ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

GRAGUATE PROJECT

STUDIES ON COPPER(II) COMPLEX DERIVED FROM THIOSEMICARBAZIDE AND NINHYDRIN

BY: GUADIE ABATE

July 2007

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

STUDIES ON COPPER(II) COMPLEX DERIVED FROM THIOSEMICARBAZIDE AND NINHYDRIN

By

GUADIE ABATE

Department of Chemistry

Faculty of Science

Approved by the Examining Board:-

	Signature
Dr. Yonas Chebude	_____
Prof. Nigussie Retta	_____
Prof. V.J.T. Raju	_____

July 27, 2007

Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried out by me under the supervision of my advisor in the Faculty of Science, Department of Chemistry, Addis Ababa University in the academic year 2006 - 2007. No part of this work shall be published in scientific journals or reported in the media or presented at a conference without the knowledge and consent of my advisor, who is the principal scientist responsible for any publication. Furthermore if the work is published the international address given should be that of the Chemistry Department, AAU.

Name: Guadie Abate Signature: _____

This project work has been submitted for examination with my approval as a university advisor.

Advisor: _____

Signature: _____

Place and date of submission: School of Graduate Studies

Addis Ababa University

.

ACKNOWLEDGMENTS

I am very grateful to my advisors Dr.Yonas Chebude and Prof.V.J.T Raju for their invaluable and stimulating interest, unreserved support, innovative ideas and scientific knowledge they have inspired me profoundly during the whale course of the project.

I wish to gratefully acknowledge Dr.Wondmagegn Mammo for his willingness to record all the NMR Spectra.

I am very thankful to Dr.Endalkachew Sahle Demessie from USEPA, Office of Research and Development, National Risk Management Research Laboratory Sustainable Technology Division Cincinnati OHIO for running the mass spectrum.

My sincere thank is also due to Ato Sehalessilassie, W/t Medhanit and Ato Haile who stood beside me during the course of this project.

Finally I am very grateful to the department of chemistry, AAU, for arranging all the facilities and giving me this opportunity.

List of figures

List of Schemes

List of tables

Lists of Appendices

Symbols and abbreviations

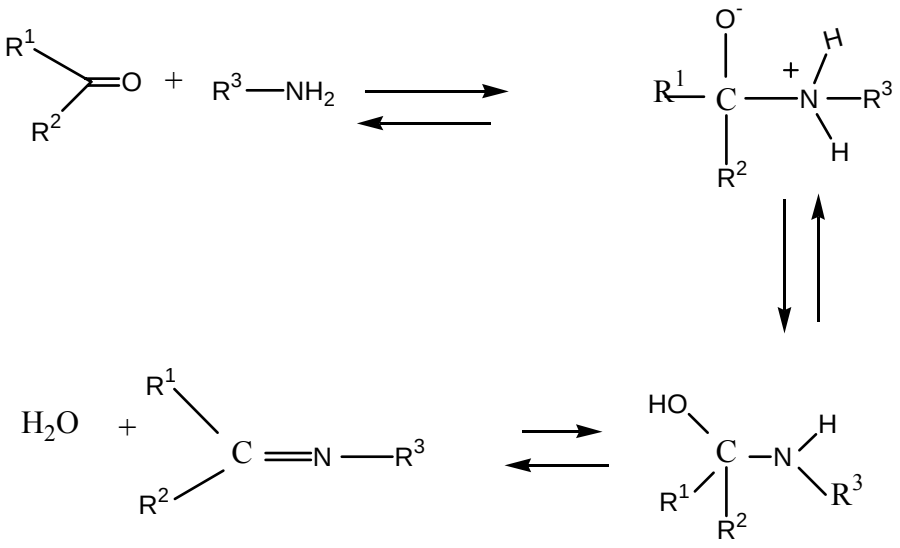
υ	Stretching
λ	Wave length
δ	bending
IR	Infrared
nm	nanometer
M .pt	melting point
Λ	Molar conductance
DMSO	Dimethyl sulfoxide
DMF	Dimethylformamide
TMS	Tetramethylsilane
$DMSOd_6$	Hexadeutrated dimethylsulfoxide
TLC	Thinlayer chromatography
UV-Vis	Ultraviolet-visible
^{1}H NMR	Proton Nuclear Magnetic resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance.
PLTSC	Pyridoxal thiosemicarbazone
PLTSC-H	Deprotonate pyridoxal thiosemicarbazone
OAC^{-}	Acetate ion
Pph_3	Triphenyl phosphine
ECCT	Carbazolcarboxaldehyde thiosemicarbazone
HSQC-	Hetronuclear single coherence.
HMBC	Hetronuclear Multiple Bond Coherence)
Me	Methyl
SALTSC	Salicylic Aldehyde Thiosemicarbazone
TSC	Thiosemicarbazone
DEPT.	Distortion less Enhancement by Polarization Transfer.

Abstract

Anew ON donor ligand was synthesized by reacting ninhydrin with thiosemicarbazide. MS, NMR (^{1}H, ^{13}C, DEPT, HSQC, and HMBC) and UV-VIS spectra proved the formation of a new heterocyclic compound initially due to 1:1condensation between ninhydrine and thiosemicarbazide and subsequently due to hydration /tautomerization and cyclization of the thiosemicarbazone. The ligand formed a pale green Cu(II) complex in ethanoic medium.The complex was characterized by analytical, conductance, spectral (IR,UV-Vis) and magnetic susceptibility data . The data suggested the formation of a 1:2 (Cu(II) : ligand) complex with the formula $[CuL_2]Cl_2$ in which the ligand behaved as a neutral ON donor involving exocyclic carbonyl oxygen and heterocyclic ring nitrogen as coordinating centers. Square planer geometry was proposed for the complex

1. Introduction

Compounds containing imines or azomethine groups (R-C=N-) are usually formed by the condensation of primary amine with carbonyl containing compound. The condensation of primary amine with carbonyl compounds was first reported by Schiff and the condensation products are often referred to Schiff bases [1] Reaction to prepare Schiff base is reversible and progressing through carbinol amine intermediate and requires the removal of water.



R^1,R^2,R^3 =alkyl or phenyl

Scheme 1 Formation of Schiff base.

Schiff bases which are effective as coordinating ligands have functional groups such as OH, NH_2, SH etc. sufficiently near the site the azomethine function which is obtained through condensation reactions in the presence of metal ions .[2]

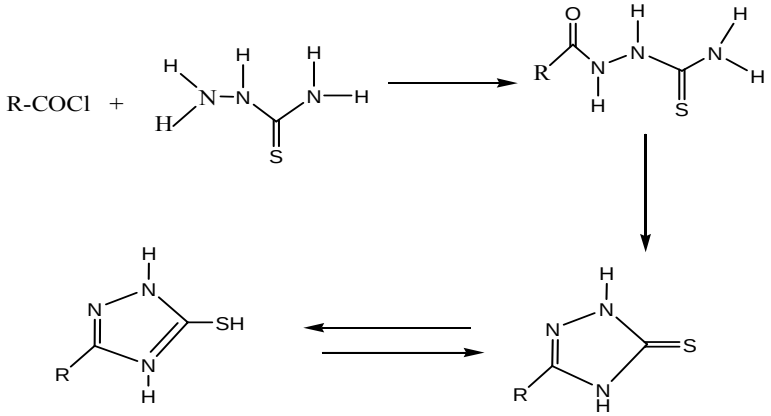
Metal complexes of Schiff bases possess considerable potential in many applications including biological, clinical, analytical, catalytic, microbial, insecticidial, antibiotics, tumor inhibitors [2, 3] Numerous researchers have attempted to synthesize a number of metal complexes of Schiff bases from different carbonyl and amine containing compounds. The present project is proposed to develop a new Schiff base from the condensation between thiosemicarbazide and ninhydrin.

1.1. Chemistry of thiosemicarbazides and their derivatives

Thiosemicarbazides belong to the family of hydrazine-based ligands with multifunctional donor centers. The application of thiosemicarbazide based ligands for bonding to transitional metals is of fundamental importance in understanding the metal organic hydrazine and also enhances scope and utility of nitrogen family of ligands in transition metal chemistry[4]

These compounds are easily cyclized by the action of acids, bases, or oxidants, and they are valuable building blocks for the synthesis of five membered heterocycles.[5] Biologically active compounds such as 1, 3,4 thiadiazoles, as anti bacterial and antifungal agents [6,7]; 1,3,4 thiadozolium-2-amidins as anticonvulsants ,antimicrobials and anti tumor agents.[8]; mercapto and thione-substituted 1,2,4-triazoles anti bacterial, antifungal, anti tubercular agents [9] are synthesized from thiosemicarbazides.

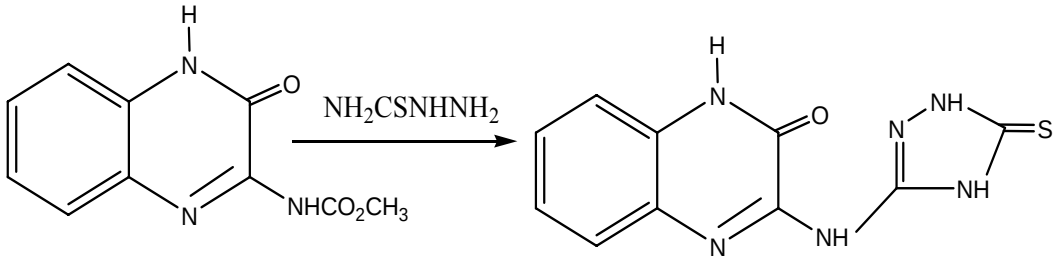
The following reactions describe the formation of some biologically active compounds from thiosemicabazides.The reaction of carboxylic acid chlorides with thiosemicarbazide in alkaline media give the corresponding mercapto and thione substituted 1, 2, 4 triazols.[10]



R=H, CH_3, CF_3, C_6H_6

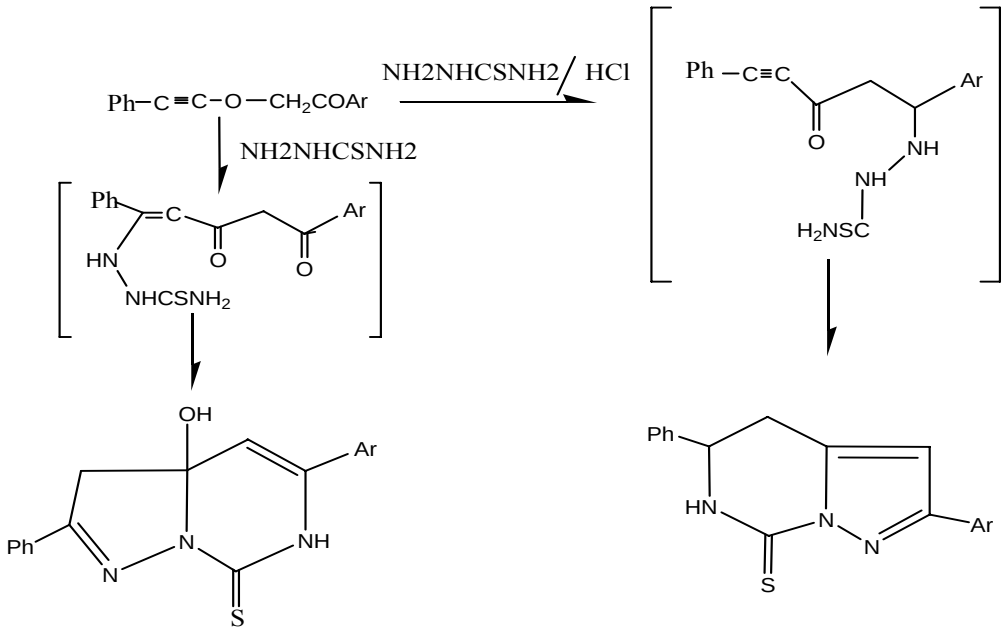
Scheme 2 Formation of 1, 2, 4-triazol-3-thiones

Condensation of carbamate with thiosemicarbazide in boiling pyridine gives triazolylquinoxaline via nucleophilic attack of the amino group of the ester carbonyl without attack at the carbonyl of the pyrazine ring followed by intramolecular cyclization reaction[11].



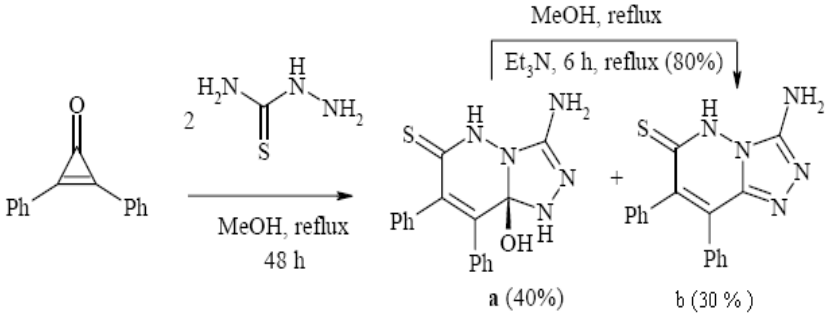
Scheme 3 Formation of triazolyl quinoxaline

Depending on the nature of the constituents as well as the reaction conditions thiosemicarbazide reacted with acetylenic ß-diketones to give different pyrazolo pyrimidinethiones.11



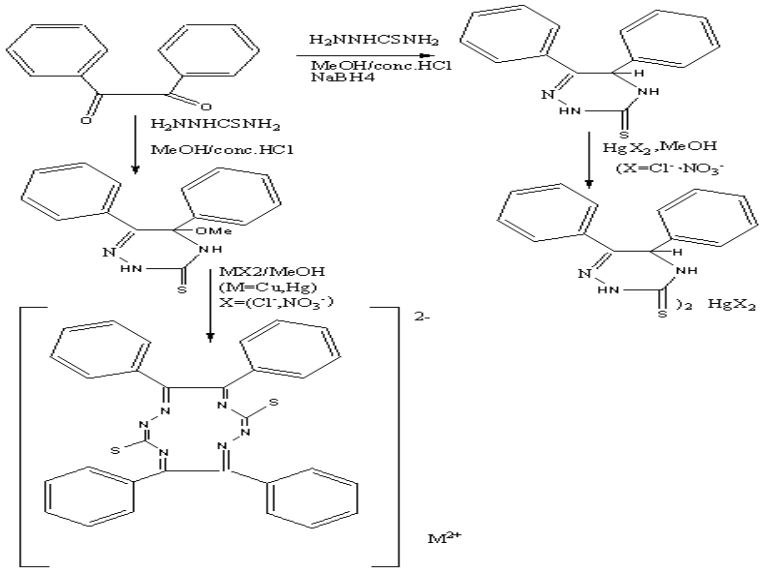
Scheme 4 Formation of pyrzolo pyrimidine thiones

Recent studies on the reaction of thiosemicarbazide and 2, 3-di phenylcyclopropenone indicates 1, 2, 4 triazolo pyridazines thions have been synthesized via cyclization reaction. The researchers commented that compound b (scheme **5)** was formed by refluxing compound in methanol containing a few drops of triethylamine and 80 percent yield was obtained. But during the period of refluxing in the absence of diethylamide gave only 30 percent.



Scheme 5 Reaction of thiosemicarbazide and 2, 3-di phenylcyclopropenone

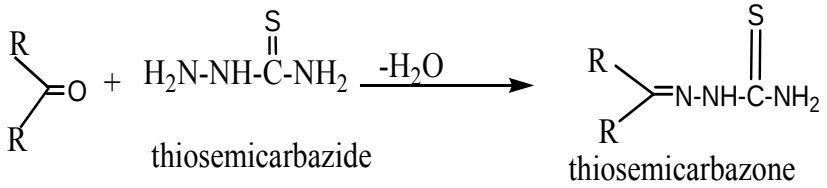
The reaction of thiosemicarbazide was also used for the synthesis of different Schiff base macro cyclic ligands. The macro cyclic ligands allow selective complexation and extraction of metallic cations and anions of environmental importance [12]



Scheme 6 Formation of macrocyclic ligands and metal complexes from thiosemicarbazides and benzil

The thiosemicarbazide are essential materials for the formation of thiosemicarbazone through condensation reaction with suitable carbonyl compounds.[13, 14, 15]

.

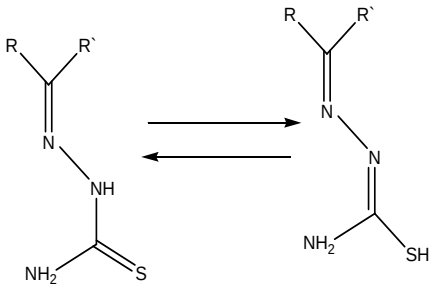


R=alkyl, H or Aryl

Scheme 7 Formation of thiosemicarbazon from thiosemicarbazide and carbonyl compounds

Thiosemicarbazones are multidentate ligands which usually coordinate to metal centers through the sulfur and azomethine nitrogen atoms to give a five-membered chelates ring with the metal. [13, 16]

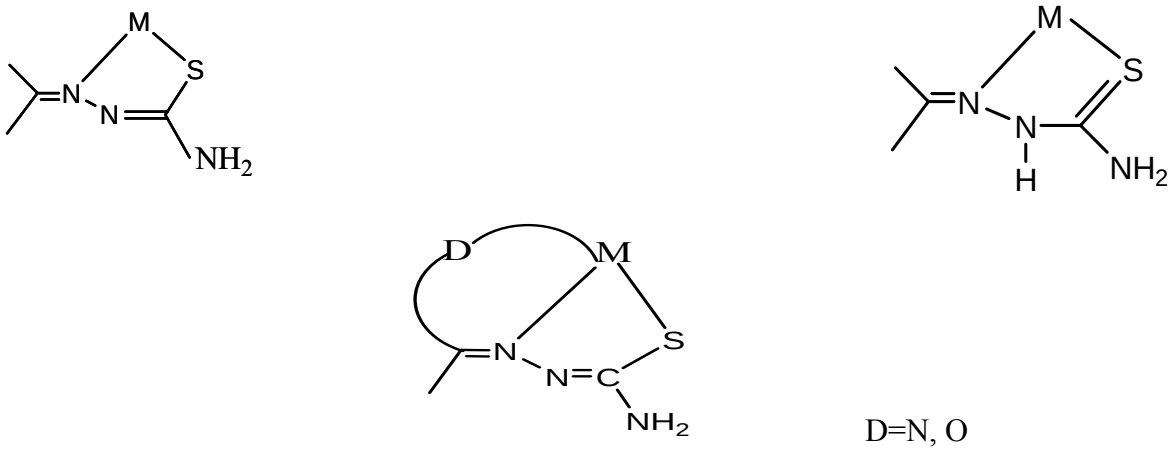
Thiosemicarbazones in the solid state are in an approximately planar arrangement with a *Z* configuration around the C=N bond and the terminal thioamide sulfur atom in a *trans* disposition with respect to the azomethine nitrogen **(**Scheme 8). The fact that this system undergoes *thione–thiol* tautomerism makes the thiosemicarbazones versatile ligands for a wide range of metal ions both in the neutral and anionic forms [17, 18.]



Scheme 8 Thautomeric form of thiosemicarbazones

1.1.1. Coordination modes in thiosemicarbazones.

The thiosemicarbazone ligands can be coordinated either in neutral or anionic form. The anionic form is formed by dissociation of the acidic protons resulting the formation of a five membered chelat ring. When an additional donor site D is incorporated in such ligands,linked to the carboxylic carbon via one or two intervening atoms ,D,N,S tricoordination usually takes place.[19] The tricoordination system is most common in heterocyclic thiosemicarbazide.

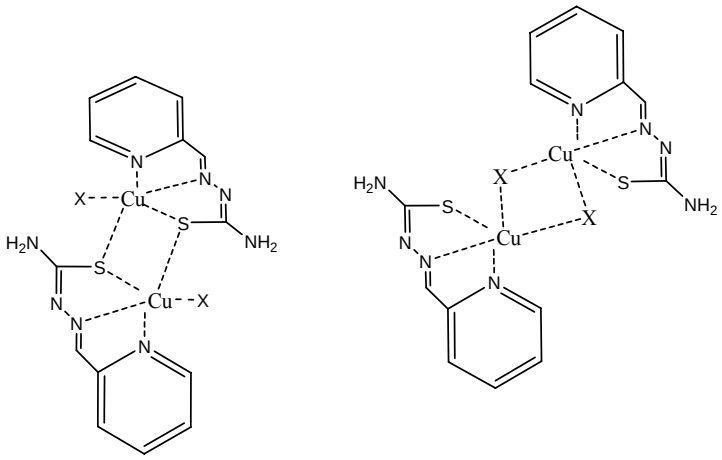


.

Figure 1 Complexing modes of thiosemicarbazones

The form of the coordinated ligand will depend on the pH and the nature of the metal ion .Namely the presence of proton accepter (NH_3,OAC^- Py) etc will facilitate the formation of complexes with anionic form of the ligands. For example studies showed that ,with $[Zn(NO_3)_2$ and $Ni(NO_3)_2$ pyridoxal thiosemicarbazone give $[Zn(PLTSC)\ (NO_3)_2].H_2O$ and $[Ni(PLTSC\text{-}H)\ NO_3].2H_2O$ Where as with $M(OAC)_2.H_2O$ (M=Zn,Ni) it forms $[Zn(PLTSC\text{-}H)\ AOC^-].H_2O$ and $[Ni(PLTSC\text{-}2H)\ ].2H_2O$.[20]

Other interesting mode of thiosemicarbazones has been found in the formation of dimeric and polymeric complexes. In these complexes, the metal ions like Cu (II) are bridged by either sulfur or non thiosemicarbazone coligands such as nitrite, formayto [21]etc.



X= Cl^-, Br^-, NSC x=oxygen containing coligands.

Figure 2 Dimeric structure of Cu (II) pyridine-2-carbaldehyde thiosemicarbazone

In recent studies some interesting coordination modes were also observed for some tridentate thiosemicarbazones. For example in the Ti (III) complex of the formula Ti Me(PLTSC-H)(H_2O)$_2$ anion in it's thiol form is bidentately coordinated via the enolyzed sulfur atom and unexpectedly via the oxygen atom of un deprotonated phenolic hydroxyl forming the nine member metallocycle and neither of the hydrazine nitrogen atoms participates in the coordination [13]

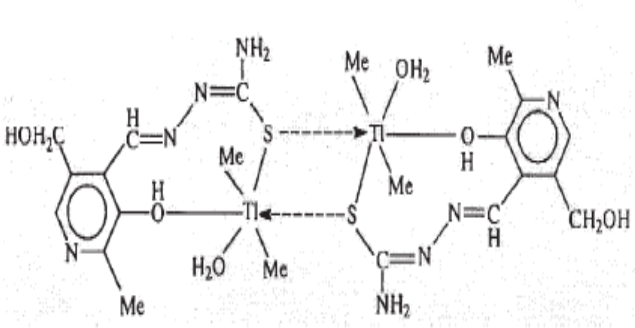


Figure 3 Structure of Ti Me_2 (PLTSC-H) (H_2O) $_2$.

Binuclear Zn^{2+} complex has also been reported with the 2, 6-diacetyl and 2, 6 diformyl pyridine bis (thiosemicarbazone) ligands. In the complexes each Zn^{2+} is coordinated to two sulfur and two nitrogen atoms from two different 2,6 diacetyl and 2,6-diformyl bis (thiosemicarbazones) ligands.,and the two pyridine nitrogens have interaction that bridge the Zn centers of the binuclear units to bring the coordination number to six.[22]

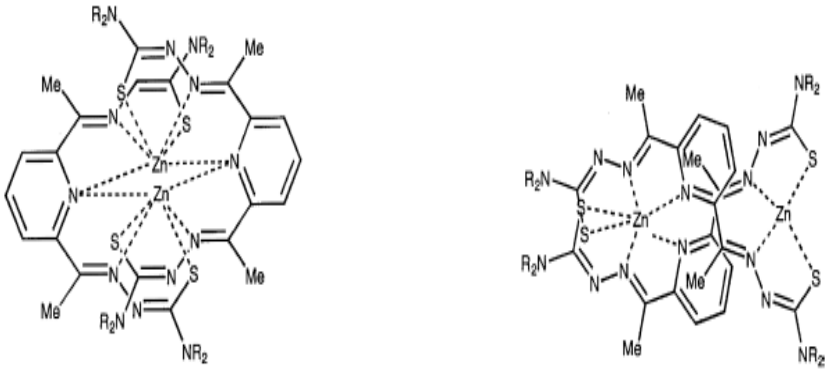
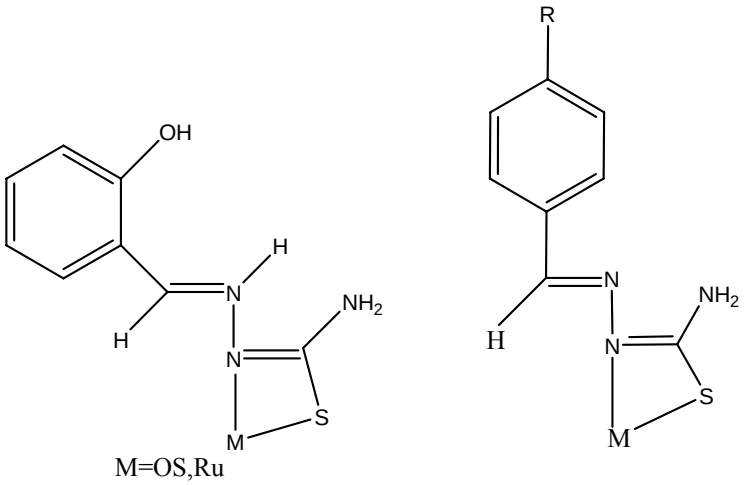


Figure 4 Structures of dinuclear Zn (II) complexes

Further studies also showed that some thiosemicarbazones showed unusual mode of coordination to the metal ions. For example tridentate ONS salicyl aldehyde thiosemicarbazone (SALTSC) in complex of the type [M (pph_3) $_2$ (SALTSC-H) $_2$ M= Ru(II) ,Os (II) is coordinated as a bidentate (NS) moiety, the TSC residue in this case is not coordinated via the azomethine nitrogen but via the hydrazine nitrogen.Thus forming a four-member ring. The same coordination mode of the coordinating thiosemicarbazone residue has also been found in the case of complexes with some other thiosemicarbazone,such as Ti (III) and Ru (II) complexes with bidentate thiosemicarbazones derivatives of acetylferrocene andbenzaldehyde[23,24]

.



R =OCH_3, H, NO_2

Figure 5 **Unusual coordination mode of thiosemicarbazones**

1.1.2 Application of thiosemicarbazones

A. Analytical application

Many thiosemicarbazones form stable colored metal complexes. These complexes are then used in selective and sensitive determinations of metal ions, From this stand point some transition metal ions have been determined by using thiosemicarbazones some of them have been proposed as analytical reagents. For example, a kinetic spectrophothometric method was used in several cases to determine binary metal ions mixtures with pyridoxal thiosemicarbazone. Studies showed that the method was successfully used to determine iron(III) and copper(II) in serum samples and copper(II) and cobalt(II) in steel samples, Fe (III) in some standard alloys

and pharmaceutical samples by pyridoxal N4-phenylthiosemicarbazone.[25]. Recent studies have also shown that N-ethyl carbazolcarboxaldehyde 3-thiosemicarbazone (ECCT) can be use as a sensitive reagent for the determination of Zn (II) containing samples. The ECCT forms yellow colored species of Zn (II) at a pH range 3.0-5.5 and the complex shows maximum absorbance at 420nm.[26] .In addition many researchers showed that thiosemicarbazides are good analytical reagents to determine the amount of acetaldehyde in blood.

B. Biological activities of thiosemicarbazones

Nowadays it is known that thiosemicarbazones and their metal complexes show anti tumors anti-virals, anti-fungal and anti-bacterial and anti-malarial activities. More over metal complexe of thiosemicarbazone often display enhanced activities when compared with the uncomplexed thiosemicarbazon activity. For example p-acetamodo benzealdehyde thiosemicarbazone (trivial name thioacetazones or Tibon) is employed in the clinical treatment of tuberculosis and functioned as a potential N, S-bidentate ligand and.3-aminopyridine-2 carboxaldehyde thiosemicarbazone (Triapine) is used for treating iron overload disease[13].

The thiosemicarbazone based metal complexes (for example Cu(II) and Zn (II) are more efficient inhibitors of cancer cell growths. [13] Platinum (II) and palladium (II) complexes of pyridine-2-carbaldehyde thiosemicarbazones as an anti herpes simplex virus agents[27]; ribose bis (thiosemicarbazanto) copper (II) as nitric oxide scavenger[28]; acetyl acetonate bis(thiosemicarbazone) Cu(II) for radiopharmaceutical application[29] also documented.

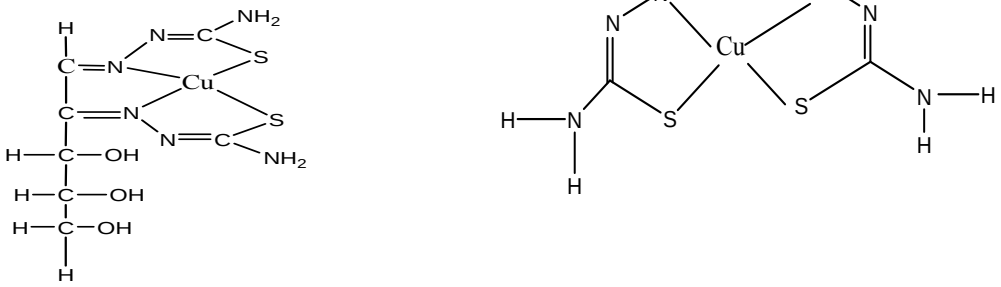


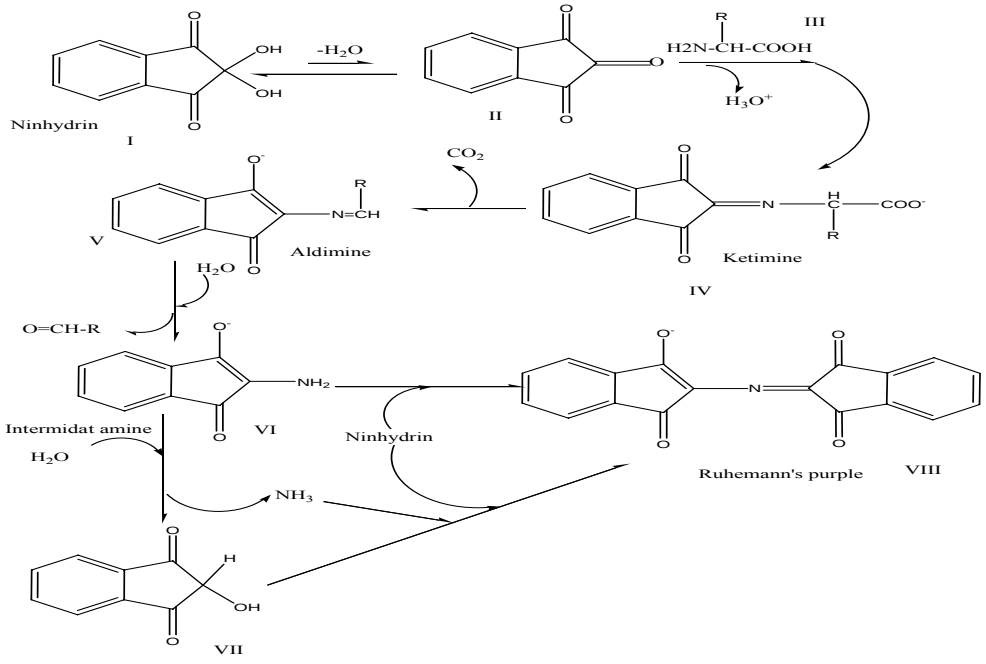
Figure 6 Structure of ribose and acetyl acetonate bis(thiosemicarbazanto) copper (II) complexes

1.2 Metal complexes of Ninhydrin derivatives.

Ninhydrin (1, 2, 3- triketohydrin hydrate) is an important reagent and analytical tool It was discovered by an English chemist Siegfried Ruheman in 1910. It has long been used as coloring material in the paper chromatographic separation of amino acids as a reagent to make them visible.

It is also used in the quantification of amino acids because of its stiochiometric reaction with free amino groups .In the reaction ninhydrin (2,2-dihydroxy -1,3-indane or 1,2,3 triketohydrindine hydrate reacts with amino acids except proline and hydroxyproline to give characteristically blue (purple, colored compound) popularly known as Ruhemans purple [30,31.32] .The λ max. for the blue compound is around 570 nm and this forms the basis for the spectroscopic quantitative determination of amino acids that can detect as little as one microgram quantity[30],[33,]

The chemistry of the reaction between ninhydrin and amino acids has been extensively studied by several workers. The mechanism how ever was not well understood until very recently and this gave rise to a series of theories as reviewed by McCaladine .A simplified form of the mechanism proposed by Filippovich and McCaladine is shown in the scheme 9. It has a condensation step that leads to Schiff base formation followed by decarboxlation, hydrolysis and finally further condensation with other ninhydrin molecules to give the final product Ruhemann's purple.[32, 33, 34,]



Scheme 9 Mechanism of ninhydrin reaction with amino acids

Among the reaction products of ninhydrin with aminoacids the ketimins is a potential Schiff base ligand that can act as a tridentate forming two stable five member rings on complexation. Studies on the coordination of Schiff bases derived from ninhydrin with glycin[34] , L-valine, L-Alanine [35], L-Histidine[36] to some transitional metal ions (Ni^{2+},Co^{2+},Zn^{2+}) showed that if a metal ion is present before the reaction begins , the reaction does not always proceed to the final product (Ruhemann's purple) ,.but stops at the first step (at ketamine stage) and the metal ions form highly stable complexes with the Schiff bases. Most of these metal complexes have octahedral geometry in which the ligands behave as monobasic tridentate (ONO) donors.[36]

The reaction of ninhydrin is applicable for the detection, isolation and analysis of numerous compounds across a broad of spectrum of disciplines such as agricultural, biochemical, medicinal, nutritional, plant science and in forensic science to study finger prints.[37]

1.3. The Chemistry of Cu (II) complexes

Cu (II) has d^9 configuration. The stereochemistry of Cu (II) is very variable. The most common observed arrangements of ligand atoms are square planer and distorted octahedral. Elongation distortion is the most common in many Cu(II) octahedral complexes[40,41] Octahedral complexes with out any distortion are expected to have only one d-d absorption band corresponding to $^2Eg \rightarrow {}^2T_2g$ transitions. For distorted octahedral complexes several weak absorption bands are observed around 16000 cm^{-1} and often abroad band in the near IR region .In the axially elongated tetragonal distortion three absorption bands corresponding to the transition $^2B_1g \rightarrow {}^2A_1g$, $^2B_1g \rightarrow {}^2B_2g$, and $^2B_1g \rightarrow {}^2Eg$ are observed. In addition distorted tetrahedral species, such as $[Cu(C_6H_{11}NH_2)_4][NO_3]_2$,Schiff base complexes with bulky constituents on N and some diphyromethane species are also mentioned.[38]

Tetrahedral complexes are expected to give a single broad band corresponding to $^2T_2 \rightarrow {}^2E$ transition in the near IR regions. The extreme limiting distortion both in octahedral and tetrahedral geometry is square planer. True square planer coordination is observed in the Cu (II) complexes of phatalocyanines, but in some square planer complexes, the molecules are stacked, so that there is weak interaction between the Cu (II) and the middle carbon atom of the neighboring molecule.[39]

The ground term in the square planer geometry is ^2B_1g and three d-d bands corresponding to the transition $^2B1g \rightarrow {}^2B_2g$, $^2B1g \rightarrow {}^2A_1g$, and $^2B_1g \rightarrow {}^2Eg$ are observed. In both square planer and tetrahedral geometries the transition are not well resolved.

Five coordination of Cu (II) is also observed in some other compounds in which there are four N-donors such as bis dipyridyl copper(II), terphyridyl copper(II) complexes.[39]

1.4 Objectives and scope of the present investigation

Literature survey reveals that metal complexes derived from thiosemicarbazide and different carbonyl containing compounds received much attention by researchers .This is due to their wide application in different fields such as medicine, analytical, catalytical, microbial growth factors ,tumor inhibitors etc .How ever there is no significant attempt appeared in literature for the synthesis of metal complexes derived from ninhydrin and thiosemicarbazide. In view of this, it was aimed to synthesis Cu (II) complexes derived from ninhydrin and thiosemicarbazide. It was also aimed to elucidate the structure of Cu (II) complex. .It will be of interest to study the reaction between ninhydrin and thiosemicarbazide and examine whether the reaction is straight forward 1:1 Schiff base formation or of 1:2, 1:3 stoichiometry or a cyclization product formation is possible. It will also be a worthwhile investigation to understand the complexing or chelating ability of the product formed. In view of the versatility of Cu (II) ion in its structural characteristics it is aimed to attempt the synthesis of Cu (II) complex using the product between ninhydrin and thiosemicarbazide

Analytical, spectral (IR, ^{1}H NMR, ^{13}C NMR, UV-Vis, Mass), AAS, conductance and magnetic susceptibility studies will be appropriately applied for the characterization of the ligand and the Cu(II) complex.

2. Experimental

2.1. Chemicals

Most chemicals used in the investigation were of AnalaR grade. The salt used was $CuCl_2.6H_2O$. Other chemicals include ninhydrin,thiosemicarbazide $AgNO_3$. Absolute ethanol, absolute methanol, tetrahyderofuran, dimethylsulfoxide(DMSO), dimethylsulfonamide (DMF), HNO_3,acetonitril,petroleum ether, acetone, chloroform, distil water, deionized water, deutrated DMSO, D_2O water were also used as a solvent . Nitric acid and ammonia solution were also used.

2.2 Instrumentation and method

Electronic spectra of the compounds were recorded in solution (10^{-5}) using supertonic Genesys 2PC UV-VIS spectrometer with a1cm cell at room temperature. Melting points /decomposition temperatures of the products were determined on BUCK MODEL SCIENTIFIC 210VGP digital melting point apparatus. Infrared spectra were recorded on a perkin Elmer spectrum BX FT-IR spectrometer in the range of 4000-400 cm^{-1} in KBr medium.

^{1}H and ^{13}C-NMR were recorded on BRUKER Advanced 400 MHZ Spectrometer with TMS internal reference in DMSO-d_6. Mass spectrum of the ligand was recorded on FINIGAN high temperature insertion probe instrument. The sample was ramped from RT up to 300 ^{0}c .Molar conductivities of the complex in a mixture of acetnitrile and methanol was recorded at room temperature with freshly prepared 0.39 mM solution using JENWAY 4330 conductivity and pH meter. Metal content of the complex was determined using a Flam Atomic Absorption Spectrometer. Magnetic susceptibility measurement of the complex in the solid state was carried out on MSB-AUTO (Sherwood scientific). Chloride from the samples was determined as AgCl by gravimetric method using standard methods. The purity of the ligand and the complex was also tested by thin layer chromatography.

2.3 Synthesis part

2.3.1. Synthesis of the ligand

To a hot ethanol solution of thiosemicarbazide (0.255 gram or 2.5 mmol) 0.5 gram (2.5 mmol) of an ethanol solution of ninhydrin was added .The resulting solution was stirred for 12 hours by magnetic stirrer. The whitish yellow precipitate was filtered off using suction and washed with cold ethanol. Finally the product was dried and stored in desiccator.

2.3.2 Synthesis of the Cu (II) complex

To a hot magnetically stirred absolute ethanol (30 ml) solution containing 1.5 mmol of the ligand, 0.75 mmol of $CuCl_2$.$6H_2O$ dissolved in 20 ml of ethanol was added. The resulting solution was then stirred for six hours and allowed to stand over night .The green precipitate was filtered off under section and then washed with cold ethanol, dried and stored in desiccator.

3. Results and discussion

3.1 Physical characteristic of the ligand

Some of the physical properties of the ligand are listed in Table **1.** The ligand is soluble in DMSO, DMF, acetonitrile, warm methanol and hot ethanol. However, it is insoluble in water and in most non-polar solvents like diethyl ether, petroleum ether, benzene, nitrobenzene, dioxane

Table 1 Some physical properties of the ligand

compound	Mol. formula	Mol. Weight (g/mol)	**A**ppearance	color	Melting (decomposition) Point	Yield %
ligand	$C_{10}H_9O_2N_3$ S	251	microcrystaline	Whitish yellow	197-200 0_C	55

3.2. Spectroscopic result of the ligand

Since the reaction was carried out in one to one (1:1) molar ratio, theoretically the following structures can be expected.

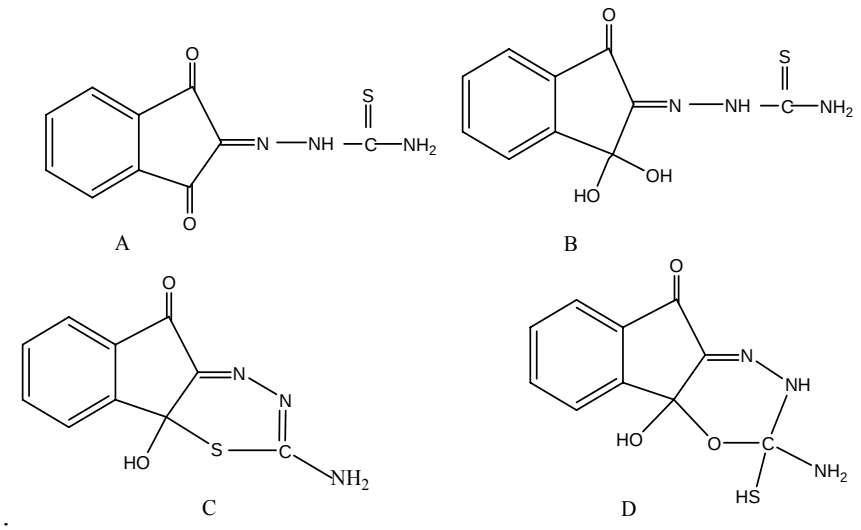


Figure 7 **Theoretically expected structures**

Spectral studies (IR, NMR) indicate a 1:1 condensation between ninhydrin and thiosemicarbazide with subsequent cyclization resulting in a new ON heterocyclic system appeared to the condensed ninhydrin function. The most likely mechanism of its formation is shown in scheme 11.

3.2.1. Infrared spectral studies.

The Infrared data are given in Table 1 and the spectrum is attached on **appendix 1.**The strong bands observed at 3320 and 3274 cm^{-1} in the free ligands have been assigned to $\upsilon(NH_2)$ asymmetrical and symmetrical stretching frequencies respectively. As it can be seen on the spectrum the weak band at 3421cm^{-1} may be due to OH stretching. This band has similar feature to the OH stretching of the ninhydrin attached on appendix 2. The presence of this group further supported by ^1H NMR spectrum. The presence of the hydroxyl group was also supported from the weak bands at 720 and 1378 cm^{-1} assignable to out of plane and in plane bending respectively. The strong band observed at 3122 cm^{-1} has been assigned to NH stretching. υ(SH) appears as a weak band at 2741 cm^{-1}.

The absorption band at 1720 cm^{-1} is attributed to the C=O stretching of the exocyclic carbonyl group remained in the ninhydrin moiety. Free ninhydrin shows three bands in the C=O stretching region: 1768, 1754, 1720 $cm^{-1.}$ The 1754, 1720 cm^{-1} bands are characteristics of it's 1, 3-dicarbonyl functional groups and the 1768 cm^{-1} band is characteristics of the intermediate carbonyl in the tricarbonyl species which is in equilibrium with the dihydroxy species.[37] In the synthesized ligand only one absorption band is observed in the free ninhydrin C=O stretching region, which shows the derivitization or conversion of the two carbonyl groups. A number of bands in the range of 1648-1467cm^{-1} in the spectra of the ligand are assigned to C=C and C=N stretching. The stretching frequencies such as 1289, 1249,1195,1168,1110 cm^{-1} may be contributed from υ(C-N), υ(C-O) of the alcohol and υ(C-O-C) of the cyclic ether.

Table 2 Selected vibration bands (cm^{-1}) of the ligand and its copper (II) complex.

Chemical groups.	Stretching /bending frequencies in cm^{-1}	
	Ligand	Cu (II) complex.
υ(NH_2)	3320 3274	3204
υ(OH)	3421	3409
υ (NH)	3122	3109
υ (C=O)	1720	1707.91
υ(C-N)+υ(C-O)+ υ (C-O-C)	1289,1249,1195,1168,1110.	1298,1238,1208,1122
δ(OH)	720,1378	719,1395
υ (N-N)	1009	1025
υ (S-H)	2741	2741
δ(CH)	772	772
υ(C=C)+υ(C=N)	1600,1648,1500	1601,1517.
υ (C-S)	686	680
Cu-O, Cu-N	--	500,666

3.2.2 ^{1}H and ^{13}C NMR spectra studies

Both ^{1}H and ^{13}C NMR spectra of the ligand were recorded in $DMSOd_6$.

Table 3 lists the ^{1}H NMR chemical shifts of the ligand. The assignment is also supported by the two dimensional NMR spectroscopy techniques. From the proton NMR spectrum there are nine protons with different chemical shift. The HSQC data which is helpful to assign the proton is given on Table 4 and appendix 4. The data indicates the type of proton attached to each carbon atom[40]. From the spectrum there are only four protons directly attached to four carbons. Hence there are a total of nine protons the remaining five protons are attached to oxygen, nitrogen and sulfur.

Table 3 ^{1}H NMR data of the ligand

Type of proton	Number of proton	δ H NMR/ppm	Appearance
OH	1	10	singlet
NH	1	7.0	singlet
NH_2	2	4.5	singlet
SH	1	7.3	singlet
H of C-5	1	7.8-7.85	doublet
H of C-6	1	7.90-7.86	triplet
H of C-7	1	7.65-7.60	triplet
H of C-8	1	7.80-7.75	doublet

Table 4. HSQC data of the ligand.

Carbon number	Chemical shift	Proton chemical shift and appearance
C-6	137	7.90-7.86 (Triplet)
C-7	131	7.65-7.60 (Triplet)
C-8	125	7,80-7.85 (Doublet)
C-5	123	7.80-7.75 (Doublet)

As it can be observed from the DEPT ^{13}C spectrum **(appendix 6), t**here are four CH carbons. The four CH carbons are assigned for C-5, C-6, C-7 and C-8 of the aromatic or benzene ring carbons. There for the protons appeared in the HSQC spectrum are protons of the benzene ring. Specifically based on the HSQC data δ = 137 is assigned for C-6, δ = 131 is assigned for C-7, δ = 125 is assigned for C-5 and δ = 123 is assigned for C-8. The remaining protons which are not attached to carbon atoms are assigned in the following manner. The singlet at δ = 10 is may be due to the OH proton, the singlet at δ = 4.5 is assigned for the two protons of NH_2 and the peaks at δ = 7.0 and 7.3 are assigned to N-H and S-H respectively. The peaks at δ=4.5, 7.3, 7.00 and 10 were disappeared from the D_2O exchange spectrum **(appendix 5).** This confirms that the protons were attached to O, N, S and they easily exchanged with deuterium.

From ^{13}C NMR (**Table 5 and appendix 7**) there are ten carbons in different chemical shifts. The four carbons at δ =137, 131, 125, 123 are already assigned from the Dept ^{13}C spectrum and they belong to proton bearing carbons of the benzene ring. In addition to this, there are six additional quaternary carbons. From the spectrum δ = 194 is assigned for C-3.This carbon is the carbonyl carbon remained at the ninhydrin moiety. The δ = 150 and δ = 132 are assigned for C-4 and C-9 of the benzene ring respectively. The peak at δ = 179 is assigned for C-2 and this carbon is the azomethine carbon. The peaks at δ = 90 and 87 are assigned for C-10 and C-1 respectively. The formation of the two peaks at lower chemical shift may be due to the presence of two aliphatic quaternary carbons due to the nucleophilic attack of one of the carbonyl group of the ninhydrin by water followed by intermolecular cyclization reaction.

The assignment is also supported by the HMBC (Hetronuclear Multiple Bond Coherence) technique which is important to determine long range ^{1}H - ^{13}C correlation or coupling[40]. The HMBC data and spectrum are given in **Table 6 and appendix 8**. From the spectrum, except one proton having δ = 7.30, all protons have correlation with two and three carbon atoms in the compound. From the spectrum the protons at a δ = 4.5 which is assigned for NH_2 has correlation with the azomethin carbon (C-2) and the aliphatic carbon (C-10). The NH proton at δ = 7.00 has also correlation with the aliphatic carbon (C-10), the benzene carbon (C-4) and the azomethine carbon (C-2). The OH proton has also correlation with two quaternary aliphatic carbons (C-1 and C-10) and the azomethin carbon (C-2). Especially the presence of correlation between OH and the two aliphatic carbons may be due to the presence of bond between them.

Table 5 ^{13}C NMR data of the ligand.

Type of carbon	Number of carbons	δ ^{13}C NMR/ppm
C1	1	87
C2	1	180
C3	1	190
C4	1	150
C5	1	123
C6	1	137
C7	1	131
C8	1	125
C9	1	132
C10	1	90

Table 6 HMBC data of the ligand.

Proton chemical shift	Chemical shifts of correlated carbons
7.65-7.60 (triplet)	132,137,125
7.86-7.90 (triplet)	123,150,137
7.75-7.80 (doublet)	190,150,137
7.80-7.85 (doublet)	131,132,87
7.00 (singlet)	90,179,150
4.5 (singlet)	90,179
10 (singlet)	87,90,179
7.30 (singlet)	---

Generally based on the spectral data structure A is not preferable due to the presence of only one carbonyl group from the IR and the asymmetrical nature of the carbons and protons from the NMR spectra. Again structure B is not appropriate due to the absence of N-H and S-H protons in the structure. These protons are appeared in the proton ^{1}H NMR spectrum. Structure C is also not appropriate due to the presence of two aliphatic carbons in the ^{13}Cspectrum. How ever structure D agrees with both the NMR and IR data. There for the most likely structure of the ligand is shown in the following figure.

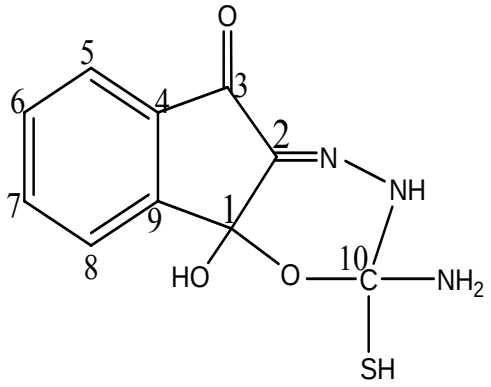
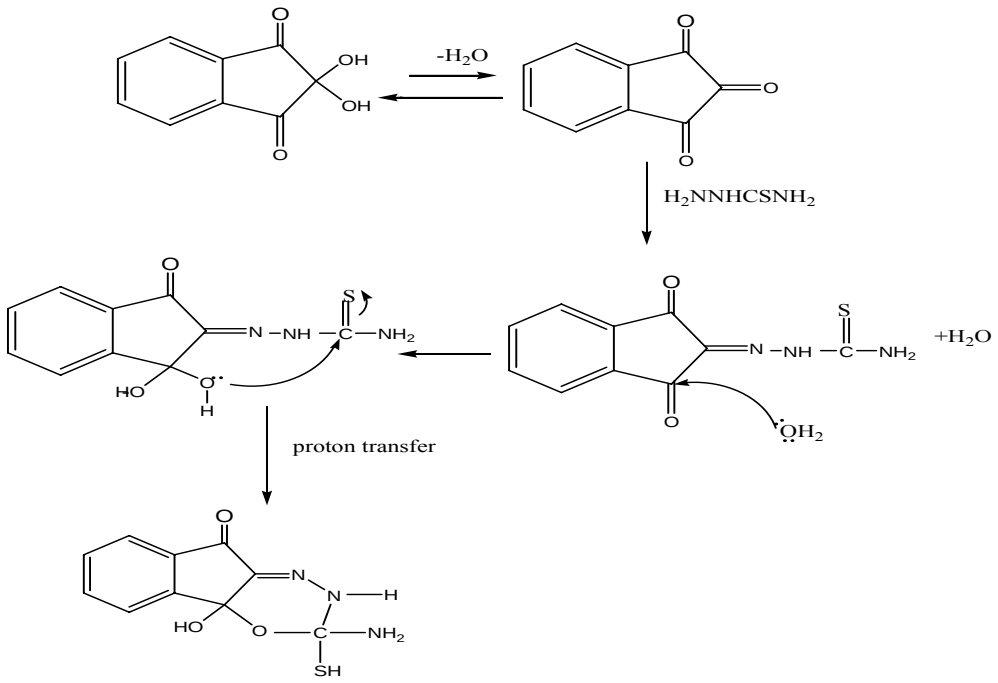


Figure 8. Structure of the ligand

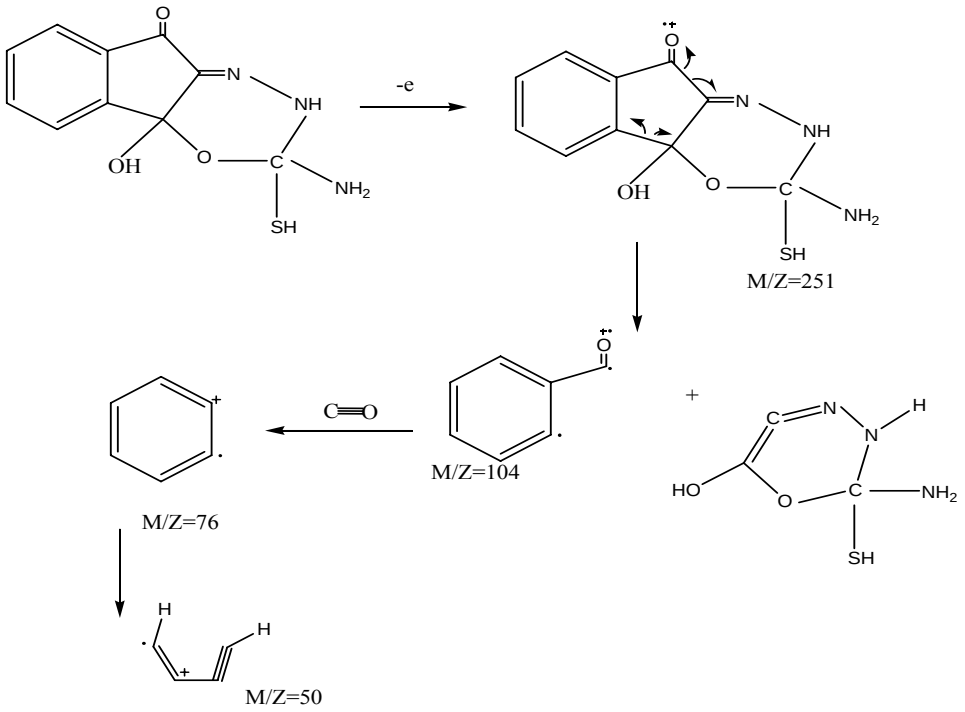
Based on the spectra data the following mechanism is proposed for the formation of the ligand.



Scheme 10 . Proposed mechanism for the formation of ligand.

3.2.3 Mass spectrum of the ligand

As can be observed in the mass spectrum of the ligand m/z=251 corresponds to the molecular ion peak. The peak at M+1 (m/z=252) shows the presence of isotopic carbon and nitrogen. The peak at m+2 (m/z=253) shows the presence of isotopic sulfur and oxygen. From the spectrum the base peak is m/z=104. The base peak can be formed from the molecular ion in different ways. The following partial fragmentation pattern can be involved for the formation of different peaks.



Scheme 11 Partial fragmentation pattern of the ligand.

In addition to the above partial fragmentation pattern, the peak at m/z = 233 (m-18) may be formed by the removal of water from the cyclic ether oxygen and the hydroxy group. The peak at m/z = 217 (m/z = 233-16) may be formed by the loss of NH_2.The peak at m/z = 192 (m-59) may be formed by the loss of CSNNH. The peak at m/z=176 (m/z = 192-16) may be formed by the removal of NH_2 .The other peak at m/z = 132 (m/z = 176-44) may be due to the loss of CO_2.

3.3 Characterization of Cu (II) complex

3.3.1 Physical characteristics

Some of the physical characteristics of the Cu(II) complex are listed in **Table 7.**

In addition to these properties the compound is soluble in DMSO and in an equal molar ratio mixture of acetonitrile and methanol. However, it is insoluble in water, ethanol, methanol and in most non polar solvents. More over the synthesized metal complex is not completely soluble in cold concentrated nitric acid

Table 7 some physical properties of the Cu (II) complex

compound	Mol. formula	Mol. Weight (g/mol)	**A**ppearance	color	Melting (decomposition) Point	Yield %
Cu(II) complex	$C_{20}H_{18}O_6N_6S_2CuCl_2$	**637**	microcrystal	Pale green	232-235 0_C	45

3.3.2 Analytical data

3.3.2.1. Metal estimation by (AAS)

The percentage of Cu (II) in the complex was found to be 9.30.This is close to the calculated value and agrees with the proposed metal to ligand ratio of 1:2.

3.3.2.2. Chloride estimation

The percentage of chloride in the complex was found to be 10.71.The value agrees with the calculated value

Table 8 Metal and chloride content of Cu (II) complex

complex	%Cu(II)		% of Cl	
	Calc.	found	Calc.	found
Cu(II)L_2	10.04	9.30	11.01	10.71

3.3.3. Infrared spectra

Comparison of the infrared spectra of the ligand and the Cu (II) complex is important to know the groups coordinated to the metal. The strong absorption band observed at 1720 cm^{-1} in the ligand has shifted to 1707 cm^{-1} in the complex. This is attributed to the involvement of the ninhydrin carbonyl group in the complex formation.[41]. 1648, 1500 cm^{-1} bands are completely modified, and the complex IR shows distinct down ward shift of 1648 cm^{-1} band which is merged with the band at 1600 cm^{-1} .There is also a positive shift in the 1500 cm^{-1} band to 1516 cm^{-1} .These features can be correlated with the involvement of heterocyclic ring nitrogen corresponding to azomethin group in coordination.

The N-H band observed at 3122 cm^{-1} in the ligand is shifted to 3109 cm^{-1} in the metal complex. The NH_2 symmetrical stretching frequency also shifted from 3273 to 3205 cm^{-1}.The weak and broad peak observed at 3409 cm^{-1} may be due to the over lap of NH_2 asymmetric stretching frequency and the O-H stretching frequencies.

The stretching frequencies of C-N + C-O +C-O-C in the region of 1289 to 1110 cm^{-1} showed positive shift by 10 to 15 cm^{-1}. The υ (N-N) also showed a change of 16 cm^{-1} in the complex. This also confirms the coordination of the azomethin to the metal[13, 15]. There is no significant change for the S-H stretching frequencies between the ligand and the complex. The presence of the S-H stretching in the ligand and the complex with out significant change indicates the absence of coordination between the metal and the sulfur. The new bands at 667 and 500 cm^{-1} in the metal complex are assigned to Cu-N and Cu-O stretching respectively.

Generally, in addition to the shift of the carbonyl and the azomethin stretching frequencies, the presence of the new bands in the spectra of the metal complex are evidences for the formation of the expected product. Based on the IR data it is proposed that the ligand behaves as a neutral bidentate ON donor involving the carbonyl oxygen and the azomethine nitrogen.

3.3.4. Conductivity data

The specific conductance of the copper complex was determined by using JENWAY 4330 conductivity and pH meter. The solution was prepared by dissolving 0.01 gm of the metal complex in 40 ml solvent, which was prepared by mixing equal volume of methanol and acetonitrile. The molar conductance of the complex is derived from the specific conductance by the following formula.

$\Lambda = k \times 1000 / C$ .Where k=specific conductance C=concentration,

Λ=molar conductance.

The measured k value is 90 (μS/cm)

By using the above formula the molar conductance is calculated as 229 $Scm^2mole^{-1.}$ The molar conductance value reveals the Cu (II) complex dissociates in the prepared solvent and behaves 1:2 electrolytes. From the quantitative chloride determination the synthesized compound has two chloride ions. Since the compound behaves 1:2 electrolytes, the two chlorides are found in the outer sphere the Cu(II) complex

3.3.5 Magnetic susceptibility

The magnetic susceptibility data is crucial to know the magnetic properties of the complex and the number of unpaired electrons on the metal ion which can correlate with the complex. The effective magnetic moment of the complex was calculated using the formula $\mu eff = 2.824 (XmT)^{1/2}$ where Xm is the molar susceptibility . The molar susceptibility is derived from the gram susceptibility by the following formula.

$Xm = Xg .Mwt.$

Where Xm=molar susceptibility, Xg=gram susceptibility,

Mwt=molecular weigh of the complex.

The Cu (II) complex has a magnetic moment of 1.85 B.M indicating that it has one unpaired electron and consistent with square planer geometry around the central metal ion.

3.3.6. The electronic spectra

The electronic absorption spectra are often very helpful in the evaluation of results furnished by other methods of structural investigations. The electronic spectra measurement is used for assigning the type of coordination of metal ions in the complexes based on the position and the number of d-d transition bands.

The electronic spectra and data of the ligand and the Cu (II) complex are given in table-9 and appendixes 10**.** The electronic spectrum of the ligand was recorded in DMSO and methanol. But the Cu (II) complex was recorded in DMSO. The ligand shows two bands both in DMSO and methanol. The bands in methanol are well resolved. In DMSO the bands are appeared at 254 nm (39370 cm^{-1}) and 350 nm (28571 cm^{-1}). The band at 254 nm (39370 cm^{-1}) is assigned to $\pi \rightarrow \pi^*$ transition of benzene moiety and the band at 350 nm (28571 cm^{-1}) is assigned for $n \rightarrow \pi^*$ transition of the carbonyl and /or azomethine group[41]. The Cu (II) complex shows some common bands of the ligand at 269 nm (37174 cm^{-1}) and 369 nm (27100 cm^{-1}) .The d-d transition of the complex was also recorded in visible region by concentrating the solution **(appendix 12**). The broad band in the region of 850-550 nm (11764-18184 cm^{-1}) with maximum absorbance at 679 nm (14727 cm^{-1}) is assigned to a merges of $^2B_1g \rightarrow {}^2Eg$, $^2B_1g \rightarrow {}^2A_1g$ transition in square planer geometry. The very weak band appeared at 918 nm (10893) may be $^2B_1g \rightarrow {}^2B_2g$ transition in square planer geometry.

Table 9 Electronic spectral, magnetic moment and molar conductance data

compound	$\lambda_{max.}$ in nm		Assignment of transition.	Magnetic moment (B.M)	Molar conductance (Scm^2 $mole^{-1}$
	In methanol	In DMSO			
Ligand	295, 345	254	π-- π^*	-	-
		350	n-- π^*		
Cu(II) complex		269	π -- π^*	1.85	229
		369	n-- π^*		
		679 918	d-d		

4. Conclusion

Copper(II) complex has been synthesized from the ligand derived from thiosemicarbazide and ninhydrin. Because of both condensation and cyclization reactions taking place simultaneously instead of the expected ONS tridentate ligand an ON bidentate ligand was formed. Based On the analytical and spectroscopic data, it is concluded that ligand behaves as an ON neutral bidentate and square planer geometry has been proposed for the copper (II) complex with the formula $[CuL_2]\ Cl_2$.

The proposed structure of the complex is given in fig.8.

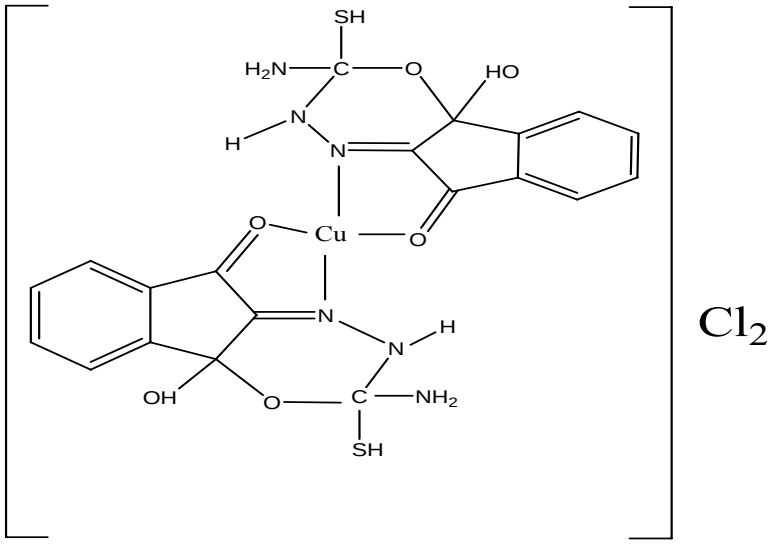


Figure 9 Proposed structure of the Cu(II) complex.

5. References:

1. Smith, J.W. *The chemistry of the carbon–nitrogen double bond*, John Wiley and sons, Interscience: London, **1970**, 63-68

2**.** Suseela, T.; *Structural studies on transition metal complexes derived from some Symmetrical and unsymmetrical bis chelating ligands,* Thesis submitted to the Osmania University for the degree of Doctor of Philosophy in chemistry, **1996.**

3. Nandi, A.K.; Chudhuri, S.; Mazumda, S.K .*J.Chem.Soc.Perkin II*,**1964**, 1729-1735.

4. Kotti, V.K; Singh,.P.R.; Barnes,L.C. *J.Chem.Soc. Dalton Trans* **1993**. 2153-2156.

5. Katritzky,A.R.; Khashab,N.M; Gromova,A.V *Center for Heterocyclic Compounds* University of Florida Department of Chemistry. Arkivoc **2006,** ISSN 1424-6376.

6 Karabocek, N.;Mazium, H.; Degirmencioglu, I. Turk J.Chem.**2004**, 87-94.

7 .Yamamoto, I.; Ikui, A.; Muneharu, N.; Kotani, M.; Motoyoshiya, J.; Gotoh, H.; *J. Chem. Soc.* **1983**, 2297.

8. Dasilva, E.F.; Braz,V.R; Canto-Cavalheio ,M.A.; J. Med.Chem. 2002, 979-982.

9**.** Shaker, R.M.; *The chemistry of Mercapto and thiones substituted 1, 2, 4-triazoles and their utility in heterocyclic synthesis, chemistry department.*EL-Minya University,ARKIVOC 2006, ISSN 1444-6376.

10. Moskowitz, H.; Mignot, A.; Miocque, M. *J. Heterocyclic Chem.* **1980**, 1321.

11. Leovac,V.M.; Jevtovic,L. .*J.Serb.Chem.Soc*.**2005.** 70:3, 123-127

12. Blanco, M.A; Torrese.E.L; Mendiola,M.A. *Tetrahedron*, **2002,** vol.58, 1525-1531

13 Cases, J.S.;Castaño, M.S;Garcia-Tasende, M.S. *J. Chem. .Soc.Dalton Trans*.**2004** , 2019- 2026.

14. Belichi, F.M.; Gsperie, F.G.; Leporati, E.; Pelizzie,C. *J.Chem.Soc. Dalton. Tran*.**1986**, 2455- 2460.

15. Ataly,T.; Akgemic,G..T. *J. Chemistry*.**1998**, vol 22, 123-127.

16 . Agarwal, R.K.; Prasad.S. *Turk. J. Chem.,* **2004**, vol.28, 691-701

17. Agarwal, R.K; Prasad.S. *Turk. J. Chem.,* **2005**, vol.29, 289-297.

18 Matesanz, A.I.; Perez, M.J.; Navarro.; J Inorganic Biochemistry , **1999**, 29-37.

19. Gomez-saiz, P.;Garcia-Tojal.;Mendia, A.;Lezoma,L.; *Eur.J.Inorg.Chem*.**2003**, 515-527

20 Belicchi, M.F;Gaspari, G.F.; Pelizzi, C. *J. Chem.soc. Dalton T*ran.**1992**, 2153.

21 Gomez-Saiz, P.; Garcia-Tojial.J; Mendia,A; Lezoma,L.Eur.J.Inorg.Chem.**2003**, 518- 527.

22. Brown, C.A.; Kamensky, W.; Claborn, K.A.; Goldberger, K.I. ; *J.Braz.Chem.Soc*, **2002,** 13:1,10- 18.

23 Gursoy, A.; Terzioglu,N.;Turk J.Chem.**2005**, 247-245.

24. Vukadine, M.L.; Violetas,J.; Gorn, A.B. *J.Serb.Chem.Soc*.**2005,** 70:3

25. Samala, L.S.; Ramesh.G.N.; Kumer, C.J.; Reddy.A.V.; *J.Indian Chem.soc*.**2000,** 77:405.

26. Inardhan, K R.; Regish, J.K.; Ramachanderiah,C.T.; *J. Food .Chem*.**2007**, 1ssue 2.vol.101.

27. Kovala-Demertizzi,D ; Varadinova,T.; Genova, P.; Souza.P;Demertzi, A.M.; *Bioinorganic Chemistry and application*, Hindawi publishing corporation vol.**2007.**doi; 10.

28. Makonone, S.C. « ^{64} *Cu Production, Ligands and biomedical* application," Pro gradu dissertation, University of Helsinki, **2006**, 37-41

29. Cowley, A. R.; Dilworth, J.R.; Donnelly, P.S.; DaltonTrans.2004, 2404-2412.

30. Kendaol, P.A.; Letters to Nature, Nature 197, **1963**, 1305-1306.

31. Holm, D.J Peck, *Analytical Biochemistry,* 3rd Ed. Addison Wesley, Longman ltd: New York, **1998**

32. Nicolas, D.K.; Gloria, P.; Jennifer, J.J. *J. Forensic science*, **2006,** 51:6, 230-234

33. Lamenth, P.J.; McCormick, P.G.; .J. Anal. Chem., **1972, 821**-827

34 .Rao,N.S.R.R.M.M.K.; Reddy, M.G.R *Biol.Met*.**1990**, 3:19.

35. Muhabaw, G.D.; Raju.V.J.T.; Negussie, R.; *Bull.Chem.Soc.Ethiopia*, **2002**, 16:1,53-64.

36. Shimeles, T.; *M. Sc Thesis" Synthesise and characterization of some metal complexes of Schiff bases derived from amino acids (L-Histidine and L- Lysine) and ninhydrine* Addis Ababa University **2002.**

37. Friedman, M.; J. *Agric. Food Chem*. **2004**, 52:3, 385-406.

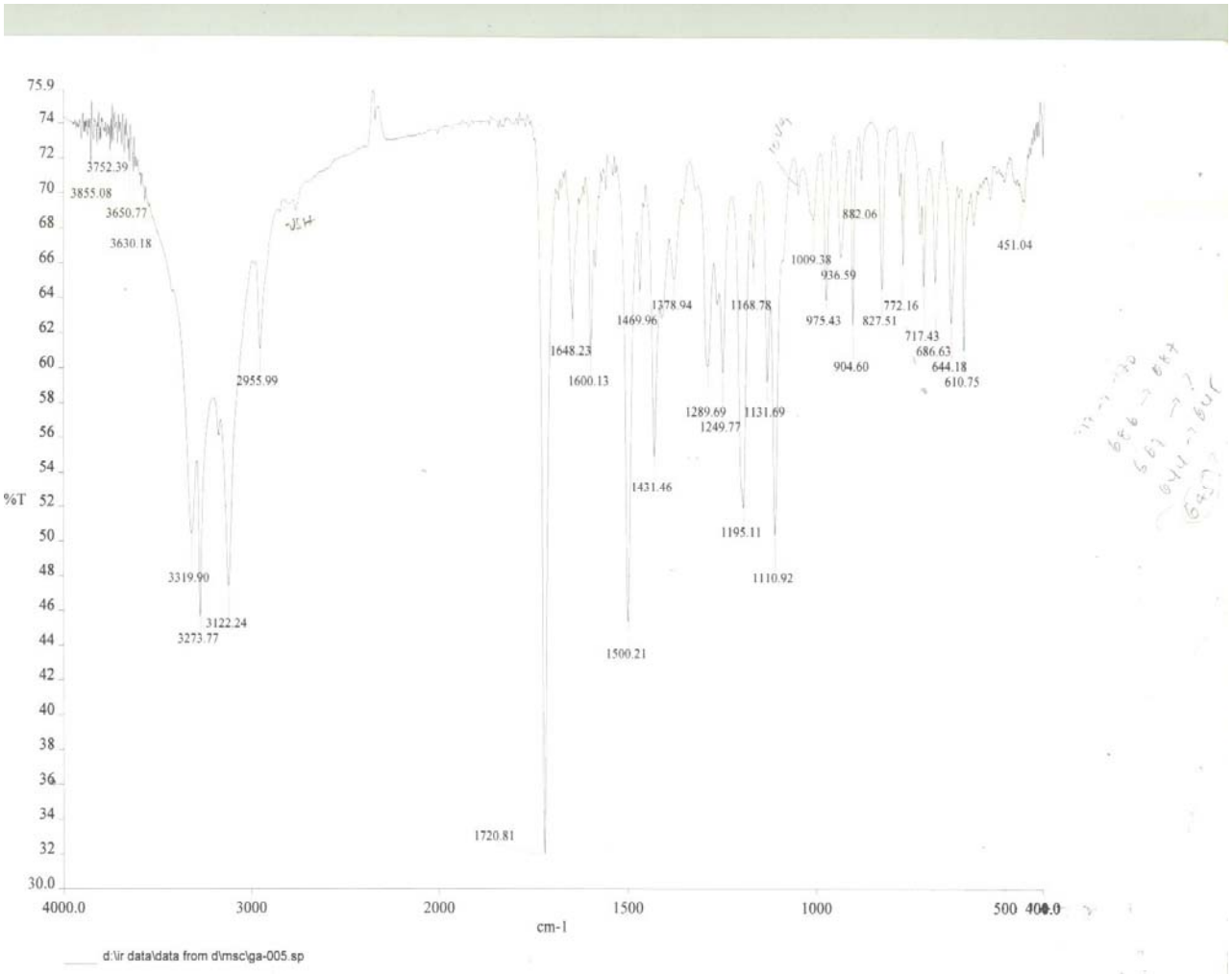
38. Cotton, F.A.; Wilkson, G. *Advanced Inorganic Chemistry*, *5th ed*. John Wiley and Sons, 5th ed. USA, **1988,** 811-817.

39. Grad dons, D. P. *An introduction to Coordination chemistry*, Pergone press, London: vol.3, **1968**

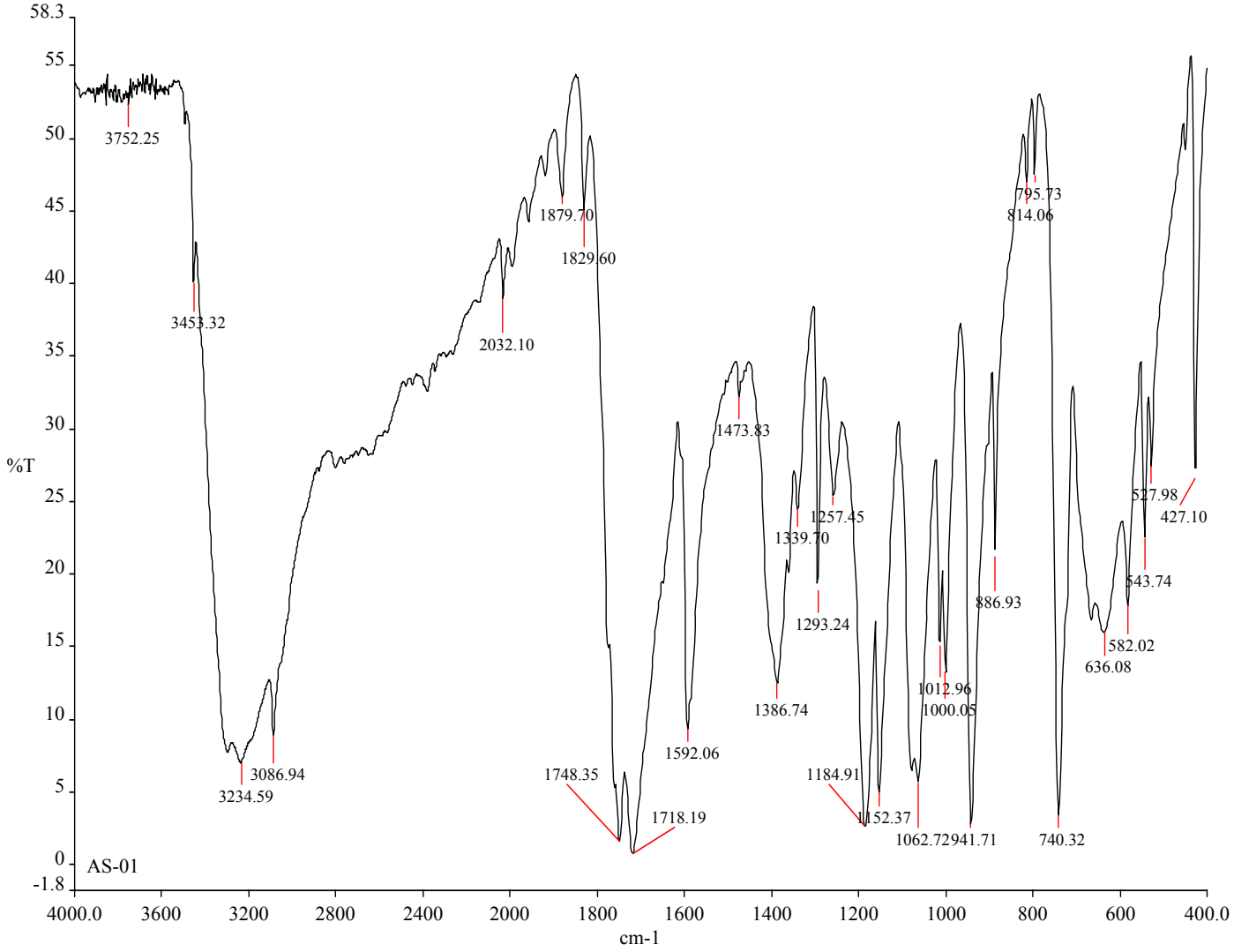
40. Silverstein, M.; Webster, F.X. Spectroscopic Identification of Organic Compounds, 6th ed., John Wiley: New York, **1998,** 255-265.

41. Rao, C.N. *Ultraviolet and visible spectroscopy, Chemical, Application*, 3rd ed., Butterworths: London, **1975**.

Appendix 1: IR spectra of the ligand

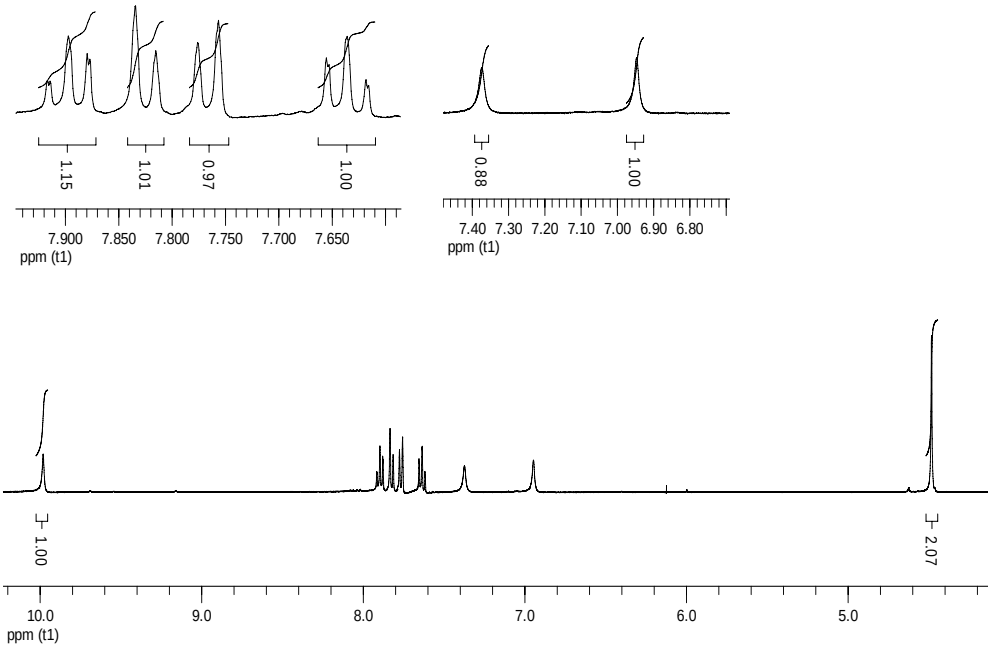


Appendix 2: IR spectra of ninhydrine

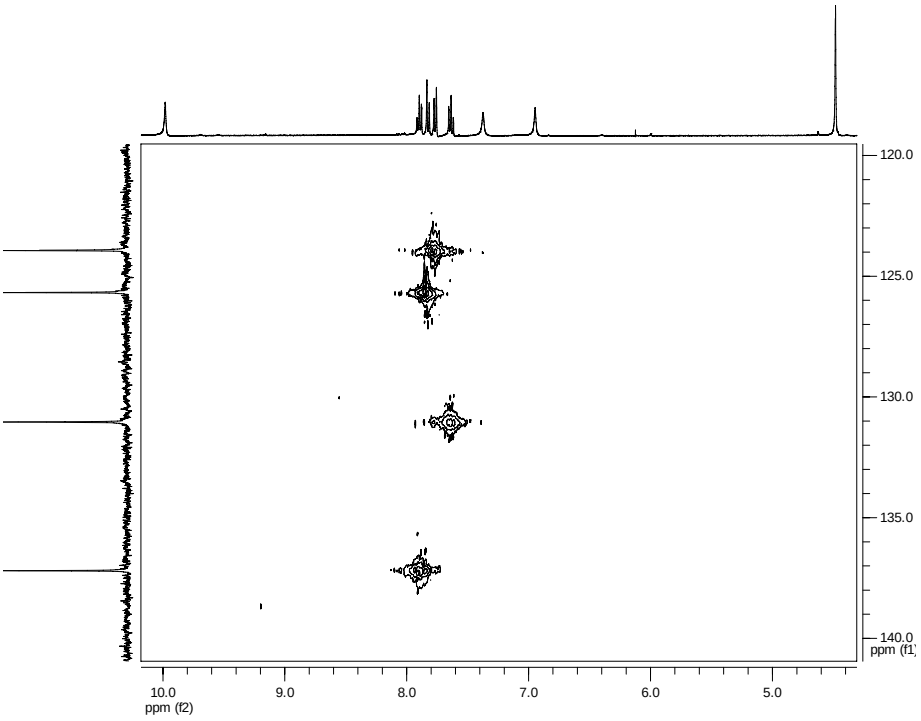


.

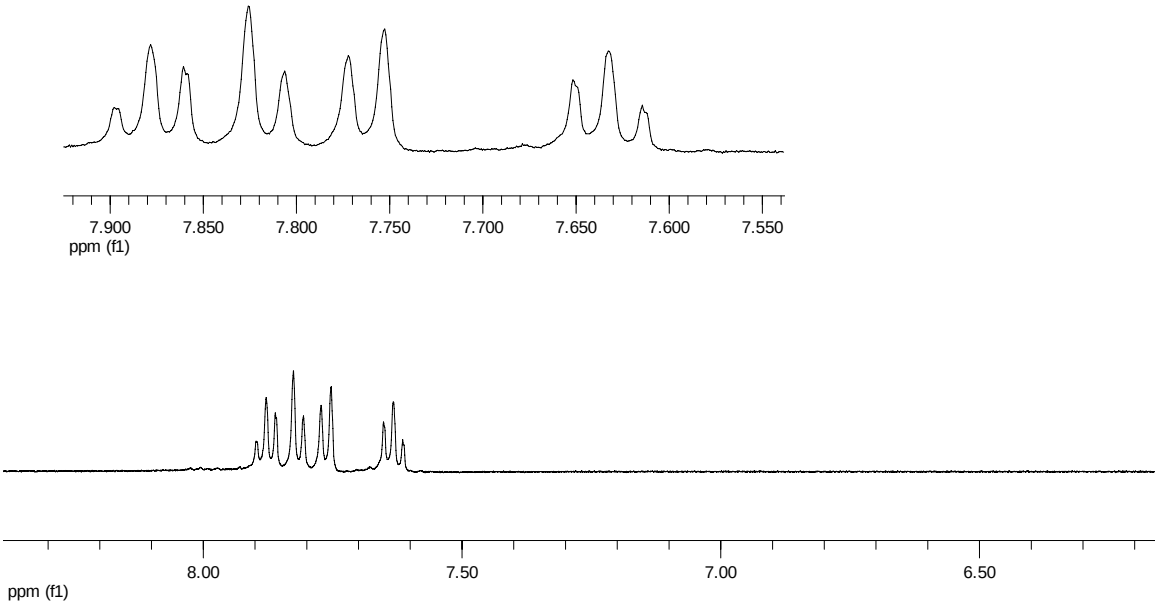
Appendix 3: ^{1}H NMR spectrum of the ligand



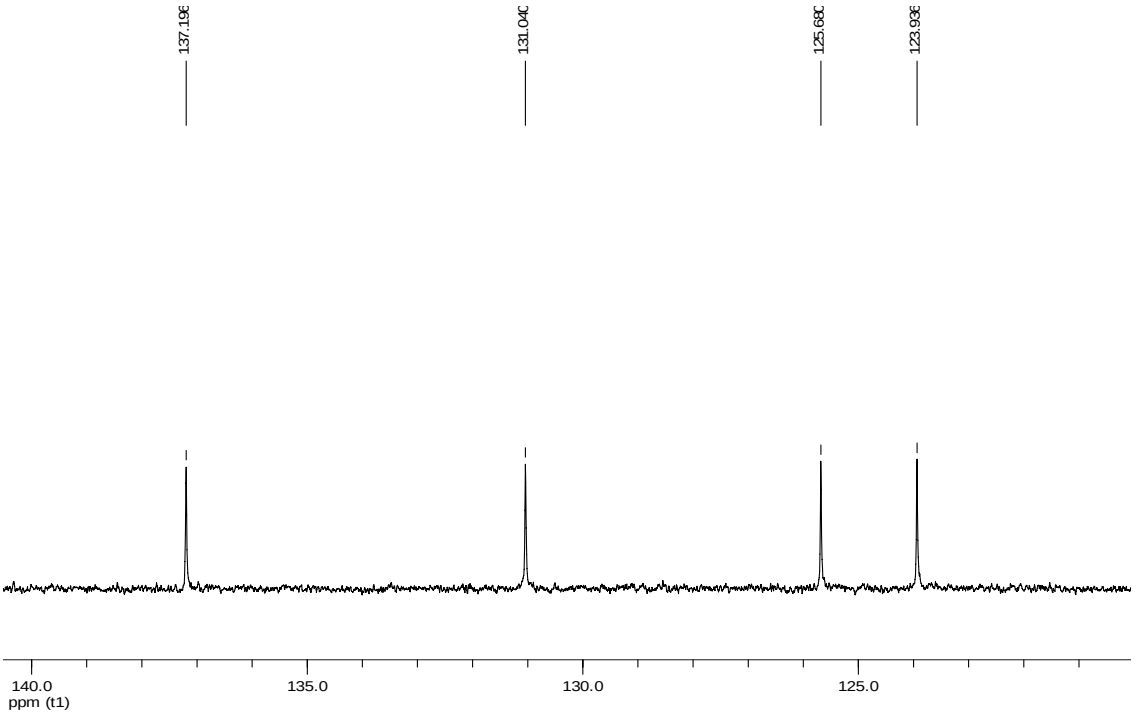
Appendix 4: HSQC- spectrum of the ligand.



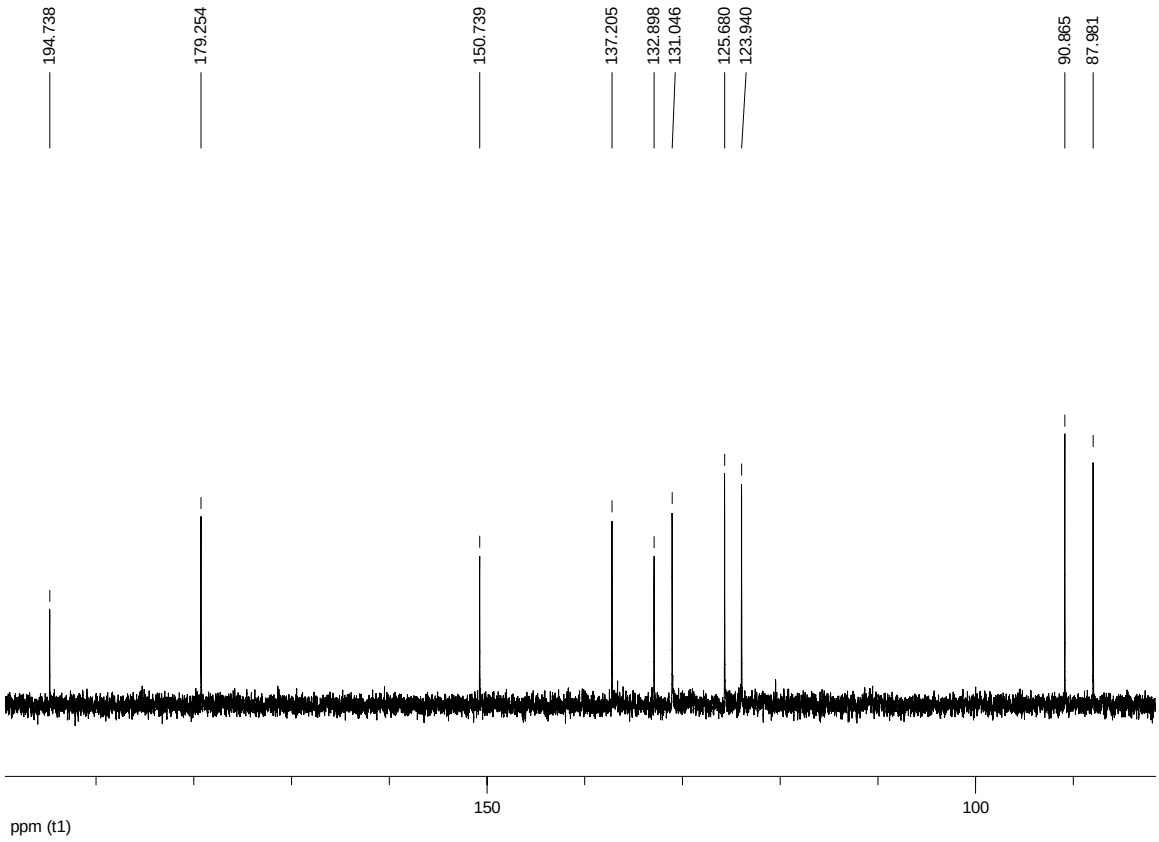
Appendix 5: .D_2O exchange NMR spectrum



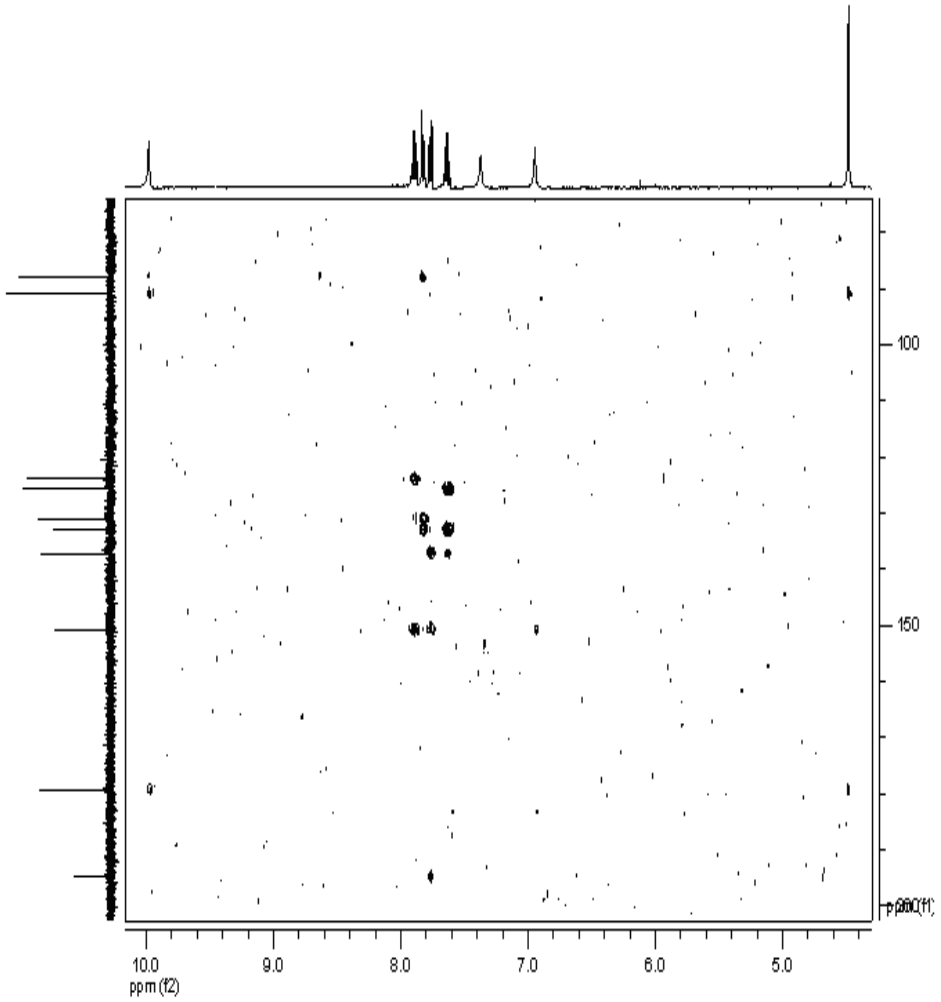
Appendix 6: Dept ^{13}C spectrum of the ligand



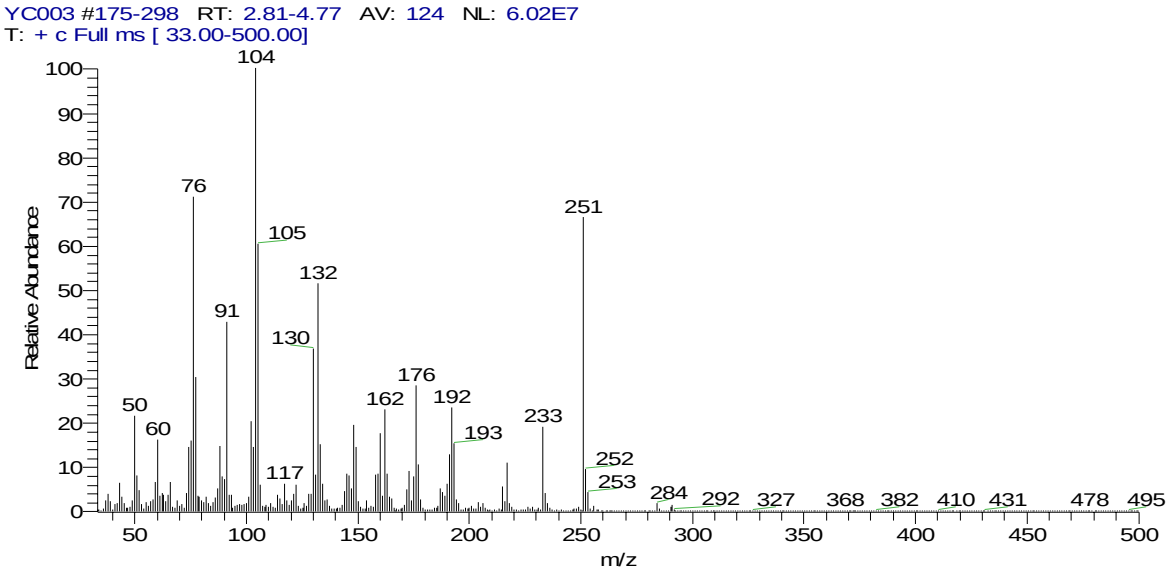
Appendix 7 : ^{13}C NMR spectrum of the ligand



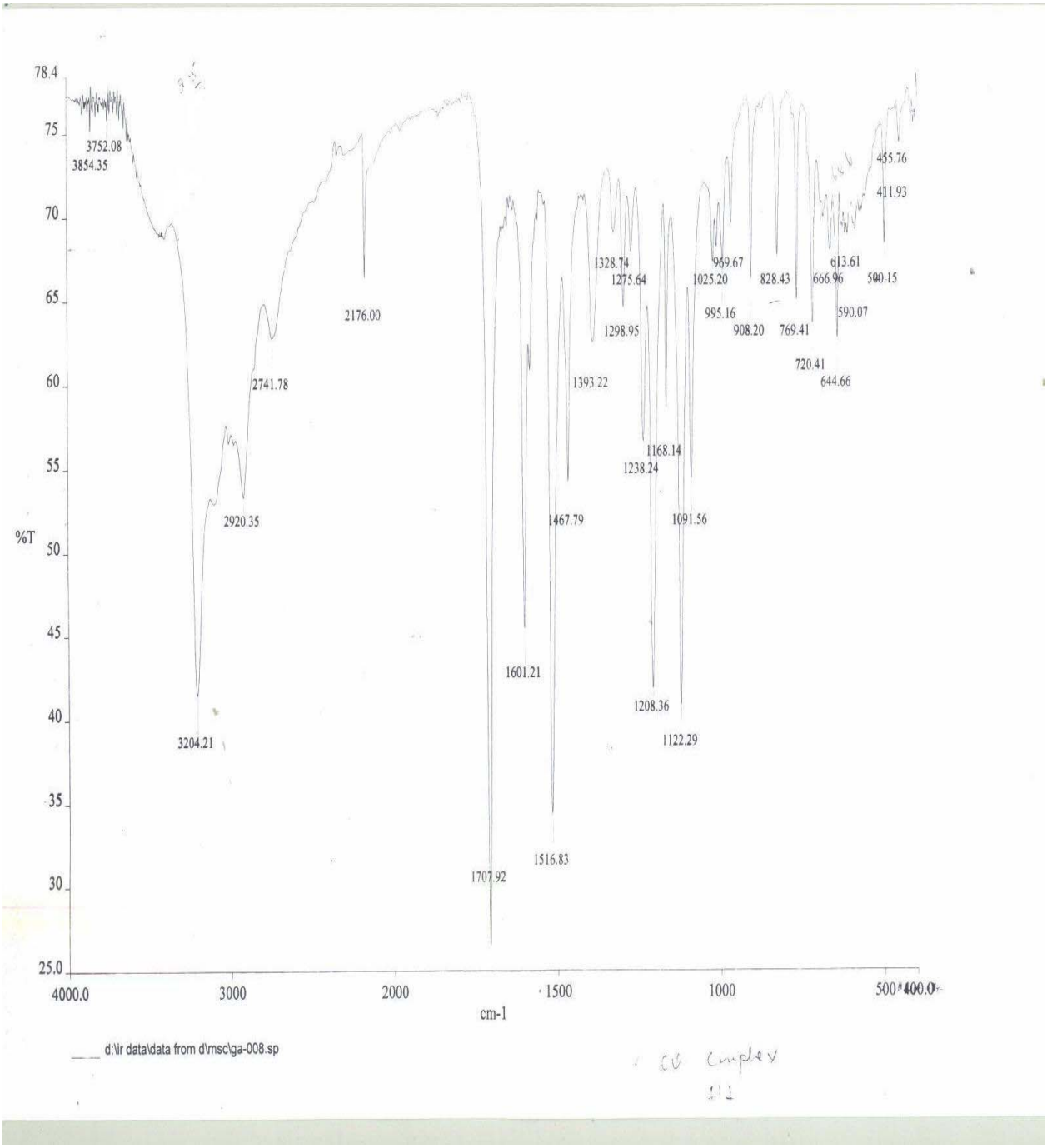
Appendix 8: HMBC spectrum of the ligand



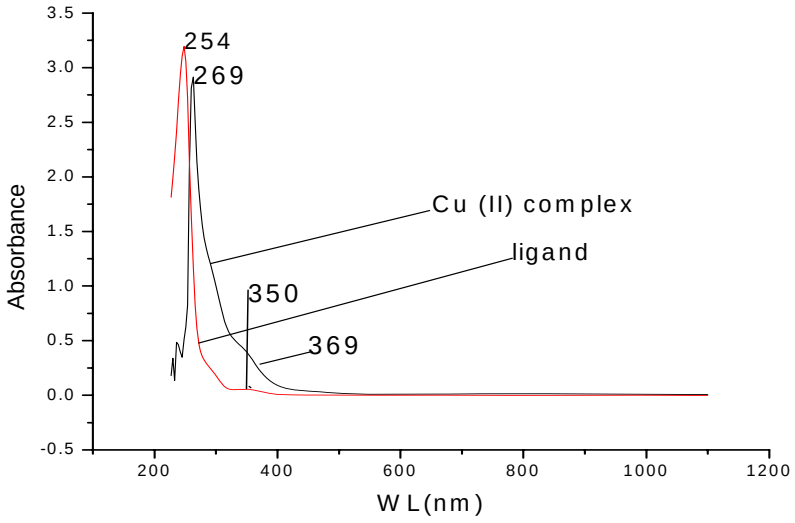
Appendix 9: Mass spectra of the ligand



Appendix 10: IR spectrum of Cu (II) complex.



Appendix 11: Electronic spectra of the ligand and Cu (II) complex



Appendix 12: Electronic spectra of Cu(II) complex the in concentrated solution.