). Parameters from Langmuir adsorption isotherm

Conc. ppm	**24 hours**	
	K_{ads}	**$\Delta G°_{ads}$(kjmol^{-1})**
50	191130.29	-40.750
100	167474.28	-40.426
150	119735.75	-39.580
200	104976.8	-39.240

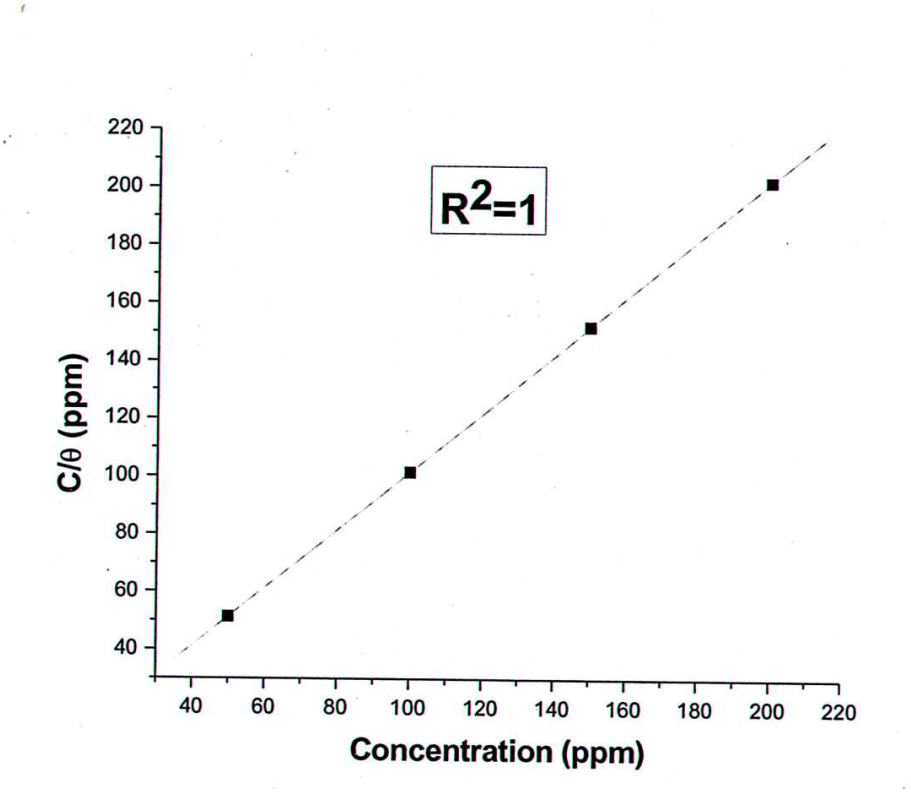


Figure 7.

Langmuir adsorption isotherm

3.4. Scanning electron microscopy (SEM) studies

SEM was employed to confirm the nature of interaction of the inhibitor molecules on the metal surface. The scanning electron micrographs of mild steel specimen before and after exposure to 1M HCl are shown in Figures 8,9 and 10. Figure 8 gives the micrographs of plain mild steel specimen before immersing in 1M HCl. Figure 9 clearly shows that the surface is covered with pits due to corrosion, when immersed in 1M HCl. The influence of addition of 200 ppm of ω-B AacdMPTCH$_2$ in 1M HCl decreases the corrosion rate due to the formation film on the metal surface. This is evident from the Figure 10.

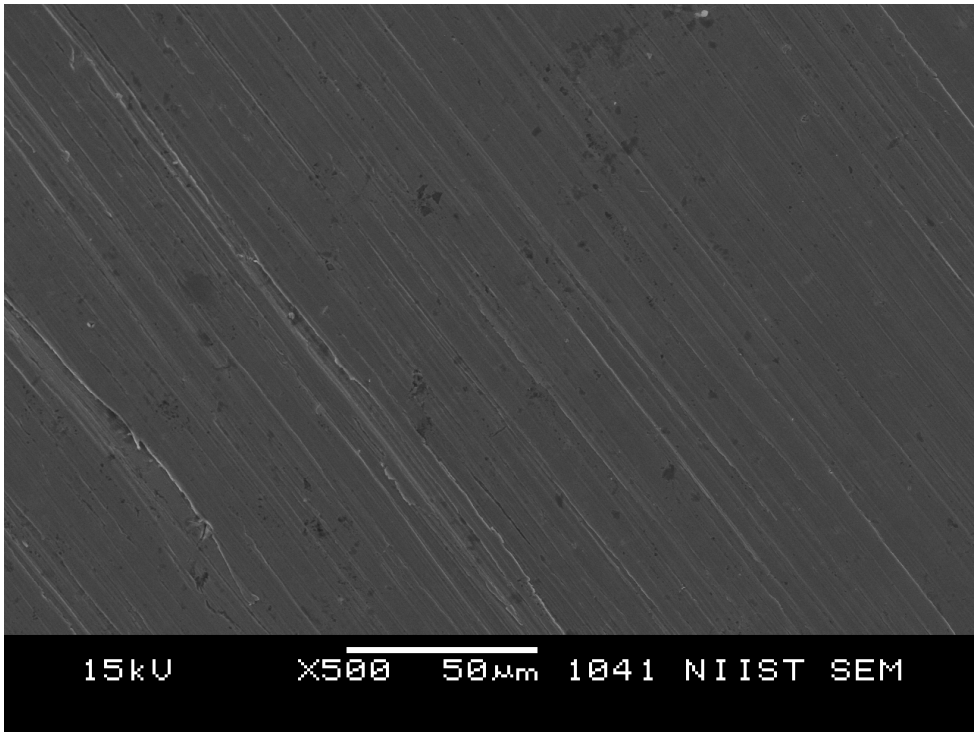


Figure 8.

Plain metal piece

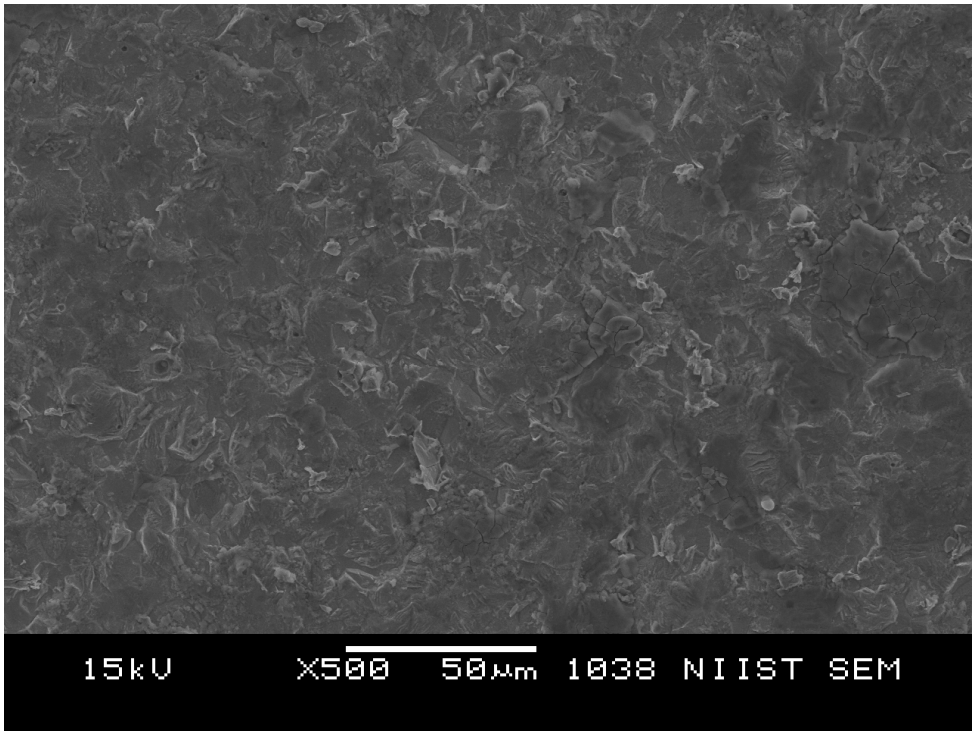


Figure 9.

Plain metal piece in 1M HCl

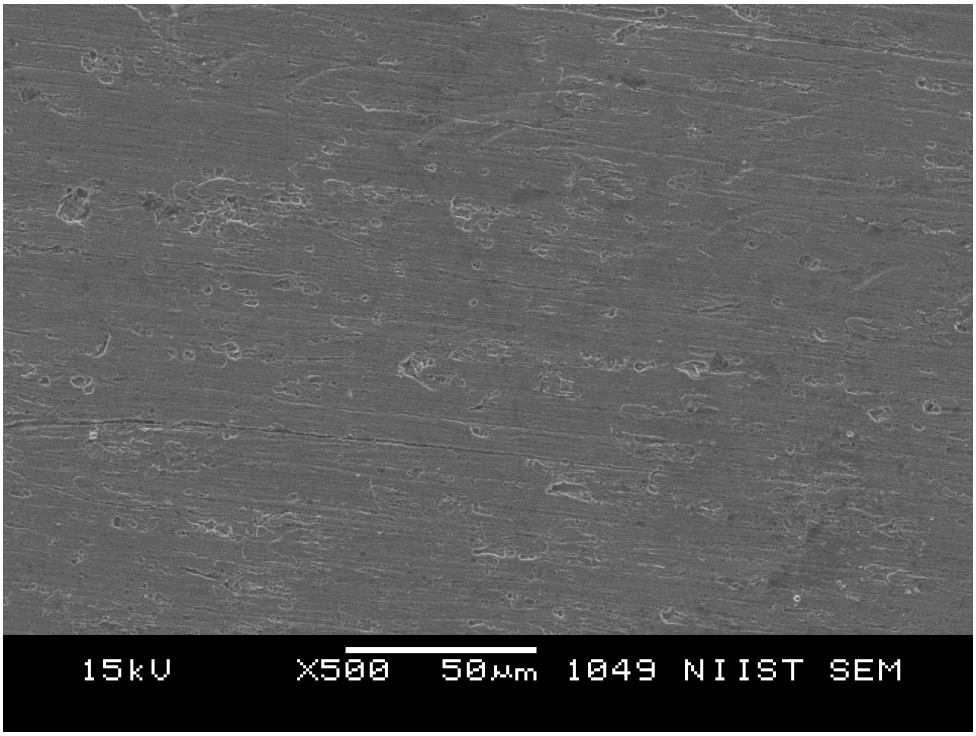


Figure 10.

Plain metal piece in 1M HCl with inhibitor

3.5. Conclusion

The conclusions drawn on the basis of the results obtained from the corrosion inhibition studies of ω-BAacdMPTCH$_2$ in 1M HCl using mild steel are given below.

i. The compound ω-BAacdMPTCH$_2$ in 1M HCl possesses excellent inhibition efficiency for mild steel corrosion at room temperature.

ii. The percentage of inhibition efficiency increases with the concentration of the ligand and decreases with exposure time.

iii. As the concentration of the inhibitor increases, corrosion inhibition efficiency and charge - transfer resistance increases.

iv. The inhibitor molecule affects both anodic- and cathodic processes and hence it is considered as a mixed type inhibitor.

v. The adsorption of the inhibitor obeys Langmuir adsorption isotherm model.

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CHAPTER I

METAL COMPLEXES AS ANTIMICROBIAL AGENTS

I. Introduction

Thiosemicarbazones and their metal complexes show wide range of biological activities and therefore, they are given a special importance in the pharmaceutical field. For the last fifty years, different types of thiosemicarbazones have been evaluated as antiviral-, antibacterial-, antifungal-, and antimalarial agents[1]. The biological activities of thiosemicarbazones are found to be enhanced on complex formation with metal ions[2-9].

1.1 Transition metal complexes as antimicrobial agents

The studies on N-methylacetoacetanilide isonicotinylhydrazone and its metal chelates revealed that they are active against several pathogenic fungal strains[10]. It is concluded that, eventhough both ligand and metal chelates were active against all fungal strains, the chelates were found to be more active than the ligand. Synthesis and *in vitro* study of Co(II), Ni(II), Cu(II) and Zn(II) complexes of β-diketone were done by Javed *et al*.[11] As reported by Vinod and Anshu, the transition metal complexes of acetone- and p-aminoaceto- phenone benzoylhydrazone showed significant antifungal activity against Aspergillus and Penicillium[12]. *In vitro* antimicrobial studies

of transition metal complexes of cyclohexylamine-N-dithiocarbamate have been reported to be promising fungicides[13]. The mixed ligand complexes of lomefloxacin drug with transition metals were found to exhibit enhanced activity on bacteria and fungi[14].

1.2 Schiff bases as antimicrobial agents

A large number of Schiff bases and their complexes have been studied for their important biological properties. Schiff bases posess some unique properties like the ability to bind with oxygen reversibly[15], transfer of an amino group[16] and photochromic effect[17]. Biological screening studies of complexes of Schiff bases derived from salicylidine-4-aminoantipyrine and-2-aminophenol were carried out by Raman *et al*[18]. Metal complexes of Schiff bases derived from 2-furanocarboxaldehyde and o-phenylenediamine were screened for their antibacterial activity against Escherichia coli[19]. The biological activities of Schiff bases derived from 2-thiophenecarboxaldehyde and 2-aminobenzoic acid and their metal complexes were tested against bacterial species and the metal complexes were found to be more potent than the parent Schiff bases[20]. Antimicrobial activity of copper(II) complexes of 1,10- phenanthroline have been tested against Bacillus magaterium and a candidatropicalis[21]. Studies on antimicrobial activity of Co(II), Ni(II) and Zn(II) complexes of Schiff base derived from ninhydrine and glycine[22] showed that the free ligands are highly active against E-coli and moderatively

active against P.mirabilis[23]. The mixed ligand complexes containing N, O and S donor atoms are important due to their significant antifungal and antibacterial activities[24]. The relevant antibacterial - and antifungal activities of a new series of compounds, 3-amino-2methyl-4- (3H)- quinazolinone prepared by the condensation reaction of different substituted aromatic aldehydes were reported by Hosakere *et al*[25]. The antibacterial- and antifungal activities of Schiff bases of sulphanilamide were studied by Santosh Kumar *et al*[26] and concluded that among these, compounds bearing tri-methoxy group, methoxy group and furan ring have shown higher activity against microbes. The ligand and the metal complexes of Schiff bases derived from 2-furanocarboxaldehyde and 2-aminobenzoic acid showed activity against some bacterial species[27]. The literature survey revealed that Schiff bases and their metal complexes are versatile class of compounds due to their important medicinal-, biological-, analytical-, clinical- and industrial properties.

1.3 Thiosemicabazones as antimicrobial agents

Thiosemicarbazones and their complexes are given immense importance due to their pharmacological- and biological activities[28]. Literature survey showed various interesting activities of thiosemicarbazones like antibacterial-,[29] antiviral-,[30] antifungal-,[31] and other biological properties[32]. Studies on salicylaldehyde -4-phenylthiosemicarbazone by

Novak *et al*[33] revealed that it posess antiviral-, antibacterial- and antiinflamatory activities. Prathima *et al*[34] established the biological activities of Mn(II) and Co(II) complexes of benzyloxybenzaldehyde -4-phenyl-3-thiosemicarbazone and concluded that the metal complexes were more biologically active than the ligand. However, the ligand has exhibited higher antioxidant activity than its metal complexes. Sugam *et al*[35] conducted antimicrobial studies of the ligand synthesized by the condensation of thiosemicarbazide with benzyl. They prepared several transition metal complexes of this ligand and observed that all the complexes were more powerful antibacterial agents than the ligand. The biological studies of Cu(II) and Ni(II) complexes of benzyloxybenzaldehyde -4-phenyl-3-thiosemicarb-azone revealed that the antibacterial activity of the ligand increased upon complexation, particularly with Cu(II)[36,37]. The relationship connecting the structure and the antimicrobial activity of 2-acetylpyridine thiosemicarbazone was suggested by Collins *et al*[38]. The antimicrobial activities of a series of stable complexes of 4-hydroxycoumarin -3-thiocarbohydrazone with Cu(II), Ni(II), and Co(II) ions were carried out by Mosa *et al*[39]. Baldini *et al*[40] reported the synthesis and biological evaluation of α-ketoglutaricacid thiosemicarbazone and its Cu(II) and Zn(II) complexes. Otero *et al*[41] conducted a study on *in vitro* growth inhibition activity of Pd(II) complexes of 5-nitrofuryl-3-acroleine thiosemicarbazone against Trypansoma cruzi.

1.4 Scope of the present investigation

Scientists were interested on the biological properties of thiosemicarbazones even at the beginning of 20th century. They suggested metal complexes as medicines against leprosy and tuberculosis[42,43]. The antiviral properties of the thiosemicarbazones were discovered and methisazone, a drug for small- pox was introduced[44]. Followed by this, the anticancer drug, Triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) was discovered[45]. Presently, thiosemicarbazones are considered as versatile, and promising compounds with antitumor-, antiprotozoal-, antibacterial-, antiviral- and antifungal activities.

In our present investigation, the antibacterial- and antifungal activities of some thiosemicarbazones and their complexes were studied and the results were analysed. The methods and the observations are presented in the following chapter.

CHAPTER II

ANTIMICROBIAL STUDIES OF THIOSEMICARBAZONES AND THEIR METAL COMPLEXES

In view of the increasing interest of organic-, inorganic- and coordination compounds from the biological point of view, it has been decided to evaluate the anti bacterial- and anti fungal activities of some of the thiosemicarbazones and their metal complexes that we have synthesized during this programme. For the sake of convenience, those studies are presented in the following three sub-sections in this chapter.

SUB-SECTION I

I. ANTIBACTERIAL ACTIVITY OF ACETYLACETONE N(4)-METHYL (PHENYL)THIOSEMICARBAZONE ($AacMPTCH_2$) AND ITS METAL COMPLEXES

Evaluation of antibacterial activities of the ligand and its Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes was carried out using Kirby Bauer or Disc Diffusion Method. The structure of the ligand, $AacMPTCH_2$ is given in Figure 1.

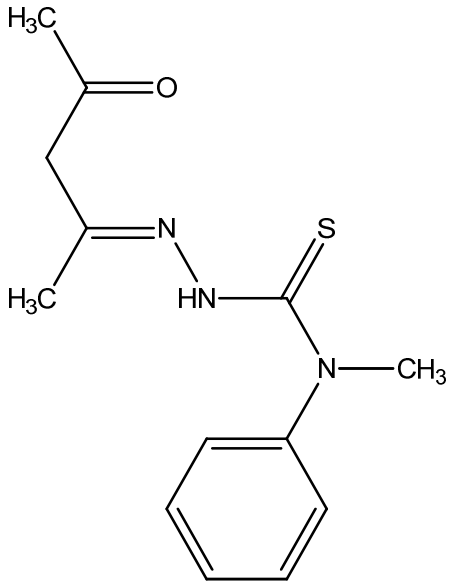

Figure 1.

Acetylacetone N(4)-methyl(phenyl)thiosemicarbazone ($AacMPTCH_2$)

1. Materials and methods

1.1. Bacterial cultures used

Three Gram positive bacteria; *Staphylococcus(*MTCCNO 3103), *Streptococcus (*MTCCNO 1938), *Bacillus(*MTCCNO 869) *sp* and three Gram negative bacteria; *Klebsiella (*MTCCNO 2653), *Escherichia coli (*MTCCNO 68) *and Pseudomonas (*MTCCNO 2642) *sp.* were used in this study. The pure cultures were purchased from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

1.2. Media used

Nutrient Broth

(HiMedia Laboratories Pvt Ltd, Mumbai)

Peptic digest of animal tissue : 5g

Sodium chloride : 5g

Yeast extract : 1.5g

Distilled water : 1000ml (pH – 7.4)

Nutrient Agar

(HiMedia Laboratories Pvt Ltd, Mumbai)

Peptic digest of animal tissue : 5g

Sodium chloride : 5g

Yeast extract	: 1.5g
Distilled water	: 1000ml (pH – 7.4)
Agar	: 15 g

1.3. Antibacterial activity by Kirby Bauer or Disc Diffusion Method

Antibacterial tests were carried out by disc diffusion method[46] (with some modifications). The bacterial cultures (mentioned above) were maintained in nutrient broth. Each culture was uniformly distributed on Nutrient agar plates using sterile swabs. Sterile filter paper discs of 3mm diameter were placed on the surface of Nutrient agar plates at a distance of 2cm using sterile forceps. DMSO (2 %) was used to dissolve the drug, which was found to have no adverse effect on the bacterial cultures. Drugs of different concentrations [100, 250, and 500 µg/ml] were added on each disc with a micropipette. Disc with DMSO but without drug was used as a control. The plates were then incubated at 37^{o}c for 24 hrs. After incubation, the zone diameter was measured. The greater diameters shown by the compounds than the control indicated their antibacterial activity.

2. Results

After incubation, the diameter of zone inhibition was measured. The results of disc diffusion method are shown in Tables 2.1. - 2.5 and Figures 2 and 3. The experimental results showed that the complexes of acetylacetone N(4)-methyl(phenyl)thiosemicarbazone, $AacMPTCH_2$ showed activity

against the tested strains of bacteria in a dose dependent manner. However, the ligand was not found to possess any antibacterial activity.

Table 2.1. Antibacterial activity of $[Co(L2H)_2X_2]$

DRUG (µg/ml)	**ZONE DIAMETER (mm)**					
$T_{4(1)}$	***E.coli***	***Klebsiella***	***Pseudomonas***	***Streptococcus***	***Staphylococcus***	***Bacillus***
DMSO	NA*	NA*	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	NA*	NA*	NA*
250	7	12	16	5	10	6
500	15	14	22	8	16	10

NA*= No activity

Table 2.2. Antibacterial activity of $[Ni(L2H)_2X_2]$

DRUG (µg/ml)	**ZONE DIAMETER (mm)**					
$T_{4(1)}$	***E.coli***	***Klebsiella***	***Pseudomonas***	***Streptococcus***	***Staphylococcus***	***Bacillus***
DMSO	NA*	NA*	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	NA*	NA*	NA*
250	10	9	11	9	10	9
500	15	12	14	14	14	13

NA*= No activity

Table 2.3. Antibacterial activity of [Cu(L2H)(OH)$_2$]

DRUG (µg/ml)	**ZONE DIAMETER (mm)**					
$T_{4(1)}$	***E.coli***	***Klebsiella***	***Pseudo-monas***	***Streptococcus***	***Staphylococcus***	***Bacillus***
DMSO	NA*	NA*	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	NA*	NA*	NA*
250	16	8	20	6	15	5
500	20	12	26	9	24	9

NA*= No activity

Table 2.4. Antibacterial activity of [Zn(L2H)$_2$X$_2$]

DRUG (µg/ml)	**ZONE DIAMETER (mm)**					
$T_{4(1)}$	***E.coli***	***Klebsiella***	***Pseudomonas***	***Streptococcus***	***Staphylococcus***	***Bacillus***
DMSO	NA*	NA*	NA*	NA*	NA*	NA*
100	NA*	NA*	NA*	6	NA*	NA*
250	11	13	9	9	21	14
500	15	18	12	12	25	18

NA*= No activity

Table 2.5. Antibacterial activity of [Cd(L2H)$_2$X$_2$]

DRUG (µg/ml)	**ZONE DIAMETER (mm)**					
$T_{4(1)}$	***E.coli***	***Klebsiella***	***Pseudomonas***	***Streptococcus***	***Staphylococcus***	***Bacillus***
DMSO	NA*	NA*	NA*	NA*	NA*	NA*
100	5	13	14	7	18	19
250	8	16	18	16	21	23
500	13	20	23	22	29	26

NA*= No activity

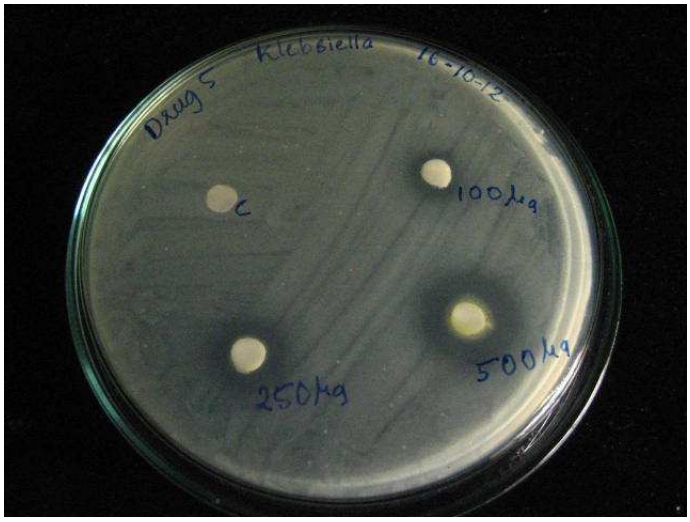

Figure 2.

Antibacterial activity of Cd(II) complex on the Klebsiella species

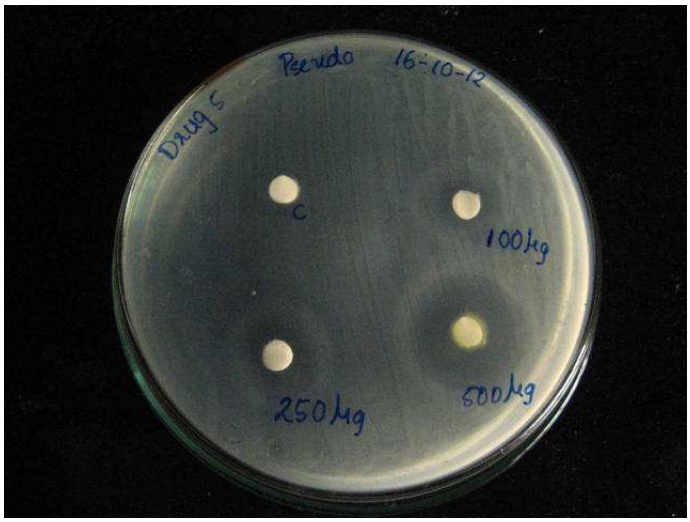

Figure 3.

Antibacterial activity of Cd(II) complex on the Pseudomonas species

3. Discussion

The antibacterial activities of the complexes were compared on the basis of the consolidated Table 3.1. choosing a concentration of 500(µg/ml).

Table 3.1. Consolidated antibacterial activities of the complexes, Conc. 500 (µg/ml)

Sl. No.	Compound	Zone Diameter (mm)					
		E. coli	***Klebsiella***	***Pseudo-monas***	***Strepto-coccus***	***Staphylo-coccus***	***Bacillus***
1	Ligand AacMPTCH$_2$	NA*	NA*	NA*	NA*	NA*	NA*
2	[Co(L2H)$_2$X$_2$]	15	14	22	8	16	10
3	[Ni(L2H)$_2$X$_2$]	15	12	14	14	14	13
4	[Cu(L2H)(OH)$_2$]	20	12	26	9	24	9
5	[Zn(L2H)$_2$X$_2$]	15	18	12	12	25	18
6	[Cd(L2H)$_2$X$_2$]	13	20	23	22	29	26

Of the complexes, those of Cu(II) and Cd(II) exhibited higher inhibition than Co(II), Ni(II) and Zn(II) complexes. However, for the different species of bacteria, the complexes showed variation in their activities. For eg. Cd(II) complex exhibited greater activity for Bacillus species but lesser activity towards E.coli. It was found that all the complexes showed greater activity than the ligand. Preliminary studies showed that most of the complexes were active towards all the six bacterial species and hence a detailed study in this regard will be interesting.

SUB-SECTION II

ANTIBACTERIAL ACTIVITY OF ω-BROMOACETOACETANILIDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (ω-BAacdMPTCH$_2$) AND N-ETHYLACETOACETANILIDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (EAacdMPTCH$_2$) AND THEIR METAL COMPLEXES

Evaluation of antibacterial activity of Co(II), Ni(II), Cu(II) and Zn(II) complexes of ω-bromoacetoacetanilide N(4)-methyl(phenyl)-thiosemicarbazone, ω-BAacdMPTCH$_2$ and Co(II), Ni(II), Cu(II) and Zn(II) complexes of N-ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone, EAacdMPTCH$_2$ were carried out using Kirby Bauer or Disc Diffusion Method[46]. The structures of the ligands, ω-BAacdMPTCH$_2$ (Figure 1.) and EAacdMPTCH$_2$ (Figure 2.) are given below.

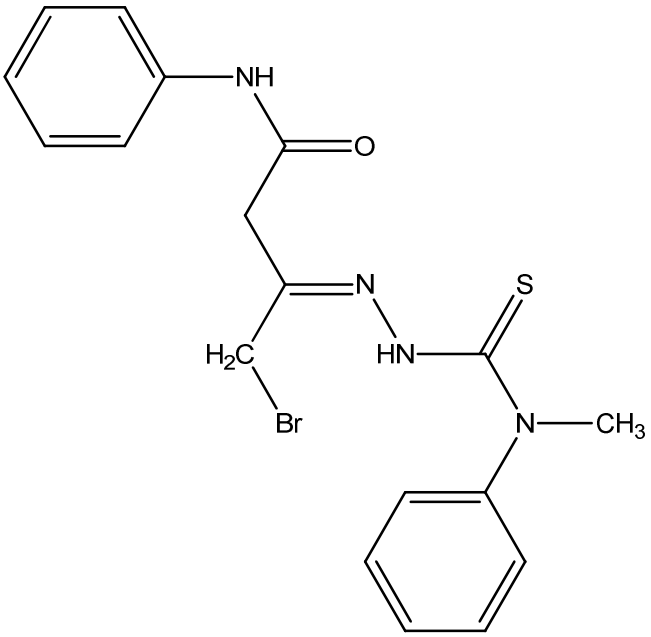

Figure 1.

ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone

ω-BAacdMPTCH$_2$

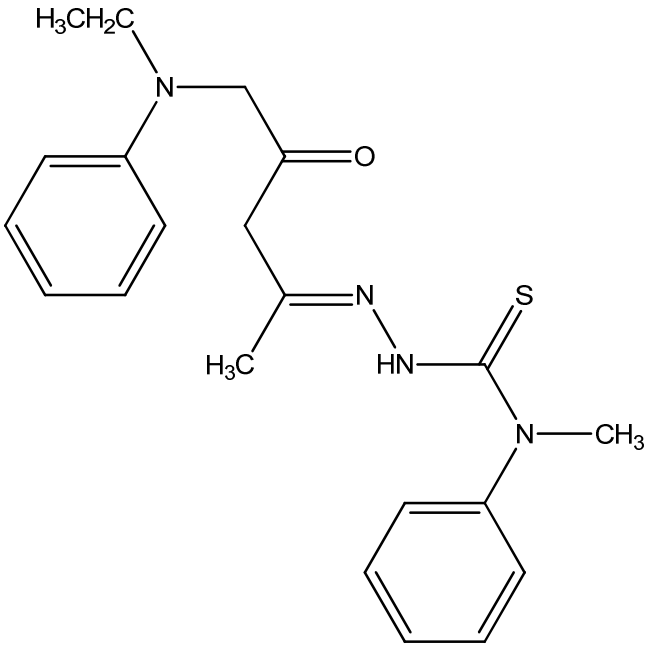

Figure 2.

N-ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone

EAacdMPTCH$_2$

1. Materials and methods

The bacterial cultures used, media and procedure used for the antibacterial activity of all the complexes by disc diffusion method were already mentioned in 1.1, 1.2, and 1.3 sections in this chapter.

2. Results

Antibacterial activities of the samples were tested against a set of clinically important bacterial strains. The samples showed activity against all the bacterial strains studied. Some of them showed low activity. The results are given in Tables 2.1- and 2.2.

Table : 2.1. Antibacterial activity of ω-bromoacetoacetanilide N(4)methyl -(phenyl)thiosemicarbazone and its complexes

Sl. No.	Organism	$[Co(LH)_2]$	$NiL(H_2O)_3$	$[CuL(H_2O)]$	$[ZnL(H_2O)]$	Ligand	S	D
1	Proteus Vulgaris	-	-	-	8	12	14	-
2	Bacillus cereus	11	9	11	9	14	15	-
3	Eschericia coli	-	-	-	-	12	10	-
4	Serratia marcescens	10	12	9	-	12	16	-
5	Pseudomonas aeruginosa	13	-	-	-	9	14	-
6	Bacillus suitilis	20	17	-	19	21	11	-
7	Salmonella typhi	-	-	-	-	-	12	-
8	Staphylococcus aureus	14	-	19	-	20	21	-
9	Klebsiella pneumonia	23	18	-	-	-	10	-

+ve control S = Streptomyces
-ve control D = DMSO

Table : 2.2. Antibacterial activity of N-ethylacetoacetanilide N(4)-methyl-(phenyl)thiosemicarbazone and its complexes

Sl. No.	Organism	$[CoL(H_2O)_3]$	$[NiL(H_2O)_3]$	$[CuL(H_2O)_3]$	$[ZnL(H_2O)_3]$	Ligand	S	D
1	*Klebsiella pneumonia*	8	-	-	12	10	14	-
2	*Eschericia coli*	9	14	-	14	7	15	-
3	*Salmonella typhi*	-	-	-	12	9	10	-
4	*Serratia marcescens*	11	10	-	12	-	16	-
5	*Pseudomonas aeruginosa*	-	13	-	-	7	14	-
6	*Proteus vulgaris*	19	20	-	21	12	11	-
7	*Bacillus suitilis*	8	7	-	18	11	12	-

+ve control S = Streptomyces

-ve control D = DMSO

3. Discussion

It was found that the ligand, ω-bromoacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone exhibited greater activity than the complexes. Another observation is that the Co(II) complex showed greater activity for most of the samples. However, Cu(II), Zn(II) and Ni(II) complexes exhibited medium inhibition, less than that of the ligand.

The results of the antibacterial activity of N-ethylacetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone and its complexes revealed that they possess considerable activity. It was observed that the ligand exhibited almost same inhibition effect as that of the complexes. However, the complexes of Ni(II) and Zn(II) exhibited higher inhibition effect than the parent ligand towards most of the bacterial strains. On the other hand, the Cu(II) complex exhibited no activity towards any one of these species.

SUB-SECTION III

ANTIFUNGAL ACTIVITY OF ACETYLACETONE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE (AacMPTCH$_2$) AND ITS METAL COMPLEXES

Evaluation of antifungal activities of Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) complexes of AacdMPTCH$_2$ was carried out using Kirby Bauer or Disc Diffusion Method.

1. Materials and methods

1.1. Fungal cultures used

Three fungal cultures : *Penicillium, Fusarium and Aspergillus species* were used in this study. The pure cultures were taken from the stock culture maintained in the Department of Microbiology, St Mary's College, Thrissur, Kerala, India.

1.2. Media used

Sabouraud Dexrose Broth

(HiMedia Laboratories Pvt Ltd, Mumbai)

Mycological peptone	: 10g
Dextrose	: 40g
Distilled water	: 1000ml (pH – 5.6 ± 0.2)

Sabouraud Dexrose Agar

(HiMedia Laboratories Pvt Ltd, Mumbai)

Mycological peptone	: 10g
Dextrose	: 40g
Agar	: 15g
Distilled water	: 1000ml (pH – 5.6 ± 0.2)

1.3. Antifungal activity by Kirby Bauer or Disc Diffusion Method

Antifungal activity was studied by disc diffusion method[46]. The fungal cultures were maintained in Sabouraud's dextrose broth. From this, 0.1 ml of each culture was uniformly distributed on Sabouraud's Dextrose Agar (SDA) plates. Sterile filter paper discs of 2mm diameter were placed on the surface of SDA plates. Drugs of different concentrations [100, 250, and 500µg/ml] were added on each disc using a micropipette. DMSO (2%) was used as control. The plates were kept at room temperature for 2 days. After incubation, the zone diameters were measured.

2. Results

The antifungal activities were evaluated against the following three species, *Penicillium, Fusarium and Aspergillus* by disc diffusion method. After incubation, the diameters of zones of inhibition were measured. The results of disc diffusion method are presented in Tables 2.1. - 2.5 and Figures

1 and 2. Acetylacetone N(4)-methyl(phenyl)thiosemicarbazone (AacdMPTCH$_2$) was not found to possess any antifungal activity. However, the complexes showed antifungal activity in a dose dependent manner.

Table- 2.1. Antifungal activity of [Co(L2H)$_2$X$_2$]

DRUG (µg/ml)	**ZONE DIAMETER (mm)**		
	Penicillium	***Fusarium***	***Aspergillus***
DMSO	NA*	NA*	NA*
100	NA*	5	NA*
250	6	8	NA*
500	8	11	NA*

NA*= No activity

Table 2.2. Antifungal activity of [Ni(L2H)$_2$X$_2$]

DRUG (µg/ml)	**ZONE DIAMETER (mm)**		
	Penicillium	***Fusarium***	***Aspergillus***
DMSO	NA*	NA*	NA*
100	NA*	NA*	6
250	NA*	NA*	7
500	NA*	NA*	9

NA*= No activity

Table- 2.3. Antifungal activity of [Cu(L2H)(OH)$_2$]

DRUG (µg/ml)	ZONE DIAMETER (mm)		
	Penicillium	***Fusarium***	***Aspergilus***
DMSO	NA*	NA*	NA*
100	6	6	16
250	8	9	22
500	14	11	28

NA*= No activity

Table. 2. 4. Antifungal activity of [Zn(L2H)$_2$X$_2$]

DRUG (µg/ml)	ZONE DIAMETER (mm)		
	Penicillium	***Fusarium***	***Aspergillus***
DMSO	NA*	NA*	NA*
100	5	NA*	NA*
250	8	NA*	NA*
500	10	NA*	NA*

NA*= No activity

Table 2. 5. Antifungal activity of of [Cd(L2H)$_2$X$_2$]

DRUG (µg/ml)	ZONE DIAMETER (mm)		
	Penicillium	***Fusarium***	***Aspergillus***
DMSO	NA*	NA*	NA*
100	18	NA*	20
250	25	NA*	25
500	30	NA*	33

NA*= No activity

Figure 1.

Antifungal activity of Ni(II) complex on the Aspergillus species

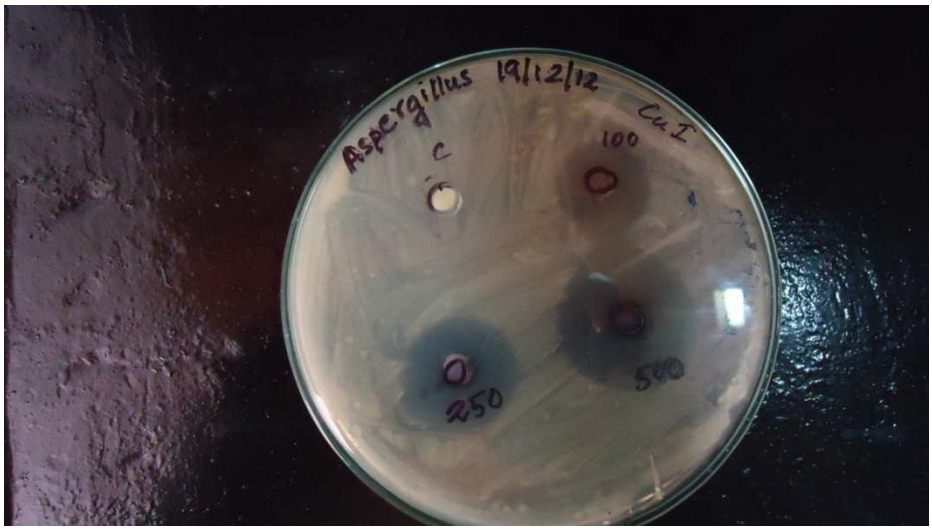

Figure 2.

Antifungal activity of Cu(II) complex on the Aspergillus species

3. Discussion

The antifungal activities of the complexes were compared on the basis of the consolidated Table 3.1 choosing a concentration of 500(µg/ml).

Table 3.1. Consolidated antifungal activity of complexes, Conc. 500 (µg/ml)

Sl. No.	Compound	ZONE DIAMETER (mm)		
		Penicillium	***Fusarium***	***Aspergillus***
1	Ligand, AacdMPTCH$_2$	NA*	NA*	NA*
2	[Co(L2H)$_2$X$_2$]	8	11	NA*
3	[Ni(L2H)$_2$X$_2$]	NA*	NA*	9
4	[Cu(L2H)(OH)$_2$]	14	11	28
5	[Zn(L2H)$_2$X$_2$]	10	NA*	NA*
6	[Cd(L2H)$_2$X$_2$]	30	NA*	33

The antifungal studies revealed that the metal complexes were more potent than the parent ligand. The studies showed that all the complexes were active towards three fungal stains in different manner. From the studies, Cu(II) and Cd(II) complexes were found to have maximum activity, while Co(II), Ni(II) and Zn(II) complexes exhibited lesser activity.

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CHAPTER I

METAL COMPLEXES AS ANTITUMOUR AGENTS

1. Introduction

Now a days cancer, medically know as malignant neoplasm, is one of the most dangerous diseases in the world. Eminent British oncologist, Sir Ruport Willis defined cancer as an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same direction in an excessive manner even after the cessation of the stimuli which evoked the change[1]. The word cancer was suggested by Hippocrates, a Greek physician. Cancer is a broad group of various diseases, all involving unregulated cell growth due to the change or mutation in DNA. Each mutation alters the behavior of the cell. Normal cells will be transformed to a cancer cells due to the genetic accumulation which is called carcinogenesis. The substances that are capable of producing tumours in any test species by any route and at any dose level are called carcinogens[2]. There are two types of tumours. The first type which is dangerous and cancerous is called Malignant and the second type, that can be removed surgically and not life threatening is called Benign[3] . There are over 200 different known cancers that affect almost every organ of the human bodies.

1.1. Causes of cancer

Determination of causes of cancer is complex and difficult as most of the cancers have multiple possible causes. An attempt was made in 1895 for the identification and observation of cancer by a German urologist, Rehn. He observed three men with Urinary bladder cancer working in a dye manufacturing factory. Cancer causation can also be studied in experimental settings. Such studies are complex and difficult to perform, but many permit a straight forward judgment of causation of cancer. The causes of cancers are as diverse as the nature of disease. They include environmental, life style, physical, chemical, biological and genetic factors.

More than 90% of cancer is due to the environmental factors which include air, water, ground, etc. Our environment is polluted with carcinogens like asbestos fibers, tobacco, smoke, benzene, etc. Benzene exposure at 50 to 100 ppm/year in the environment will cause leukemia in animals[4]. About 45% of cancer is attributed to life style factors and two-third of these results from smoking, which is one of the most important factors shown to cause lung cancer. Cancer of oral cavity, larynx, stomache, etc, will be caused by the use of tobacoo[5.6]. Habit of chewing tobacoo is one of the main reasons for oral cancer[7]. According to Moore *et al*[8], oral cancer is one among the ten common cancers. Alcohol is a typical chemical carcinogen[9]. Physical inactivity will produce negative effect on immune- and endocrine system

which increases body weight and contribute to cancer risk[10]. Chemical carcinogens include organic- and inorganic chemicals which may induce changes in DNA. (Examples are benzypyrene, aflatoxin, etc). Pure metals like selenium, aluminium, zinc, mercury and the ligand tryptophan are reported to cause cancer[11].

Certain viruses called Oncovirus cause cancer. (eg. cervical carcinoma, T-cell leukemias, etc). Similarly, certain bacteria like Helicobacterpylom induce gastric carcinoma[12]. Parasitic infections can also cause cholangiocarcinoma[13].

Radiations, including both ionizing (radon gas) and non-ionizing (ultraviolet) cause about 10% of invasive cancer[14]. Effect of radiation becomes more dangerous when it is combined with other cancer causing agents[15].

Diets play a prominent role in causing cancer. Diet with excess salt may produce gastric cancer. Diet consisting of charred animal fats, red meat and low quality fruits, vegetables and grains are connected with different types of cancers.

Cancer is a genetic disease too. Approximately 5 to 10% of cancers are hereditary which are caused by inherited genetic defects.

1.2. Treatment of cancer

Cancer is one of the major causes of death in the world. According to world Cancer Report from the International Agency for Research on cancer, cases of cancer doubled globally between 1975 and 2000, will double again by 2020, and will nearly triple by 2030[16]. Therefore, cancer is regarded as diseases that must be fought to end. Here lies the importance of cancer research, which includes the intense scientific efforts to understand the disease processes and to discover the possible therapies. Surgery, chemotherapy, radiation therapy and palliative care are the main options for the treatment of cancer. In the case of solid cancers, if the diagnosis and biopsies are perfect, surgery play an important role for prolongation of the survival. Chemotherapy is very useful for different types of cancers like breast cancer, pancreatic cancer, ovarian cancer, etc. In radiation therapy, the symptoms of cancer will be removed particularly in head and neck cancers. Sometimes radiation is used in addition to surgery and chemotherapy. There are various forms of therapy that are viewed as complementary and alternative cancer treatment which include acupuncture, homeopathy, etc. Instead of conventional medicine, palliatives are often used in the treatment of cancer, when people approach the end of life. In this, the medicine gives temporary relief from the symptoms of the disease but does not actually cure the disease.

1.3. Meal complexes as anticancer agents

Coordination compounds play an important role as antitumour agents. Their use is mainly due to the fact that may of them are soluble and thus make the metal available to the biological systems which may not be otherwise easily available. Literature survey reveals certain metal complexes show anticancer activities due to their toxicity towards cancer cells. Although metals were used in the medical field[17], the actual roles and potentials of metal based anticancer agents have been fully understood after the discovery of the biological activity of *cis*-platin, *cis*-dichlordiammninePt(II) compound[18]. Since the discovery of tumour inhibiting property of *cis*-platin in 1960's[19], it and carboplatin still play prominent role in cancer chemotherapy[20.21]. But it is noted that *cis*-platin is inactive towards some tumours that are very common (eg. lung tumours). In addition, it is having neuro-hepato- and nephrotoxicity[22]. As the therapeutic efficiency of platinum compounds are limited by cellular resistance, scientists were persuaded to develop other anticancer drugs with new metals. Walter Berger *et al*[23] studied the cytotoxic activity of drugs of gallium, lanthanum, bismuth, ruthenium, etc, prepared at the Institute for Inorganic chemistry, Vienna. The anticancer nature of half sandwich, piano-stool ruthernium(II) and osmium(V) arene complexes were suggested earlier[24]. Previous studies of ruthenium complexes show that they are good anticancer agents [25-27]. Ruthenium complex based drugs showed particular efficiency to control the growth of metasis of mouse

carcinomas and human xenografts[28]. Gold(III) complexes are isoelectronic and isostructural with Pt(II) complexes and are used in anticancer studies[29]. Novel gold(III) complexes, due to their low toxicity and more stability, showed *in vitro* pharmacological properties[30,31]. *In vitro* inhibitory effect of gold(I) antiarthritic drug, triethylphosphinegold(I) tetracetatothioglucose (auranofir) was confirmed by Teikink *et al*[32]. The *in vivo* anti tumour studies of 5 - fluorouracil and its Cr(III), Fe(III) and A1(III) complexes were carried out on C3H/He mice against P 815 murine mastocytoma[33]. The complexes of coinage metals [Cu(I)[34-39], Ag(I)[40,41] and Au(I)[42-48]] with aromatic tertiary phosphines and disphosphines were investigated for their *in vitro* anti tumour activities. The structure-activity relationships of diphosphine ligands and their complexes were ascertained. Studies on the anticancer properties of gallium(III) salts have been started since 1975[49,50]. Gallium nitrate was proved to be an effective drug for the treatment of hypercalcemia of malignancy.

1.4. Thiosemicarbazones as anticancer agents

Literature survey revealed that transition metal complexes of thiosemicarbazones have been ascribed special importance due to their antitumour properties[51-53]. The antitumour activities of Co(II), Ni(II), Cu(II) and Zn(II) complexes of the thiosemicarbazones derived from 3-acetylumbelliferone were studied. It was confirmed that Co(II) and Cu(II) complexes produced more inhibitory effects compared to the other

complexes[54]. Previous studies reported that a series of di-2-pyridylketone thiosemicarbazone (HDPT) complexes showed selective antitumour activity[55]. It has been reported that the divalent Ni(II), Cu(II) and Zn(II) complexes of thiosemicarbazone dissociate inside the cell liberating the respective free thiosemicarbazone which is responsible for the inhibition of tumour growth[56]. Thus, the divalant complexes of thiosemicarbazone act as transport vehicles of the ligand in to the cell, where they decompose to liberate the free chelator which show the antitumour properly[57]. The metal complexes of Triapine (3-aminopyrdine-2-carboxaldehydethiosemicarbazone) and similar thiosemi-carbazones were accepted as the strong antitumour agents due to their ability to inhibit ribonucleotide reductase (RNA) enzyme which promotes the DNA synthesis[58-63]. The antitumour activity of palladium(II) and platinum(II) thiosemicarbazone complexes have been reported[64-72]. The effect of these complexes against leukemia P 388 was confirmed[73]. The antitumour property of novel gallium(III) complex of 2–acetylpyridene N-dimethylthiosemi-carbazone was reported by Vladimir *et al*[74]. The bis-thiosemicarbazone of gold(I) and gold(III) showed anticancer activity against Jurkat, HL-60 and MCF-7 human cancer cell lines[75]. The cytotoxic activities of iron(III) and nickel(II) complexes of S-methyl thiosemicarbazones were studied. Anticancer activity of iron(III) complex of N^1-3-methoxysalicylidene – N^4-4-methoxysalicylidene –S – methyl- thiosemicarbazone against K 562 Chronic myeloid leukemia cell was confirmed[76]. Thus the literature survey revealed

that thiosemicarbazones posess a wide range of antitumour activities due to their ability to inhibit the biosynthesis of DNA by blocking the enzyme ribonucleotide diphosphate-reductase and binding to the nitrogen base of DNA. They also block base replication and make lesions in DNA helix by oxidative rupture[77,78].

1.5. Copper complexes of thiosemicarbazones as antitumour agents

Since the cost of platinum is very high, cheaper metals like copper, iron, etc, were tried in cancer chemotherapy. According to Petering, copper(II) alone will not have any antitumour activity[79]. But will act as an inhibitor of tumour growth in the chelate form[80,81]. Even though the actual pathway by which the copper chelate inhibits the cancer growth is not known, it is believed that this will be on the basis of structure - activity correlation as in the case of *cis*-platin (DNA intercalation)[82]. Earlier studies, based on the inhibition of DNA biosynthesis showed that copper complexes were more active as antitumour agents than Fe(III) compounds due to reversible reduction processes at accessible electrode potential of copper compounds[83]. Secondly, four coordinate planar copper(II) complexes will get attached to nitrogen base of DNA (nitrogen adduct formation), thus blocking base replication[84] leading to the inhibition of tumour growth. Previous studies showed that the copper(II) complex of 2-formylpyridene thiosemicarbazone is more powerful antitumour agent than the free ligand[85]. Copper(I)

diphosphine complex also showed cytotoxic activity in these tumour models in mice[86]. Literature review showed that a large number of compounds in chelation with copper were found to be of therapeutic use with promising results[87]. This is because the cancer cells have shown the tendency of taking more amount of copper than normal cells[88,89]. Moreover, copper metabolism is having connection with angiogenesis[90] and metastasis[91].

2. Scope of the present investigation

Cancer is one of the major causes of death in the world. Therefore, the research and the discovery of new compounds for its treatment is an important area in medicinal chemistry. Thiosemicarbazones and (bis)-thiosemicarbazones are famous for their cytotoxic activity against tumour cells both *in vitro* and *in vivo*[92-94]. This property is due to the capacity of thiosemicarbazones to inhibit ribonucleotide reductase, the enzyme used in the synthesis of DNA[95]. The pharmacological properties of thiosemicarbazones are enhanced when they are coordinated to metal ions[96]. This inhibitory action is thought to be due to the co-ordination of metal ions to thiosemicarbazones with N-N-S-ligating system. The therapeutic effect of thiosemicarbazone is stimulated when copper gets attached to it. As per the previously published papers the mechanism behind the anticancer property of copper(II) complex is due to the intracellular generation of reactive oxygen species (ROS) through thiol induced reduction of Cu(II) to Cu(I); even though

it is mentioned that this is not the major determining factor[97]. The reduction potential of the copper complex, the strength of the ligand field and various spectral properties account for the inhibitory activity of copper(II) complexes of thiosemicarbazones. Literature survey described a variety of copper complexes of thiosemicarbazones with antitumour properties which persuaded me to conduct the cytotoxic and antitumour studies of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (AacdMPTCH_2 Ligand) and its complexes with a special emphasis on its copper complex. The copper(II) complex was also subjected to the *in vivo* antitumour studies and long term cytotoxicity (MTT assay) studies.

CHAPTER II
MATERIALS AND METHODS

A brief account of the chemicals used and the procedures adopted for the evaluation of antitumour activities are given in the following pages.

1. Materials

1.1. Chemicals

All chemicals used were of AnalaR quality and purchased from Merck. Commercial solvents used for the synthesis were purified by standard methods[98]. Hydrated metal salts were used as such for the preparation of the complexes.

1.2. Synthesis of acetoacetanilide thiosemicarbazone ($AacdMPTCH_2$)

The ligand, $AacdMPTCH_2$ was prepared by refluxing an equal molar mixture of acetoacetanilide and N (4)-methyl(phenyl)thiosemicarbazide for 4 h (Figure 1). The detailed procedure for the synthesis of the ligand is described elsewhere[99].

1.3. Synthesis and characterization of thiosemicarbazone complexes

The complexes of Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) were prepared by refluxing $AacdMPTCH_2$ with the respective metal salt in 2:1 ratio

for 4 h (Figure 1). The compounds were characterized by elemental analysis, magnetic moment measurements, IR-, UV-visible- and ^{1}H NMR spectral studies[99].

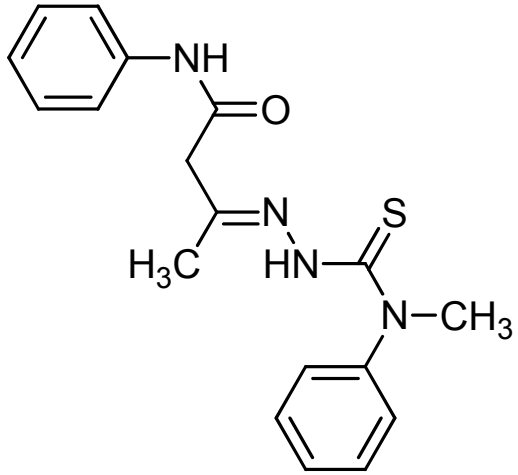


Figure 1.

Acetoacetanilide N(4)methyl(phenyl)thiosemicarbazone ($AacdMPTCH_2$)

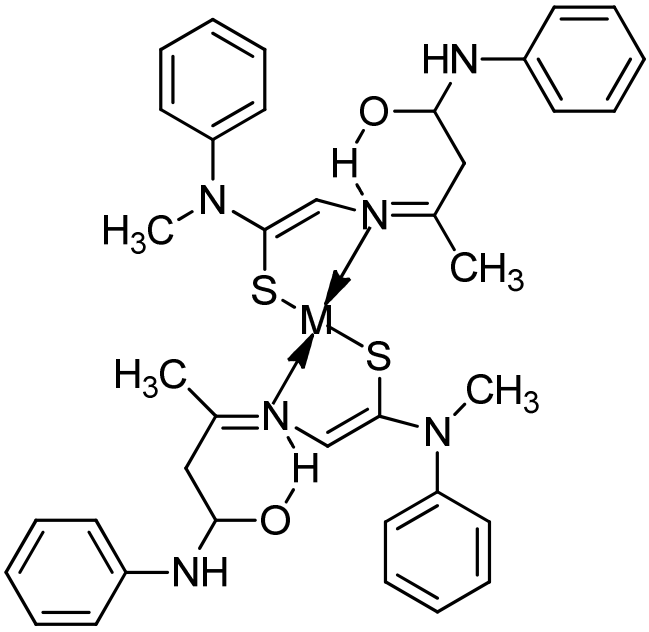


Figure 2.

The complex of $AacdMPTCH_2$

(M = Fe(II), Co(II), Ni(II), Cu(II), Zn(II) or Cd(II))

1.4. The preparation of drug

Fifty milligram of the compound was dissolved in 1 ml of dimethyl sulphoxide (DMSO). For *in vitro* studies, the drug was dissolved in DMSO and for *in vivo* studies 50 mg of drug was first dissolved in 1 ml DMSO and further it was diluted using distilled water to desired concentrations.

1.5. Animals

Swiss albino female mice (20-25 g) were obtained from the Small Animal Breeding Station (SABS), Mannuthy, Thrissur, Kerala. They were kept under standard conditions of temperature and humidity in animal house of Amala Cancer Research Centre. The animals were provided with standard mouse chow (Sai Durga Feeds and Foods, Bangalore, India) and water and libitum. All the animal experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division, Government of India.

1.6. Cell lines

Mouse lung fibroblast (L929 cells) were cultured in DMEM medium supplemented with FBS (10% v/v), streptomycin (100µg/ml) and penicillin (100µg/ml) and kept at 37°C in an incubator with 5% CO_2. Dalton's

Lymphona Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells maintained in intraperitoneal cavity of mouse were used for the study.

Ehrlich ascetic tumour is a rapidly growing carcinoma with very aggressive behaviour[100]. It is able to grow in almost all strains of mice. The Ehrlich ascetic tumour implantation induces a local inflammatory reaction with increasing vascular permeability which results in an intense edema formation, cellular migration and a progressive ascetic fluid formation[101]. The ascetic fluid is essential for tumour growth since it constitutes a direct nutritional source for tumour cells[102].

1.7. Preparation of phosphate buffered saline (PBS)

NaCl (8.00g), KC1 (0.20 g), $Na_2HPO_42H_2O$ (1.44g) and KH_2PO_4 (0.20 g) were dissolved in distilled water and the solution was made up to 1 lit. (pH=7.2).

1.8. Preparation of PBS-EDTA

EDTA (0.02g) was dissolved in 100 ml PBS. The solution was autoclaved and used for experiments.

1.9. Preparation of Trypsin (0.2%)

Dissolved EDTA (0.02g), glucose (0.02 g) and trypsin (0.2 g) in 100 ml PBS. The solution was filtered under sterile conditions and used for trypsinization.

1.10. Preparation of normal saline (0.85 %)

It was prepared by dissolving AR Nacl (0.85 g) in 100 ml distilled water.

1.11. Preparation of Minimum Essential Medium (MEM)

MEM (5.37 g) and 50 ml of goat serum were added to 450 ml of autoclaved double distilled water. The mixture was stirred well on a magnetic stirrer and the pH was adjusted to 7.2 by adding small quantities of sodium bicarbonate. It was sterilized by filteration and transferred to culture bottles.

2. Methods

2.1. Short term *in vitro* cytotoxic analysis – Trypan blue exclusion method

AacdMPTCH$_2$ and its metal complexes (test compounds) were studied for short term *in vitro* cytotoxicity using Dalton's lymphoma ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Cell viability was determined by Trypan blue exclusion method. Viable cell suspension (1 x 10^6 cells in 0.1 ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using Phosphate Buffered Saline (PBS). Control tube contained only cell suspension. These assay mixtures were incubated for 3 h at 37°C. Further, the cell suspension was mixed with 0.1 ml of 1% Trypan blue and kept for 2-3 minutes and

loaded on a haemocytometer. Dead cells take up the blue colour of Trypan blue while live cells do not take up the dye. The stained and unstained cells were counted separately. The percentage of dead cells was determined using the haemocytometer.

2.2. Long term *in vitro* cytotoxic analysis – MTT assay

For long term cytotoxicity, L929 cells were used. The cells were seeded in to 96 well flat bottom titre plates (5000 cells/well) containing 200µ*l* MEM with 10% FCS (Fetal Calf Serum) and incubated for 24 h at 37°C with 5% CO_2 atmosphere for the attachment of cells. After incubation, various concentrations of the test compounds were added to the wells in triplicates and the incubation was continued for 48 h. 20µ*l* of MTT (5 mg/mL in PBS) was added to each well before 4 h of the completion of incubation. After the incubation period, the plates were centrifuged, supernatant liquid was removed and 100 ml of DMSO was added to each well. The plate was then incubated at room temperature for 15 minutes and the optical density (O.D) was measured at 540 nm, using ELISA reader. The percentage of dead cells was determined using the formula,

% of dead cells = (1 – O.D of drug treated / OD of control) x 100.

2.3. Toxicity studies with metal complexes

Twenty four Swiss albino mice divided into 4 groups (6 animals / group). Animals were divided as follows:

Group 1 : 1 mg/kg b.wt, treated, Group 2 : 5 mg/kg b.wt, treated

Group 3 : 10 mg/kg b.wt, treated and Group 4 : 25 mg/kg b.wt, treated

The drug was administrated once daily (I.P) and continued for 10 weeks. The animals were observed for their mortality.

2.4. *In vivo* Studies

2.4.1. Effect of copper complex of AacdMPTCH$_2$ on the survival rate of ascites tumour bearing animals

Animals (female, 6-8 weeks old) weighing 28-30 g were divided into 5 groups of 6 animals each. Viable EAC cells (1×10^6) in 0.1 ml of phosphate buffered saline (PBS) were injected in to the peritoneal cavity.

Group 1 : control, Group 2 : 1 mg/kg b.wt, treated,

Group 3 : 5 mg/kg b.wt, treated and Group 4 : 10 mg/kg b.wt, treated,

Group 5 : Standard drug (cyclophosphamide)

Drugs (cyclophosphamide for group 5) were given by intraperitoneal injection from the first day of tumour induction. The death pattern of the

animals due to tumour burden was noted and the percentage of increase in life span (%ILS) was calculated as follows :

$$\%ILS = [T-C/C \times 100],$$

where 'T' and 'C' are mean survival of treated and control mice, respectively.

2.4.2. Effect of copper complex of AacdMPTCH$_2$ on solid tumour development

Swiss albino mice (female, 5-6 weeks old) weighing 20-25 g were divided into 5 groups comprising of 6 animals in each group for the above studies. Tumour was induced by injecting DLA cells (0.1 ml of 1 x 10^6 cells per mouse) in to the right hind leg of mice. Group 1 was kept as control and groups 2, 3 and 4 were treated with copper complex of AacdMPTCH$_2$. Group 5 was treated with cyclophosphamide. The tumour development in animals of each group was determined by measuring the diameter of tumour growth in two perpendicular planes using a digital vernier caliper starting from seventh day of toumor induction, up to 34th day. The tumour volume was calculated using the formula, $V=4/3\pi r_1^2 r_2$, where r_1 was the minor diameter and r_2 was the major diameter[103].

CHAPTER III

CYTOTOXIC AND ANTITUMOUR STUDIES OF ACETOACETANILIDE N(4)-METHYL(PHENYL)THIOSEMICARBAZONE AND ITS TRANSITION METAL COMPLEXES

In 2008, approximately 12.7 million cancer patients were diagnosed and 7.6 million people died of cancer. The most common being, lung cancer, stomach cancer, liver cancer and breast cancer. Though many diseases (such as heart failure) may have worse effect on the society, it is believed that cancer is the most dangerous, deadly disease reflected in the society.

Bearing in mind that the N-N-S or O-N-O donor system is a common feature for many of the compounds with carcinostatic potency, we proceeded with the antitumour studies of the complexes of ligands with same nature i.e. AacdMPTCH$_2$ and its complexes. We got promising results.

Cancer is a fatal disease rating at the top of the three causes of death due to the lack of effective drugs[104]. The unique properties of metal ions are very useful in the preparation of new drugs. Due to the toxic side effect of the common chemotherapeutic agent *cis*-platin, several other new metal based anticancer agents were developed by the chemists. Because of their biological activity, including antitumour and anticarsinogenic[105] properties, metal complexes of thiosemicarbazones with transition metals have been given

much importance. The most active compounds of non-platinum antitumour agents include the copper complexes of thiosemicarbazones. The previous studies showed that copper complexes of 3-ethoxy-2-oxobutyraldehyde thiosemicarbazones, (CuKTS)[106] and N-heterocyclic thiosemicarbazones[107] are important compounds of this class. Keeping all these facts in mind, copper complex of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone has been selected for the antitumour studies.

1. Results

The results obtained from the short term *in vitro* cytotoxic analysis, long term *in vitro* cytotoxic analysis (MTT Assay) solid tumour reduction experiment and the survival rate of ascites tumour are presented here.

1.1. Short term *in vitro* cytotoxic analysis

AacdMPTCH$_2$ ligand and its complexes of Fe, Co, Ni, Cu, Zn and Cd showed marked cytotoxic activity for DLA cell line, (Table.1, Figure 1). The copper complex showed maximum activity and the concentration required for 50% death (IC_{50}) was found to be 46 µg/ml. The AacdMPTCH$_2$ ligand showed only very low cytotoxic activity. Figures 2, 3, 4, 5, 6, and 7 shows the cytotoxic activity of Fe, Co, Ni, Cu, Zn and Cd complexes respectively, in comparison with the ligand.

Table 1.1. In vitro cytotocicity of ligand and its different complexes

Concentr-ations (µg/ml)	**Percentage cytotoxicity of complexes**						**Ligand**
	Fe	**Co**	**Ni**	**Cu**	**Zn**	**Cd**	**(AacdMPTCH_2)**
10	0	0	3	18	7	2	0
20	2	0	7	24	14	9	0
50	6	5	11	58	20	12	0
100	10	8	20	70	35	36	2
200	20	18	33	92	58	42	4

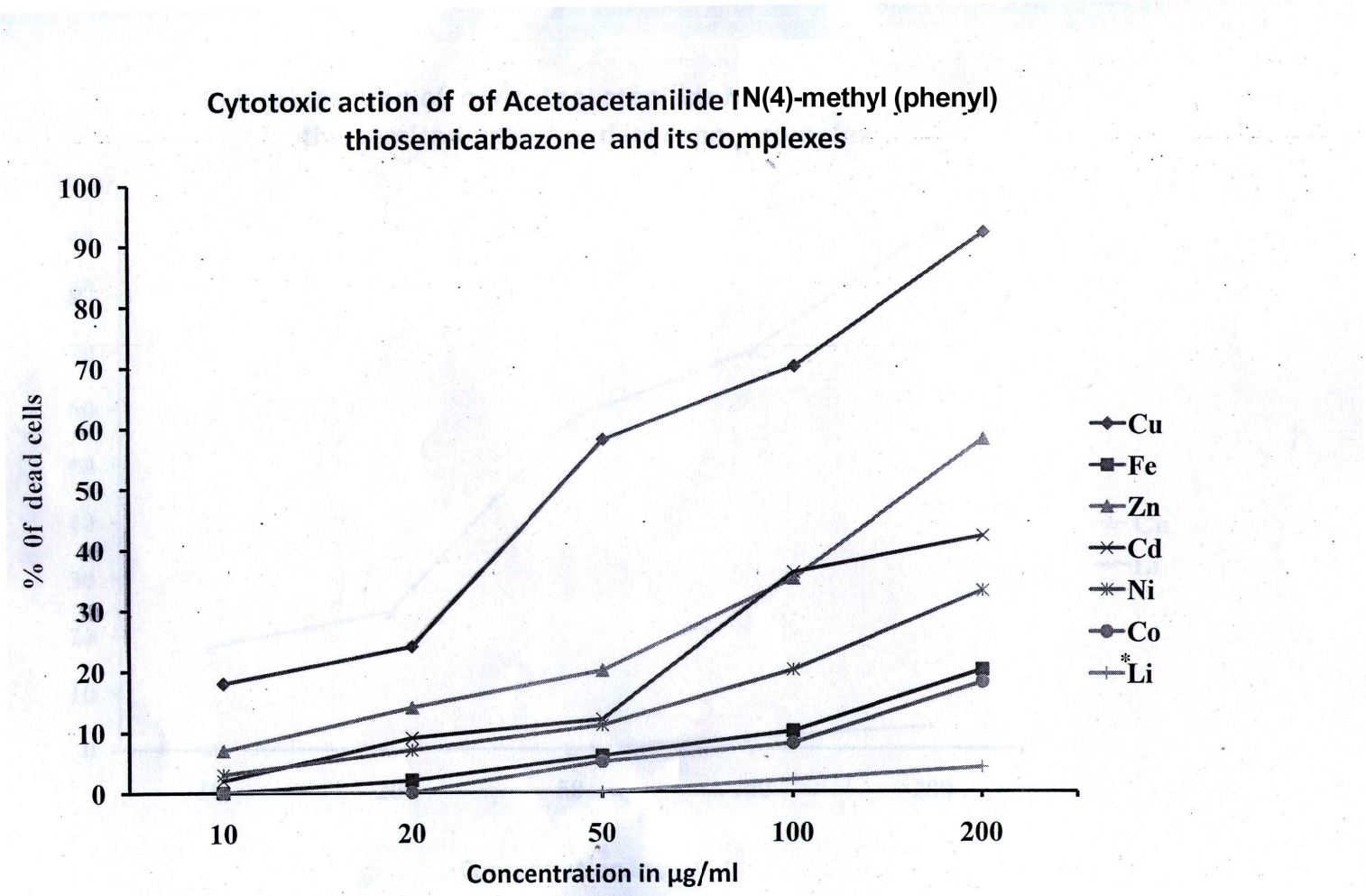


Figure 1.

*Li=ligand(AacdMPTCH$_2$)

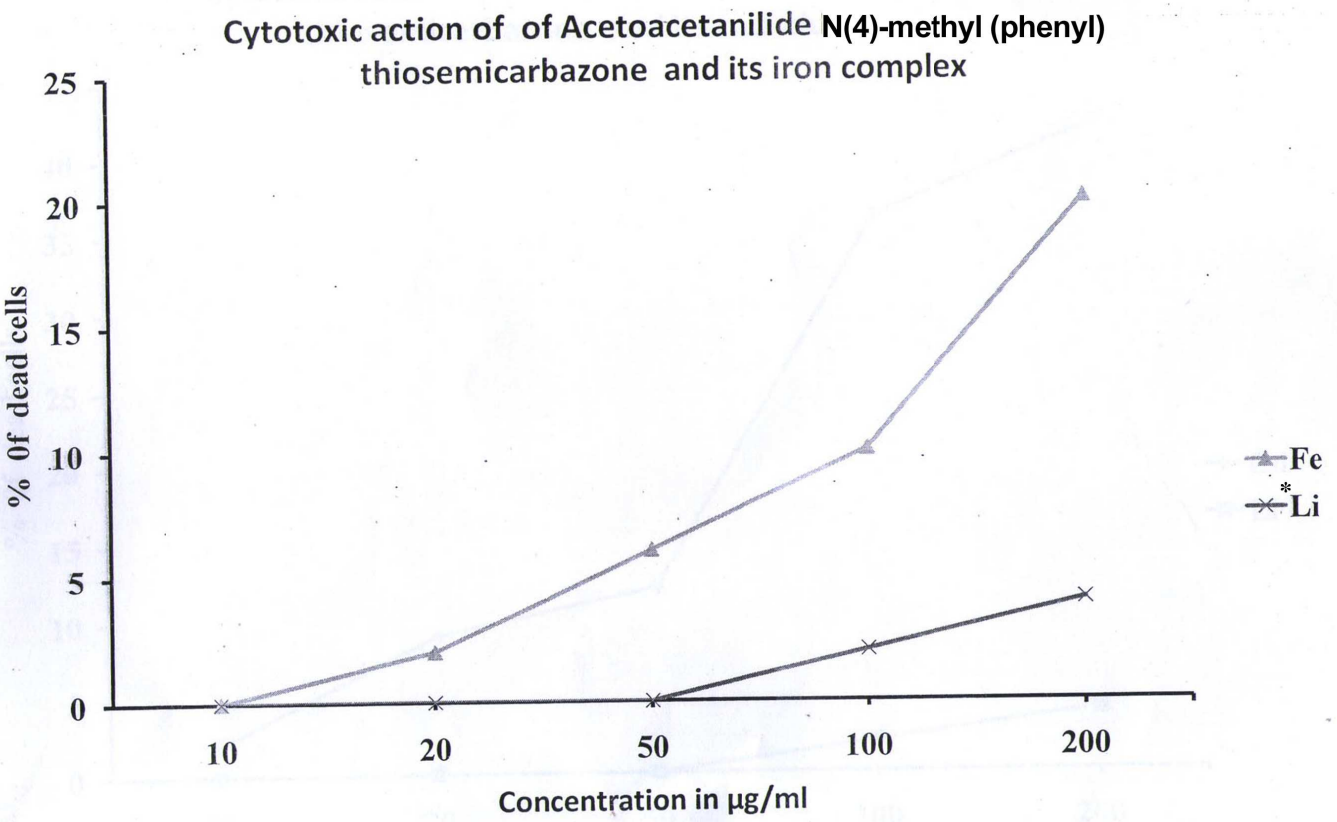


Figure 2.

*Li=ligand($AacdMPTCH_2$)

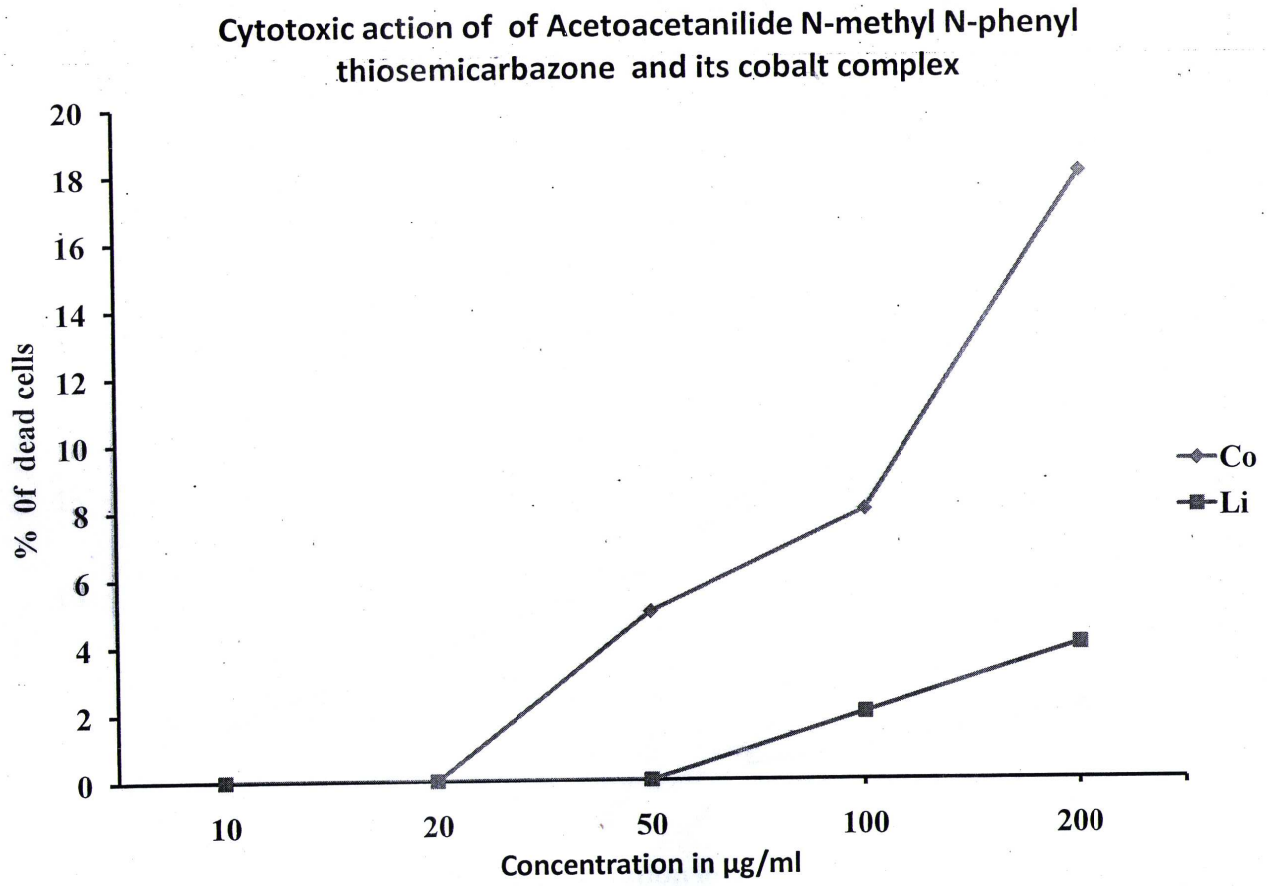


Figure 3

*Li=ligand(AacdMPTCH$_2$)

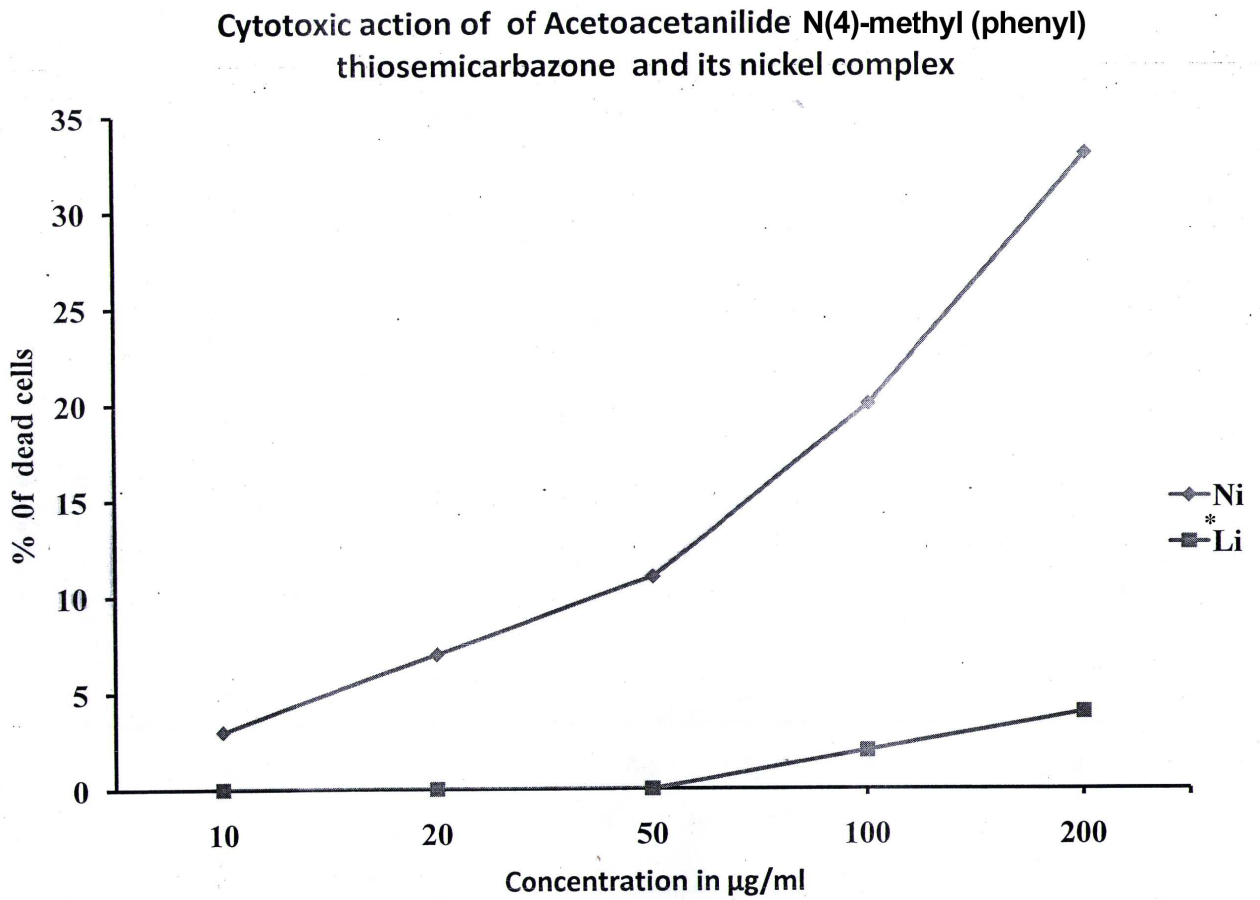


Figure 4.

*Li=ligand(AacdMPTCH$_2$)

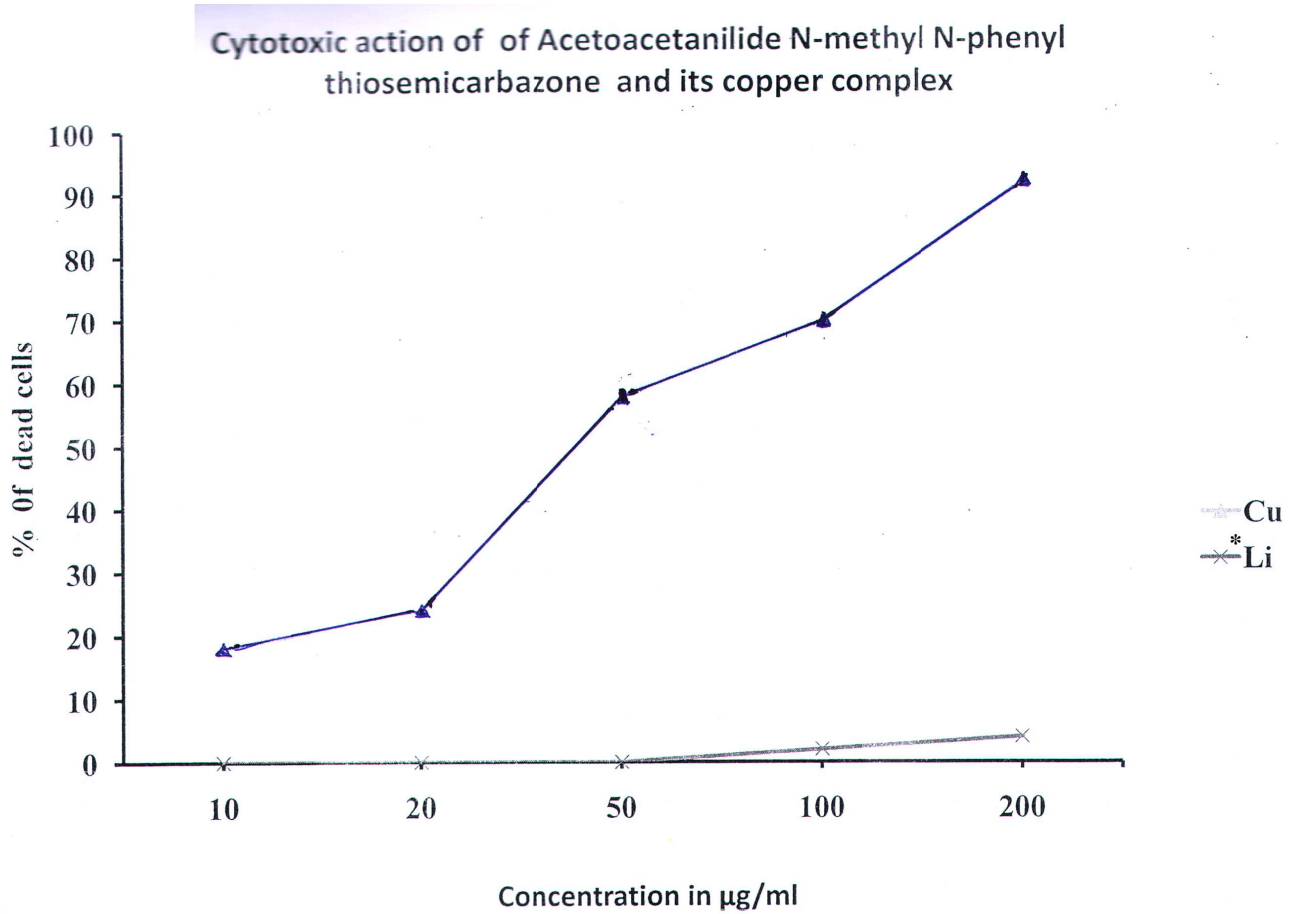


Figure 5.

*Li=ligand($AacdMPTCH_2$)

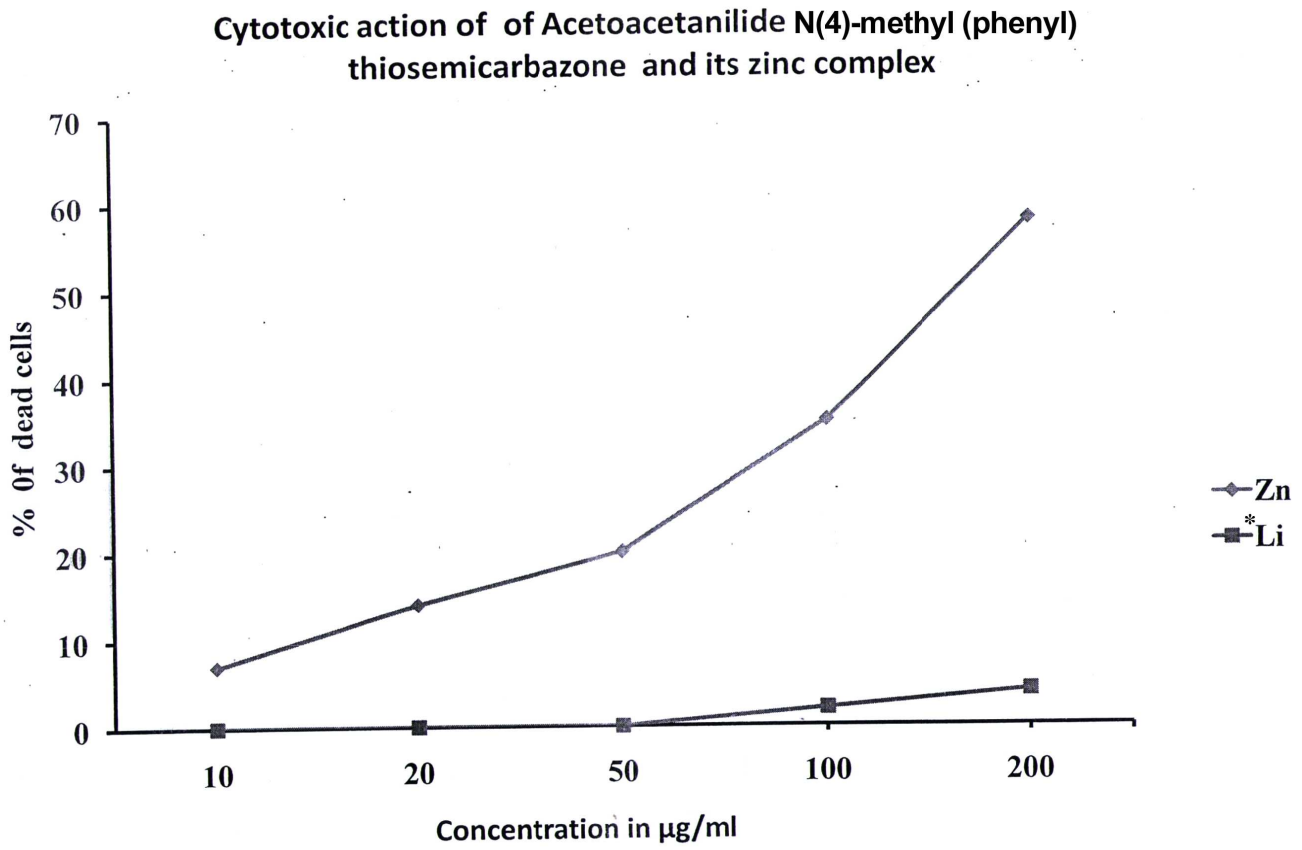


Figure 6

*Li=ligand(AacdMPTCH$_2$)

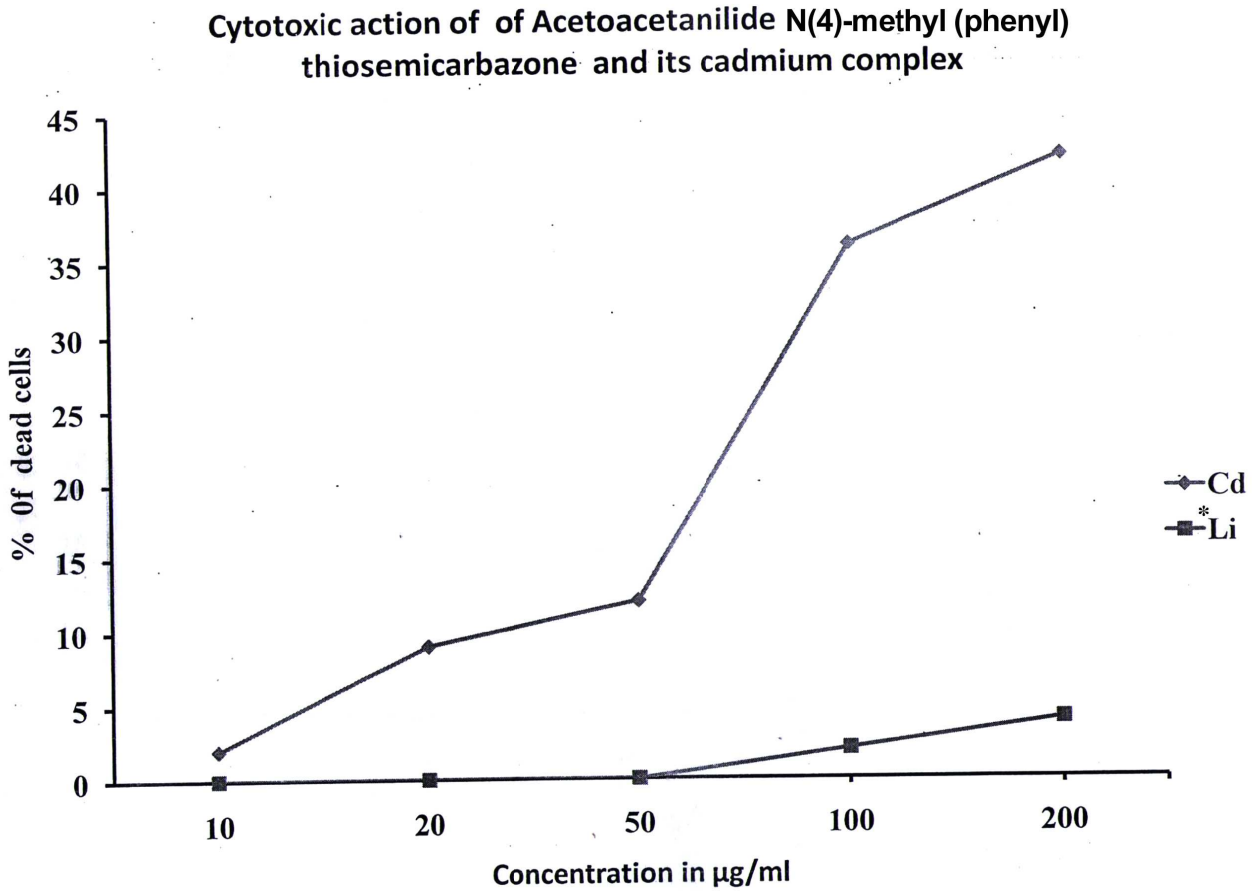


Figure 7.

*Li=ligand($AacdMPTCH_2$)

1.2. Long term *in vitro* cytotoxic analysis (MTT Assay)

The results of long term *in vitro* cytotoixicity of the copper complex of AacdMPTCH$_2$ showed that it is non- toxic up to 0.5µg/ml towards L929 cells (Table 1.2).

Table 1.2. Long term in vitro cytotoxicity (MTT) of copper complex of AacdMPTCH$_2$

Concentrations (µg/ml)	**% cytotoxicity**
Control	0
0.05	0.37±0.03
0.1	0.51±0.03
0.25	0.61±0.03
0.5	1.05±0.03

1.3. Toxicity studies

The result of toxicity studies of copper complex, AacdMPTCH$_2$ on 24 Swiss albino mice, 4 groups, at four different concentrations (25, 10, 5 and 1mg/kg body weight) showed that 25 mg/kg body weight is toxic to the animals. And this concentration was avoided and 10, 5 and 1 mg/kg body weight were selected for *in vivo* studies.

1.4. *In vivo* Studies

1.4.1. Effect of copper complex on ascites tumour development

The animals of the tumour control group survived for a period of 15.8±0.65days. The animals treated with cyclophosphamide survived for 26.63±1.8 days and those treated with the copper complex at 10, 5 and 1 mg/kg body weight increased the survival rate of animals by 21±2.1 days. 19.6±1.4 days, and 18.6±1.2 days, respectively [Table 1.4.1 (a)]. Thus the copper complex was also found to be effective in increasing the average life span of animals by 32.9, 24.05 and 17.7% days, respectively, at 10, 5 and 1 mg/kg body weight [Table 1.4.1.(b)]. The pictures of the normal mice and ascites tumour bearing mice are shown in figures 8 and 9 respectively.

Table 1.4.1(a). Effect of copper complex of AacdMPTCH$_2$ on the mean survival rate of ascites tumour bearing mice.

Treatment	**Mean survival rate**
Control	15.8±0.65
10mg/kg b.wt	21±2.1
5mg/kg b. wt	19.6±1.4
1.5mg/kg b.wt	18.6±1.2
Standard (Cyclophosphamide)	26.63±1.8

Table 1.4.1(b). Effect of copper complex of AacdMPTCH$_2$ on the life span of ascites tumour bearing mice

Treatment	**Increase in life span (%)**
Control	---
10mg/kg b.wt	32.90
5mg/kg b. wt	24.05
1mg/kg b.wt	17.70
Standard-Cyclophosphamide (10mg/kg b.wt)	68.40

Normal mice

Figure 8.

Ascities tumour bearing mice

Figure 9.

1.4.2. Effect of copper complex on solid tumour development

In control animals, the volume of tumour was increased to 2.973 cm^3 on 34^{th} day while in copper complex treated animals; there was a significant reduction of tumour volume. (Figure10). At 10 mg/kg body wt, the volume was 1.1605 cm^3, while at lower concentrations (5 and 1 mg/kg body wt) the tumour volumes were 1.4692 and 2.1011 cm^3, respectively, Treatment with cyclophosphamide reduced the tumour volume to 0.583 cm^3 (Table 1.4.2). The pictures of the control- and treated mice are shown in the figures 11and 12 respectively.

Table 1.4.2. Effect of copper complex of AacdMPTCH$_2$ on the reduction of tumour volume.

	No of days observation									
Treated mg/kg : b.wt	**Initial**	**10**	**13**	**16**	**19**	**22**	**25**	**28**	**31**	**34**
Mean volume	0.0986	0.6594	0.8353	1.3440	2.0350	2.2830	2.5140	2.6360	2.8300	2.9730
10mg/kg b.wt	0.0747	0.4361	0.5759	0.7555	0.8338	0.8899	1.0316	1.05845	1.1053	1.1605
5	0.0799	0.5113	0.8774	1.1676	1.2633	1.4080	1.3512	1.4402	1.4553	1.4692
1	0.0832	0.7139	0.8850	1.3141	1.4945	1.7766	1.9121	1.9293	1.9806	2.1011
Std.	0.0800	0.2200	0.2600	0.2900	0.3700	0.4000	0.4700	0.5100	0.5700	0.5830

Effect of copper complex on solid development

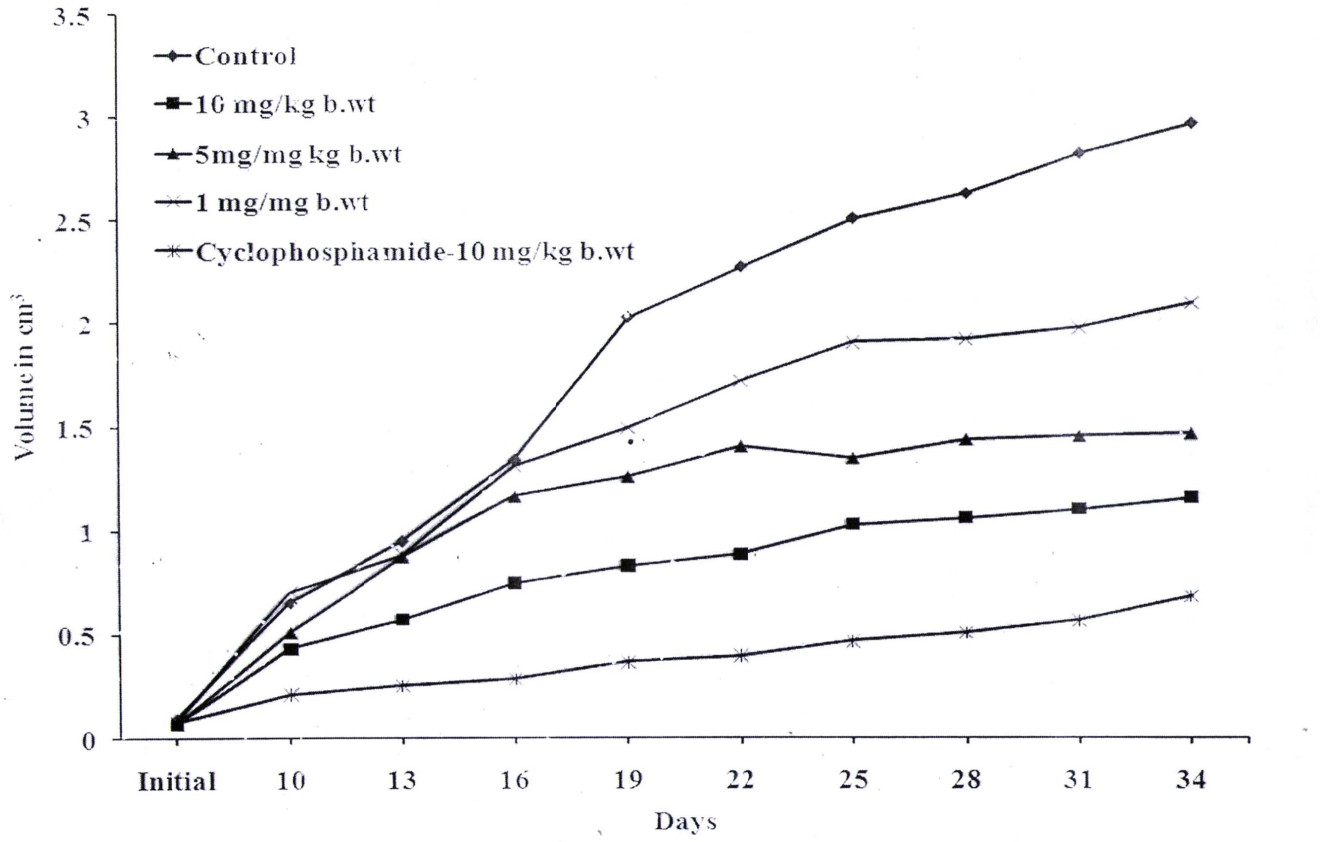
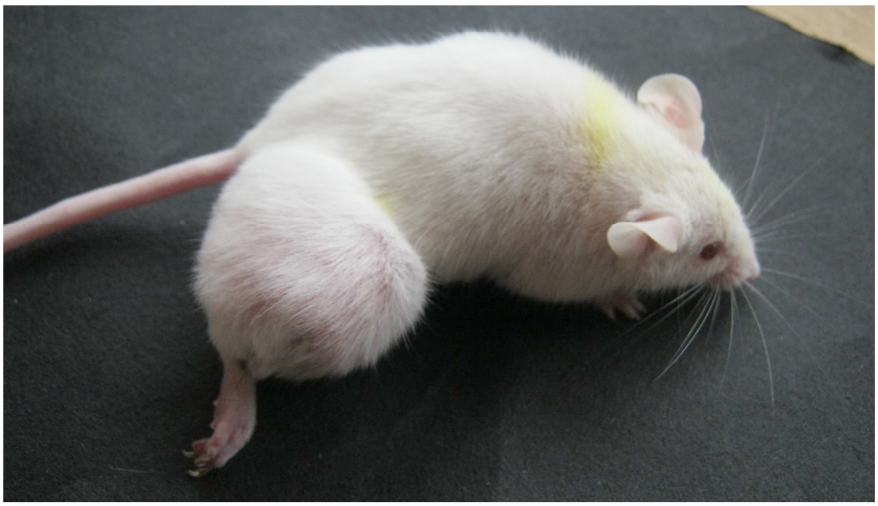


Figure 10.



Control

Figure 11.

Copper complex treated (10mg/kg b. wt)

Figure 12.

2. Discussion

In vitro studies on AacdMPTCH$_2$ ligand and different complexes showed cytotoxicity against DLA cell lines. The copper complex showed maximum cytotoxicity and its IC_{50} value was found to be 46 µg/ml.

The copper complex of AacdMPTCH$_2$ was found to be effective against DLA induced solid tumour and EAC induced ascites tumour. The 10 mg/kg body weight was more effective than the other two concentrations (5 and 1 mg/kg b.wt) in both the cases.

The increased activity of the copper(II) complex in comparison with the ligand may be due to lipophilicity of the drug, as a result of metal coordination. Hydrogen bonding also plays a role in antitumour mechanism of the drugs. Adenine, guanine and cytocine have amino groups, which are capable of forming hydrogen bonds with the drugs, thus reducing normal cell metabolism. Ehrlich ascetic tumour is a rapidly growing carcinoma with very aggressive behavior[108]. It is able to grow in almost all types of mice. The Ehrlich ascetic tumour implantation induces a local inflammatory reaction with increasing vascular permeability which results in an intense edema formation, cellular migration, and a progressive ascetic fluid formation[109]. The ascetic fluid is essential for tumour growth since it constitutes a direct nutritional source for the tumour cells[110].

3. Conclusion

The present study indicates the *in vitro* cytotoxic and antitumour properties of the copper complex of acetoacetanilide N(4)-methyl(phenyl)thiosemicarbazone (AacdMPTCH_2) suggesting its potential use as an anticancer agent.

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