

# Chapter One

## 1. Introduction and Literature review

### 1.1. Introduction:

#### 1.1.1. General Introduction of Schiff Base:

Schiff bases are condensation products of primary amines with carbonyl compounds and they were first reported by Schiff <sup>(1)</sup> in 1864. The common structural feature of these compounds is the azomethine group with a general formula  $\text{RHC}=\text{N}-\text{R}^1$ , where R and  $\text{R}^1$  are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. These compounds are also known as anils, imines or azomethines. Several studies <sup>(2,3)</sup> showed that the presence of a lone pair of electrons in  $\text{sp}^2$  hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance. Because of the relative easiness of preparation, synthetic flexibility, and the special property of C=N group, Schiff bases are generally excellent chelating agents,<sup>(4-7)</sup> especially when a functional group like –OH or –SH is present close to the azomethine group so as to form a five or six membered ring with the metal ion. Versatility of Schiff base ligands and biological, analytical and industrial applications of their complexes make further investigations in this area highly desirable.

Schiff bases have been known since 1864 when Hugo Schiff reported the condensation of primary amines with carbonyl compounds <sup>(1)</sup>. Nowadays, the research field dealing with Schiff base coordination chemistry has expanded enormously. The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed <sup>(8)</sup>.

Schiff bases resulted from aromatic aldehydes *ortho-substituted* with a hydroxyl group have initially arouse the researchers' interest because of their ability to act as bidentate ligands for transitional metal ions <sup>(9-11)</sup>. Later, in studies concerning quantitative structure-

antitumor activity relationship of a series of Schiff bases derived from variously substituted aromatic amines and aldehydes, it has been shown that azomethines from salicylaldehydes gave the best correlation <sup>(12)</sup>. Schiff bases of salicylaldehydes have also been reported as plant growth regulators and antimicrobial or antimycotic <sup>(13)</sup> activity. Schiff bases also show some analytical applications <sup>(14)</sup>. Schiff Bases are characterized by the -N=CH- (imine) group which imports in elucidating the mechanism of transamination and rasemination reaction in biological system <sup>(15,16)</sup>. Schiff bases are active against a wide range of organisms for example; *Candida Albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus polymxa*, *Trychophyton gypseum*, *Mycobacteria*, *Erysiphe graminis* and *Plasmopora viticola*.

## **1.2. Literature review:**

### **1.2.1. Schiff's Base Complexes:**

Schiff bases derived from an amino and carbonyl compound are an important class of ligands that coordinate to metal ions via azomethine nitrogen and have been studied extensively <sup>(17)</sup>. In azomethine derivatives, the C=N linkage is essential for biological activity, several azomethine has been reported to possess remarkable antibacterial, antifungal, anticancer and antimalarial activities <sup>(18,19)</sup>. The complexes of copper with Schiff bases have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical, anti-inflammable activity, antiradical activities and biological activities <sup>(20)</sup>. Schiff-base complexes are considered to be among the most important stereo chemical models in main group and transition metal coordination chemistry due to their preparative accessibility and structural variety <sup>(21)</sup>. Copper (II) complexes show distorted octahedral and tetrahedral symmetries due to d<sup>9</sup> configuration (Jahn Teller effect). The distortion is usually seen as axial elongation consistent with the lability and geometric flexibility of the complex. Therefore, typical Cu (II) complexes have square planar or square pyramidal geometries with weakly associated ligands in the axial position (s), but some copper (II) complexes possess trigonal bipyramidal geometry. The fundamental role of copper and the recognition of its complexes as important bioactive compounds in vitro and in vivo aroused an ever-increasing interest in these agents as

potential drugs for therapeutic intervention in various diseases. The vast array of information available for their bioinorganic properties and mode of action in several biological systems combined with the new opportunities offered by the flourishing technologies of medicinal chemistry, is creating an exciting scenario for the development of a novel generation of highly active drugs with minimized side effects, which could add significantly to the current clinical research and practice. A considerable number of Schiff's base copper complexes have potential biological interest, being used as more or less successful models of biological compounds <sup>(22)</sup>. Not only they have played a seminal role in the development of modern coordination chemistry, but also they can also be found at key points in the development of inorganic biochemistry, catalysis and optical materials<sup>(23)</sup>.

### **1.2.2. Antimicrobial activities:**

#### **1.2.2.1. Antibacterial activities:**

Tetradentate Schiff base ligands derived from Knoevenagel condensation of  $\beta$ -ketoanilides and furfural with o-phenylenediamine and diethylmalonate and their Cu (II) complexes showed antibacterial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by disc diffusion method. It has been reported that complexes have higher antibacterial activity than that of free ligand <sup>(24,25)</sup>. Bidentate Complexes of Cu (II) with Schiff bases derived from 2,6-diacetylpyridine ( $L^1$ ), 2-pyridine carboxaldehyde, 3-amino-5-methyl isoxazole with 5-methyl furan-2-carboxyaldehyde, 5-methyl thiophene-2-carboxaldehyde and pyridine-2-carboxaldehyde coordinate through the azomethine nitrogen, furfural oxygen, thiophene sulphur and pyridine nitrogen, respectively show antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Mycobacterium Smegmatis*, *Pseudomonas aeruginosa*, *Enterococcus cloacae*, *Bacillus megaterium* and *Micrococcus leteus*. The results showed that ( $L^1$ ) ligand has a greater effect against *E. coli* than the other bacteria, while it has no activity against *S. aureus*. Metal complexes have a greater effect against almost all bacteria <sup>(26,27)</sup>. Schiff base of aroyl-hydrazone and its copper complexes has been screened for antibacterial activity against two Gram positive bacterial strains (*B. subtilis*

and *S. aureus*) and two Gram-negative bacterial strains (*E. coli* and *P. fluorescence*) by the (MICs) method. Schiff base showed significant activity against two Gram-negative bacterial strains with MIC but inactive against two Gram positive bacterial strains <sup>(28)</sup>. Bidentate Schiff base complex derived by the condensation of Cinnamaldehyde hydrazone with different benzaldehyde Cu (II) compounds screened more active against gram positive bacteria *Bacillus subtilis* than gram negative bacteria *E. Coli* <sup>(29)</sup>. Azo Schiff's base ligand (N'E)-N'-(5-((4-chlorophenyl)diazenyl)-2-hydroxybenzylidene)-2-hydroxyl benzohydrazide complexes with VO (II), Co (II), Ni (II), Cu (II), and Zn (II) has been studied against several microorganisms by the well diffusion method. In general, the activity order of the synthesized compounds can be represented as Cu (II) > Co (II) > Ni (II) > Zn (II) > VO (II) > Ligand <sup>(30)</sup>.

Bidentate complexes of Co (II), Cu (II) and Cd (II) with benzofuran-2-carbohydrazide and benzaldehyde [BPMC] or 3,4-dimethoxybenzaldehyde [BDMePMC] showed biological activities but Cu (II) complex of [BPMC] and [BDMeOPMC] are more active against *S. aureus* as compared to Co (II) and Cd (II) complexes. Bidentate ligand of 2-[(2-[1-(hydroxyphenyl) ethyl] aminophenyl) ethanimidoyl] phenol derived from o-phenylenediamine are effective against <sup>(31)</sup> *Bacillus cereus*, *Staphylococcus aureus* and *E. Coli*. Cu (II) complexes with o-phenylenediamine (L<sup>1</sup>) and 2-hydroxyacetophenone (L<sup>2</sup>) has been screened for antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus* and *E. Coli* and tested by MuellerHinton agar plates. *E. coli* resistance L<sup>2</sup> was given best results due to the presence of hydroxyl group (2 OH) in the compound and L<sup>1</sup> as free ligand is biological less effective than that of its coordinate with copper due to the presence of (Cu<sup>2+</sup>) ion <sup>(32)</sup>. Antibacterial activity of the Clomiphene citrate copper complex has been determined against *E.coli*, *Staphylococcus aureus* and *Xanthomonas vesicatoria* by Disk diffusion method <sup>(33)</sup>.

The mixed ligand complexes of Cu (II) with Schiff bases N-(2-hydroxy-1-naphthylidene)-4chloroaniline (L<sup>1</sup>) and N-(2-hydroxybenzylidene)-2,3-dimethylaniline (L<sup>2</sup>) reported to show some antibacterial activity to certain extent against *E.coli*, *S. aureus*, *B. subtilis*, and *S. typhi*., but their complexes exhibit comparatively greater amount of activity against these bacteria <sup>(34)</sup>. Mixed ligand complexes of Cu (II) Schiff base ligands derived from o-hydroxy benzaldehyde and amino phenols has been reported to show antibacterial activities <sup>(35)</sup>.

Schiff bases derived from 2-formylindole, salicylaldehyde and N-amino rhoda nine and their copper complexes have been screened for antimicrobial activities against *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. The results indicated that the free ligands do not have any activity, where as their complexes showed more activity against same bacteria <sup>(36)</sup>. Mixed ligand complexes of o-vanillidene-2-aminobenzothiazole and 1,10phenanthroline has been screened for their in vitro biological activities against bacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella Typhi* and *Vibrio parahaemolyticus* by well diffusion method using agar nutrient <sup>(37)</sup>.

It has been reported that the novel 14-membered macro cyclic Schiff base derived from 3-cinnamalideneacetanalide and o-phenylenediamine, gave good results in the presence of copper ion in the ligand system <sup>(38)</sup>. Twenty Schiff bases of 2-amino-5-aryl-1,3,4-oxadiazoles were synthesized with different aromatic aldehydes were investigated against *Proteus mirabilis* and *bacilius subtilis* <sup>(39)</sup>. Two thiocyanatobridged dinuclear copper (II) complexes derived from 2, 4-dibromo-6-[(2-diethylaminoethylimino)methyl] phenol and 4-nitro-2-[(2-ethylaminoethylimino) methyl]phenol showed wide range of antibacterial activity <sup>(40)</sup>. Complexes of a tridentate Schiff base from the condensation of S-benzylidithiocarbazate with salicylaldehyde showed very strong activity against bacteria <sup>(41)</sup>. Copper (II) complexes of Schiff's base derived from o-phenylenediamine and 5-bromosalicylaldehyde possess growth inhibitory activity against *E. coli* and *staphylococcus aureus* <sup>(42)</sup>. Schiff base, synthesized from 5-bromo-3-fluorosalicylaldehyde and benzidine and its complexes with transition metal ions such as; Cu (II) and Ni (II) has been tested against four pathogenic bacteria (*S. aureus* and *B. subtilis*) as Gram-positive bacteria and (*E. coli* and *K. pneumoniae*) as Gram-negative bacteria. Copper complex showed higher activity than Ni complex <sup>(43)</sup>.

The metal complexes show higher activity than the free ligand against the same organism under identical experimental conditions, such increased activity of the metal chelates can be explained on the basis of chelation theory <sup>(44)</sup>. Antibacterial activity studies of Cu (II) complex of Schiff base derived from 3,3'-{1,2phynylenebis-[nitrilo(E) methylylidine]} diquinolin-2-ol has been carried out. A cup plate method has been employed for the in vitro

study of antibacterial effect against, *Staphylococcus aureus* and *E. coli*. Screening data indicated that the metal chelates exhibit a higher inhibitory effect than the free ligand <sup>(45)</sup>. Complexes of chlorosalicylidene aniline with Co (II) and Cu (II) screened for antibacterial activity against several bacterial strains, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The metal complexes showed enhanced antibacterial activity as compared to uncomplexed ligands. Copper complex showed greater activity against *Pseudomonas aeruginosa* as compared Co (II) complex <sup>(46)</sup>. Schiff bases ligands and their copper complexes synthesized from sulphonamide and resacetophenone have been evaluated for their antimicrobial activity against both Gram-positive and Gram-negative bacteria *B. megaterium*, *E. coli*, *B. subtilis* and *P. fluoresces*. The comparison of antimicrobial activities of the free ligands and complexes shows that the presence of metal causes more inhibition i.e., more activity <sup>(47)</sup>. Similarly schiff base Cu (II) complexes of the type [HLMClH<sub>2</sub>O] and [HLMOAcH<sub>2</sub>O] synthesized by condensation of oxalyldihydrazide and 3-tert-butyl-2-hydroxy-3-(3-phenylpent-3-yl) benzaldehyde have been screened for their antibacterial activity against the bacteria *Staphylococcus aureus* and *Bacillus subtilis* (as gram positive bacteria) and *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi* (as gram negative bacteria). The results compared with standard drug (Imipenem) have indicated that compounds were more active than the standard drugs <sup>(48)</sup>. Copper (I) 4-benzylimino-2,3-dimethyl-1-phenyl pyrazal-5-one complex showed remarkably active against *Staphylococcus aureus* as compared with zinc complex. The standard drug used (ciprofloxacin) did not show any inhibition <sup>(49)</sup> against *Staphylococcus aureus*. Schiff base derived from sulfonamide and their copper (II) complexes have been screened for their in vitro antibacterial activity against bacterial strains, *Escherichia coli*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Salmonella typhi* <sup>(50)</sup>. Copper (II) complexes with Schiff bases derived from pyrrole-2-carboxaldehyde and 2-hydroxy-1-naphthaldehyde and 2,3-diaminopyridine showed antibacterial activity <sup>(51)</sup>.

#### 1.2.2.2. Antifungal activities:

The antimicrobial activity of the N-(2-hydroxy-1-naphthylidene) phenylglycine and its copper complexes has been investigated. The antifungal screening data indicated that the activity of the ligand has increased upon complexation. Cu (II) complexes have shown

better antifungal activity compared to the ligand and the corresponding metal salts <sup>(52)</sup>. Tetradentate Schiff base and their Cu (II) complexes showed antifungal activity against *Aspergillus niger*, *Rhizopus stolonifer*, *Aspergillus flavus*, *Rhizoctonia bataticola* and *Candida albicans* by well diffusion method. Antifungal activity of the Clomiphene citrate Cu compounds has been determined against two fungi, *A. flavus* and *A. niger*, by Batemann poisoned food technique <sup>(33)</sup>.

The fungicidal effect of salicylaldehyde containing formaldehyde and piperazine moiety and its metal polychelates has been determined against *Candida albicans*, *Aspergillus*. Cu (II) polychelate exhibited high activity against *Candida albicans* and the other show mild activity. The presence of N and O donor groups in the ligand and its metal polychelates inhibited enzyme production because enzymes that require free hydroxyl group for their activity appear to be especially susceptible to deactivation by the metal ion of polychelates. All the metal polychelates are more toxic than the ligand <sup>(53)</sup>. Neutral complexes of Cu (II) with Schiff bases derived from 3-nitrobenzylidene-4-aminoantipyrine and aniline or p-nitroaniline or p-methoxyaniline showed antifungal activity. A comparative study of the MIC values for the ligands and their complexes indicates that the complexes exhibit higher antifungal activity. Such increased activity of the complexes can be explained on the basis of overtone's concept and Tweedy's chelation theory <sup>(54)</sup>. Inhibition is enhanced with the introduction of an electron withdrawing nitro group in the phenyl ring <sup>(55)</sup>. Cu (II) complexes with Schiff base 3,3-thiodipropionic acid bis (4-amino-5-ethylimino-2,3-dimethyl-1-phenyl-3pyrazoline showed antifungal activity against *Alternaria brassicae*, *Aspergillus niger* and *Fusarium oxysporum* and results indicate that the complexes show the enhanced activity in comparison to free ligand <sup>(56)</sup>.

Copper Schiff base ligand like, 3-amino-5-methyl isoxazole with pyridine-2-carboxaldehyde, 5-methylfuran-2-carboxyaldehyde and 5-methylthiophene-2-carboxaldehyde were screened for antifungal activity <sup>(26)</sup>. Copper (II) complexes of Schiff base ligands derived from 2-substituted anilines and salicylaldehyde exhibit good broad-spectrum antifungal in vitro <sup>(57)</sup>. Cu (II) complexes has been synthesized by the addition of Cu (II) acetate to ligand, i.e., 1,10-phenanthroline and 2,2-bipyridyl, which led to the precipitation of binary complexes. The complex has been tested for antifungal activity

against the human and plant fungal pathogens. The [Cu(Sala)bpy].H<sub>2</sub>O complex has been found to have some inhibitory effect against *Candida sp.* and *Mucor sp.*, *Alternaria alternate*, *Fusarium sp.*, *Penicillium sp.* Copper (II) complex with binuclear Schiff base, synthesized from benzidine and 5-bromo-3-fluorosalicylaldehyde has been tested against stone pathogenic fungi; *A. fumigates* <sup>(43)</sup>. Mixed ligand complexes of o-vanillidene-2aminobenzothiazole and 1,10-phenanthroline has been screened for their in vitro antifungal activities against fungus: *Aspergillus niger*, *Penicillium* and *Trichoderma viride*<sup>(37)</sup>.

Antifungal activity studies of Cu (II) complexes of Schiff base derived from 3,3-{1,2phynylenebis[nitrilo(E) methylylidine]} diquinolin-2-ol have been carried out. A cup plate method employed for the in vitro study of antifungal effect against, *C. albicans* and *A. niger* and the screening data showed that the metal chelates exhibit a higher inhibitory effect than the free ligand <sup>(45)</sup>. Schiff's base synthesized from sulphonamide and resacetophenone and its metal complexes showed antifungal activity against *A. awamori*. The comparison of antimicrobial activities of the ligands and complexes shows that the presence of metal causes more inhibition i.e., more activity <sup>(49)</sup>. Copper (II) complexes of oxalyldihydrazide and 3-tert-butyl-2-hydroxy-3-(3-phenylpent-3-yl) benzaldehyde ligand show a significant degree of antifungal activity against *Aspergillus sp.*, *Rizoctonia sp.* and *Penicillium sp.* The complexes are highly effective against *Aspergillus sp.* All the metal complexes exhibited greater antifungal activity against *Aspergillus sp.* as compared to the standard drug Miconazole <sup>(48)</sup>. Schiff base ligands derived from sulfonamide and their copper (II) complexes have been tested antifungal activity against fungal strains, *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata* <sup>(50)</sup>.

#### 1.2.2.3. Antimalarial activity:

It has been reported that thiosemicarbazones and its derivatives show antimalarial activities <sup>(58)</sup>. The primary task of researchers is to investigate new compounds in respect to their activity against malaria. A correlation between the structure and biological activity has



been established indicating that the tridentate (NNS) 2-(N)-heterocyclic thiosemicarbazones and its complexes are most efficient as therapeutic agents <sup>(59)</sup>.

Schiff base ligand 2-acetylpyridine thiosemicarbazones and their copper complexes possess significant antimalarial activities <sup>(60)</sup>. The presence of a 2-pyridylalkylidene moiety or selenocarbonyl group has been shown to be essential for antimalaria activities <sup>(61)</sup>. These features would also be expected to promote effective transition-metal chelating properties <sup>(62)</sup>. It has been reported that the presence of certain bulky groups at position N4 of the thiosemicarbazones moiety greatly enhances the antimalarial activity <sup>(63)</sup>. Heterocyclic ligands based on ferrocene, pyridine and thiosemicarbazones derivatives and these compounds as ligands for the preparation of transition metal complexes and test their biological activities against malaria <sup>(64,65)</sup>. Acetylferrocenyl-4-phenyl thiosemicarbazone, acetylferrocenyl-4-methyl thiosemicarbazone, acetylferrocenyl-2-thiophene carboxyl semicarbazone, acetylpyridine-2-thiophenecarboxyl semicarbazone and their copper (II) complexes has been screened against malaria parasite *Plasmodium falciparum* <sup>(66)</sup>. Cu(II) complexes of buparvaquone 3-trans (4-tert.-butylcyclohexyl) methyl-2-hydroxy-1, 4-naphthoquinone has been tested for their in vitro antimalarial activity against *Plasmodium falciparum* strains <sup>(67)</sup>.

#### 1.2.2.4. Pesticidal activity:

Copper complexes of semicarbazones and phenolic hydrazone have been used as herbicides, insecticides, nematocides, and rodenticides <sup>(68)</sup>. Phenoxyacetic acid and its derivatives act as herbicides <sup>(69)</sup>. Aryloxyacetate anions as acid ligands in the corresponding (carboxylato) copper (II) complexes possess herbicidal activities <sup>(70)</sup>. Pesticidal activities of Schiff's bases like; N-(1-phenyl-2-hydroxy-2phenylethylidene)-2,4-dinitrophenyl hydrazine], N (1-phenyl-2-hydroxy-2-phenyl ethylidene)-2-hydroxy phenylimine and [N-(2-hydroxybenzylidene)-2-hydroxy phenyl amine] derived from benzoin, salicylaldehyde, 2-aminophenol and 2,4-dinitrophenyl hydrazine and their copper complexes has been studied against *Tribolium castaneum* <sup>(71)</sup>. 2-Hydroxybenzalmethylene-O,O-diethyl phosphorohy drazono thionate (HL) and its complexes with copper (II) possess some insecticides activities <sup>(72)</sup>. The complex of copper and N-salicylidene-O,S-dimethylthio

phosphorylimine (HSMa) showed much higher pesticidal rates on poisoning *Aphis fabae* and *Metatetranychus ulmi*, compared with methamidophos a commercial pesticide <sup>(73)</sup>.

### **1.2.3. Thiosemicarbazones and their transition metal complexes:**

The coordination chemistry of thiosemicarbazones (TSCs) with transition metals <sup>(74,75)</sup>, has been more intensively investigated as compared to that of main group elements <sup>(76)</sup>. Thiosemicarbazones are thiourea derivatives and the studies on their chemical and structural properties have received much attention due to the widespread application in the chemotherapeutic field <sup>(75)</sup>. Thiosemicarbazones have been a subject of interest to researchers of different profiles. In view of the fact that these compounds form complexes with many metals are of diverse chemical, physical and structural characteristics, they are of special interest to coordination chemists <sup>(74)</sup>. Thiosemicarbazones, with the general formula  $R^1R^2C=N-NH-CS-NR^3R^4$  usually react as chelating ligands with transition metal ions by bonding through the sulfur and hydrazinic nitrogen atom. The group  $N-C=S$  is of considerable chemotherapeutic interest and is responsible for the pharmacological activity. Thiosemicarbazones of *or*-(N)-heterocyclic aldehydes and ketones possess a broad spectrum of potentially useful chemotherapeutic activities such as antimalarial, antibacterial, antiviral activities <sup>(77,78)</sup>. Heterocyclic thiones and thiosemicarbazones, which contain chemically active  $N(H)C(S)$  or  $=NN(H)C(S)$  chromophores, are useful model compounds for sulfur containing analogues of purine and pyrimidine bases, and thus have invited considerable interest in their coordination behavior <sup>(74,75,79)</sup>. Chemically heterocyclic thiosemicarbazones are of interest because of their great versatility as ligands, which derives from the presence of several potential donor atoms, their flexibility and their ability to coordinate in their neutral or deprotonated forms. The chemistry of transition metal complexes of thiosemicarbazones have been receiving considerable attention largely because of their pharmacological properties. Moreover, metal complexes of thiosemicarbazones often display enhanced activities when compared to the uncomplexed thiosemicarbazones.

Thiosemicarbazones (TSCs) are prepared by the condensation of thiosemicarbazides with aldehydes or ketones. Among the different NS donors, thiosemicarbazides and thiosemicarbazones, as well as their metal complexes have been studied because of the

large number of potentially useful biological properties, particularly the antitumor activity <sup>(80)</sup>. Thiosemicarbazides and thiosemicarbazones are well known ligands, coordinating through the sulfur and one of the hydrazinic nitrogen atoms <sup>(81)</sup>. Coordination through hydrazinic nitrogen atom results in a five-membered chelate ring.

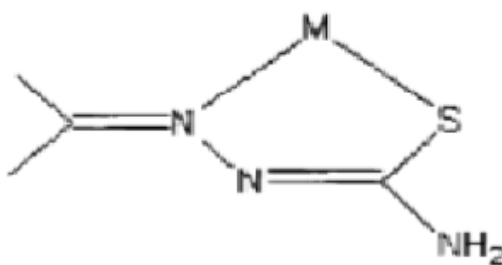


Fig 1.1. A five-membered chelate ring

Thiosemicarbazones are versatile ligands in both neutral (HL) and anionic ( $L^-$ ) forms

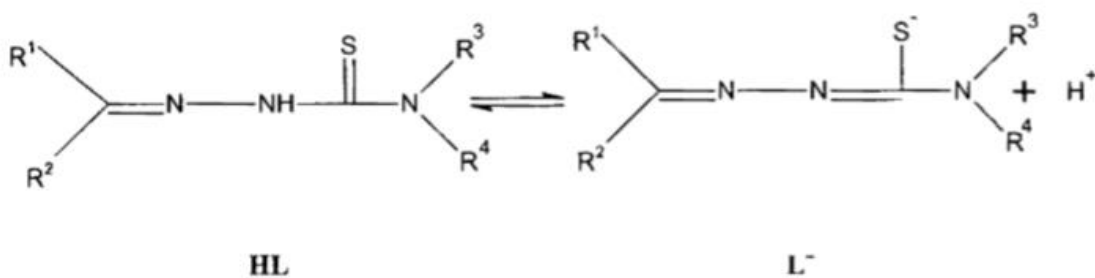


Fig 1.2. Ligands in both neutral (HL) and anionic ( $L^-$ ) forms

The  $R^1$  and  $R^2$  groups may provide additional donor atoms and  $R^3$  and  $R^4$  are N (4)-substituents.

Although the proton lost by the anions formally belongs to the hydrazinic —NH group, the anion is usually presented in the canonical form I, II and III and usually in the Z-configuration <sup>(81)</sup>.

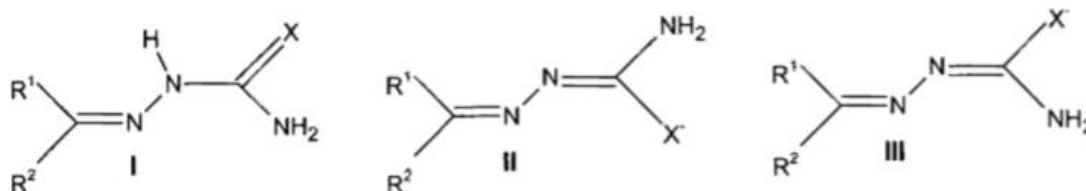


Fig 1.3. The canonical form I, II and III of ligand in the Z-configuration

The IUPAC numbering scheme of the ligand is shown in fig 1.4.

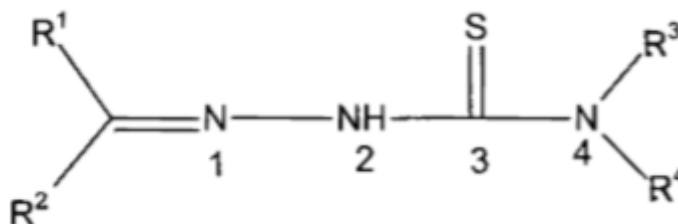


Fig 1.4. IUPAC numbering scheme of the ligand

#### 1.2.3.1. Bonding and stereochemistry:

The thiosemicarbazone moiety acts as a chelating agent for metal ions by bonding through the S atom and the hydrazine N atom. The thiosemicarbazones exist in the thione form (IV) in the solid state and in solution, they are known to tautomerize to exist in a mixture of thione (IV) and thiol (V) forms. Fig 1.5.

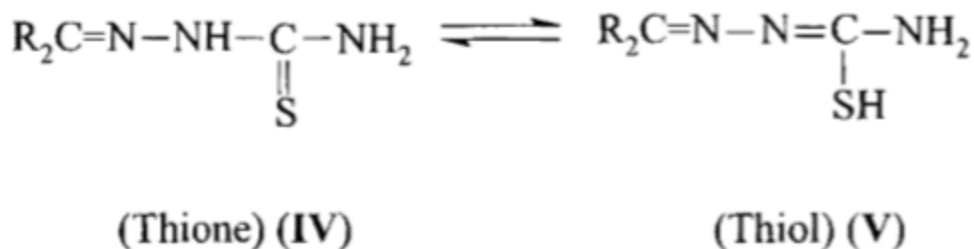


Fig 1.5. Tautomerize of thione and thiol

(IV) Acts as a neutral bidentate ligand, while the loss of the thiol proton from (V) yields a singly charged bidentate ligand due to the loss of its proton. Therefore depending upon the preparative conditions (especially pH), the complex unit can be cationic, neutral or anionic. However, most investigations of metal thiosemicarbazone complexes have involved ligands in the uncharged (IV) or thiolate form, while definitive data on the complexes containing thiosemicarbazone in the thiol form (V) are generally lacking. Furthermore, it is possible to isolate complexes containing both tautomeric forms of the ligand. For e. g. Garg et al. <sup>(82)</sup> have prepared a copper (II) chelate of 2-acetylpyridine thiosemicarbazone containing neutral and anionic forms of the ligand.

When an additional donor site D is incorporated in such ligands, linked to the carbonylic carbon via one or two intervening atoms D, N, S tricoordination usually takes place. Scheme

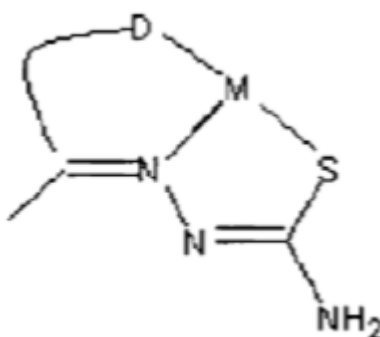


Fig 1.6. Additional donor site D to a five-membered chelate ring

If the additional functionality can also lose a proton (e.g. phenolic group), anions of greater negative charge are formed. There are instances reported, however, where the heterocyclic

atom and the azomethine nitrogen are involved in bidentate coordination <sup>(83)</sup>, and the sulfur atom is considered not to be coordinated, weakly coordinated to the same metal center, or coordinated to an adjacent metal center <sup>(84)</sup>.

The thiosemicarbazone moiety (Figure 1.1) without substituents attached to the thione sulfur coordinates as either a neutral or anionic NS bidentate ligand, depending on the method of complex preparation <sup>(85)</sup>, a third coordinating atom often gives ONS (e.g. 2-hydroxybenzaldehyde thiosemicarbazones) <sup>(86)</sup> or NNS (e.g. 2-acetylpyridine thiosemicarbazones) donor tridentate ligands. A few examples of higher denticity involving one or more thiosemicarbazone moieties, as well as monodentate coordination have been reported <sup>(84)</sup>.

Due to the presence of C=N bond, the thiosemicarbazones can exist in three isomeric forms Z, E, and E' <sup>(87)</sup>. The TSC may generally exist in the E form (Trans) but in such situation the compound may act as a unidentate ligand, by bonding through sulfur only <sup>(88)</sup>. In case the sulfur center is substituted, the bonding may occur through the hydrazine nitrogen and the amide nitrogen <sup>(85)</sup>. Z, E and E' isomeric forms of 2-formylpyridine thiosemicarbazone are shown below. E' is the bifurcated E-hydrogen bonded ring isomer.

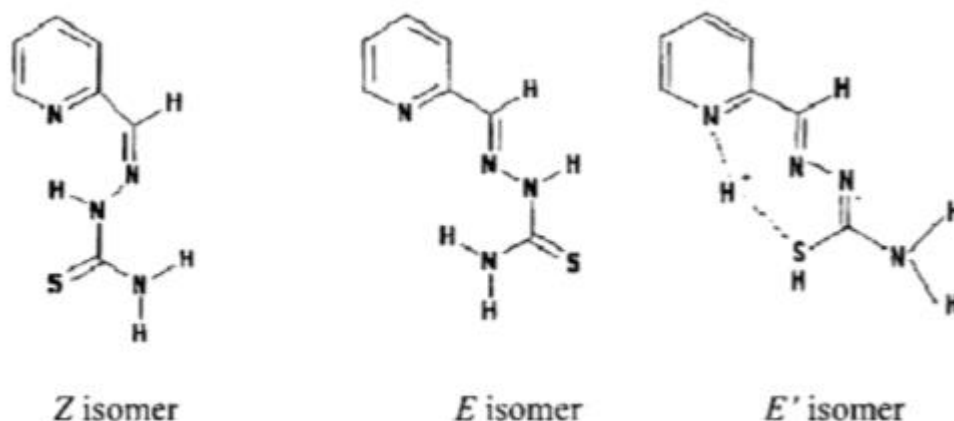


Fig 1.7. Isomeric forms of 2-formylpyridine thiosemicarbazone

Thiosemicarbazones can coordinate to the metal either as a neutral ligand or as a deprotonated ligand through the NNS <sup>(84)</sup>, ONS <sup>(86)</sup> or through the NNO atoms <sup>(81,89)</sup>.

Thiosemicarbazones obtained by condensation of thiosemicarbazides with benzaldehyde, salicylaldehyde and substituted salicylaldehydes <sup>(75,90)</sup> form a class of versatile NS/NSO chelating ligands. They also stabilize uncommon oxidation states <sup>(91)</sup>, generate unfamiliar coordination numbers in the resultant transition metal complexes and participate in various types of redox reactions <sup>(92,93)</sup>. These ligands can act in a dianionic tridentate manner by deprotonation of both the phenol and the thioamide functions. They can also behave as monoanionic tridentate ligands coordinating to a metal centre through the deprotonated phenolic oxygen, thione sulfur and the azomethine nitrogen <sup>(91)</sup>. The importance of the coordination chemistry of such ONS donors increased markedly after the presence of ONS donor environment was detected at the active sites of some metalloenzymes <sup>(92,93)</sup>. West et al. <sup>(94)</sup> have shown that the nature of substituents attached at <sup>4</sup>N of 2-formyl- and 2-acetylpyridine thiosemicarbazones can influence the stereochemistry and stoichiometry of metal complexes. An important feature in the chemistry of thiosemicarbazones and their metal complexes is the acid character of the <sup>2</sup>NH; this allows for either neutral or anionic ligands. When coordinated as anionic ligands, the conjugation is extended to include the thiosemicarbazone moiety (i.e. C=N-N=C(S)-N). If <sup>2</sup>N is alkylated, the thiosemicarbazone will function as the thione isomer without extended conjugation. Earlier reports suggest that stereochemistries adopted by 2-heterocyclic thiosemicarbazones of transition metal complexes often depend upon the anion of the metal salt used and the nature of the <sup>4</sup>N-substituents. Further, as indicated previously, the charge on the ligand is dictated by the thione-thiol equilibrium which in turn is influenced by the solvent and pH of the preparative medium.

Common stoichiometries encountered with 2-heterocyclic thiosemicarbazones are six coordinate having the general formula  $ML_2^{n+}$ , where M= Cr (III), Fe (III), Co (III) and Ni (II); L= tridentate, anionic ligand and n = 0, 1 and planar with the stoichiometry of  $MLX$ , where M= Ni (II) or Cu (II), L=tridentate, anionic ligand and X is generally a halo or pseudohalo ligand.

The stereochemistries adopted by thiosemicarbazone ligands while interacting with transition metal ions depend essentially upon the presence of additional coordination centre in the ligand moiety and the charge on the ligand, which in turn is influenced by the thione-

thiol equilibrium. For e.g. benzaldehyde thiosemicarbazone is generally found to act as neutral bidentate ligand, depending upon the pH of the synthetic medium yielding complexes of the type  $[ML_2X_2]$  (where M= Co (II), Ni (II), Cu (II) and Fe (II); L= the ligand in the thione form and X= the monoanionic ligand where as salicylaldehyde thiosemicarbazone is found to act as a tridentate uninegative ligand yielding compounds of type  $[M(HL)_2]$ , which may be spin-free or spin-paired. As a result of the above considerations, the most common stereochemistries encountered in thiosemicarbazone complexes are tetrahedral and square planar. On rare occasion's five coordinated structures also obtained, as in the case of Co (II), Fe (II) and Ni (II) complexes of acetone thiosemicarbazone <sup>(95)</sup> and the Fe (II) complex of 2-acetylpyridine thiosemicarbazone <sup>(96)</sup>.

Health and Social Action Bureau (HSAB) considerations dictate that the degree of softness character of the metal depends upon the oxidation state of the metal, and it is found to be stronger for transition metals in low oxidation states. Thus the low spin  $d^8$  ions Pd (II), Pt (II) and Au (III) and  $d^{10}$  ions Cu (I), Ag (I), Au (I) and Hg (II) exhibit higher stability constants with this class of sulfur ligands because of the formation of strong  $\sigma$  bonds as well as  $d_{\pi}$ - $d_{\pi}$  bonds by donation of a pair of electrons to ligands <sup>(97)</sup>.

Thiols but not thioethers cause spin-pairing of Co and Ni. Thiosemicarbazones are not capable of spin-pairing of Fe (III) ions, unlike other soft bases such as  $CN^-$ , diarsine and certain charged sulfur ligands <sup>(98)</sup>. Consequently, intermediate spin states are found to be stabilized <sup>(96)</sup>.

#### **1.2.4. Spectrophotometric uses of Thiosemicarbazone complexes:**

The low cost of thiosemicarbazones as well as their easy preparative methods could provide a major attraction for the development of these reagents in analytical applications. The tendencies of thiosemicarbazone ligands to form chelating complexes with the metal ions have been exploited for the determinations of these metals via spectrophotometric <sup>(99)</sup>, stripping voltammetric <sup>(100)</sup> and potentiometric technique. Thiosemicarbazone complexes have also shown interesting biological properties such as anticancer <sup>(101)</sup>, antibacterial and antifungal agents <sup>(102)</sup>. In the last few years, a great deal of research has been aimed to



design compounds highly selective to toxic metal ions like  $\text{Hg}^{2+}$ , in order to detect, and even if possible to remove them from the environment. The reaction of thiosemicarbazone derivatives with  $\text{Hg}^{2+}$  has formed stable as well as labile complexes through mercury (II) thiol bond <sup>(103)</sup>. Generally, thiosemicarbazones are good chelating agents for the mercury (II), but only a limited number of Hg (II)-thiosemicarbazone complexes are known <sup>(104)</sup>.

In industry, they have been used for optical computing, optical storage and optical information processing <sup>(105)</sup>. The present communication reports their application as antiwear additives to paraffin oil as lubricant. Zinc dialkyl dithiophosphates have been frequently used as effective commercial antioxidant and antiwear additives for a number of lubricating motor oils <sup>(106-108)</sup>. Since zinc, sulfur and phosphorus damage catalytic exhaust convertors and poison the catalyst thereby reducing the life of the emission control system <sup>(109,110)</sup>. Organic borate esters have been studied as antiwear additives since they possess wear resistance and reduce friction. These have shown synergistic effects with zinc dialkyl dithiophosphates and improve their performance as antiwear additive. Keeping in view the absence of phosphorus, metallic component and low sulfur content in salicylaldehydeN(4)-p-substituted phenyl thiosemicarbazones, it was thought worthwhile to evaluate their antiwear properties and study their synergistic/antagonistic behavior with organic borate esters (Vanlube 289) in paraffin oil as a lubricant and zinc dibutyl dithiophosphate (ZDDP) as a reference additive.

Nickel is present in small amounts in soils, plants and animal tissues. The main source of nickel comes from the hydrogenation of oils, iron factories, from the combustion of coal, diesel and residual oils, tobacco smoke, chemicals and catalysts <sup>(111)</sup>. The strength, high melting point and anti-corrosive nature of nickel make it an attractive component in steel and non-ferrous alloy manufacturing industries. However, nickel is toxic and the primary route for nickel toxicity is mainly due to its exposure through inhalation and contaminated food and water. The soluble nickel complexes are more toxic than insoluble complexes. Nickel in human body <sup>(112)</sup> causes dermatitis, pneumonia, lung and nose cancer. The formation of extremely toxic  $\text{Ni}(\text{CO})_4$  causes giddiness, headache, nausea and vomiting. Considering the toxic effects of nickel, it has become necessary to determine the nickel content in soil and industrial effluent samples. Among the various instrumental analysis

methods available for the determination of Ni (II), spectrophotometry is preferred as a versatile technique due to its low cost and easy experimental procedures. The successful application of such procedures mainly relies on the stability of the colored complex that usually formed between the analyte metal ions and the organic complexing agent. Thio- and phenyl thiosemicarbazones find a wide range of applications in medicine <sup>(113,114)</sup> and agriculture <sup>(114)</sup>. Owing to the ability of these reagents in forming intense colored complexes with various metal ions, they are widely employed in spectrophotometric and extractive spectrophotometric analysis, atomic absorption spectrometry (AAS) and solid-liquid separation. The nickel (II)-phenyl thiosemicarbazone complexes have intense colors and high molar absorptivities when compared with the analogous thiosemicarbazone complexes. A thorough literature survey indicated that very few phenylthiosemicarbazones have been explored for the extractive spectrophotometric determination of nickel (II) <sup>(115,116)</sup>. Hence it is proposed to develop extractive spectrophotometric methods for nickel (II) by using phenyl thiosemicarbazones.

An important use of nickel in the food industry as a catalyst is evident from its use in the hydrogenation of oils. Nickel is an important element due to its high strength and resistance to corrosion in many media <sup>(117)</sup>. It is used in nickel plating and also in the manufacture of alloys along with iron, copper, aluminum, chromium, zinc and molybdenum. Nickel containing steels are highly resistant to corrosion. Because of its high melting point (14531°C), nickel is also used in the production of heat-resistant steels and cast iron. Nickel-plated steels are used in the manufacture of some food processing vessels and many other pieces of equipment. Nickel (II) is present in small amounts in most soils, plants and animal tissues. The interest in the determination of nickel has grown considerably in recent years, owing to its involvement in some essential metabolic processes <sup>(118)</sup>.

Nickel is relatively non-toxic and does not cause any serious human health hazard, despite the fact that acid foods take up nickel during cooking. The nickel deposited in the human body from nickel vessels is not readily absorbed and causes no detectable hazard. However, a high incidence of respiratory tract neoplasia among workers in nickel refineries and carcinogenic properties of this metal have been reported <sup>(117)</sup>. Thio- and phenyl thiosemicarbazones have a wide range of applications in medicine and agriculture. Owing

to the ability of these reagents to form intense colored complexes with various metal ions <sup>(119,120)</sup>, they are widely employed in spectrophotometric and extractive spectrophotometric analysis, atomic absorption spectrometry and solid–liquid separation. The nickel (II)–thiosemicarbazone complexes have intense colors and high molar absorptivities when compared with the analogous thiosemicarbazone complexes. A literature survey indicated that only a few thiosemicarbazones <sup>(119,121-124)</sup> have been explored for the extractive spectrophotometric determination of nickel (II) and N-ethyl-3-carbazolecarboxaldehyde-3 thiosemicarbazone (ECCT) has so far not been used as an analytical reagent for the extraction of nickel (II). In the present work, ECCT has been examined in order to evaluate its usefulness as an extractive spectrophotometric reagent for nickel (II). Further, this method has been applied successfully for the analysis of nickel (II) in environmental matrices like soil and industrial effluents. For the determination of nickel at micro levels there are several frequently adopted methods using analytical techniques such as AAS, ICP–OES, ICP–AES, ICP–MS, X-ray fluorescence spectroscopy, spectrophotometry, spectrofluorometry and other such techniques. Among these, the spectrophotometric methods are preferred as they are cheaper and easier to handle and have comparable sensitivity formed <sup>(125)</sup>.

Copper is one of several metal ions that play an important role in the biological system. It plays a key role during cell respiration, in the blood of invertebrate animals, and in the formation of hemocyanin, an important respiratory protein, found in the lymph of most animals belonging to Phyla Mollusca and Arthropoda. From the standpoint of human health, its role in three physiological functions is of prime importance. Copper is involved in hemopoiesis and in maintenance of vascular and skeletal integrity in addition to the structure and function of the central nervous system. Copper occurs naturally in most vegetables, meats, and grains. The study of copper in food items is of great concern, because it plays a definitive role in the intrinsic mechanisms regulating vital biological processes <sup>(126)</sup>. Overexposure to copper causes metallic taste, ptyalism, nausea, vomiting, epigastric burning, and diarrhea. Heavy doses of copper cause a series of systematic toxic effects such as hemolysis, hepatic neurosis, gastrointestinal bleeding, oliguria, azotemia, hemoglobinuria, hematuria, proteinuria, hypertension, tachycardia, convulsions, and coma. When a congenital deficiency in the homeostatic mechanism for copper exists, the metal

accumulates in the liver, discrete areas of the brain, the cornea of the eye, and other tissues, causing Wilson's disease. A wide variety of clinical disorders have been associated with a dietary deficiency of copper, which respond to copper therapy. They include anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, and gastrointestinal disturbances <sup>(126)</sup>. In view of this, the separation and determination of copper from associated elements is indispensable.

For the determination of copper at micro levels there are several frequently adopted methods using analytical techniques such as AAS, ICP-OES, X-ray fluorescence spectroscopy, spectrophotometry, spectrofluorometry, and other such techniques. Among these, the spectrophotometric methods are preferred as they are cheaper and easier to handle and have comparable sensitivity. A number of spectrophotometric reagents have been used for the determination of copper (II), but a very few number are used for the separation and determination of it. Thio and phenyl thiosemicarbazones are important sulfur- and nitrogen-containing organic reagents, where copper coordinates with these reagents to form stable complexes. As it is more stable in its divalent state, it is extracted into organic solvents such as chloroform, n-butanol, and others as a divalent complex. The metal chelates of these sulfur- and nitrogen-containing organic reagents find a wide range of applications in medicine <sup>(127)</sup> and agriculture. The reviewed <sup>(128)</sup> literature revealed that only a few thio and phenyl thiosemicarbazones were employed for extractive determination of copper (II) <sup>(129)</sup>. Hence, the authors introduced a new reagent, pyridoxal-4-phenyl-3-thiosemicarbazone (PPT), for the selective and spectrophotometric determination of Cu (II) in leafy vegetable, pharmaceutical, and Bureau of Analyzed Samples. Previous studies of transition metal ions such as zinc, cobalt, and palladium extracted from biological and environmental samples using PPT were established <sup>(130-133)</sup>.

### **1.3. Aim of the study:**

The objectives of the present study can be summarize as follows:

- The first part of the study includes the preparation of ligand and different ligand metal complex by refluxing in absolute methanol.
- The second part to characterize the formed of ligand and different ligand metal complex using IR, UV and AAs.
- Finally to study the biological activity of ligand and different ligand metal complex.

## Chapter Two

### 2. Experimental

#### 2.1. Materials and Methods:

##### 2.1.1. Materials:

##### 2.1.1.1. Solvents:

- Absolute methanol  $\text{CH}_3\text{OH}$ .

##### 2.1.1.2. Chemicals:

- Thiosemicarbazide  $\text{NH}_2\text{CS.NH.NH}_2$ .
- Salicylaldehyde for synthesis  $\text{C}_7\text{H}_6\text{O}_2$ .
- Cobalt (II) Chloride (hexahydrate)  $\text{CoCl}_2.6\text{H}_2\text{O}$ .
- Nickel (II) Chloride (hexahydrate)  $\text{NiCl}_2.6\text{H}_2\text{O}$ .
- Cupper (II) Sulphate (pentahydrate)  $\text{CuSO}_4.5\text{H}_2\text{O}$ .
- Chromium (III) Chloride (hexahydrate)  $\text{CrCl}_3.6\text{H}_2\text{O}$ .

All chemicals used in this research were purified grade.

##### 2.1.2. Instruments and Equipment:

- **pH Meter:** HANNA instruments, pH 221, Microprocessor pH meter, Serial No. 659158. Made in Muaritus.
- **Conductivity Meter:** HANNA instruments, EC 214, Made in Romania.
- **Infra-Red Spectroscopy (IR):** JASCO, FT/IR-4100 LE Serial No. B18736106, protect: Class1, Made in Japan.
- **Ultra Violet-Visible Spectroscopy (UV-VIS):** SHIMADZU, UV-VIS Spectrophotometer, Model: UV-2700 240V EN, Serial No.A11675100378 LP, Made in Japan.
- **Melting Point:** Melting Point device Made in UK, BIBBY Sterilin L.T.D., Serial No. R 000102810.

- **Atomic Absorption Spectroscopy (AAS):** Savanta AA, Serial No. AA7638 GBC. Made in Australia. A7703
- **Water Bath:** Digital Water Bath, Daihan Labtech Co. L.T.D., Model; LWB-122 D, Serial No. 2014022849.
- **Sensitive Balance:** SHIMADZU, Electronic Balance, Type: AY120, NO. D432711233, Made in Japan.

### 2.1.3. Glass wares:

All glass ware were Pyrex type.

### 2.1.4. Methods:

#### 2.1.4.1. Preparation of Ligand:

The ligand was prepared by mixing equimolecular amounts of thiosemicarbazide and salicylaldehyde in absolute methanol and refluxing on a water bath for two hours, the condensation products was filtered, crystallized form methanol and dried, the percentage yield of product was calculated using the following equation :

$$\text{Yield \%} = \frac{W_{\text{Prac.}}}{W_{\text{Theo.}}} \quad (1)$$

Where:

$W_{\text{Prac.}}$  = Practical Weight.

$W_{\text{Theo.}}$  = Theoretical Weight.

#### 2.1.4.2. Preparation of Complexes:

The metal complexes were prepared by mixing 1:1 molar ratios of Cu (II), Co (II), Ni (II) and Cr (III) salts and the ligand respectively, in absolute methanol. The mixtures were refluxed on a water bath for a time depending on the nature of metal cation used. The formed complexes were filtered, washed several times with pure dry methanol, and dried, the yield of complexes calculated as equation above.

#### **2.1.4.3. Preparation of Complexes in pH 4 Condition:**

The metal complexes were prepared by mixing 1:1 molar ratios of Cu (II), Co (II), Ni (II) and Cr (III) salts and the ligand, respectively, in absolute methanol the pH controlled by buffer solution of ammonia. The mixtures were refluxed on a water bath for a time depending on the nature of metal cation used. The formed complexes were filtered, washed several times with pure dry methanol, and dried, the yield of complexes calculated as equation above.

#### **2.1.4.4. Preparation of Complexes in pH 8 Condition:**

The metal complexes were prepared by mixing 1:1 molar ratios of Cu (II), Co (II), Ni (II) and Cr (III) salts and the ligand, respectively, in absolute methanol the pH controlled by buffer solution of ammonia. The mixtures were refluxed on a water bath for a time depending on the nature of metal cation used. The formed complexes were filtered, washed several times with pure dry methanol, and dried, the yield of complexes calculated as equation above.

#### **2.1.4.5. Antimicrobial Assay:**

The following organisms were used to test the antimicrobial activity using disc diffusion method: Fungus - *Candida albicans* MTTC 227, Gram positive bacteria - *Staphylococcus aureus* MTCC 96, Gram negative bacteria - *Escherichia Coli* MTCC, All cultures were obtained from International African University, department of microbiology. Inoculums of each bacterial strain was inoculated in 3ml of Mueller Hinton Broth and incubated at 37°C for 24h. After incubation period the culture was diluted.

Bacteria inoculums were prepared by growing cells in Mueller Hinton Broth (Himedia) for 24 h at 37° C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about  $10^{-4}$  CFU/ml. Yeast was grown on Sabouraud Dextrose Broth (SDB) at 28° C for 48 h. Antimicrobial activity was carried out using disc diffusion method. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures (100µl of suspension containing  $10^8$  CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min.



The tests were conducted at 1000 µg/disc concentration of the compounds. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Gentamicin (10 mg /disc) was used as positive control. The plates were incubated for 24 h at 37° C. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

#### **2.1.5. Characterization of the formed ligand and ligand metal complexes:**

##### **2.1.5.1. Fourier Transform Infrared Spectroscopy (FT-IR):**

2 mg of the Salicylaldehyde Thiosemicarbazone and their complexes were triturated with 300 mg of dried potassium bromide. These quantities were usually sufficient to give a suitable intensity of spectrum when use a disc with diameter (10-15 mm). Carefully the mixture was grinded, spread uniformly in a suitable disc, and submitted to a pressure of about 800 MPa (8 t·cm<sup>-2</sup>). For substances that were unstable under normal atmospheric conditions, the disc had been pressed in vacuum. Several factors might be caused the formation of faulty discs, such as insufficient or excessive grinding, humidity or other impurities in the dispersion medium or an insufficient reduction of particle size. A disc was rejected if visual examination had been show lack of uniform transparency or when transmittance at about 2000 cm<sup>-1</sup> (5 µm) in the absence of a specific absorption band was less than 60 percent without compensation, unless otherwise prescribed.

Samples have been prepared by the same procedure and the spectrum was recorded between 4000- 400 cm<sup>-1</sup> (2.5-15.4 µm) under the same operational conditions. The transmission minima (absorption maxima) in the spectrum was obtained with the substance to be examined correspond in position and relative size to those in the spectrum obtained with the reference substance <sup>(134)</sup>.

##### **2.1.5.2. Ultra Violet Spectroscopy UV:**

After adjusting the instrument as directed in the apparatus and adjustment, selected and set the light source, detector mode of measurement, measuring wavelength or wavelength range, spectrum width and scanning speed. Subsequently, allow the instrument to stand for a certain time confirm the stability. The instrument was adjusted so that the transmittance

was 0% at measuring wavelength or over measuring wavelength range after the shutter was closed at the sample site of light bath. Then the shutter was opened to transmittance of 100 % (the absorbance was zero) by putting cells containing control solution, blank solvent was used. The measurement was performed with cell containing the test solution. The absorbance was readed.

#### **2.1.5.3. Atomic Absorption Spectroscopy:**

For atomic absorption spectroscopy, it recommended to use plastic apparatus, five standards and blank solutions were prepared for each element to make standard calibration curve and introduce real samples, the concentration calculated directly according to Beer and Lambert equation curve , the system of atomic absorption used is air-acetylene flame model (Savanta AA, Serial No. AA7638 GBC. Made in Australia).the wavelength of Co at 240.7 nm, Cr at 357.9 nm, Cu at 324.8 nm and Ni at 232 nm.

#### **2.1.5.4. Melting Point (M.P.):**

In this technique the melting point apparatus with capillary tube was used to determine the melting point. (210° C for ligand)

## Chapter Three

### 3. Results and Discussion

The ligand Salicylaldehyde thiosemicarbazone and their metal complexes were synthesized and characterized. The percentage of yield varies from 24% to 100%. All the ligands and metal complexes are solids.

As it is known already from salicylaldehyde thiosemicarbazone, this neutral-type of compound is obtained by simply reacting metal salts and the ligands, all dissolved in methanol. Adding a base (soft or strong) leads in general to the anionic form of the complex. The deprotonated ligand is attached to metal ion yielding  $[M II (L)]^+$  entities. Contrary to this expectation, the complex with the twice deprotonated ligand was not possible to obtain with salicylaldehyde thiosemicarbazone. The strong base may be effected by forming precipitate of metal hydroxide, instead of complexion.

Preparation of Schiff base ligand and their metal complexes can be presented by the following equation:

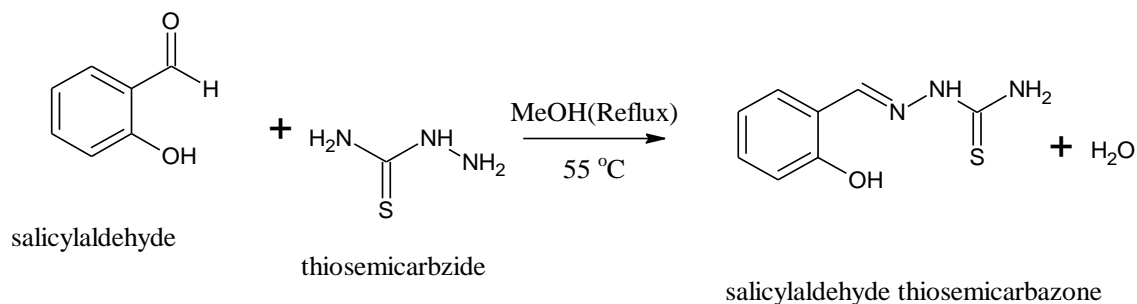


Fig 3.1. Preparation of ligand

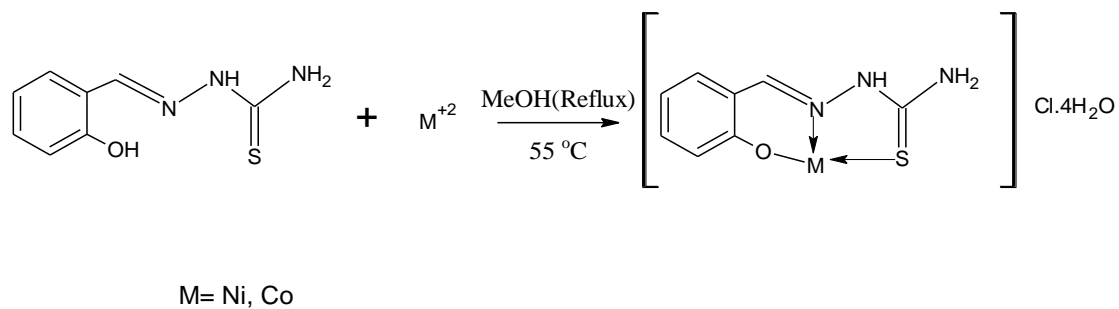


Fig 3.2. Preparation of metals (II) complexes

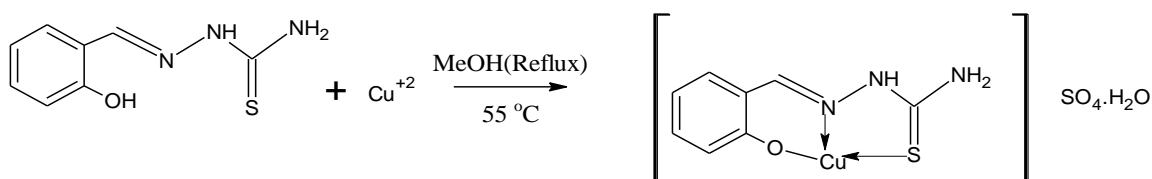


Fig 3.3. Preparation of Cu (II) complexes

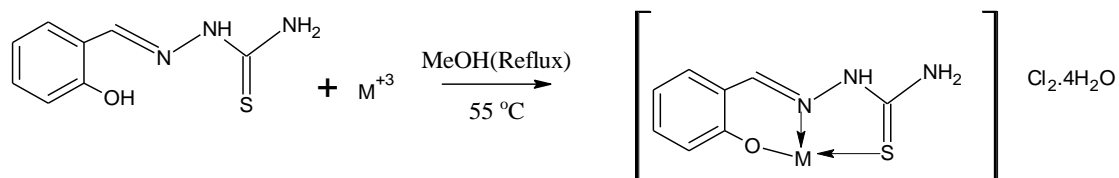


Fig 3.4. Preparation of metals (III) complexes

### 3.1. IR spectra:

The characteristic bands of the ligands and their complexes are summarized in Table (3.1). The ligand salicylaldehyde thiosemicarbazone (STSC) shows a sharp absorption band at  $1603\text{ cm}^{-1}$  corresponding to  $\text{C}=\text{S}$  frequency, and the presence of band in the region  $1600\text{--}1614\text{ cm}^{-1}$  supports the existence of  $\text{C}=\text{S}$  stretching in the metal complexes.

A sharp band at  $1536\text{ cm}^{-1}$  corresponds to C=N stretching frequency for STSC. On coordination of the azomethine nitrogen, the IR stretching frequency of C=N shows a shift and is observed in the region  $1442\text{--}1565\text{ cm}^{-1}$  in STSC metal complexes.

In the case of metal complexes, the appearance of bands in the region of  $426\text{--}474\text{ cm}^{-1}$  and  $445\text{--}551\text{ cm}^{-1}$  correspond to the M-N and M-O vibrational frequencies respectively. The appearance of the band around  $1110\text{--}1202\text{ cm}^{-1}$  corresponds to N-N stretching frequency both in ligands and in metal complexes.

Aromatic  $\nu(\text{C}=\text{C})$  value appears around  $1439\text{--}1489\text{ cm}^{-1}$ . The strong bands at  $3318\text{ cm}^{-1}$  in the spectrum of STSC have been assigned to  $-\text{OH cm}^{-1}$ . The bands around  $3114\text{ to }3396\text{ cm}^{-1}$  in ligands are due to  $-\text{OH}$  vibration.

Table (3.1) Characteristic bands of the ligands and their complexes

Compound	OH $\text{cm}^{-1}$	C=N $\text{cm}^{-1}$	C=S $\text{cm}^{-1}$	N-N $\text{cm}^{-1}$	C=C $\text{cm}^{-1}$	M-S/ M-O $\text{cm}^{-1}$	M-N $\text{cm}^{-1}$
STSC	3318	1536	1603	1110	1489	-	-
Co (II)	3396	1533	1600	1202	1470	485	464
Ni (II)	3323	1565	1607	1159	1452	556	472
Cu (II)	3114	1442	1606	1172	1442	455	419
Cu (II) f	3297	1551	1603	1157	1439	474	413
Cr (III)	3174	1538	1602	1151	1442	-	-
Cr (III) f	3172	1536	1614	1149	1463	-	-

F= filtrated

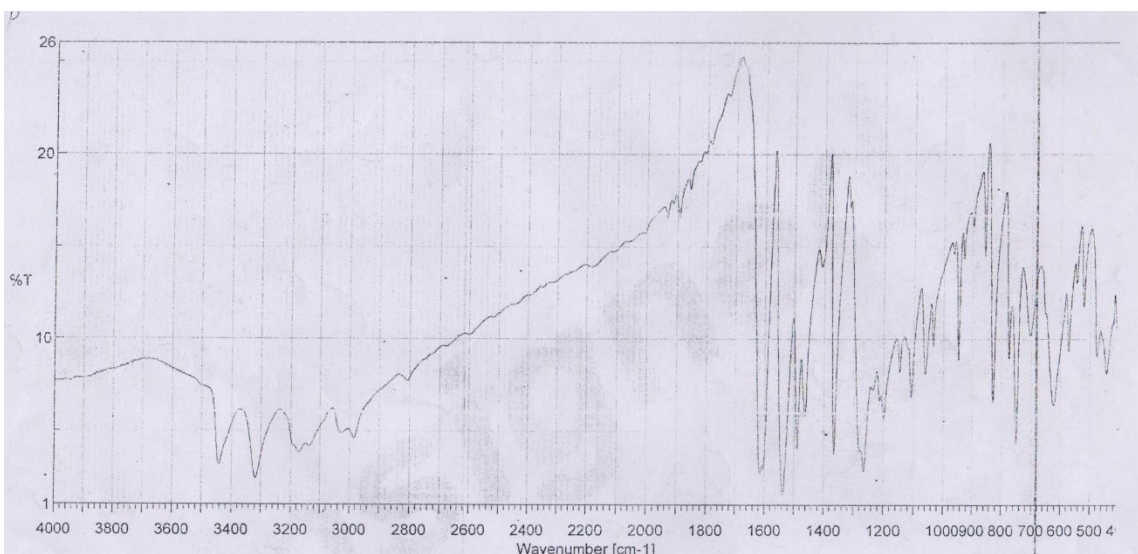


Fig 3.5. IR Spectrum of Ligand (STSC)

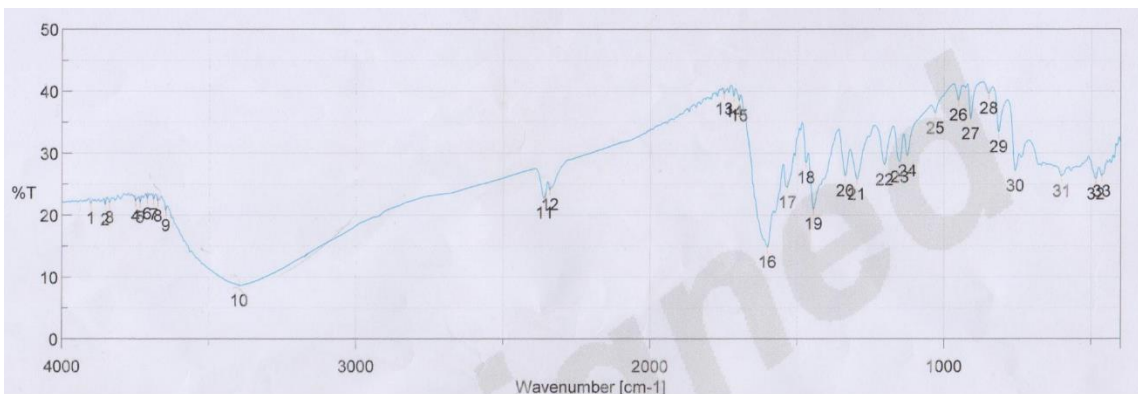


Fig 3.6. IR Spectrum of Cobalt (II) Complex

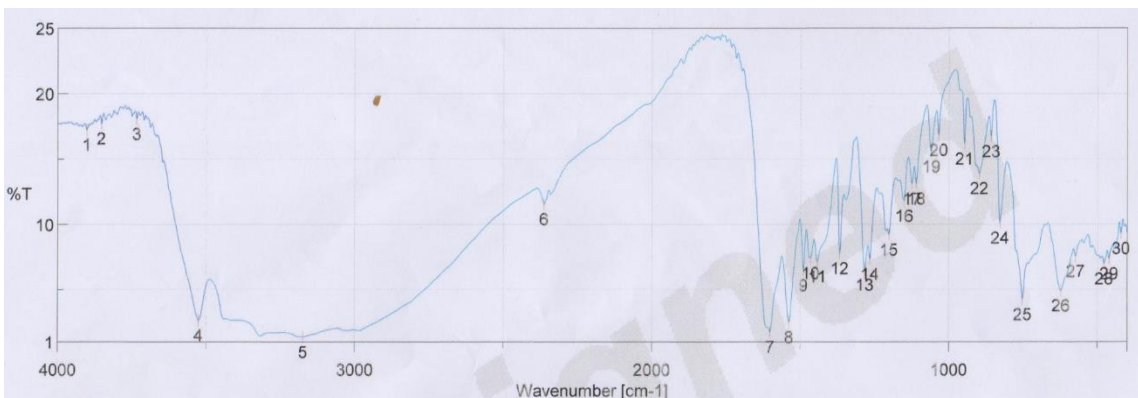


Fig 3.7. IR Spectrum of Chromium (III) Complex

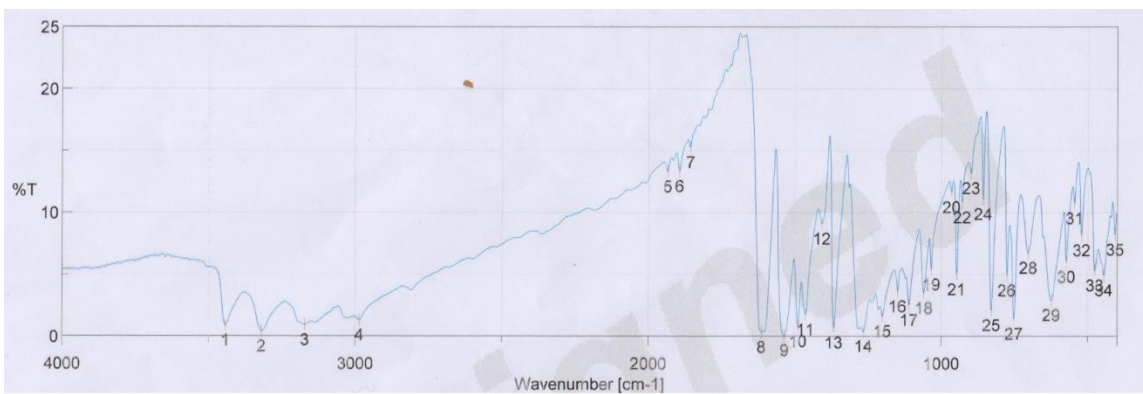


Fig 3.8. IR Spectrum of Chromium (III) Complex (f)

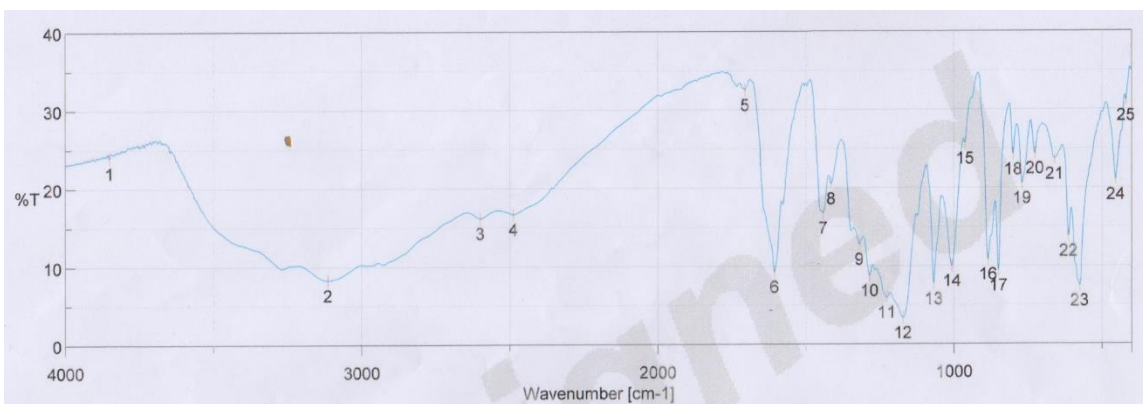


Fig 3.9. IR Spectrum of Copper (II) Complex

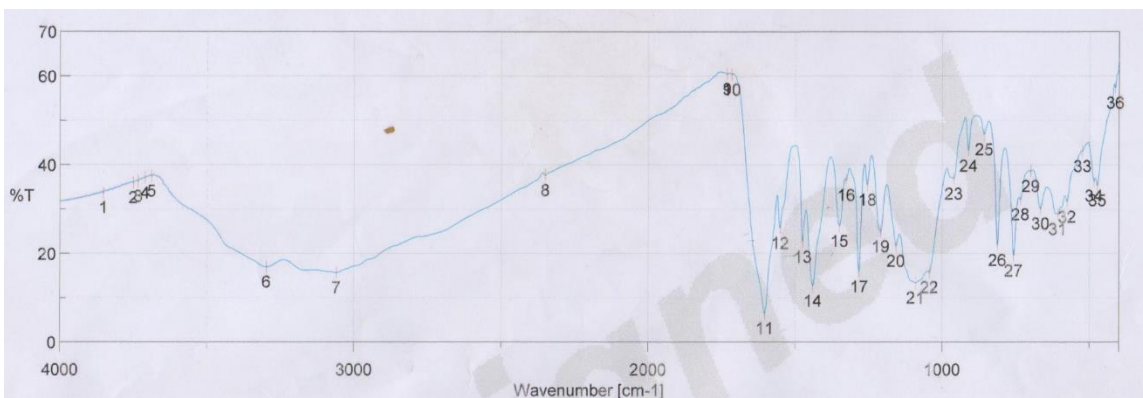


Fig 3.10. IR Spectrum of Copper (II) Complex (f)

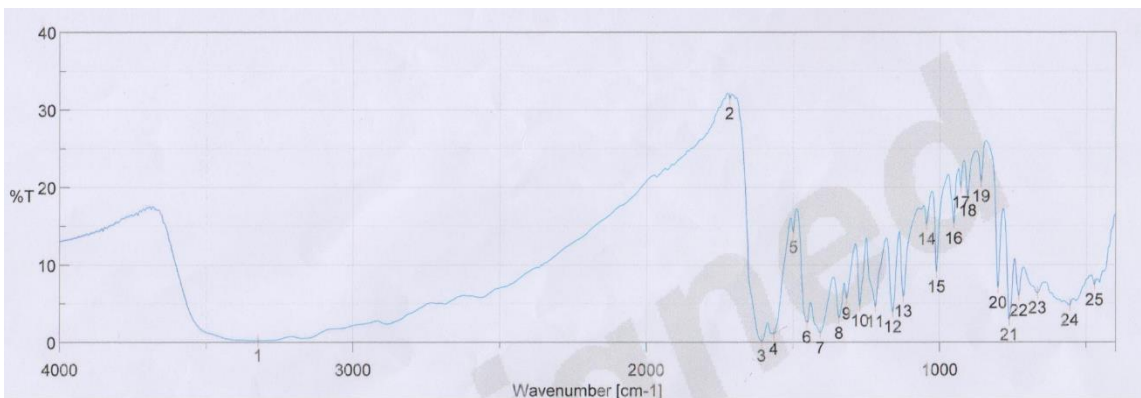


Fig 3.11. IR Spectrum of Nickel (II) Complex

### 3.2. Electronic absorption spectral bands:

U.V. of the ligand showed the  $\lambda_{\max}$  around (245 to 386 nm) it was converted to  $\text{cm}^{-1}$  according to equation  $[hc/\lambda = h\nu : 1/\lambda (\text{nm}) = \nu (\text{cm}^{-1})]$  see the table below.

Table (3.2) absorption bands of  $\lambda_{\max}$

Compound	STSC	Co (II)	Ni (II)	Cu (II)	Cu (II) f	Cr (III)	Cr (III) f
$\lambda_{\max}$	334	262	245	295	386	312	332

F= filtered



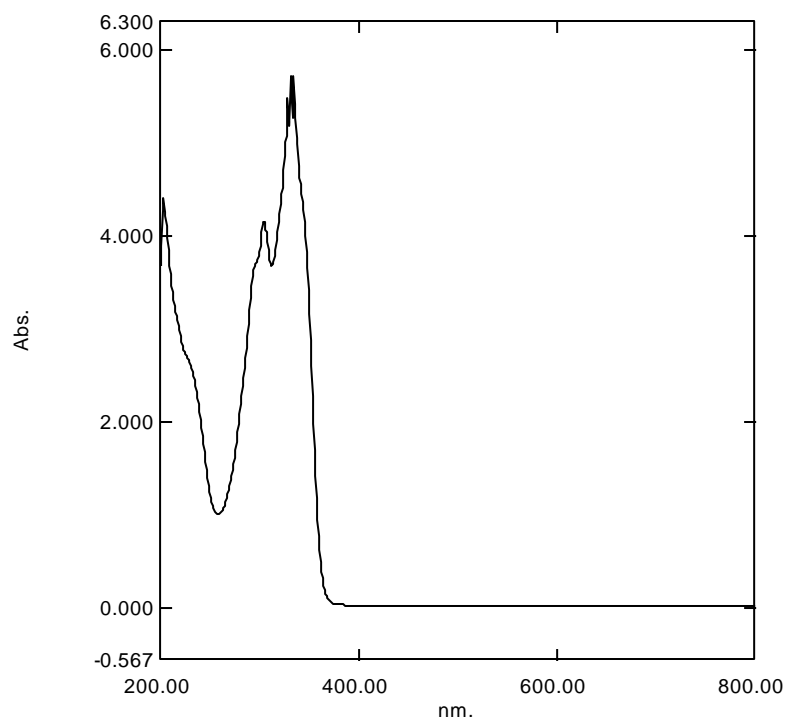


Fig 3.12. U.V. spectrum of ligand

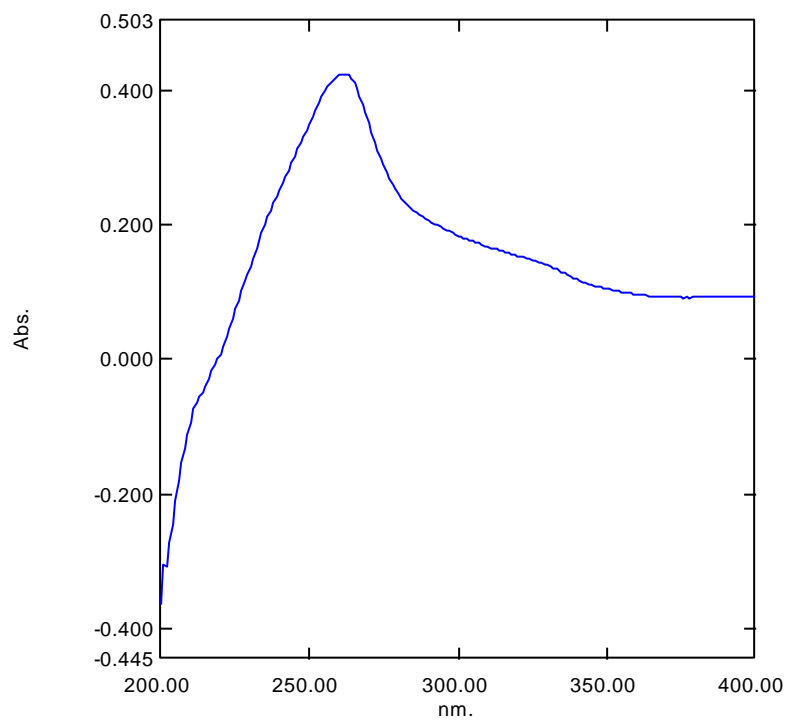


Fig 3.13. U.V. spectrum of Co (II) complex

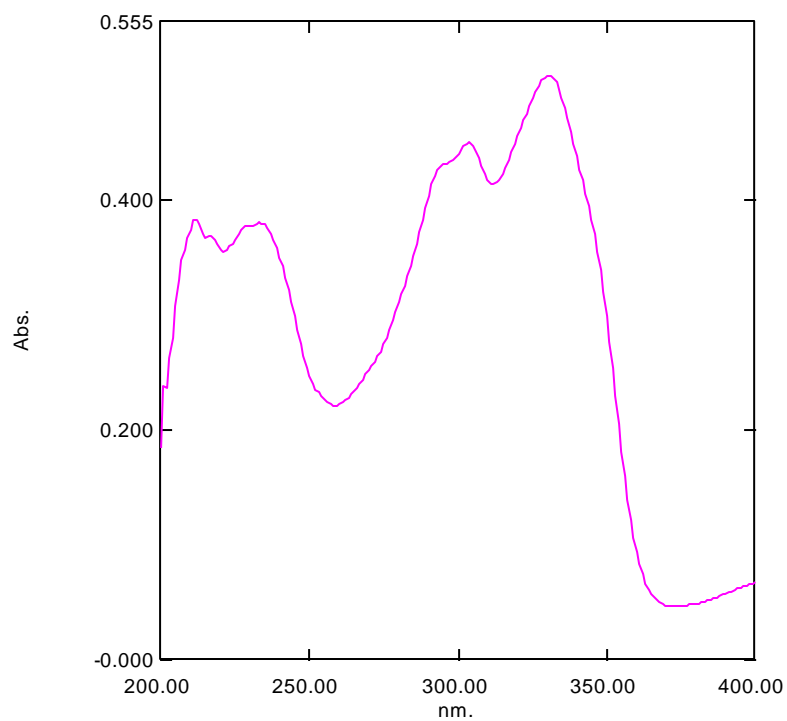


Fig 3.14 U.V. spectrum of Cr (III) complex

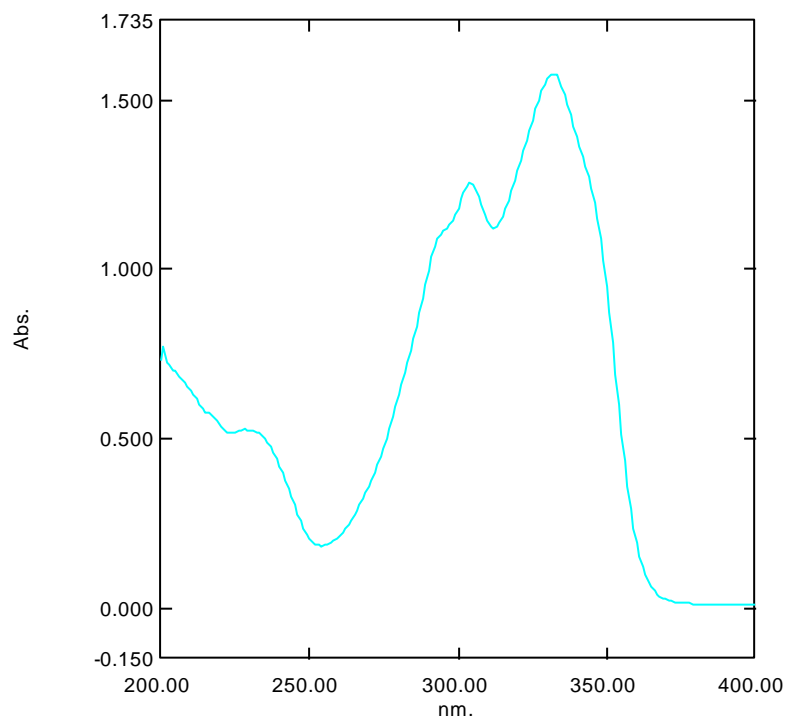


Fig 3.15. U.V. spectrum of Cr (III) f complex

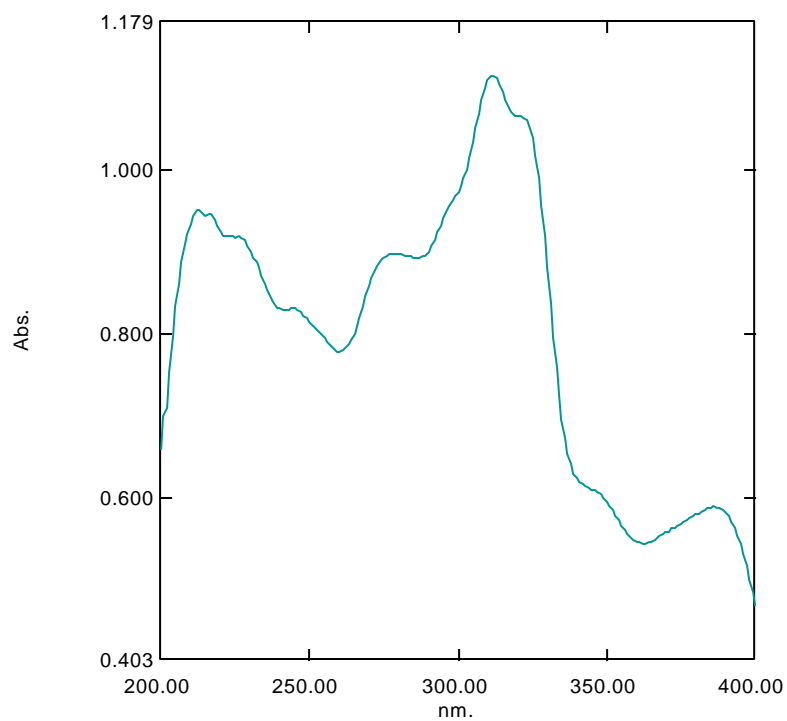


Fig 3.16. U.V. spectrum of Cu (II) complex

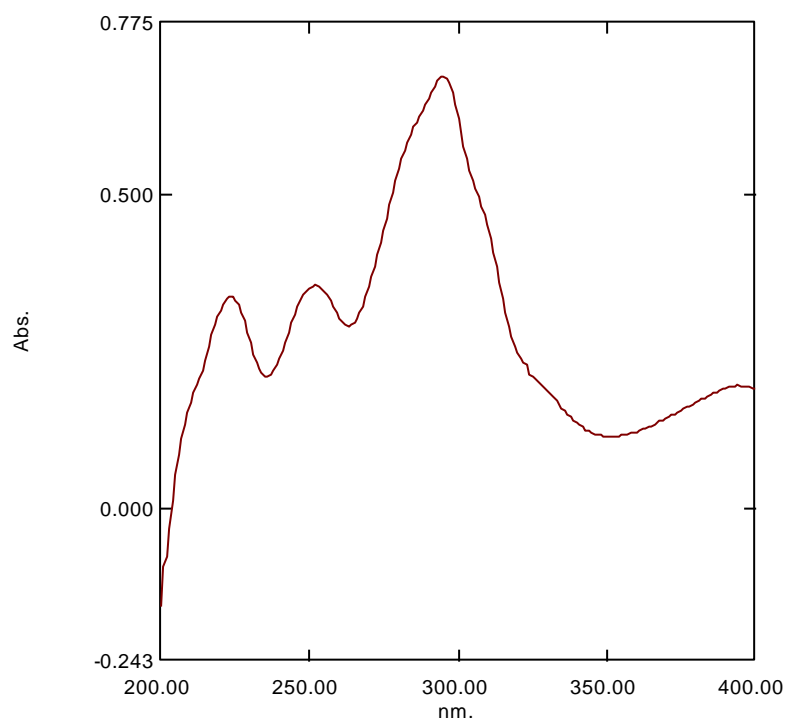


Fig 3.17. U.V. spectrum of Cu (II) f complex

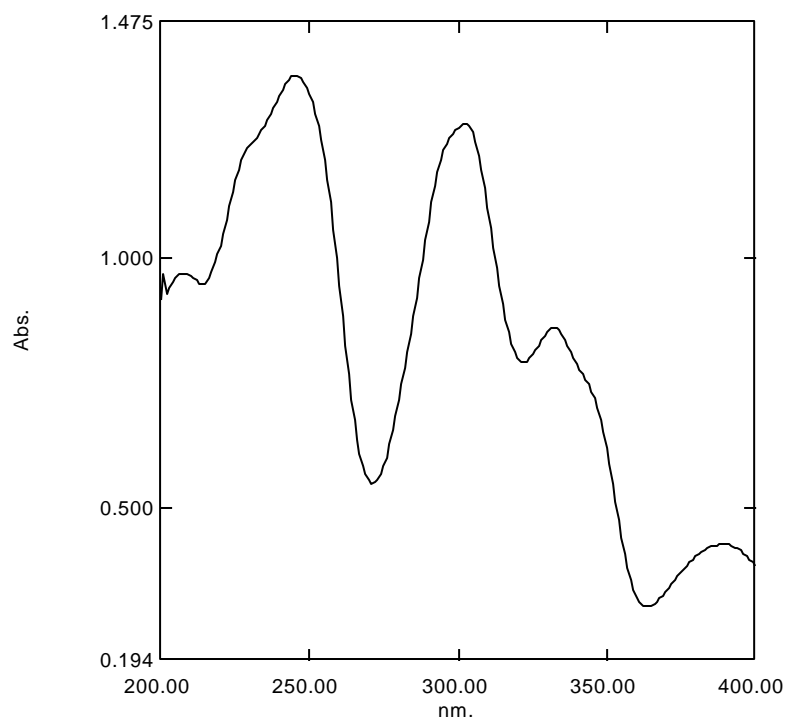


Fig 3.18. U.V. spectrum of Ni (II) f complex

Table (3.3) Absorption bands

S. No.	Sample	Absorption bands cm <sup>-1</sup>	
		n to $\pi^*$	$\pi$ to $\pi^*$
1.	STSC	29940	32787
2.	Ni (STSC)	33113	40816
3.	Co (STSC)	-	38168
4.	Cu (STSC)	32051	46948
5.	Cu (STSC) f	33898	39683
6.	Cr (STSC)	30211	32895
7.	Cr (STSC) f	30120	32895

F=filtrated

### 3.3. Antimicrobial activity:

The ligand Salicylaldehyde Thiosemicarbazone shows absent activity against fungi (*Candida albicans*), gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. Coli*). The Cu (II) complex show high activity against gram +ve bacteria (*Staphylococcus aureus*) and moderate activity against gram -ve bacteria (*E. Coli*). The Ni (II) complex have moderate activity against gram -ve bacteria (*E. Coli*) and have no activity against fungi (*Candida albicans*) and gram +ve bacteria (*Staphylococcus aureus*). The Cr (III) complex show moderate activity against gram +ve bacteria (*Staphylococcus aureus*) and no activity against fungi (*Candida albicans*) and gram -ve bacteria (*E. Coli*). The Co (II) complex shows absent activity against fungi (*Candida albicans*), gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. Coli*). The Antimicrobial activity show in table (3.4).

Table (3.4) Antimicrobial activity

Compound	Gram +ve	Gram –ve	Fungi
	Staphylococcus	E. Coli	Candida albicans
1. Ligand	-	-	-
2. Cu (II) f	21	9	10
3. Cu (II)	20	10	-
4. Ni (II)	-	8	-
5. Co(II)	-	-	-
6. Cr (III)	7	-	-
7. Cr (III) f	-	-	-

F= filtrated

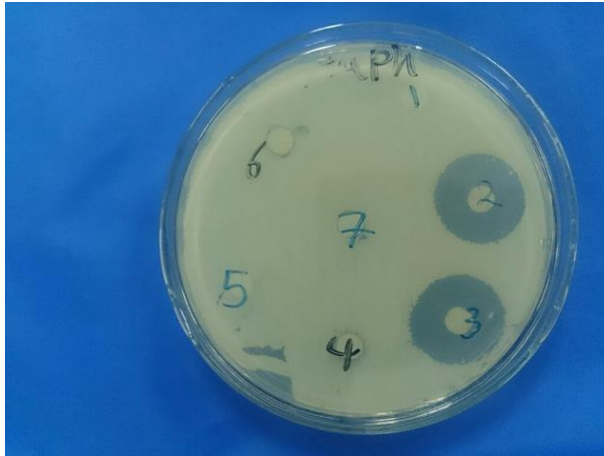


Fig 3.19 *Staphylococcus aureus*

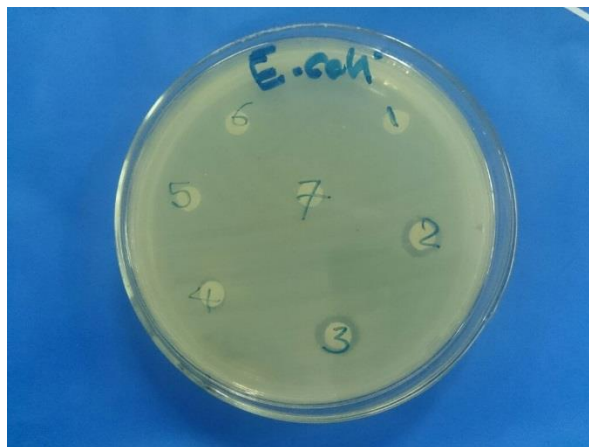


Fig 3.20. *E. Coli*



Fig 3.21. *Candida albicans*

### 3.4. Conclusion:

In the present study:

- The first part of the study include the preparation of ligand and different ligand metal complexes by refluxing in absolute methanol.
- Characterization of the formed ligand and different ligand metal complex using IR, UV and AAs.
- The ligand and different ligand metal complexes, Biological activity were performed by the disk diffusion methods, the inhibition of the microbial growth was evaluated. The ligand Salicylaldehyde Thiosemicarbazone shows absent activity against fungi (*Candida albicans*), gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. Coli*). The Cu (II) complex show high activity against gram +ve bacteria (*Staphylococcus aureus*) and moderate activity against gram -ve bacteria (*E.Coli*). The Ni (II) complex have moderate activity against gram -ve bacteria (*E.Coli*) and have no activity against fungi (*Candida albicans*) and gram +ve bacteria (*Staphylococcus aureus*). The Cr (III) complex show moderate activity against gram +ve bacteria (*Staphylococcus aureus*) and no activity against fungi (*Candida albicans*) and gram -ve bacteria (*E. Coli*). The Co (II) complex shows absent activity against fungi (*Candida albicans*), gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. Coli*).

### **3.5. Recommendation:**

The compound can use to reduce biological activity however the flowing research:

- Researching and developing studies about salicylaldehyde thiosemicarbazone (STSC).
- Applied wide application of biological activity of (STSC).
- Uses more advance analytical technique for (STSC).
- Study the effect of biological activity in silico to suggest suitable
- Use another metal and applied it.



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