ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

REACTIONS AND STRUCTURAL STUDIES OF Cu(II) AND Fe(III) IONS WITH CLOFENTEZINE DERIVATIVE OF HYDRAZINE

By

YEMESRACH ADAMU AYELE

JULY 2001

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A THESIS PRESENTED TO

THE SCHOOL OF GRADUATE STUDIES

ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN CHEMISTRY

YEMESRACH ADAMU AYELE

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ACKNOWLEDGEMENT

I am particularly grateful to my advisors Prof. Negussie Retta and Dr. Negussie Megersa for their careful guidance, critical advice and many useful suggestions that served to improve the quality of the thesis.

I greatly acknowledge Dr. Wondimagegn Mamo for NMR measurement and interpretation; Ato Mulugeta Desta for IR measurement; Ato Mulu Gebere for UV-Vis measurement; W/o Woineshet Gebeyehu for conductance measurement; Ato Yohanis Negash for antimicrobial test; and Ato Thewodros Zekarias of Ethiopian quality and standard authority for atomic absorption measurement and interpretation.

My sincere thanks are due to Dr. Yonas Chebude for his thought provoking comments, suggestions and interpreting a useful German manuscript.

It gives me great pleasure to express my deep sense of gratitude to Ato Meseret G/wold for photocopying materials and allowing me to use his computer and printer.

I wish to express my sincere appreciation to my colleques Ato Aden Tadele; Ato Tefera Entele; Ato Mehabaw Getahun; W/t Tigest Wondeson; Dereje Tsegay and staff members of department of chemistry for immediate response whenever their help was necessary.

I am pleased to appreciate Mulu Taye; Hayal Desta; Tegegn Aklilu; Wondawke Abebe; Hiwot Adamu and Abeba Kassa for their moral encouragement and positive attitude towards my success.

Last but not least I am thankful to Dilla college of teacher's education and health science for giving me this chance and sponsoring my study.

ABSTRACT

3,6-Bis(2-hydrazinophenyl)-1,2,4,5-tetrazine has been derivatized from the reaction of 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine with hydrazine hydrate. Characterization was made using UV-Vis, IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. Attempts to prepare metal complexes between metal ions such as Cu(II), Fe(III), Cr(III) and Ni(II) with the ligand, BHPT, were observed to be the decomposition of BHPT to 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine. UV-Vis, IR, ^{1}H NMR, ^{13}C NMR, AAS, conductivity measurement and analytical data have been used to characterize BHPT and 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine.

The kinetic study of the decomposition of BHPT to 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine, enhanced by the metal chlorides, revealed that the reaction is a second order with rate constant of 94.7 $mol^{-1}Lsec^{-1}$ at 4°C in acetonitrile solution. This reaction was also tested at pH = 4 (rate constant = 28.06 $mol^{-1}Lsec^{-1}$) and pH = 9.2 (rate constant = 13.7 $mol^{-1}Lsec^{-1}$) at 50°C. From the kinetic data plausible mechanism is proposed.

Antimicrobial test was conducted for the ligand on *Escherichia coli, Proteus* and *Staphilococcus aureus.* The ligand has no inhibition effect on the growth of the above bacteria.

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ABBREVIATIONS

BHPT	3,6-Bis (2-hydrazinophenyl)-1,2,4,5-tetrazine
TLC	Thin layer chromatography
UV-Vis	Ultraviolet-visible
IR	Infrared
NMR	Nuclear magnetic resonance
AAS	Atomic absorption spectroscopy
Abs/min	Absorbance per minute
BDPT	3,6-Bis (2-diaminoethane phenyl)-1,2,4,5-tetrazine
d	Density
nm	Nanometer
ppm	Parts per million
WL	Wave length
mg/L	milligram per liter
µL	microliter

To

my mother; father; brothers and sisters

1. INTRODUCTION

Heterocycles can be conveniently classified as cyclic organic compounds in which one or more of the ring carbon atoms have been replaced by another element such as nitrogen, oxygen or sulphur [1].

Heterocyclic compounds are important in the study of chemistry both for its own sake and industry. The interest in novel ring systems and the possibilities for aromatic character in these compounds provide strong driving forces for research, while the application of many of these systems to pharmaceutical, dyestuff, photographic and other products is responsible for much of the research by parts of the chemical industry [2]. Nitrogen hetero-cycles like pyrazine, triazine, tetrazole, indole, triazole, etc have attracted the attention of coordination chemists. Transition metal complexes with *N*-substituted tetrazoles have been reported [3]. Mesoionic compounds are an interesting family of heterocyclic compounds. Their unique properties, such as reaction behavior, structural characteristics, and biological activities have attracted much attention, and a wide variety of derivatives have so far been studied [4].

Metal complexes of physiologically active heterocyclic ligands have promising applications in view of enhanced solubility and modified electron distributions. Improved antimicrobial, insecticidal, insect growth regulating, plant growth regulating, weedicidal, herbicidal etc activities of metal complexes have been documented in the literature [5-9].

Tetrazines are a class of six membered ring hetero-cycles with four nitrogen atoms in the skeleton of the ring system. The tetrazine ring is highly aromatic and this must be the reason for its existence as a stable entity. The 1,2,4,5-tetrazine is normally prepared by decarboxylation of 1,2,4,5-tetrazine-3,6-dicarboxylic acid, which is obtained from ethyl diazoacetate [10], Fig.1.

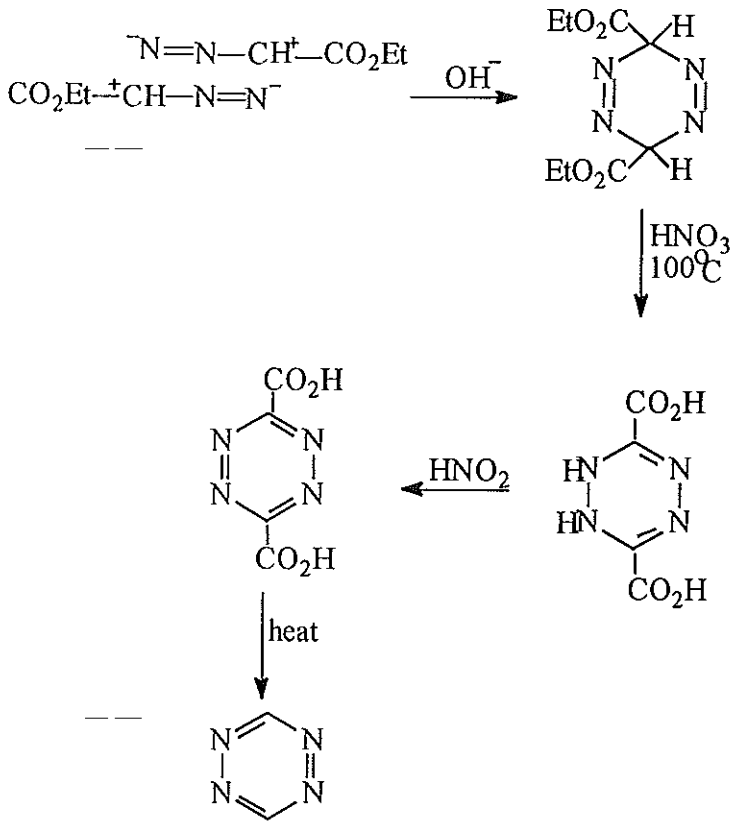


Fig.1 Preparation of tetrazine from ethyl diazoacetate

A wide variety of ligands containing azomethine centers which vary in electron density, flexibility, nature of donor atoms and electronic properties have been studied. Due to the

electronegativity difference between nitrogen and carbon, the π electrons in the ring of symmetrical tetrazines are localized in the vicinity of nitrogen atoms [11]. The boiling points clearly indicate a high degree of polarity and intermolecular association [12].

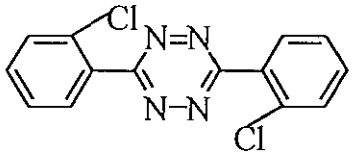


Fig. 2 Clofentezine; 3,6(2-chlorophenyl)-1,2,4,5-tetrazine

One such compound, clofentezine, is a substituted 1,2,4,5-tetrazine with chlorobenzene functions at positions 3 and 6. In table 1 are listed the code number, chemical and common name, and physical data of practical relevance [13].

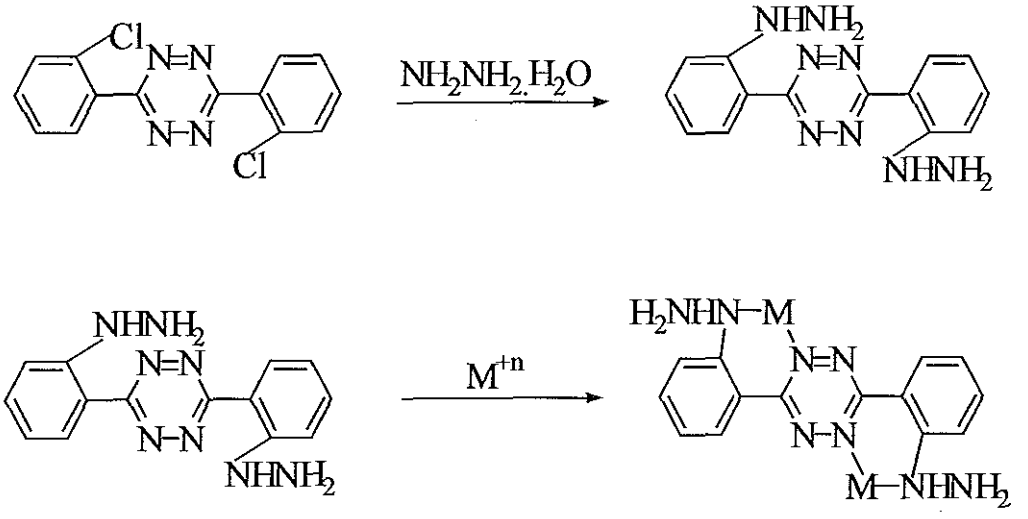
Table 1.1 Some physical properties of clofentezine

CAS N°	Chemical name	Molecular formula	Molecular weight	Melting point/°C	Water solubility at 25°C	PK_a	Vapor pressure
074115-24-5	Clofentezine	$C_{14}H_8Cl_2N_4$	303.15	182	1mg/l		9.75E-010mmHg

Clofentezine, (3,6-bis(2-chlorophenyl)-1,2,4,5-tetrazine), is used as specific acaricide with contact action, control of eggs and young motile stages of *Panonychus Ulmi* [14] and *Tetranychus* spp.[15] on pome fruit, stone fruit, nuts, vines, hops, strawberries, cucurbits, cotton, and ornamentals. Clofentezine is toxic, and may cause slight injury to glass house

glass house roses, a slight pink deposit on petals of white or pale flowers [16]. It inhibits mite growth, but the mode of action is not known [17]. Clofentezine is a possible human carcinogen [18].

Clofentezine reacts with different amines to replace the chloro-group. These amine derivatives are important *N*-hetero-cyclic- related molecules with promising chelating ability towards several transition metal ions [19].



Scheme 1 Derivatization and Complaxation reaction

Since this compound has long residual activity [16], it has been found as pesticides in food [20]. In addition, clofentezine in mammals undergoes metabolism by hydroxylation and exchange of the chlorine atoms on the rings for the methylthio group [16].

1.1 Literature Survey

Studies on the interaction of the metal ions with pesticides have shown that these compounds are widely used in agriculture and can form stable complex species with metal ions [21, 22]. Metal ions, on the other hand, may affect remarkably the fate of pesticides, eg, catalyze their decomposition [23] or detoxify them [24]. Cu(II) ion is known to be very active in influencing pesticide chemistry and biochemistry, due to its efficient binding ability [25].

1,2,4,5-Tetrazine (*s*-tetrazine) is a strongly colored (orange) molecule, which is planar [26]. *S*-tetrazines have been studied for several decades because their spectroscopic and photo dissociation properties lend themselves to modeling. Tetrazines are highly reactive towards aromatic compounds, forming cycloaddition compounds [27].

Clofentezine and benzene amine compounds of similar chemical nature [14] were the best among the new compounds for their effect on the European red mite (*Panomychus Ulmi*) and the apple rust mite (*Aculus Schlechtendali*) in an experimental apple orchards.

In experiments conducted against eggs and motile forms of *Tetranychus Urticae* and *T. cinna barinus,* clofentezine demonstrated selective toxicity against eggs. Contact mortality against motile forms was poor (< 35 %), but clofentezine was highly effective when used in combination with *dicofol* and *diazinon* [15].

1.2 Metal Complexes of Tetrazines

Documents reveal increasing interest on synthesis, structural studies and applications of transition and non transition metal complexes of substituted tetrazines. 3,6-Di(2-pyridyl)-1,4-dihydro-1,2,4,5-tetrazine reacts readily with trimethyl gallium to provide the novel pentacyclic hetero-aromatic complex [28]. Group 12 metals form complex with 1,4,5,6-tetrahydro-2,4- dimethyl-6-(2'-pyridyl)-1,2,4,5-tetrazine-3(2*H*)-one (pvd H_3) [29]. Protonation has effect on the structure and homogeneous charge transport dynamics of solid-state osmium bis(bipyridyl) tetrazine chloride films [30].

Some of the significant results form synthesis; physico-chemical and characterization studies on metal complexes are summarized in Table 1.2.

Table 1.2 A summary of literature survey on metal complexes of *s*- tetrazine

Substituted *S*-Tetrazine	Metal ion(s)	Types of study	Brief Summery of results	Reference
3,6-Di(2-pyridyl)-1,4-dihydro-1,2,4,5-tetrazine	Ga(III)	X-ray, NMR	Considerable bond localization in the tetrazine ring, and marked electronic influence of the gallium moieties on chemical shift values of the pyridine protons.	[28]
1,4,5,6-Tetrahydro-2,4-dimethyl-6-(2'-pyridyl)-1,2,4,5-tetrazine-3(2H)-one ($pvdH_3$)	Group 12 metals	X-ray, EPR, HPLC	Coordination of group 12 dications results in little perturbation of the singly occupied molecular orbital by EPR and small changes in the UV-Vis spectrum suggesting that the ligand is similar to the other polypyridyl ligand.	[29]
3,6-Bis(pyriden-3-yl)-1,2,4,5-tetrazine	Cd(II) and Zn(II)	X-ray	The molecular architecture of both Cd(II) and Zn(II) nitrates bridged by 3,3'-pytz is profoundly influenced by alcohol present in the crystallization medium.	[31]
2,2'-Bipyridyl-3,6-bis(4-pyridyl)-1,2,4,5-tetrazinme	Os(II)	Voltammetry	Mechanically attached solid state films of the compound have been formed on platinum microelectrodes. Scanning	[30]

			electron microscopy reveals that repeated voltammetric cycling in sodium perchlorate electrolyte can induce some crystallization of the material on the electrode surface	
1,2,4,5-Tetrazine with phthalocynine, tetrabenzoporphyrine, and 2,3--naphthaalocynine	Iron, cobalt and ruthenium	NMR and voltammetry	The polymeric complexes, in which the bridging ligand is bound by 2 coordinative bonds or by a coordinative bond and a σ bond, have a good to very good semi conductive properties.	[32]
3,6-Bis(2-pyridyl)-1,2,4,5-tetrazine	Ruthenium and osmium	Voltammetry	The trend observed allow the rationalization, in a consistent fashion, the stability of the mixed-valence forms toward disproportionation: the equilibrium constant K_c depends on the π-donor/π-acceptor character of the metals and on the occupancy and electron orbital.	[33]

1.3 Scope of the Present Investigation

The growth of the literature on the synthesis and application of nitrogen containing heterocyclic compound has no parallel development with respect to their coordination chemistry. Infact studies on metal binding abilities of simple or substituted tetrazine in general and chelating clofentezine in particular are relatively scanty. The present investigation aims at the preparation and structural studies on some selected metal complexes of clofentezine derivative and look in to a probable application of the complexes.

From the literature survey, it has been noted that so far no significant attempts have been made to synthesize metal complexes of clofentezine, which is active acaricide, derivative. The objectives set below are proposed to develop a new chelating *s*-tetrazine derivative by replacing the chloro group in clofentezine.

General Objectives

- Preparation of clofentezine derivative and metal complexes with clofentezine derivative.
- Characterization of the clofentezine derivative and metal complexes.
- Looking into further probable applications.

Specific Objectives

- Preparation of the complexes of some selected metals with clofentezine derivative.
- Structural studies using, IR, NMR, electronic spectra, elemental analysis, conductance measurements, and AAS techniques.
- Possible microbial activity of the complexes.

2. MATERIALS AND CHEMICALS

All chemicals used were of analar grade. Hydrazine hydrate (80% solution, d = 1.03 kg/l) was from Riedel-de Haen and used directly. Clofentezine was purchased from Aldrich Chem. Co. Vibrational (IR) spectra were recorded on a Buck Scientific Model 500 Spectrophotometer, in 200-4000 cm^{-1} range, using a potassium bromide disc as a reference material. Melting point of the compounds was determined on BOCK-MONOSCOP apparatus. The proton NMR spectra were obtained by using BRUCKER AVANCE 400 MHz NMR machine. Data were expressed in parts per million downfield shift from tetramethylsilane as an internal reference and were reported as position δ_H. All spectra were recorded in deuteriated chloroform. UV-Visible spectra (in MeOH) were determined using SPECTRONIC GENESYS 2PC with 1-cm cell at room temperature in the range 200-1100 nm. Conductance was measured on EdG conductometer using methanol solvent at room temperature. Atomic absorption spectra of compounds were recorded on a Varian SpectrAA 220 with SIPS (sample introduction pump system) component. The kinetic study was conducted using SPECTRONIC GENESYS 2PC which is fitted with thermostat Grant

LTD6G using 1-cm cell at various pHs and 50°C. A mixture of solvents was used with ratio of 1:9 (ethanol to chloroform). The pH of buffer solutions was checked by Gallenkamp pH Stick.

3. DERIVATIZATION OF THE LIGANDS

3.1 Experimental

Compounds that contain ammine functional group, such as methylamine, ammonia, 1,2-diamine ethane and hydrazine hydrate were allowed to react with clofentezine to replace the chloro-group. Of these compounds hydrazine hydrate and 1,2-diamine ethane gave promising result. Derivatization and complete characterization of clofentezine with hydrazine is presented in sections 1.1 and 1.2. The characterization of the compound, derivatized from clofentezine and 1,2-diamine ethane, was incomplete due to scarcity of sample. Some of the physical properties and spectral dates are presented in section 1.3.

3.1.1 *Synthesis of 3,6-Bis(2-Hydrazinophenyl)-1,2,4,5-Tetrazine, BHPT*

To clofentezine (500 mg, 1.65 mmol) excess hydrazine hydrate (20 ml, 412 mmol) was added and the mixture was refluxed for 3h while stirring. The yellow solid compound, which was separated, was filtered in hot condition; thoroughly washed with dichloromethane, ammonia, and water and dried *in vacuo*. The washing was continued until the chloride test became negative [34].

Yield 41%, 198.9 mg

Melting point 224-225°C

The newly synthesized compound is soluble in polar organic solvents; chloroform, acetic acid, methanol and acetonitrile.

3.1.2 *Synthesis of 3,6-Bis(2-Diamino Ethane Phenyl)-1,2,4,5-Tetrazine, BDPT*

To clofentezine (15 mg, 0.045 mmol) excess (2.5 ml, 37.4mmol) 1,2-diamine ethane was added and the mixture was refluxed for 10 min. The yellow solution and white crystals were obtained and the mixture was filtered. The yellow solution, which is assumed to be the main product was dried using Rota vapor. The yellow solid washed with hexane and diethyl ether.

Yield 43% (7.4 mg)

M.pt 245-250°C

The compound is soluble in chloroform, ethanol, methanol and acetonitrile.

3.2 Results and Discussion

3.2.1 *IR Spectrum of BHPT*

The IR spectrum of the ligand, 3,6-bis (2-hydrazinophenyl)-1,2,4,5-tetrazine, showed strong absorption band at 3260 cm^{-1} which is characteristic of $NHNH_2$ functional group [35], Fig 3.1 and Table 3.2. Multiple band in the region 1610-1400 cm^{-1} is due to $\nu_{C=N}$, $\nu_{N=N}$ along with δ_{NH} and ring stretching [36]. Strong absorption band that appears around 1350 cm^{-1} indicates

secondary amine aromatic C-N stretching [37]. Other characteristics are the medium band at 1115 cm^{-1} and weak band at around 960 cm^{-1} assignable to δ_{aNH} and $\nu_{N\text{-}N,}$ respectively [38, 39].

3.2.2 *Electronic Spectrum of BHPT*

The intense band ranging from 40,000-43,000 cm^{-1} is attributed to π-π^* transition possible with aromatic ring ($\varepsilon > 8235.5\ M^{-1}cm^{-1}$); shoulder that appeared at 35714 cm^{-1} is attributed to n-π^* transition ($\varepsilon = 2500\ M^{-1}cm^{-1}$) [40], Fig 3.2.

3.2.3 *^1H NMR Spectrum of BHPT*

The ^1H NMR spectrum of the ligand, BHPT, in $CDCl_3$ solution showed one broad signal, which is characteristic of protons bonded to hetero atoms, for the hydrazino functional group at 7.2 ppm. The signals due to aromatic protons [41] are summarized in Table 3.1. The signal at 7.4 ppm for proton attached to carbon–4 is up field compared to other protons in the structure. This might be due to the hydrazine group, at carbon–3. The triplet signals at 7.35 and 7.45 ppm are assignable to protons attached to carbon–5 and carbon–6 respectively. The symmetrical spectrum of ^1H NMR (Fig 3.3) of the ligand indicates that two hydrazine groups substitute the two chlorides, of the starting material. Furthermore the up field shifts of the ligand protons compared to that of clofentezine confirms the substitution of the two chloro groups by two hydrazino groups.

Table 3.1 Proton NMR data for:

i) clofentezine

Type	Number of proton	Pattern	Chemical shift (ppm)
H^4	2	d	7.6
H^7	2	d	8.1
$H^{5,6}$	4	m	7.5-7.4

ii) the ligand

Type	Number of proton	Pattern	Chemical shift
H^4	2	d	7.4
H^7	2	d	7.6
H^5	2	t	7.35
H^6	2	t	7.45
Hydrazine	3	br, s	7.2

Note: d = doublet; t = triplet; s = singlet and br = broad

3.2.4 *^{13}C NMR Spectrum of BHPT*

The ^{13}C NMR spectrum of the ligand, BHPT, in $CDCl_3$ solution showed three quaternary carbon atom signals at 148.6, 132.5 and 130.4 ppm for aromatic carbon attached to nitrogen atom of hydrazine functional group, azomethine carbon and carbon attached to tetrazine ring carbon, respectively. The other four bands that appeared at 132.7, 132.0, 130.7 and 127.7 ppm are assigned to carbon–5, 6, 7 and 4 respectively [48] (Fig 3.4)

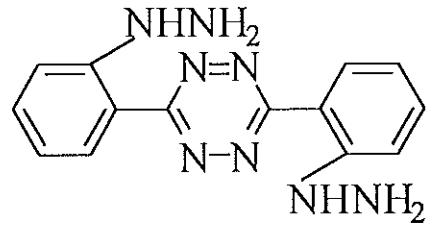


Fig 3 3,6-bis (2-hydrazinophneyl)-1,2,4,5-tetrazine, BHPT.

3.2.5 *IR Spectrum of BDPT*

A medium, multiplet bands that appeared at 3320 and 3300 cm^{-1} are due to ν_{aNH} and ν_{sNH} stretching, respectively. A weak band at about 3210cm^{-1} is due to ν_{NH} stretching. Multiple bands in the region of 1650-1500 cm^{-1} are due to the aromatic ν_{C-C} stretching. An intense band at 1290 cm^{-1} is due to secondary amine to aromatic carbon ν_{N-H}. A strong band that appeared at 720 cm^{-1} is due to C-H deformation, which is a characteristic of disubstituted aromatic ring.

3.2.6 *UV-Visible Spectrum of BDPT*

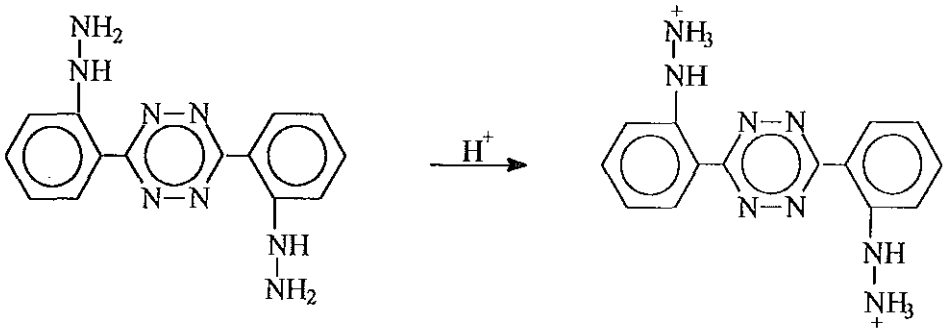
The intense bands at 48543.7 (ε = 36,000 $M^{-1}cm^{-1}$) and 32,467.5 cm^{-1} are attributed to π-π^* transition possible with aromatic ring; shoulder that appeared around 43,478.2 cm^{-1} is assignable to n-π^* transition (ε = 12,000 $M^{-1}cm^{-1}$) (Fig 3.5)

4. REACTIONS OF METAL IONS WITH 3,6-BIS (2-HYDRAZINOPHYNEYL)-1,2,4,5-TETRAZINE (BHPT)

4.1 Experimental

Attempts have been made to synthesis metal complexes of BHPT from acetic acid and acetonitrile media. The solubility of BHPT in common organic solvents showed notable increase in the presence of metal ions. The pH of the reaction medium affected the complex formation. The appropriate pH range that gave better result was 5.0 - 6.0. This is probably due to the following two reasons.

At low pH the donor atoms are bound by protons [42].



At high pH the BHPT decomposes [42]

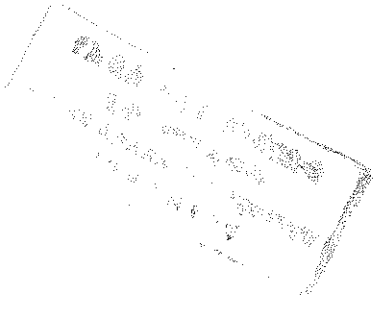
The pH range mentioned above (5.0 - 6.0) makes the lone pair of electrons on the ring as well as on the hydrazine nitrogen atoms are available for metal binding interaction. In addition, the BHPT is protected from decomposition.

4.1.1 *Attempt to Synthesis Metal Complexes in Acetic acid medium*

The metal ion complexes that have been investigated in this work are that of Cu(II), Ni(II), Cr(III), Fe(III) and BHPT. The ligand BHPT is expected to behave as neutral tetradentate system offering NN centers for metal binding interactions.

The following general method was applied for the synthesis of metal complexes of 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine.

To the acetic acid solution (15 ml) of divalent/trivalent metal chloride (0.19 mmol); 24.6 mg Ni(II); 27.3 mg Cu(II); 50.6 mg Cr(III); and 30.8 mg Fe(III) acetic acid solution (15 ml) of 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine (0.19 mmol, 57.57 mg) was added. The pH of the reaction mixture was adjusted to 5.0-6.0 by adding 25 % NH_3 (v/v) [34]. The reaction mixture for Ni(II) and Cr(III) ions were stirred over night on hot plate magnetic stirrer but for Cu(II) and Fe(III) ions stirring was not required. The colored solid product, which was separated, was filtered in cold condition and washed thoroughly with distilled water and then dried *in vacuo*. Yield and some properties of the compounds are given in table 4.1.



4.1.2 *Attempt to Synthesis Metal Complexes in Acetonitrile Medium*

The metal ion compounds that have been investigated in this section are Cu(II), Fe(III) sulphate and chromium (III) chloride. The ligand of interest is expected to behave as neutral tetradentate system offering NN centers for metal binding interactions.

The following method was applied for the synthesis of metal complexes with 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine.

To the acetonitrile solution (15 ml) of copper (II) sulphate (0.19 mmol, 47.4 mg); ferric sulphate (0.19 mmol, 75.98 mg); and chromium (III) chloride (0.19 mmol, 50.6 mg) acetonitrile solution (15 ml) of 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine (0.19 mmol, 57.57 mg) was added. The reaction mixture for Cu(II) and Fe(III) ions produce colored solution immediately after mixing the two reactants. For Cr(III) ion the reaction mixture was refluxed for 3 h and pink solution was obtained. The solutions were kept open to air for the solvent to evaporate. The pink solid was washed thoroughly with distilled water and then dried *in vacuo.* Yield and some properties of the compounds are given in Table 4.5. The products isolated have pink color and are soluble in polar organic solvents, such as dichloromethane, chloroform, methanol, acetonitrile and ethanol. These compounds melt in the range 181-194°C.

4.1.3 *IR Measurement*

1 mg of the isolated product was mixed with 100 mg of potassium bromide, used as a reference, and the mixture was crushed. Vacuum pump was used to make pellet of the crushed mixture. Finally the sample was placed in one beam of double beam infrared spectrophotometer and the spectra were recorded.

4.1.4 *Conductivity Measurement*

Measurement of conductance took place by dissolving a known amount of the solid isolated in methanol so as to find the final volume of a solution to be 25 ml and a concentration of 1 mM.

4.1.5 *Atomic Absorption Spectroscopy*

The atomic absorption spectra of the compounds were taken by digesting the sample (10 mg) in a mixture of perchloric acid and nitric acid (3 ml, 2:1) and the samples were heated in water bath for 3 h and diluted to 50 ml with distilled water.

Lassaigne's test was used to convert organic compounds in to ionisable inorganic salts [46] and; chloride and sulphur were determined gravimetrically (Table 4.1 and 4.5).

4.2 Results and Discussion

4.2.1 *Conductance Result*

The conductivity measurement is one of the simplest and easily available techniques used to study the nature of complexes. It gives direct information regarding whether a given compound is ionic or covalent.

The low values of molar conductivities (less than 10 $ohm^{-1}cm^2mol^{-1}$) of both compounds, which are prepared in acetic acid and acetonitrile media, in methanol solution at room temperature (21°C) indicate a non-electrolytic nature for all of the compounds [43] (Table 4.1 and Table 4.5).

4.2.2 *Purity of the Isolated Compounds*

The purity of all the isolated compounds were checked by TLC using different solvent mixtures 9:1 and 6:1 hexane to ethanol to compounds prepared in acetic acid and acetonitrile respectively. Only one spot was observed in each case after developing in an iodine chamber, indicating that the compounds were pure.

Table 4.1 Analytical data of the isolated compounds in acetic acid medium found (calcd.)

Compound	Color	M.P/Dec. temp. (°C)	Yield (%) (mg)	*Chlorine (%)	Metal (%)	λ_m ($\Omega^{-1}cm^2mol^{-1}$)
Ni(II) compound	Pink	175-176	36 (30)	23.50 (23.00)	< 1	1.96
Cu(II) compound	Pink	178-180	68 (48)	23.40 (23.00)	< 1	1.40
Cr(III) compound	Pink	179-180	62 (33)	23.10 (23.00)	< 1	2.60
Fe(III) compound	Pink	181-185	73 (54)	23.80 (23.00)	< 1	8.68

*The chloride ions are not the counter ions, it was rather the chloro group of the product, clofentezine

4.2.3 *IR Spectrum of Isolated compounds in Acetic Acid Medium*

The ligand, BHPT, shows characteristic band at 3260 cm^{-1} corresponding to ν_{NH}, which is due to hydrazino functional group. The characteristic bands appeared at 1350 and 1115 cm^{-1} are assignable to ν_{C-N} of aromatic carbon-secondary amine nitrogen stretching and δa_{N-H}, deformation respectively. The other characteristic band appeared at 961 cm^{-1} is due to ν_{N-N}.

The isolated compounds do not have absorption band at 3260 cm^{-1}, which is assignable to ν_{N-H} of hydrazine functional group. The other notable feature of this compounds is the absence of bands at 1350, 1115 and 960 cm^{-1} due to aromatic carbon-secondary amine ν_{C-N}, stretching; δa_{NH} and ν_{N-N} respectively. And the appearance of new band at about 1088 cm^{-1}, which is assignable to aromatic carbon-chlorine ν_{C-Cl}, stretching [44].

4.2.4 *IR Spectrum of Isolated compounds in Acetonitrile Medium*

The IR spectra of these compounds showed strong band at 1390 cm^{-1} and medium band at 1140 cm^{-1} due to asymmetric and symmetric stretching vibrations of $\nu a_{S=O}$ and $\nu s_{S=O}$, respectively [41].

Comparison of the IR spectra of these compounds which are prepared in acetonitrile medium from the reaction of Cu(II) and Fe(III) with BHPT to that of the IR spectra of compounds prepared in acetic acid medium have the following major differences.

1. Presence of strong band at 1390 cm^{-1} and medium band at 1140 cm^{-1}, which are

characteristics of sulphate functional group.

2. Absence of band in the region of 1100-1080 cm^{-1}, which is due to aromatic C-Cl stretching.

The IR spectra for Cu(II) and Fe(III) compounds are identical; in both cases sulphate salts were used. The IR spectrum of Cr(III) compound prepared by both methods are identical and is discussed in section 4.2.3.

Comparison of the IR spectra of the isolated compounds with clofentezine reveals that the IR spectrum of clofentezine is identical with the isolated compounds Table 4.2.

Table 4.2 Characteristic infrared frequencies of the compounds (expressed in cm^{-1}).

Compound	ν_{NH}	$\nu_{C=N}$	$\nu_{N=N}$	ν_{C-N}	δ_{aNH}	ν_{N-N}	New bands
Ligand	3260	1603	1413	1350	1115	961	-
Ni(II) compound	-	1609	1402	-	-	-	1088
Cr(III) compound	-	1607	1400	-	-	-	1084
Cu(II) compound	-	1607	1403	-	-	-	1088
Fe(III)	-	1606	1403	-	-	-	1086

compound							
Fe(III) compound	-	1606	1403	-	-	-	1086

From the IR spectral data it is possible to infer that the spectra of all the isolated products identical to that of clofentezine, the starting material, Figs 2.1-2.4.

4.2.4 *^{1}H NMR of Isolated Compounds in Acetic Acid Medium*

The ^{1}H NMR of the isolated product in $CDCl_3$ showed identical spectra with clofentezine irrespective of the metal ions. Accordingly, the ^{1}H NMR of the compounds showed doublet at 7.7 and 8.1 ppm and multiplet in the range 7.5 - 7.4 ppm (Table 4.3 and Figs 4.5 and 4.6), which are characteristic signals for the aromatic protons [41]. Integration of the ^{1}H NMR signals yielded the ratio of 1:1:2, respectively. The disappearance of signals for hydrazine protons, and the chemical shift is consistent with clofentezine assignment. Comparison of the ^{1}H NMR spectra of the ligand with products isolated revealed, downfield shift.

Similarity among the ^{1}H NMR spectra of each compound and clofentezine might be due to the replacement of hydrazino groups by chlorine atoms.

Table 4.3 ^{1}H NMR data of isolated compounds in acetic acid medium

Type	N° of Protons	Pattern	Chemical shift (ppm)
H^{1}	2	d	7.7
$H^{2,3}$	4	d	8.1
H^{4}	2	d	7.5-7.4
Hydrazine proton	6	-	-

4.2.5 *Electronic Spectra of Isolated Compounds in Acetic Acid and Acetonitrile media*

Assignments for various transitions are based on the supporting literature from standard references [44, 45]. 3,6-Bis(2-hydrazinophenyl)-1,2,4,5-tetrazine is a yellow, crystalline compound and has absorption band in the UV-Visible region.

The spectra of the isolated compounds from the reaction of BHPT and metal ions are discussed below:

From the UV-Vis spectral data it was possible to conclude that all compounds isolated have major absorption bands at 18761 cm^{-1} in addition to ligand absorption band. The additional bands that appeared in compounds isolated from the reaction of Fe(III) and Cr(III) with BHPT other than at 18761 cm^{-1} might be due to impurities or traces (Figs 4.7-4.10). The

starting material, Clofentezine, 3,6-bis (2-chlorophenyl)-1,2,4,5-tetrazine has also showed an absorption band at 18761cm^{-1}.

4.2.6 *AAS of Isolated Compounds in Acetic Acid and Acetonitrile Medium*

The atomic absorption spectroscopy study reveals that all the isolated compounds both in acetic acid and acetonitrile media showed that the percentage composition of metal ions in all case is < 1% (Figs 4.11 - 4.14)

The Cu(II) and Fe(III) salts oxidize aryl hydrazine according to the following chemical equation [42]:

$$C_6H_5\text{-}NH\text{-}NH_2 + 2Cu^{2+} \longrightarrow C_6H_6 + N_2 + 2Cu^{+} + 2H^{+} \qquad [1]$$

If chloride salts were used there would be a formation of an aromatic chloride in acidic medium [42].

The reaction of Ni(II) and Cr(III) with the ligand is slow relative to the other metal ions of interest.

4.2.7 *^{1}H NMR Spectra of Isolated Compounds in Acetonitrile Medium*

The ^{1}H NMR spectra of the reaction product from the reaction of Cu(II) and Fe(III) with BHPT showed identical spectra (the Cr(III) compound is the same as discussed in section 4.2.4). Accordingly, the ^{1}H NMR of the compounds showed (Table 4.4 and Fig 4.15) doublet at 8.1 and 7.6 ppm and multiplet in the range 7.5-7.6 ppm which are characteristic signals for aromatic protons [41]. Integration of the ^{1}H NMR signals yielded the ratio of 1:1:2, respectively. Comparison of the ^{1}H NMR spectra of this compounds with the spectra of compounds discussed in section 4.2.4 showed a slight down field shift due to the SO_4^{2-}.

Table 4.4 Proton NMR of isolated compounds in acetonitrile medium

Type	Number of protons	Pattern	Chemical shift (ppm)
H^{1}	2	d	8.1
H^{4}	2	d	7.65
$H^{2,3}$	4	m	7.5-7.6

4.2.8 *^{13}C NMR of Isolated Compounds in Acetonitrile Medium*

The ^{13}C NMR spectra of isolated compound from the reaction of Cu(II) and Fe(III) ions with BHPT in acetonitrile medium showed three quaternary carbon atom signals at 165.3, 134.3 and 131.9 ppm for aromatic carbon attached to oxygen, azomethine carbon and carbon attached to tetrazine ring carbon respectively. The other four bands that appeared at 133.0, 132.7, 131.6 and 127.7 ppm are assignable to carbon–5, 6, 7 and 4 respectively (Fig 4.16).

4.2.9 *Electronic Spectra of Isolated Compounds in Acetonitrile Medium*

The compounds discussed in this sub-section have pink color. The electronic spectroscopy study reveals that the appearances of bands at 47,619 cm^{-1} (ε = 33,000 $M^{-1}cm^{-1}$) and at 37,037 cm^{-1} (ε = 25,000 $M^{-1}cm^{-1}$) are assignable to π-π* transition; which are characteristic bands of aromatic compounds. And there is no any band that could be assignable to d-d transitions (Fig 4.17)

The products isolated have pink color and are soluble in less polar and polar organic solvents: dichloromethane, chloroform, methanol. Melting point, conductance measurement, spectroscopic studies and elemental analysis revealed that the isolated compound is clofentezine.

Table 4.5 Analytical data of the isolated compounds in acetonitrile medium found (calcd.)

Compound	Color	M.P/Dec. temp. (°C)	Yield (%) (mg)	Sulphur/ *Chlorine (%)	Metal (%)	λ_m ($\Omega^{-}m^2mol^{-1}$)
Cu(II) compound	Pink	192-195	61 (51)	5.60 (5.70)	< 1	1.40
Cr(III) compound	Pink	181-184	63 (37)	22.60 (23.00)	< 1	2.60
Fe(III) compound	Pink	192-194	66 (39)	5.00 (5.70)	< 1	8.68

*Chloride salts of chromium (III) was used

Note: Sulphur is calculated as part of the ligand

5. ANTIMICROBIAL TEST

5.1 Experimental

The required quantity, 25 mg of the ligand, 3,6-bis (2-hydrazinophenyl)-1,2,4,5-tetrazine, was weighed and dissolved in 1 ml of chloroform. A micropipette (20 μL) was used and the amount (500 μg) was poured on three filter paper discs. The three discs used were for three bacteria: *Escherichia coli*, *Proteus* and *Staphilococcus aureus*. After pouring the sample on filter paper disc it was left on air for 30 min for the solvent to evaporate. The preserved petrdishes were dried in sterilized hood. The filter paper discs, with the sample side down, was put on the media in the petrdishes. Finally inverting to observe the possible inhibitory effect of the sample for 24 h incubated the petrdishes.

5.2 Results and Discussion

Many physiologically active herbicides have been studied for antimicrobial properties. Prometryn and cyanuric acid were studied for nucleic acid metabolism in *Escherichia Coli* [49]. Atrazine, prometryn, and simazine have been screened against *Bacillus sustillus sustilis* [50]. When these physiologically active compounds are bound to metal ions, their properties may be enhanced or reduced depending on the binding sites, the electronic distribution and the availability or non-availability of active functional groups, which are responsible for the activity.

The result of the effect of the ligand, 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine on *Escherichia coli, Proteus* and *Staphilococcus aureus* showed that it has no inhibition effect on the growth of the above bacteria. This result is in parallel with clofentezine, which is known acaricide.

6. KINETIC STUDY

6.1 Experimental

A known quantity (6.9 mg, 0.040 mmol) of copper chloride was dissolved in 1.0 ml ethanol and diluted to 5 ml with chloroform and the ligand, BHPT (2 mg, 0.0068 mmol) was dissolved in chloroform. The two solutions and the cell were kept in the refrigerator for about 4 h. 1.5 ml of each reactant was poured in to the cell and measurement was taken immediately. The spectral change in the course of the reaction was the appearance of the characteristic absorption band of clofentezine at 538.0 nm. The two reactants are practically transparent at this wavelength. The reaction temperature was about 4°C and the pH of the solution was 8.15. The rate of the above reaction was also investigated at two pHs: 4 and 9.2 at 50°C. The rate of reaction at lower pH (0.784 Abs/min) is about two times greater than that of higher pH (0.35 Abs/min).

The rate of the decomposition of the ligand, BHPT, in the absence of the Cu(II) ion at different pHs and 50°C was investigated. The rates of the reaction and rate constant at various pHs are given in Table 6.1

Table 6.1 The rate of decomposition of BHPT at 50°C

pH	Rate (Abs/Min)	Rate constant (Sec^{-1})
4	$2.6x10^{-2}$	$2.8x10^{-2}$
5	$6x10^{-3}$	$1x10^{-4}$
6	$4.3x10^{-4}$	$4x10^{-5}$
7	0.0	0.0

6.2 Results and Discussion

It has been observed that the reaction between metal ions and the ligand, BHPT is not complex formation. Kinetic measurement of the reaction between copper (II) chloride and the ligand revealed that the reaction is a second order (Fig 6.1 and 6.2) with rate of 0.736 Abs/min and rate constant of 94.7 $mol^{-1}Lsec^{-1}$ in acetonitrile solution at pH 8.15 and 4°C.

The rate of the reaction in the presence of the Cu(II) ion was investigated at two pH. Accordingly, the rate of the reaction at pH = 4.0 is 0.784 Abs/min with rate constant of 28.06 $mol^{-1}Lsec^{-1}$ and at pH = 9.2 the rate is 0.35 Abs/min with rate constant of 13.7 $mol^{-1}Lsec^{-1}$. In both cases the temperature was kept constant at 50°C. From the kinetic study of the reaction between $Cu(II)Cl_2$ and the ligand, BHPT, the rate of the reaction is higher at lower pH. This

might be explained in terms of redox reaction and the decomposition of the ligand, BHPT, in acidic medium to give the same reaction product.

The rate of decomposition of the ligand, in the absence of metal ion, decreases as pH increases. The decomposition of the ligand in acidic medium is initiated by protonation of hydrazino group [47]. At lower pH the rate of decomposition of the ligand, BHPT, is higher. The reaction is first order.

The following reaction presents the decomposition of the ligand in acidic medium [47].

$$2R\text{-}NH\text{-}NH_2 + H^{\cdot} \longrightarrow R\text{-}H + R^{+} + N_2 + 2NH_3 \qquad [2]$$

The redox reaction that takes place between $CuCl_2$ and the ligand is also confirmed by analytical method.

5 ml aqueous solution of ammonia was added to (5 ml, 2.34 mM) $CuCl_2$ that made the solution to have deep blue color. The blue colored solution is due to the formation of copper (II) ammonia complex. The blue color disappeared when the solution was reacted with the ligand and this might be due to the reduction of Cu(II) to Cu(I) ion. The colorless solution was converted to blue gradually, probably due to the oxidation of Cu(I) back to Cu(II).

In addition to the above analytical method the pH of the starting material and the reaction product was measured and compared. Accordingly, the pH of the reaction product is reduced

abruptly to 2.9. This observation further verifies the reaction between copper (II) chloride and 3,6-bis (2-hydrazinophenyl)-1,2,4,5-tetrazine is redox reaction in which hydrogen ion is produced in the course of reaction.

From the above preliminary kinetic investigation the following mechanism is proposed.

$$R\text{-}NH\text{-}NH_2 + 2Cu^{2+} \longrightarrow R\text{-}N{=}NH + 2Cu^{+} + 2H^{+} \quad [3]$$

$$R\text{-}N{=}NH + 2Cu^{2+} \longrightarrow R^{+} + 2Cu^{+} + H^{+} + N_2 \quad [4]$$

7. CONCLUSIONS

The synthesis and characterization of 3,6-bis(2-hydrazinophenyl)-1,2,4,5-tetrazine (BHPT) from the reaction of clofentezine with hydrazine hydrate has been achieved. Based on analytical, conductance and spectral studies it is concluded that the reaction between Cu(II)

ion and BHPT is not complex formation. Preliminary kinetic investigation reveals that the reaction between Cu(II) ion with BHPT is redox reaction and independent of pH. The reaction of metal chlorides with BHPT in moderately acidic to strongly acidic media produce clofentezine, which is the starting material.

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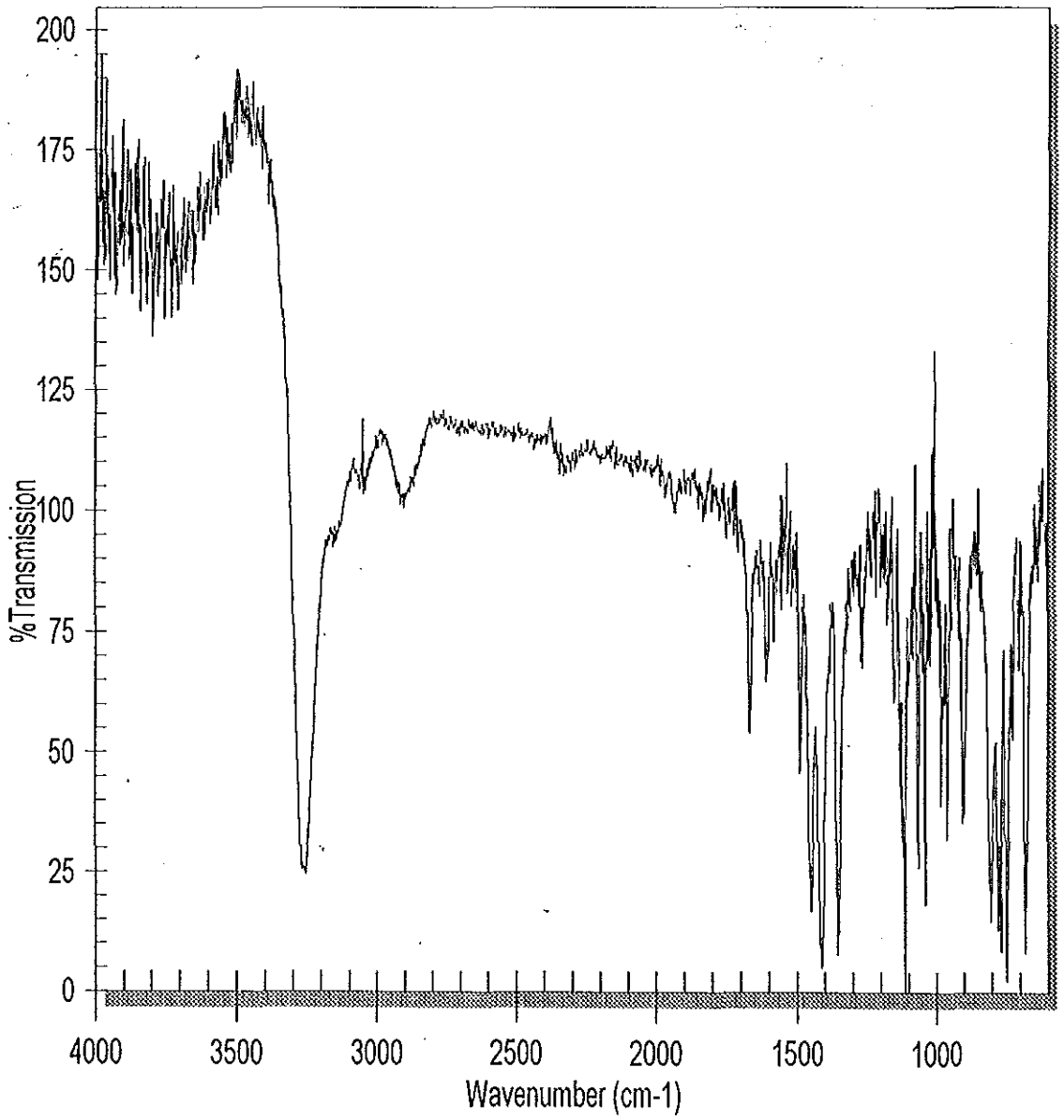
Fig 3.1 IR Spectrum of 3,6-Bis(2-Hydrazino Phenyl)-1,2,4,5-Tetrazine



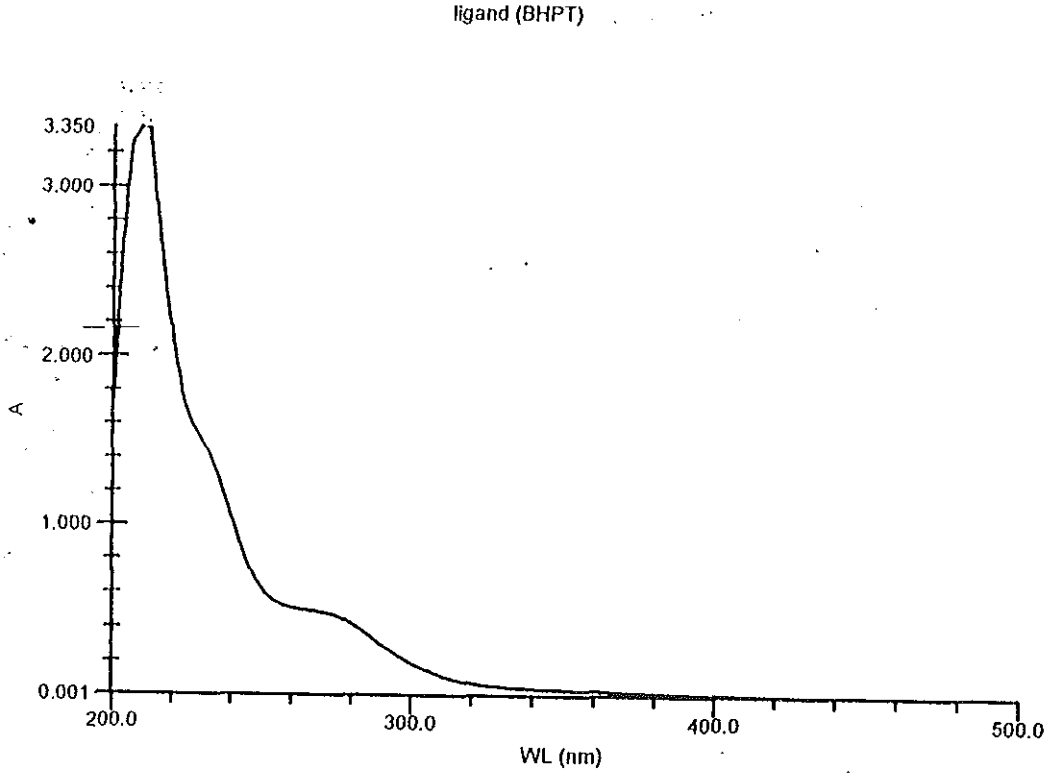


Fig 3.2 UV-Visible spectrum of BHPT

Fig 3.3 ^{1}H NMR spectrum of BHPT, $CDCl_3$

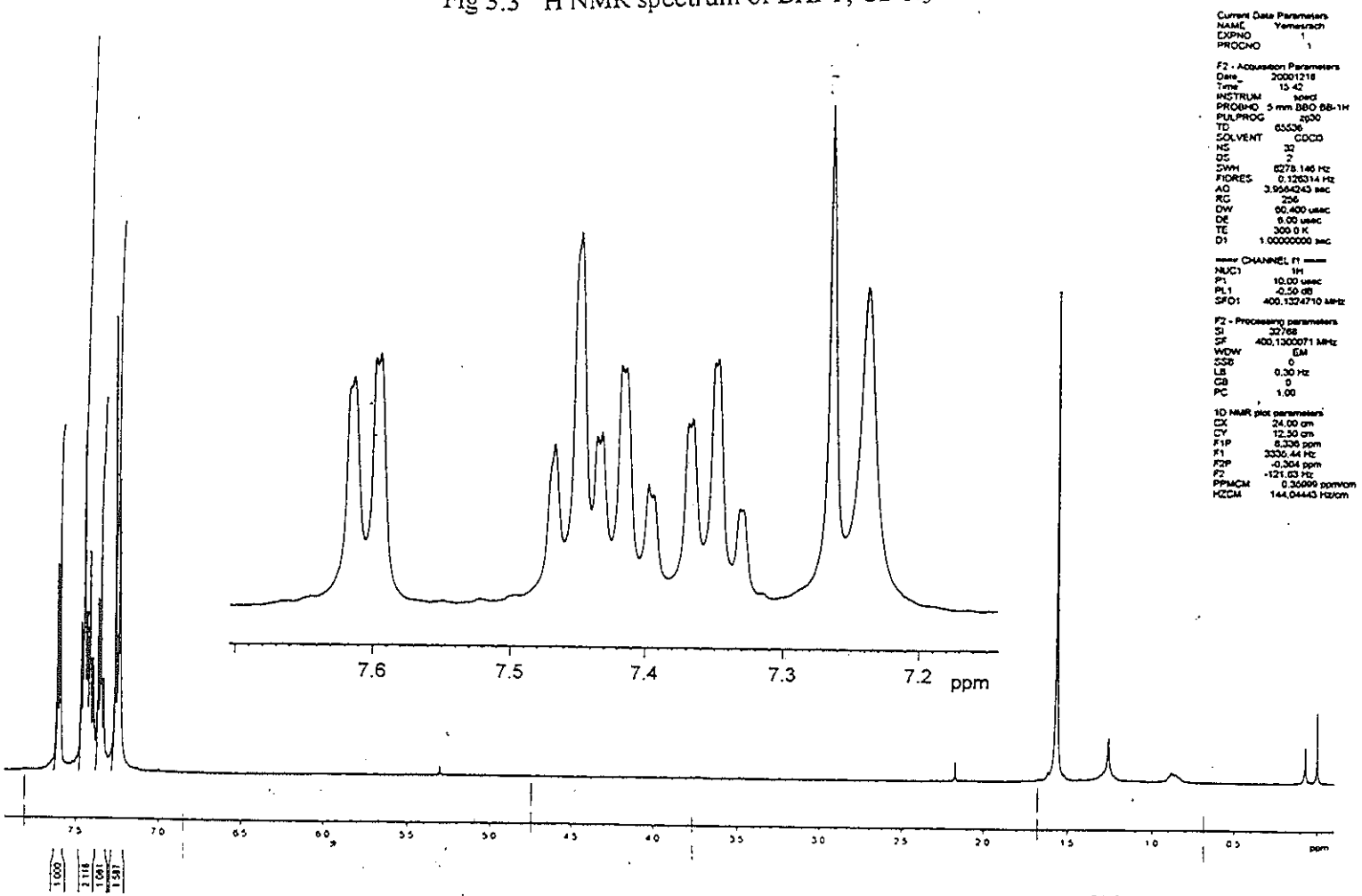
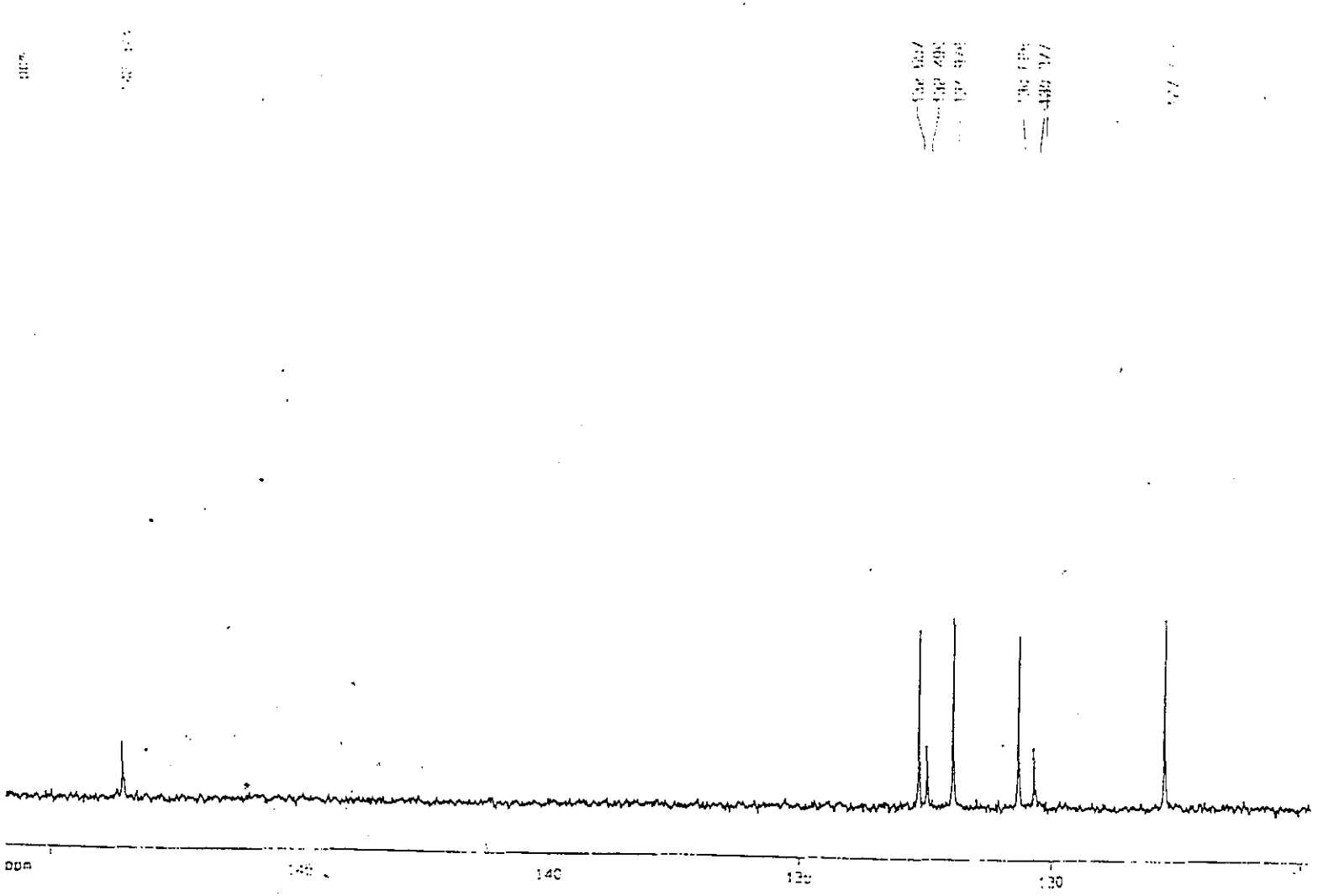


Fig 3.4 ^{13}C NMR spectrum of BHPT, 3, 6-bis (2-hyorazinopheny1) -1, 2, 4, 5-tetrazine, CDCl3



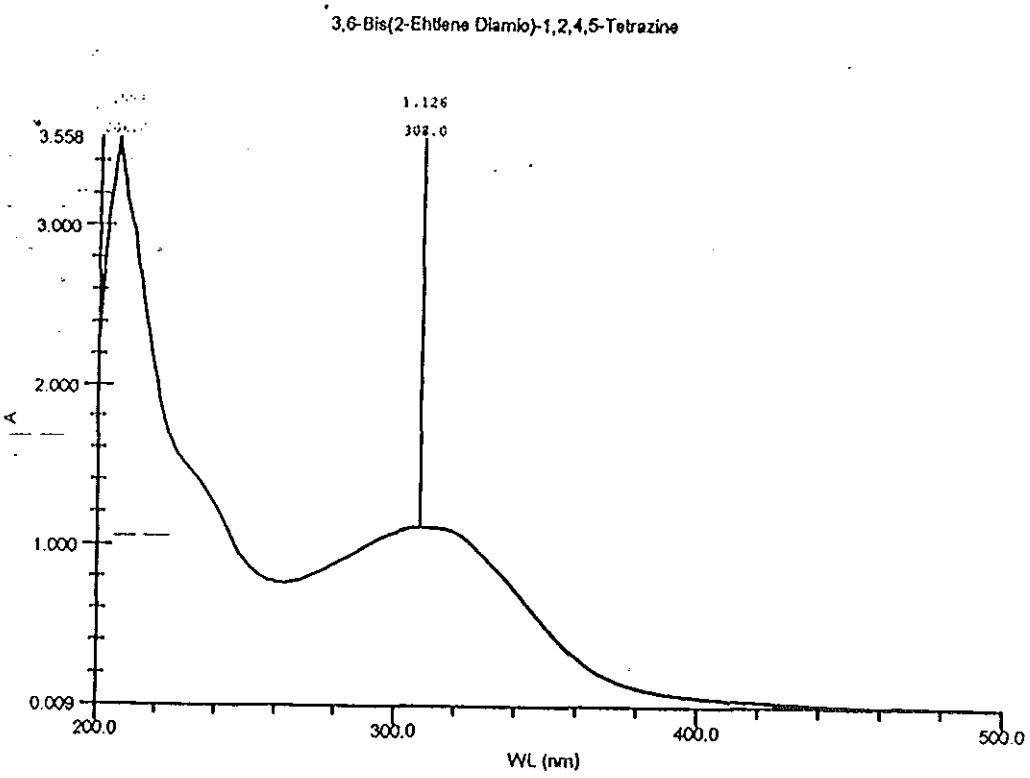


Fig 3.5 UV-Visible spectrum of 3,6-bis.(2-diamino ethane)-1,2,4,5-tetazine

Fig 4.1 IR Spectrum of 'Cr(III) Complex'

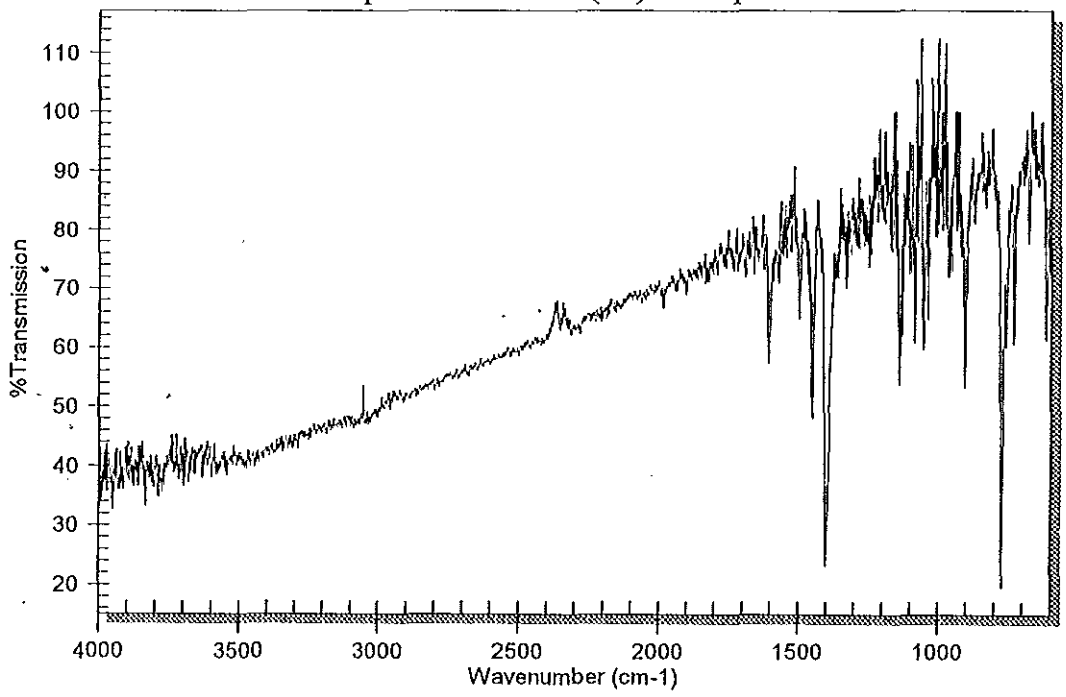


Fig 4.2 IR Spectrum of 'Cu(II) Complex'

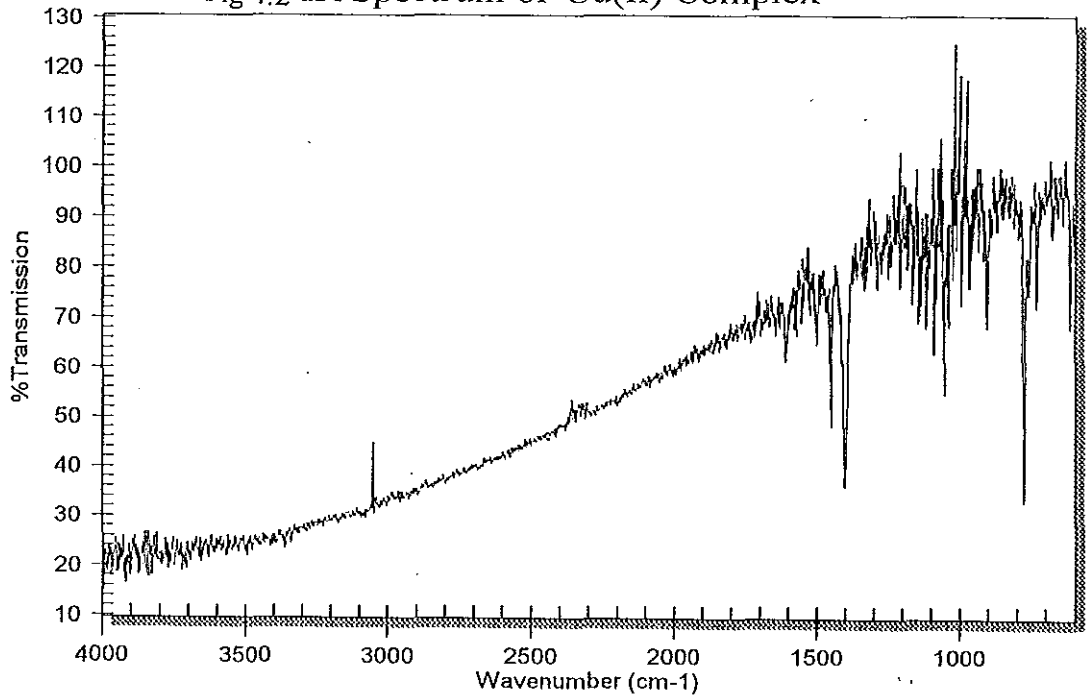


Fig 4.3 IR Spectrum of 'Fe(III) Complex'

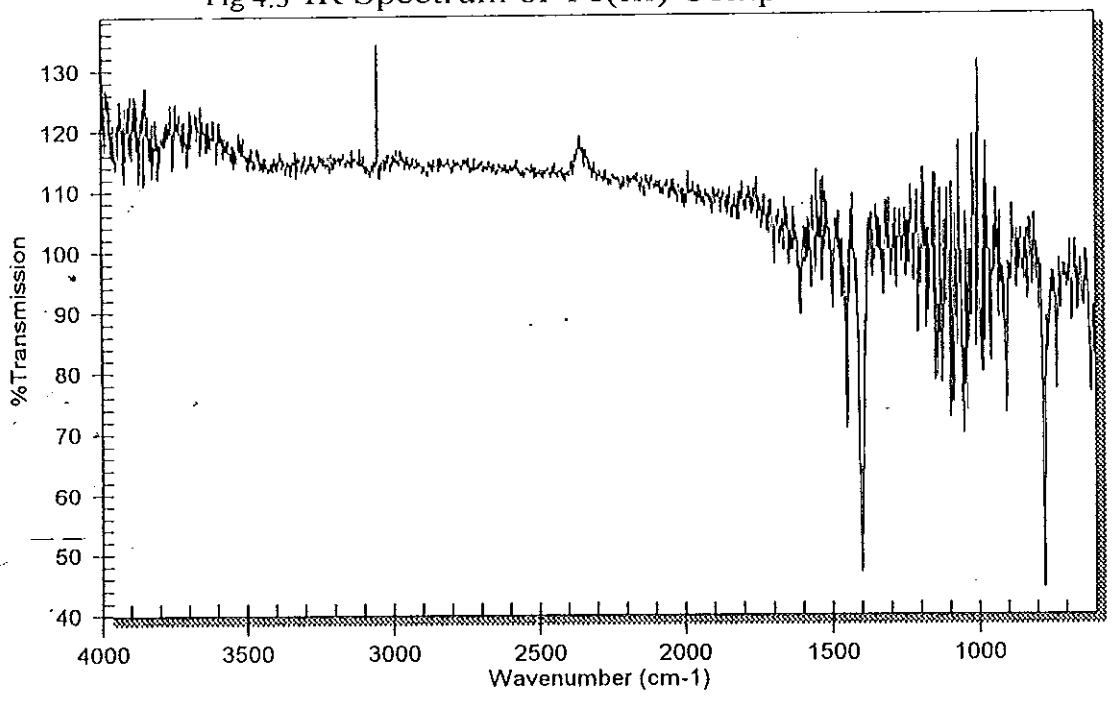


Fig 4.4 IR Spectrum of 'Ni(II) Complex'

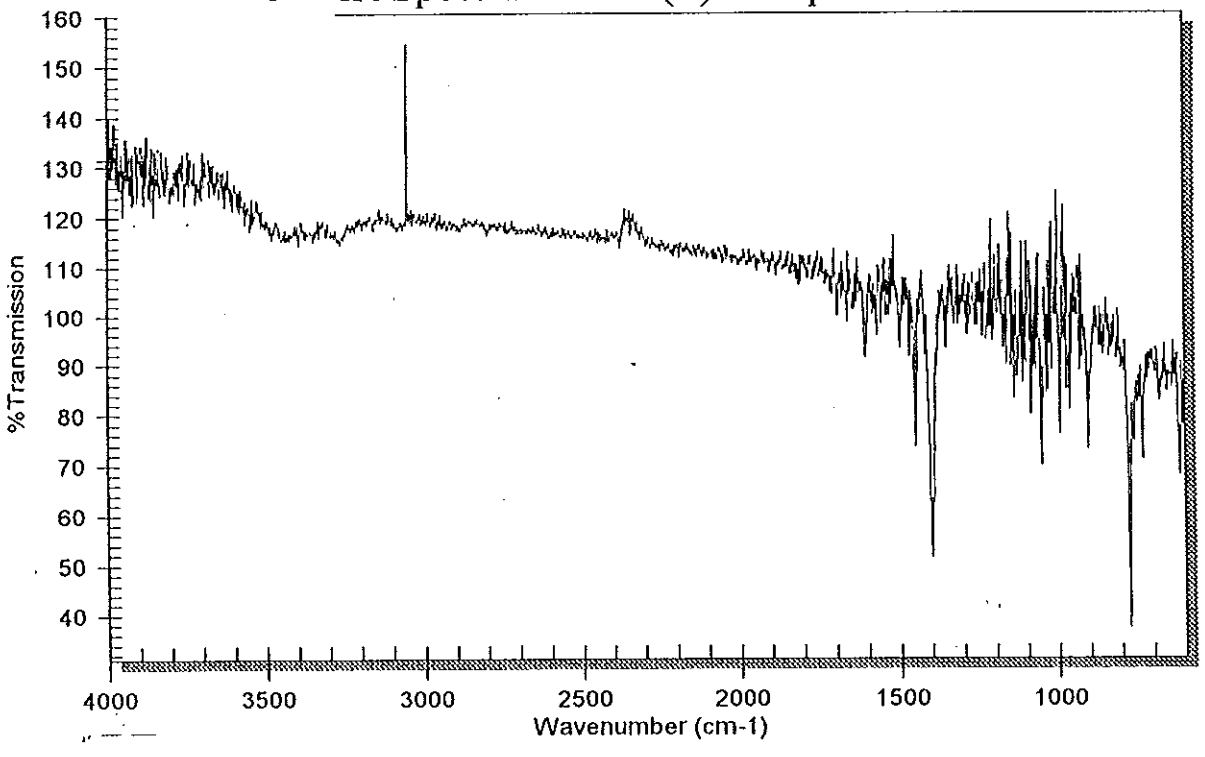


Fig 4.5 ^{1}H NMR spectrum of 'Cr (III) complex', $CDCl_3$

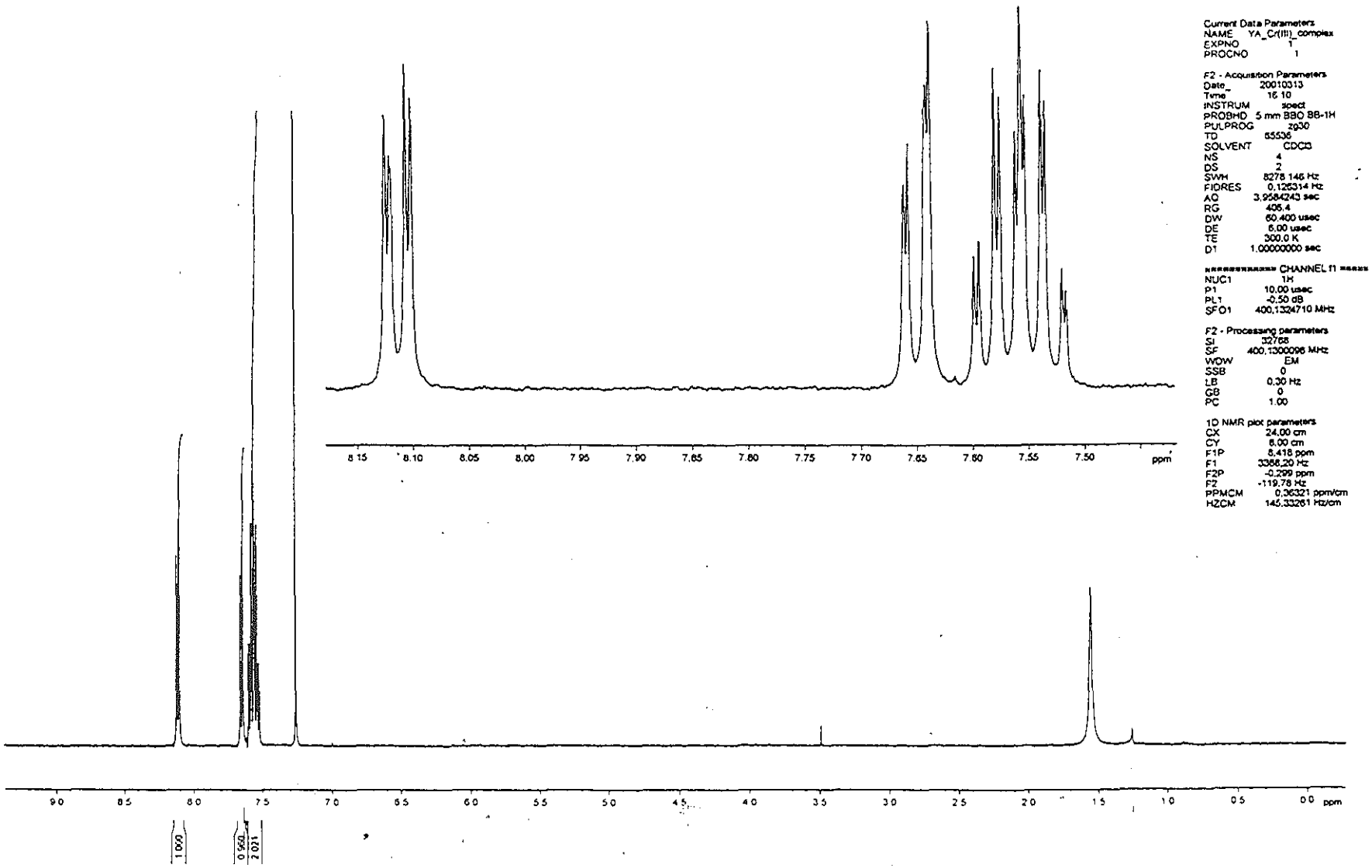
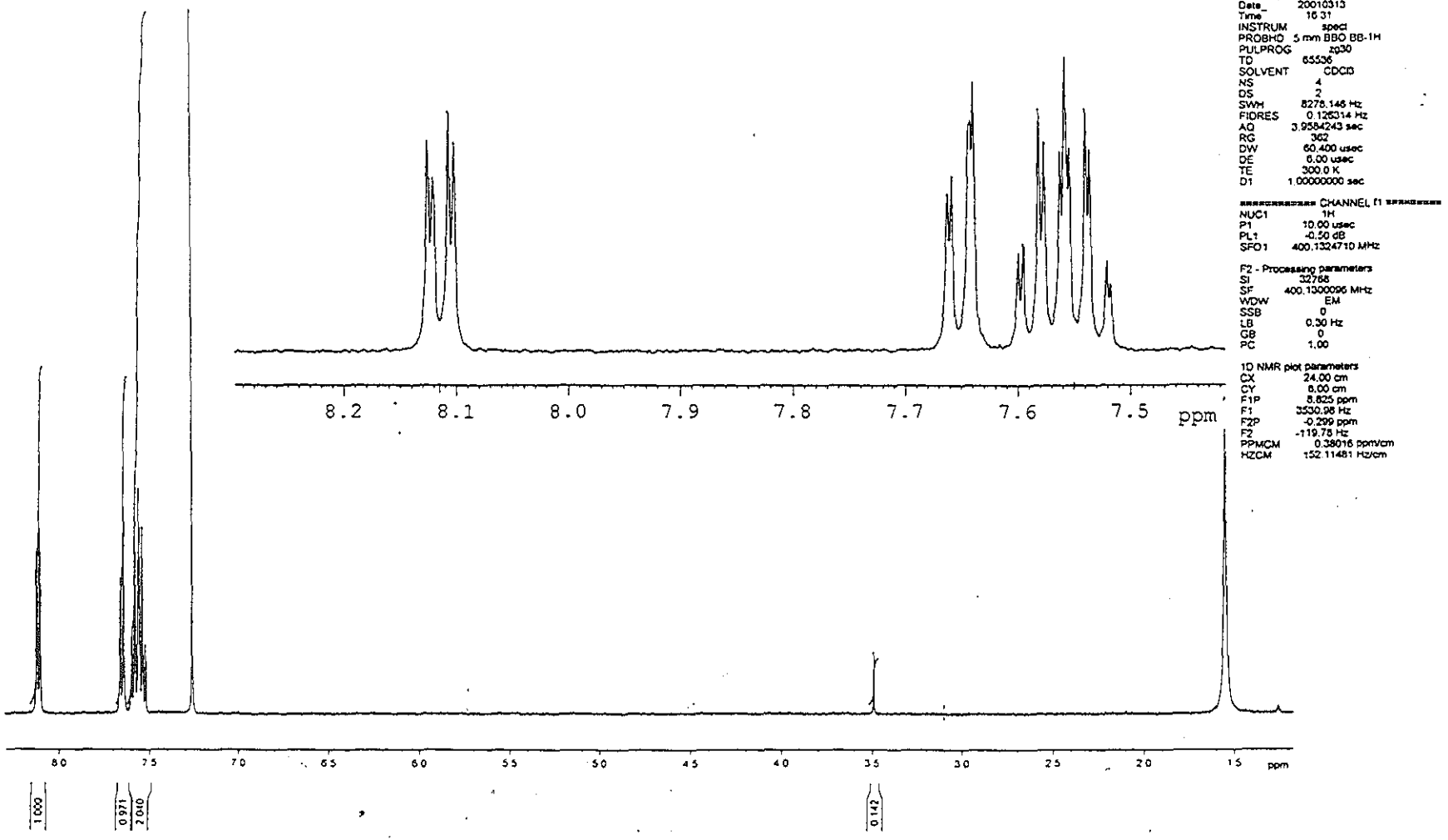


Fig 4.6 ^{1}H NMR spectrum of 'Cu (III) complex', $CDCl_3$



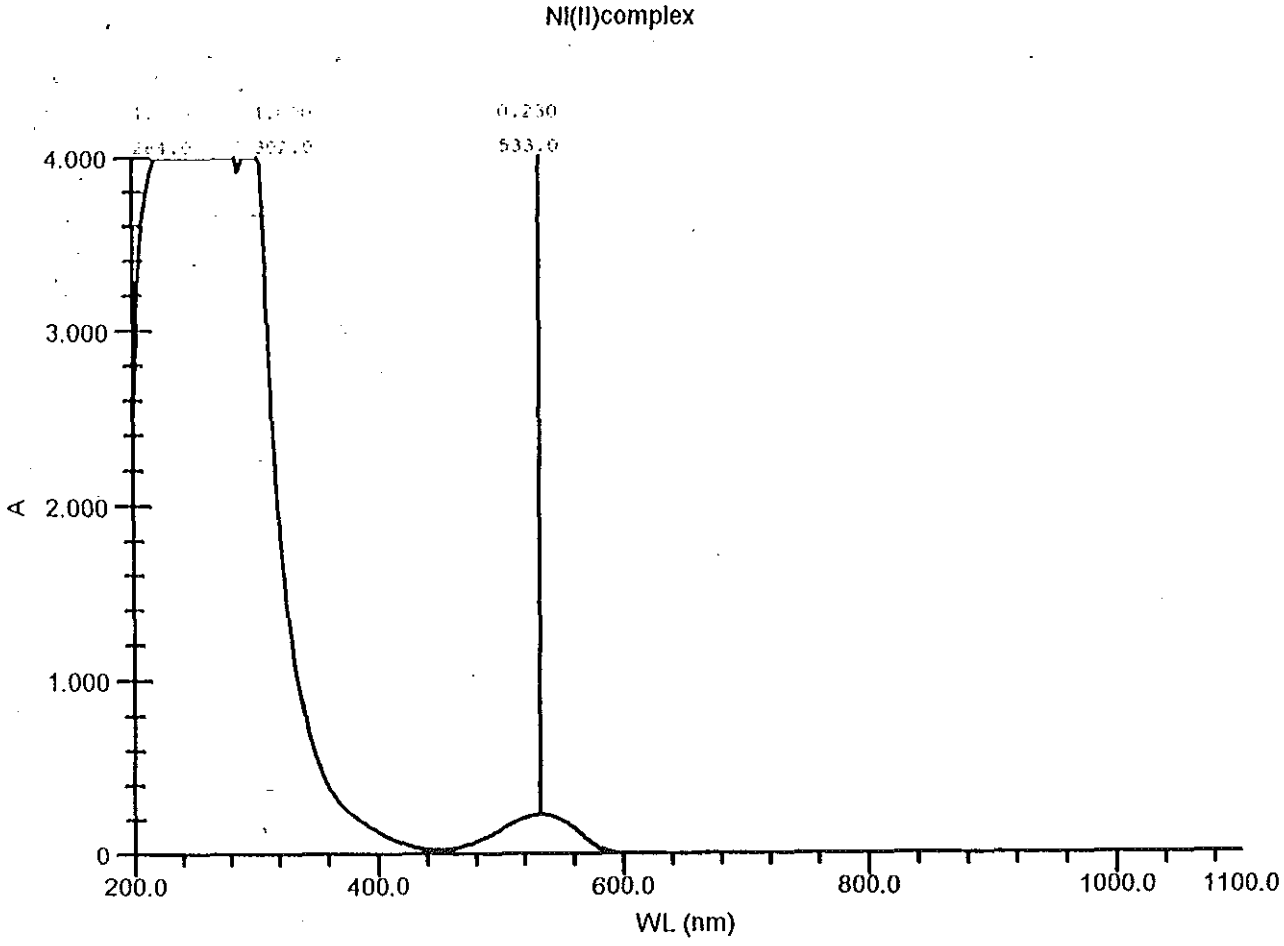


Fig 4.7 UV-Visible spectrum of 'Ni (II) complex'

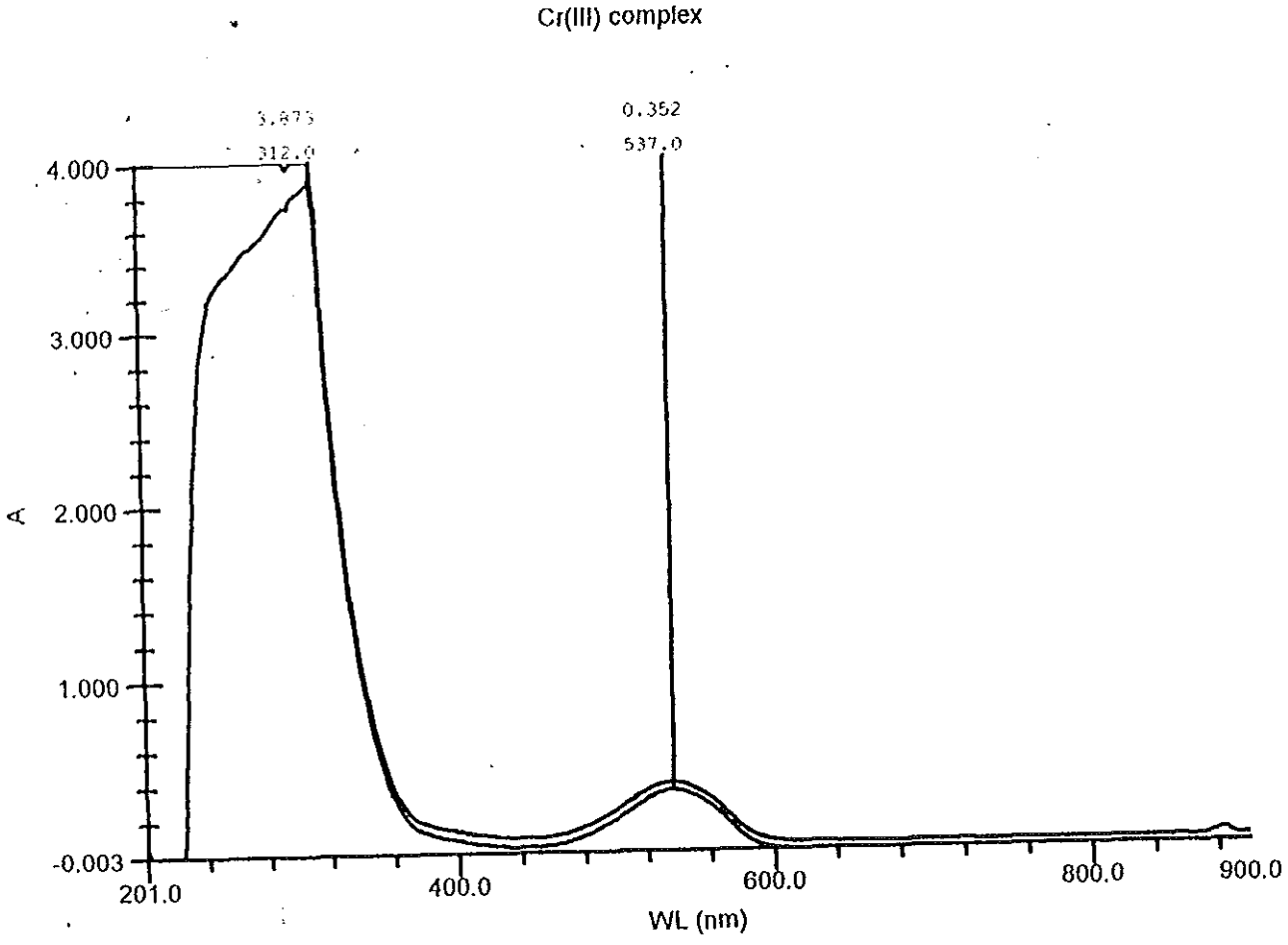


Fig 4.8 UV-Visible spectrum of 'Cr (III) complex'

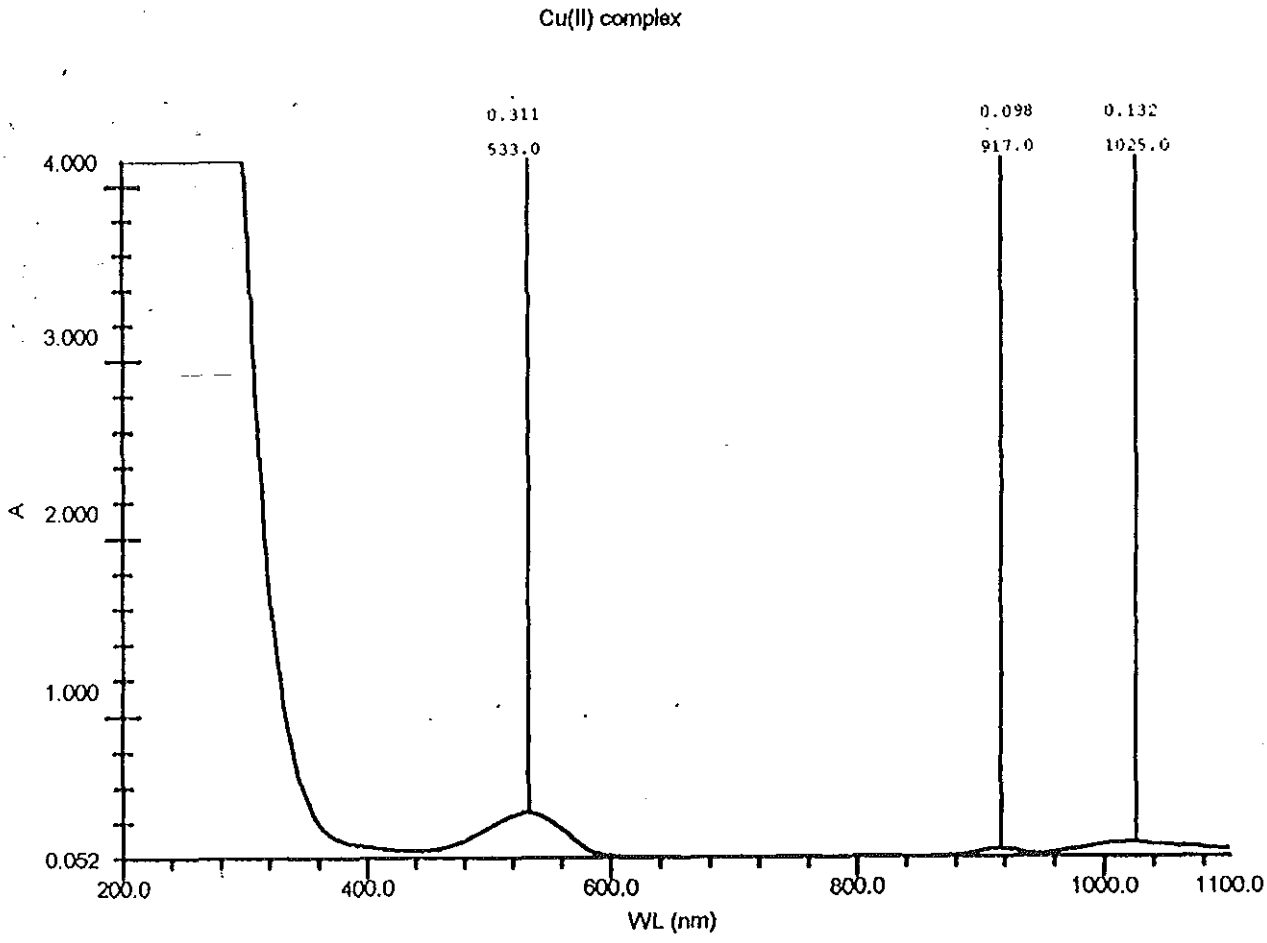


Fig 4.9 UV-Visible spectrum of 'Cu (II) complex'

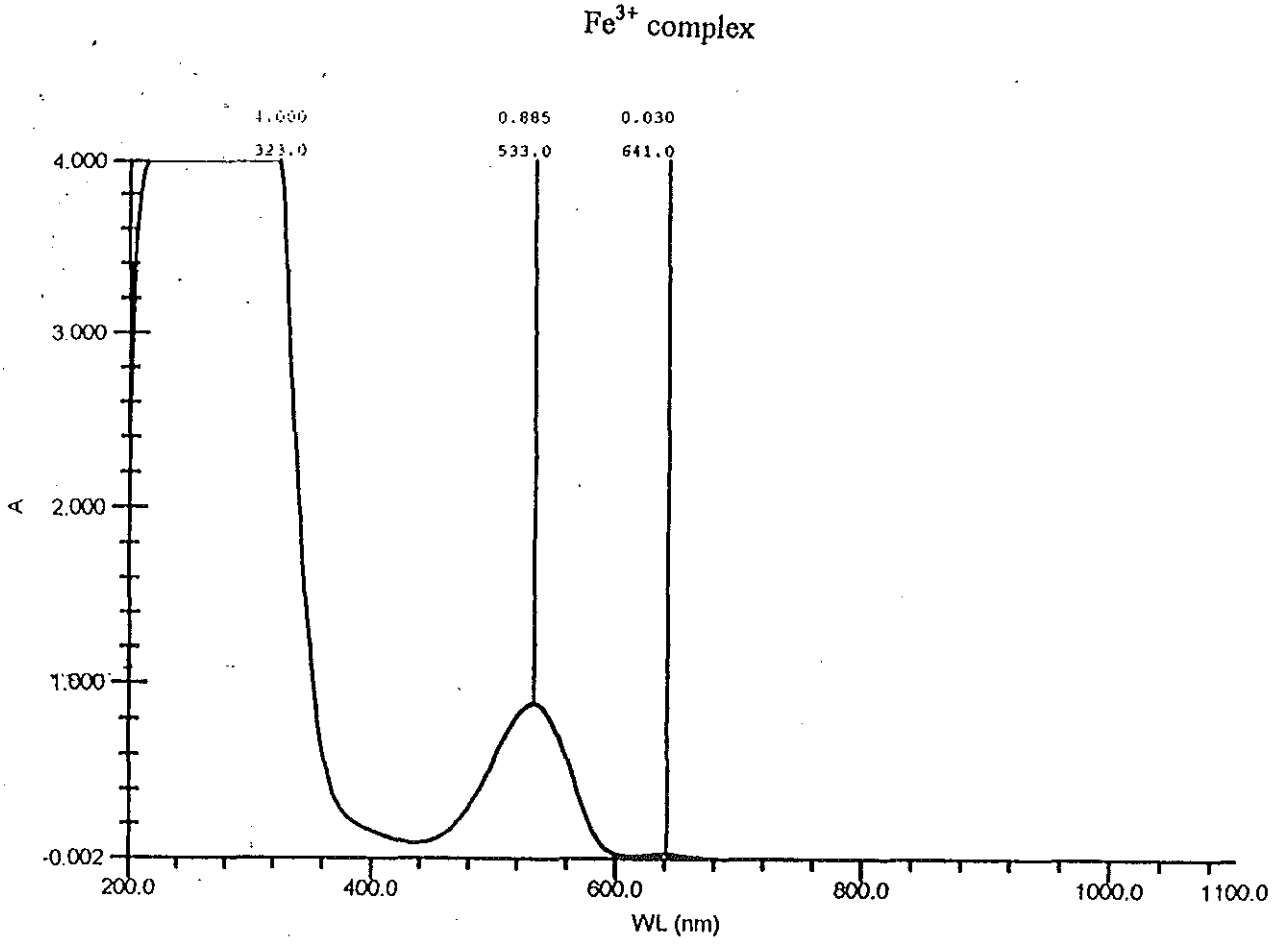
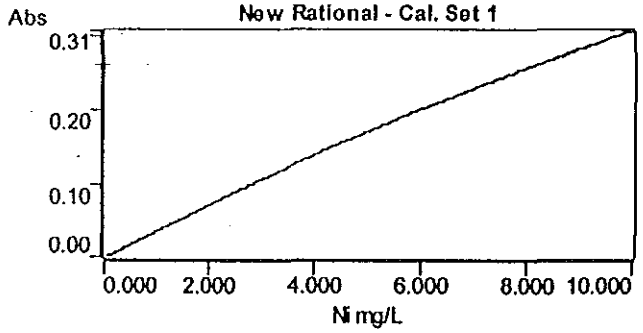


Fig 4.10 UV-Visible spectrum of 'Fe (III) complex'

Method: Ni (Flame)

Sample ID	Conc mg/L	%RSD	Mean Abs	BG Abs	Readings		
CAL ZERO	0.000	66.5	0.0005	0.0004	0.0001	0.0008	0.0007
STANDARD 1	2.000	1.0	0.0717	-0.0014	0.0709	0.0723	0.0718
STANDARD 2	4.000	0.2	0.1386	-0.0007	0.1387	0.1388	0.1382
STANDARD 3	6.000	0.1	0.1998	0.0001	0.1998	0.1995	0.2000
STANDARD 4	8.000	0.0	0.2567	0.0005	0.2567	0.2567	0.2566
STANDARD 5	10.000	0.1	0.3089	0.0005	0.3093	0.3089	0.3085



Curve Fit = New Rational

Characteristic Conc = 0.122 mg/L

r = 1.0000

Calculated Conc = 0.015 2.020 4.001 5.961 7.973

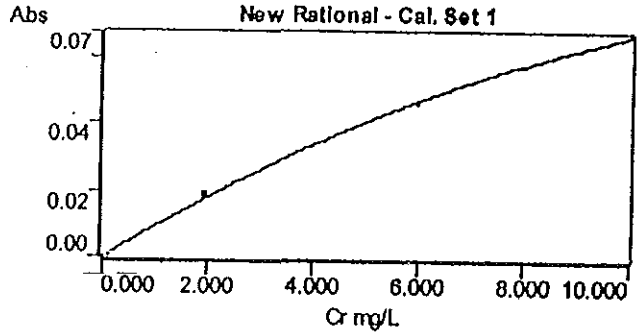
Residuals = -0.015 -0.020 -0.001 0.039 0.027

BL	0.033	9.2	0.0012	-0.0013	0.0011	0.0011	0.0013
Ni1	0.544	2.9	0.0195	-0.0008	0.0198	0.0189	0.0199
Ni2	0.123	7.3	0.0044	-0.0010	0.0041	0.0044	0.0047
	DF = 2.03						
Check(10ppm)	10.212	0.3	0.1712	0.0004	0.1715	0.1713	0.1706

Fig 4.11 Atomic absorption spectrum of 'Ni (II) complex'

Method: Cr (Flame)

Sample ID	Conc mg/L	%RSD	Mean Abs	BG Abs	Readings		
CAL ZERO	0.000	>100	0.0002	0.0001	0.0001	0.0001	0.0005
STANDARD 1	2.000	6.1	0.0189	0.0007	0.0184	0.0181	0.0202
STANDARD 2	4.000	2.2	0.0330	0.0011	0.0328	0.0324	0.0338
STANDARD 3	6.000	2.4	0.0457	0.0015	0.0446	0.0456	0.0468
STANDARD 4	8.000	1.2	0.0566	0.0016	0.0570	0.0558	0.0570
STANDARD 5	10.000	0.2	0.0675	0.0025	0.0676	0.0675	0.0673



Curve Fit = New Rational

Characteristic Conc = 0.463 mg/L

r = 1.0000

Calculated Conc = 0.023 2.103 3.932 5.860 7.853

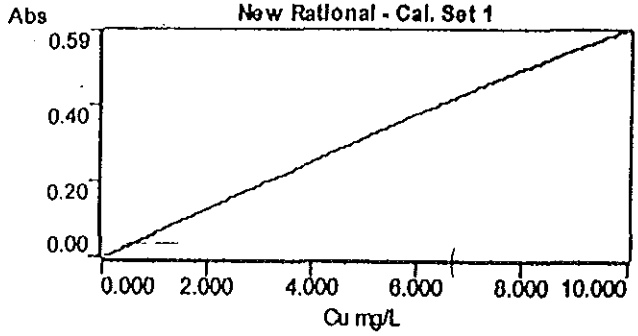
Residuals = -0.023 -0.103 0.068 0.140 0.147

BL	0.111	90.4	0.0011	0.0000	0.0021	0.0010	0.0001
Cr	0.214	39.5	0.0020	0.0013	0.0029	0.0019	0.0013
	DF = 2.10						
Check(10ppm)	10.550	2.3	0.0404	0.0018	0.0410	0.0393	0.0408

Fig 4.12 Atomic absorption spectrum of 'Cr (III) complex'

Method: Cu (Flame)

Sample ID	Conc mg/L	%RSD	Mean Abs	BG Abs	Readings		
CAL ZERO	0.000	71.7	-0.0007	0.0008	-0.0010	-0.0001	-0.0008
STANDARD 1	2.000	0.5	0.1244	0.0029	0.1240	0.1251	0.1242
STANDARD 2	4.000	0.4	0.2533	0.0043	0.2542	0.2534	0.2523
STANDARD 3	6.000	0.4	0.3730	0.0050	0.3745	0.3732	0.3713
STANDARD 4	8.000	0.3	0.4851	0.0054	0.4842	0.4867	0.4844
STANDARD 5	10.000	0.1	0.5886	0.0062	0.5894	0.5882	0.5881



Curve Fit = New Rational

Characteristic Conc = 0.071 mg/L

r = 1.0000

Calculated Conc = -0.011 1.980 4.028 6.004 7.987 9.999

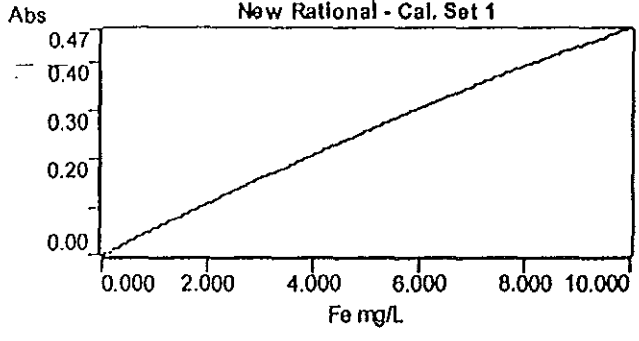
Residuals = 0.011 0.020 -0.028 -0.004 0.013 0.001

BL	0.002	>100	0.0001	0.0020	0.0004	-0.0002	0.0002
Cu	0.036	37.9	0.0022	0.0022	0.0032	0.0018	0.0017
Check(10ppm)	9.842	0.8	0.5809	0.0063	0.5859	0.5768	0.5801

Fig 4.13 Atomic absorption spectrum of 'Cu (II) complex'

Method: Fe (Flame)

Sample ID	Conc mg/L	%RSD	Mean Abs	BG Abs	Readings		
CAL ZERO	0.000	>100	0.0002	0.0003	-0.0003	0.0001	0.0008
STANDARD 1	2.000	0.3	0.1071	0.0006	0.1073	0.1072	0.1066
STANDARD 2	4.000	0.3	0.2077	0.0014	0.2070	0.2080	0.2081
STANDARD 3	6.000	0.3	0.3034	0.0018	0.3044	0.3026	0.3031
STANDARD 4	8.000	0.4	0.3903	0.0022	0.3885	0.3909	0.3916
STANDARD 5	10.000	0.3	0.4698	0.0022	0.4711	0.4703	0.4680



Curve Fit = New Rational

Characteristic Conc = 0.082 mg/L

r = 1.0000

Calculated Conc = 0.004 2.014 3.986 5.993 7.990

Residuals = -0.004 -0.014 0.014 0.007 0.010

BL	0.144	4.4	0.0077	-0.0001	0.0076	0.0074	0.0081
Fe	0.284	0.9	0.0152	0.0001	0.0151	0.0153	0.0153
Check(10ppm)	9.699	0.4	0.4579	0.0034	0.4593	0.4559	0.4585

Fig 4.14 Atomic absorption spectrum of 'Fe (III) complex'

Fig 4.15 ^{1}H NMR spectrum of 'Cu (II) complex'. $CDCl_3$

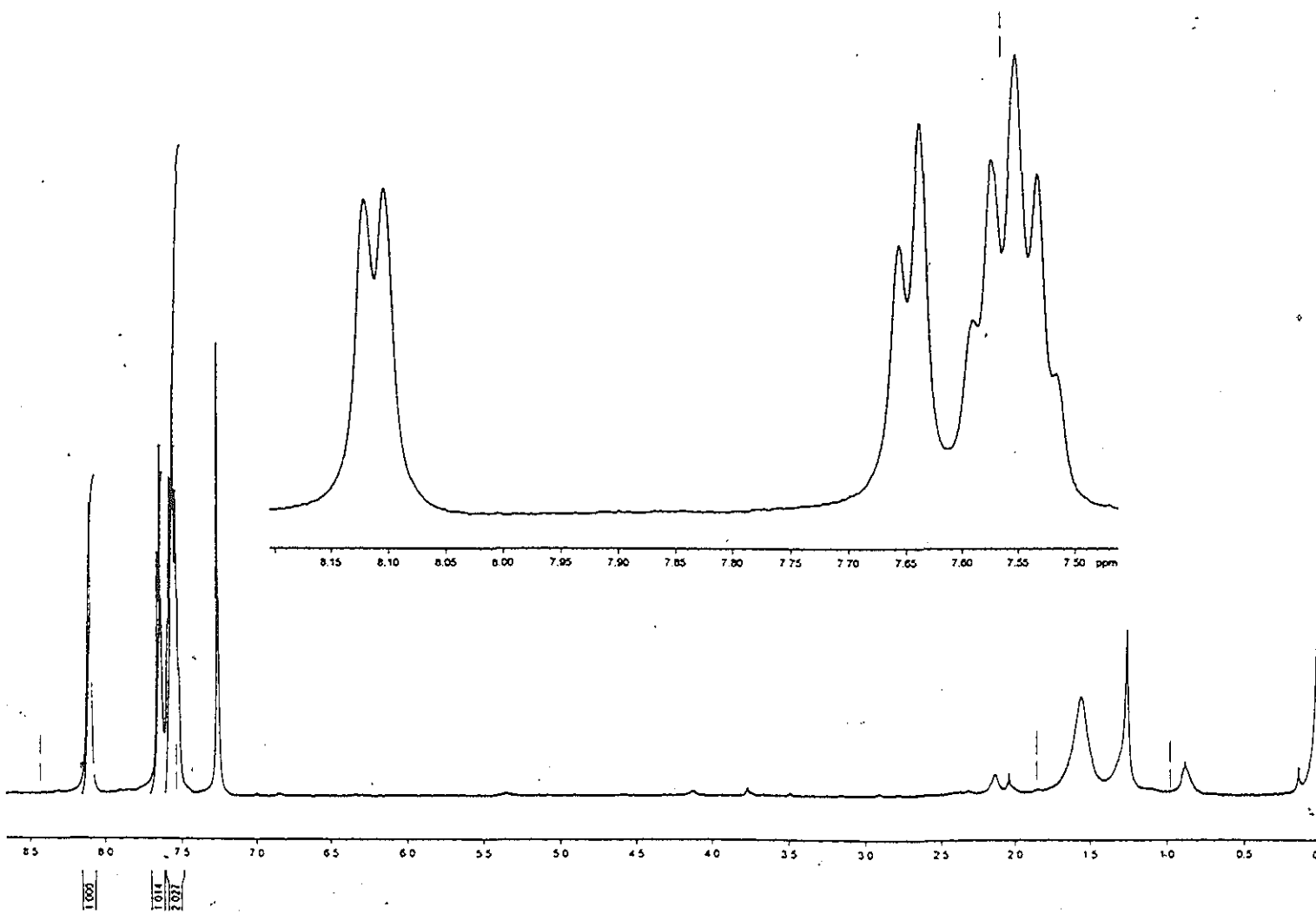


Fig 4.16 ^{13}C NMR spectrum of 'Cu (II) complex', $CDCl_3$

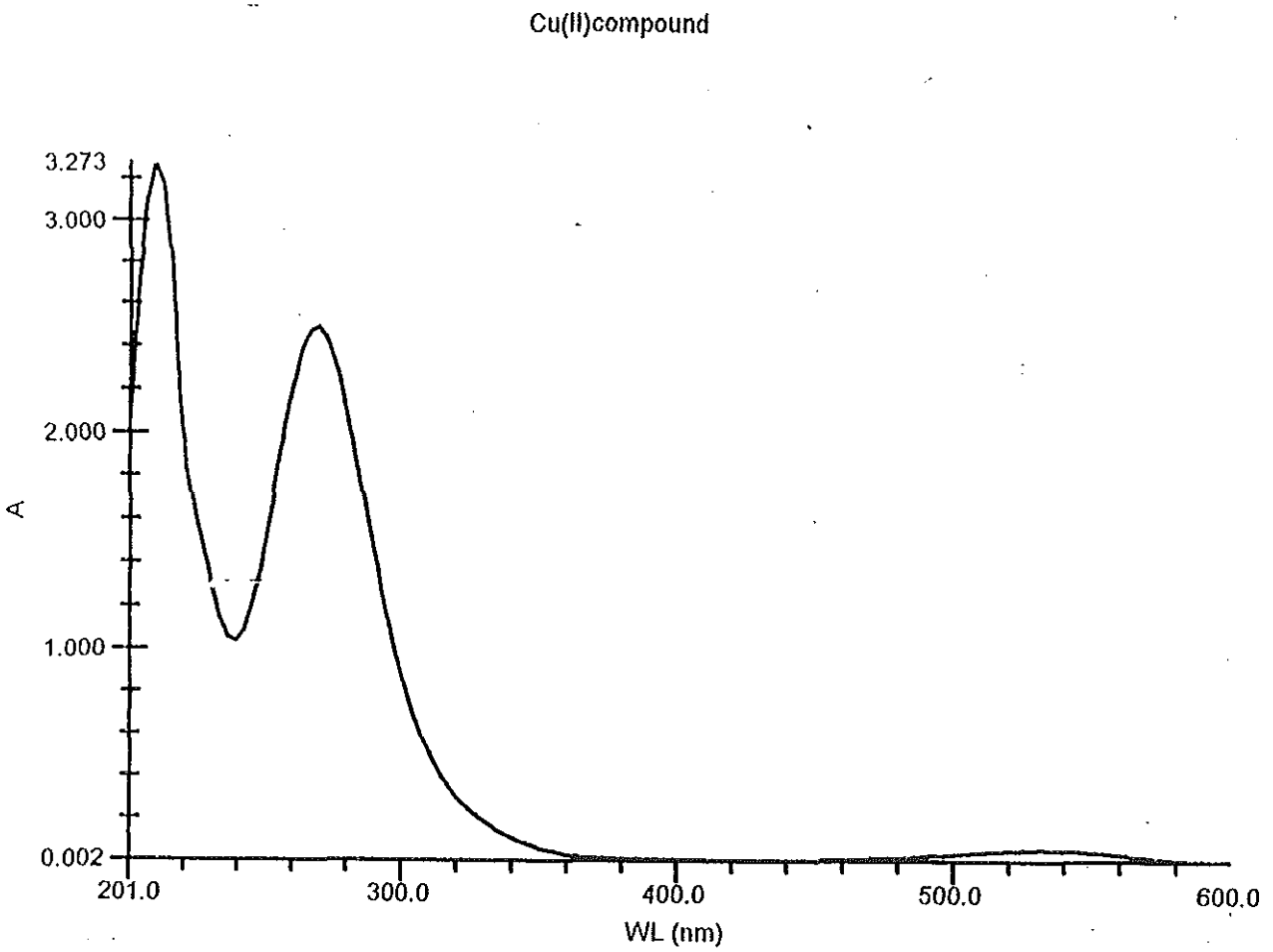


Fig 4.17 UV-Visible spectrum of 'Cu (II) complex'

Fig 6.1 Plot of absorbance *vs* time for the reaction of Cu (II) Cl_2 with BHPT at pH= 4 and 50°C

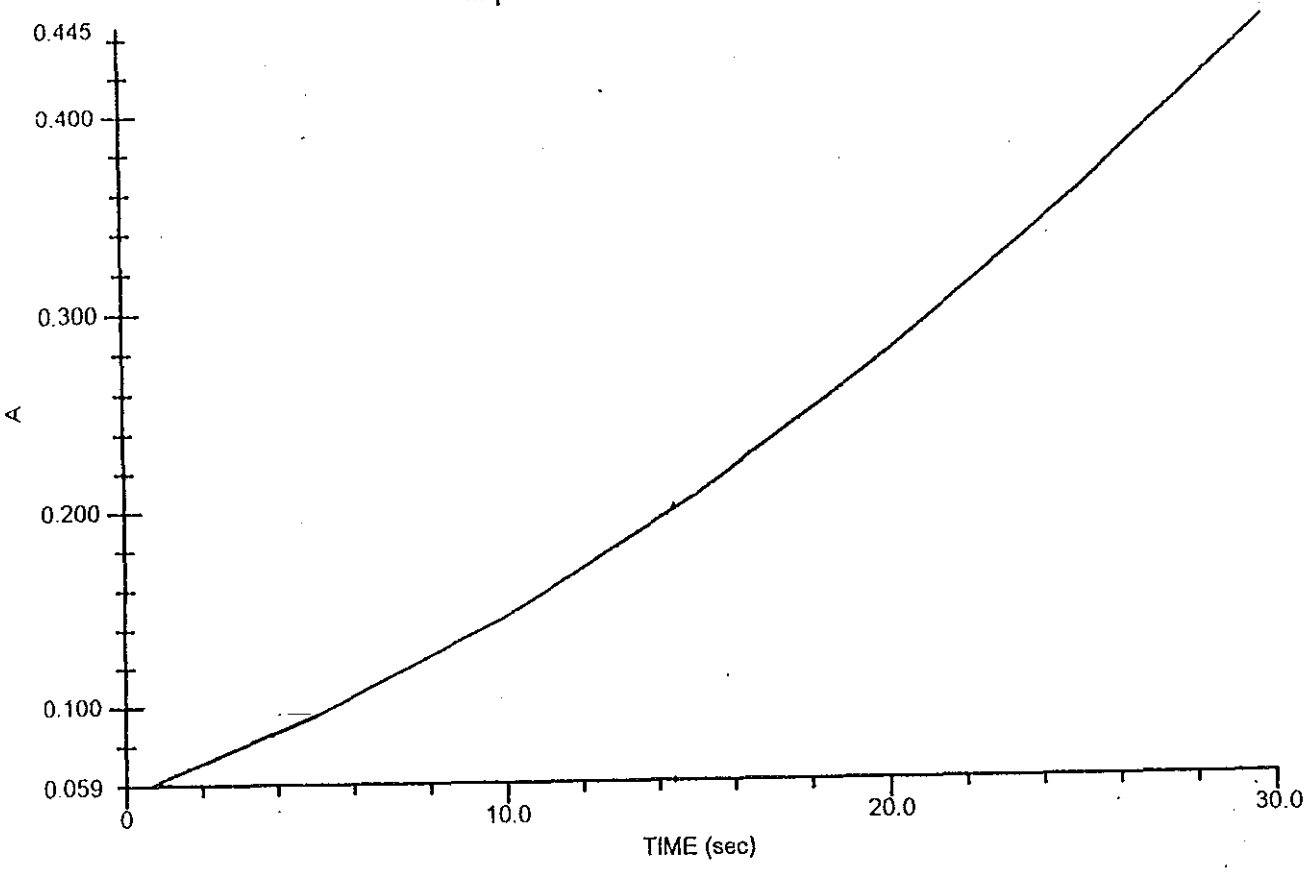
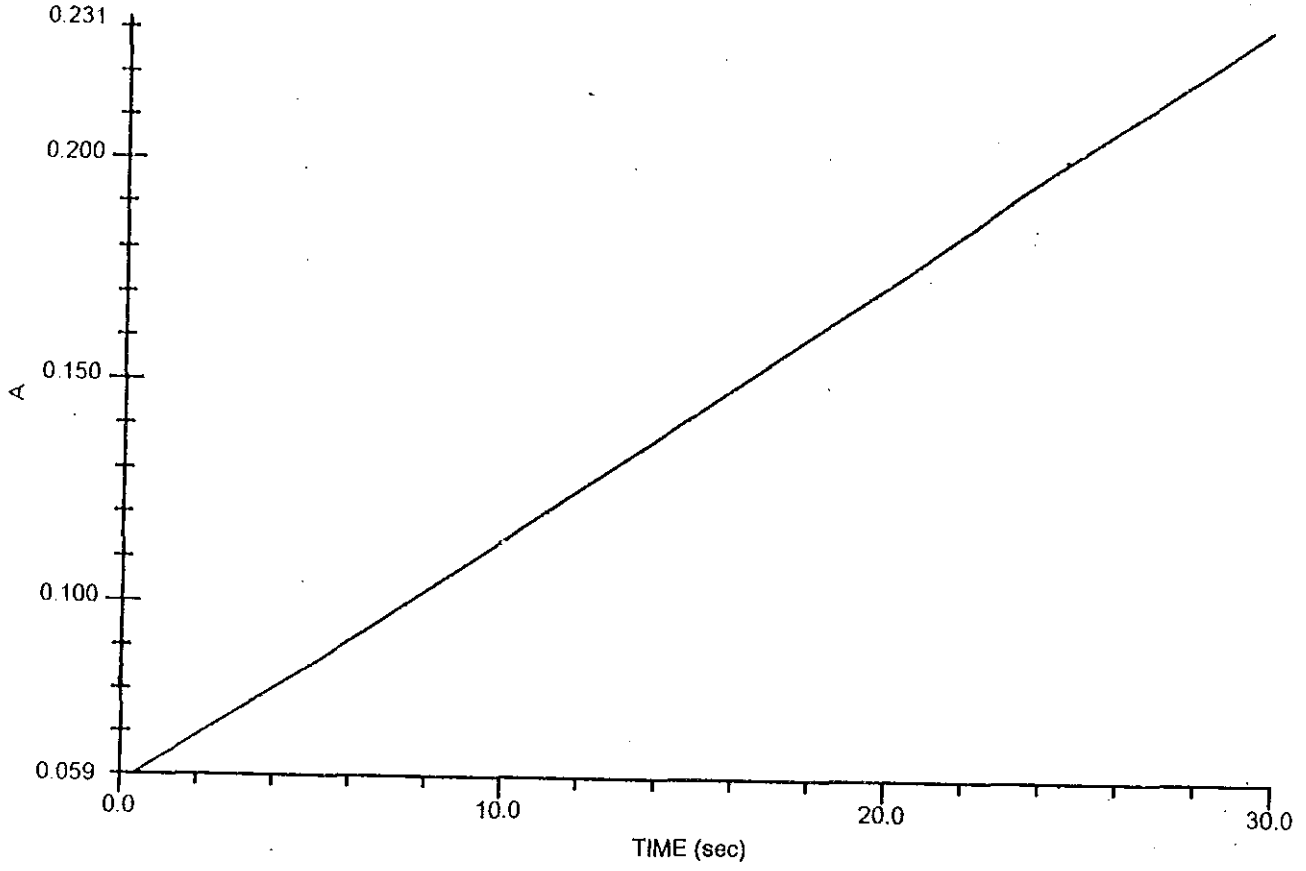


Fig 6.2 Plot of absorbance *vs* time for the reaction of Cu (II) Cl_2 with BHPT at pH= 9.2 and 50°C



DECLARATION

The thesis is my original work, has not been presented for a degree in this and any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Yemesrach Adamu Ayele

Signature: _____

This thesis has been submitted for examination with our approval as university advisors.

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1. Prof. Negussie Retta

_____

2. Dr. Negussie Megersa

Place and date of submission: School of Graduate Studies

Addis Ababa University

July 2001