

JIMMA UNIVERSITY
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY



**SYNTHESIS , CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NICKEL(II)
AND ZINC(II) COMPLEXES FROM MIXED LIGAND OF N-PHENYL AND 4-METHYL-N-
PHENYL DITHIOCARBAMATE**

BY : MESERET MELAKU

OCTOBER , 2017

JIMMA , ETHIOPIA

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**A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA UNIVERSITY IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER
OF SCIENCE IN CHEMISTRY**

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TABLE OF CONTENTS

Content	page
ACKNOWLEDGEMENT	i
TABLE OF CONTENTS.....	ii
LIST OF FIGURES.....	v
LIST OF TABLES.....	vi
ABBREVIATION AND SYMBOL.....	vii
ABSTRACT.....	viii
1. INTRODUCTION.....	1
1.1 Background information.....	1
1.2 Statement of the problem.....	3
1.3 Objectives.....	4
1.3.1 General objective.....	4
1.3.2 Specific objectives.....	4
1.4 Significance of the study.....	4
2. REVIEW OF LITERATURE.....	5
2.1 DTC Compounds.....	5
2.1.1 DTC Ligands.....	5
2.1.2 DTC Complexes.....	6
2.1.3 DTC Complexes as fungicides.....	7
2.2. Nickel(II) and Zinc(II) compounds and their applications.....	9
2.2.1 Coordination Chemistry of Nickel and Zinc.....	9
2.2.2 Nickel and Zinc DTC Complexes with biological activities.....	9
3. MATERIALS AND METHODS.....	11

3.1 Materials, Instruments and Methods.....	11
3.1.1 Reagents and chemicals.....	11
3.1.2. Instruments.....	11
3.2. Experimental Procedures.....	11
3.2.1 Synthesis of DTC Ligands.....	11
(i) Synthesis of N- Phenyl DTC Ligand.....	11
(ii) Synthesis of 4-Methyl-N-phenyl DTC Ligand.....	12
3.2.2 Synthesis of Metal Dithiocarbamate Complexes.....	12
(i) Synthesis of Ni(II) Complex.....	12
(ii) Synthesis of Zn(II) Complex	13
3.3. Test of the Purity of the Products.....	14
3.4. Chloride Analysis.....	14
3.5. Physico - chemical methods of characterization.....	15
3.6. Antimicrobial activity tests.....	16
(i) Antibacterial tests.....	16
(ii) Antifungal tests.....	16
4. RESULTS AND DISCUSSION.....	18
4.1 Synthesized Ligands and Complexes.....	18
4.2 Elemental Analysis	21
4.3 Physical Measurements of Ligands and Complexes.....	21
4.3.1 Solubility tests.....	21
4.3.2 Conductivity Measurements of Ligands and Complexes.....	22
4.3.3 Determination of Melting Point of Ligands and Complexes.....	23
4.4 FT-IR Spectra Studies of the Ligands and Complexes.....	23
4.5 Electronic Spectra of Ligands and Complexes.....	25

4.5.1 Electronic Spectra of N- Phenyl DTC Ligand ,Ni(II) and Zn(II) Complexes.....	26
4.5.2 Electronic Spectra of 4-Methyl -N- DTC Ligand , Ni(II) and Zn(II) Complexes.....	27
4.6 ¹ H NMR and ¹³ C NMR Spectra.....	27
4.6.1 ¹ H NMR Spectra of Ni(II) Complex.....	27
4.6.2 ¹ H NMR Spectra of Zn(II) Complex.....	28
4.6.3 ¹³ C NMR Spectra of Ni (II) Complex.....	28
4.6.4 ¹³ C NMR Spectra of Zn (II) Complex.....	29
4.7 Magnetic Susceptibility Measurements.....	29
4.8 Atomic Absorption Spectroscopy.....	30
4.9. Proposed Structure of dithiocarbamate ligands and their metal Complexes.....	32
4.10 Antimicrobial Studies of Dithiocarbamate Ligands and Metal Complexes.....	33
4.10.1 Anti bacterial Test.....	33
4.10.2 Anti fungal Test.....	34
5. CONCLUSION AND RECOMMENDATION.....	35
5.1 Conclusion.....	35
5.2 Recommendation.....	35
REFERENCES.....	36
APPENDICES	42

LIST OF FIGURES.

Figure 2.1 : Flow of electron from nitrogen to sulfur.....	5
Figure 2.2 : Binding forms of dithiocarbamate ligands to metal complexes.....	5
Figure 2.3 : Preparation of metal dithiocarbamate complexes by direct addition.....	6
Figure 2.4 : Preparation of metal dithiocarbamate complexes by one pot synthesis.....	7
Figure 2.5: Structure of some of the widely used metal DTC complexes and their trade name...8	
Figure 3.1: Reaction equation for the synthesis of N- Phenyl dithiocarbamate ligand.....	12
Figure 3.2: Reaction equation for the synthesis of 4-methyl-N- phenyl DTC ligand.....	13
Figure 3.3 : Reaction equation for the synthesis of Nickel(II) complex.....	14
Figure 3.4 : Reaction equation for the synthesis of Zinc(II) complex.....	15
Figure 4.1 :General synthesis routine of dithiocarbamate ligands.....	16
Figure 4.2 :Mechanism of formation of dithiocarbamate ligands.....	17
Figure 4.3 :Mechanism of formation of Nickel(II) and Zinc(II)complexes.....	19

LIST OF TABLES

Table 4.1 : Analytical data for the synthesized ligands and complexes.....	21
Table 4.2 : Solubility of ligands and complexes.....	22
Table 4.3 :Molar conductance of ligands and complexes.....	23
Table 4.4 : Selected FT-IR region of ligands and complexes.....	24
Table 4.5 : Electronic spectra data of the ligands and complexes	26
Table 4.6 : Magnetic susceptibility value of Ni(II) complex.....	30
Table 4.7 : AAS analysis Results Nickel Standards and Nickel complex.....	30
Table 4.8: AAS analysis Results Zinc Standards and Zinc complex.....	31
Table 4.9: Percent composition metal in complex.....	31
Table 4.10 : Antibacterial activities of ligands and metal complexes.....	32
Table 4.11 : Antifungal activities of ligands and metal complexes.....	33

ABBREVIATIONS AND SYMBOLS

AAU	Addis Abeba University
L₂	Ammonium 4-Methyl-N-Phenyl dithiocarbamate Ligand
L₁	Ammonium N-Phenyl dithiocarbamate Ligand
AR	Analytical reagent
AAS	Atomic Absorption Spectrophotometer
¹³C-NMR	Carbon Nuclear Magnetic Resonance Spectroscopy
δ	Chemical shift
DTC	Dithiocarbamate
DMSO	Dimethyl sulfoxide
FT-IR	Fourier Transform Infrared Spectroscopy
¹H-NMR	Hydrogen Nuclear Magnetic Resonance Spectroscopy
JU	Jimma University
LMCT	Ligand to metal charge transfer
ppm	Parts per million
spp	Species
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV-Vis	Ultraviolet -Visible Spectroscopy

ABSTRACT

Dithiocarbamate compounds have received tremendous attention for their synthesis, characterization and biological activities. Two different dithiocarbamate ligands namely N-Phenyl dithiocarbamate and 4-Methyl-N-Phenyl dithiocarbamate were prepared separately by the reaction of aniline and para methyl aniline with carbon disulfide in the presence of concentrated ammonia. Transition metal mixed-ligand complexes of Nickel (II) and Zinc (II) have been synthesized using substitution method. The structures of synthesized ligands and metal complexes were elucidated by their elemental analysis, Magnetic susceptibility measurement, FT-IR, UV-Vis, ^1H and ^{13}C NMR and Atomic Absorption Spectroscopic data. The data from the elemental analysis suggest a good agreement between the calculated and the experimental values for the elements and the FT-IR studies revealed that each of the dithiocarbamate ligand acted as bidentate through sulfur atoms in synthesized complexes. The metal complexes were isolated in good yield and are thermally stable. The molar conductance measurements of the complexes indicated their non-electrolytic nature. On the basis of spectral studies and magnetic susceptibility measurements, a square planar geometry with dsp^2 hybridization has been assigned for Nickel (II) complex and tetrahedral for Zinc(II) complex with sp^3 hybridization. A comparative anti-microbial study test was performed using the disk diffusion method against on two gram positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*,) and two gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) and four fungi strains *Aspergillus(spp)*, *Penicillium(spp)*, *Fusarium(spp)* and *Trichoderma(spp)*. It was revealed that Zinc (II) complex has a much enhanced activity relative to synthesized ligands in all selected bacteria but Nickel (II) complex showed a reduced activity against *Staphylococcus aureus*. Moreover, in antifungal study Zinc (II) complex showed better zone of inhibition against *Penicillium(spp)* and *Trichoderma(spp)* relative to standard drug. Further tests are needed to evaluate anti microbial activities especially on Zinc complex against other (additional) microbial species to explore all possibilities to evaluate the potential of Zinc complex in the development of pharmaceutical drugs.

Keywords : Dithiocarbamates, Carbon disulfide, Metal complexes, Anti-microbial.

1. INTRODUCTION

1.1 Background information

Dithiocarbamate compounds have taken a wide place in coordination chemistry and have important roles in medicine and agricultural field [1]. Special interest in the study of metal dithiocarbamates were aroused due to interesting structural features presented by this class of compounds and also due to their potential antimicrobial, antitumor and anticancer activities [2]. Moreover, their thermal stability with various transition metals are contribute for their possible applications in various areas. The main synthetic route of dithiocarbamate is based on the interaction between the corresponding amine and carbon disulfide in basic media [3]. The complexation ability of dithiocarbamates are well established and this due to two sulfur atoms which are capable to donate a lone pair of electrons to central metal to form the stable complex [4].

In the last decades microbial infection has become an extremely serious threat to the health and economic prosperity of the world and it is currently restricted to the developing countries which cannot afford to pay for the high cost of research. It is hoped that finding solutions for bacterial and fungal infection will come to reflect the major problem that represents to the world's population.

The ability of bacteria to change rapidly in response to existing drug is new challenges and often has adverse effects on humans [7]. Recently, concern has arisen over the prevalence of antibacterial drugs in the market place. They are marketed as a means of protecting from harmful bacteria; however, it is likely that their routine use will favor bacteria that have mutations making them immune to resist the available antibiotics [8]. Ultimately, extensive use of antibacterial drugs could have an adverse effect and unable to treat common bacterial infections. Bacteria cause many diseases in humans, including cholera, leprosy, tetanus, bacterial pneumonia, whooping cough, diphtheria and other infections. Tuberculosis is another bacterial disease which still a leading cause of death [9].

Plants are constantly exposed and threatened by a variety of pathogenic microorganisms and diseases caused pathogenic fungi which significantly contribute to the overall loss in crop yield in worldwide [11]. Crop losses in pre and post- harvest due to fungal diseases exceed 2.5 trillion dollar in worldwide in which the pathogen attacks nearly all types of plant under suitable conditions. Different fungicides have been used to control fungal crop diseases and there is a growing interest in research

on the possible uses of natural products for disease control but still the existence of crop pathogenic fungi has not stopped [12]. However, at present the search for new fungicide is very important due to increase of resistance and new strains of fungi. Among synthetic chemicals used as antifungal agents, dithiocarbamates compounds are common and some are commercially available [13]. Now a day, researchers turned toward inorganic substances for finding a solution for those drug resistance bacteria and fungi strains for invention metal containing new drugs called metal complexes [15].

Although different dithiocarbamate complexes including mixed ligand complexes have been synthesized and exploited for their diverse usages [16]; surprisingly reports describing chemistry of dithiocarbamate complexes from those mixed ligands are rare despite of their potential metals binding properties. This work was based on the fact that many transition metal dithiocarbamate complexes have good potency toward pathogenic bacteria and fungi[18 19]. Therefore, there is the need to continuously carrying out research that can deliver new drugs to combating these drug resistant pathogens and also to make invaluable contributions to the knowledge in this regard with respect to combating pathogenic species by carrying out study.

1.2 Statement of the problem

Chemical control has been critical in preventing losses that are due to plant diseases, especially with the development of numerous fungicides since the 1960s [21]. But still drug resistant fungal pathogenic are rising and the need for a new antifungal substances and alternative treatments is becoming increasingly obvious [22]. As the world population increases, we also need to increase food production. Despite the choice of effective fungicides available, new anti-fungal chemicals are still needed to improve the yield. For many years, a variety of different synthetic chemicals has been used as antifungal agents to inhibit the growth of plant pathogenic fungi. However, there are series of problems for the effective use of these chemicals in areas where the fungi have developed resistance; Hubel *et al*, 2000. The emerging infectious diseases and the development of drug resistance in the pathogenic bacteria and fungi at an alarming rate is a matter of serious concern. Despite the increased knowledge of microbial pathogenesis and application of modern therapeutics, the morbidity and mortality associated with the microbial infections still remains high; Navarro *et al*, 2001. Therefore, there is a pressing demand to discover novel strategies and identify new antimicrobial agents from natural and inorganic substances to develop the next generation of drugs to control microbial infection.

Although plenty of dithiocarbamate complexes including mixed ligand complexes have been synthesized and exploited for their diverse usages, surprisingly reports describing chemistry of dithiocarbamate complexes from those mixed ligands are rare ; despite of their potential metals binding properties and their antimicrobial activities. This study was proposed based on the fact that many transition metal complexes of dithiocarbamates have good potency toward pathogenic bacteria and fungi [25].

1.3 Objectives

1.3.1 General objective

- ❖ To Synthesis, Characterization and Antimicrobial Investigation of Nickel(II) and Zinc(II) Dithiocarbamate Complexes.

1.3.2 Specific objectives

- To synthesize N- Phenyl and 4-methyl-N- Phenyl dithiocarbamate ligands from corresponding amines.
- To synthesize Nickel(II) and Zinc(II) complexes from N-Phenyl and 4-Methyl N-Phenyl dithiocarbamate ligands.
- To elucidate the structure of ligands and metal complexes by using $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, IR, Elemental analysis and UV-Vis spectra.
- To determine the antifungal and antibacterial efficacy of the Nickel (II) and Zinc(II) complexes and ligands using disk diffusion method on four bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Salmonella typhi*) and four fungi strains *Aspergillus(spp)*, *Penicillium(spp)*, *Fusarium(spp)* and *Trichoderma(spp)*.

1.4 Significance of the study

In view of the growing cases of drug resistance microorganisms, there is an urgent need to search for new promising antimicrobial agents which is less toxic to the host and the environment. Therefore, understanding of the interaction of metal-based drugs with biological systems still need justification of newly synthesized metal complexes. How to make better use of the advantages of dithiocarbamate-based fungicides and avoid their weakness is a problem to be solved [27]. This study generally was focused on synthesis, characterization and antibacterial and antifungal activities of both the ligands and metal complexes.

The motivation for the current study is to contribute the effort to search for novel antimicrobial agents from dithiocarbamate complexes and possible to make invaluable contributions to the knowledge in this regard with respect to combating pathogenic species leads for the development of pharmaceutical product and to make use of the important information for further investigation.

2. LITERATURE REVIEW

2.1 Dithiocarbamate Compounds

2.1.1 Dithiocarbamate Ligands

Dithiocarbamate is a type of negatively charged compound synthesized by reaction of primary or secondary amines and carbon disulfide (CS_2) in alkaline solution [26]. Dithiocarbamates are highly versatile ligands toward transition metals [27]. They can stabilize a variety of oxidation states and they coordinate through double sulfur atoms with most transition metals [28]. Dithiocarbamate ligands can stabilize high oxidation state metal ions in metal complexes due to strong sigma bonding characteristic of these ligands. Although the sulfur atoms of dithiocarbamate ligands possess sigma donation characteristics, these ligands have special features in that there is an additional electron flow from nitrogen to sulfur through a planar delocalization pi orbital system [29].

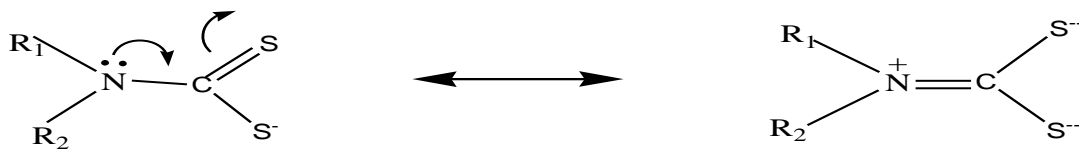


Figure 2.1 flow of electron from nitrogen to sulfur



Figure 2.2 Binding forms of dithiocarbamate ligands to metal(M) complexes(R= alkyl or aryl group)

Among the various sulfur bearing ligands, the dithiocarbamates are a class of Lewis bases capable of coordinating to a wide number of metal ions, are unique due to its S-C-S delocalized electronic system. They can stabilize a variety of oxidation states and coordination geometries, and small modifications to the ligand can lead to significant changes in the behavior of complexes formed [30-32]. Dithiocarbamate ligands exist in different resonance forms of dithiocarbamate moiety which are shown and can act as monodentate, bidentate chelating or bidentate bridging ligands due to their binding character [33].

The dithiocarbamates are the reaction product of primary or secondary amine with carbon disulfide in the presence of alkali metal (Sodium, Potassium) or ammonium hydroxides.

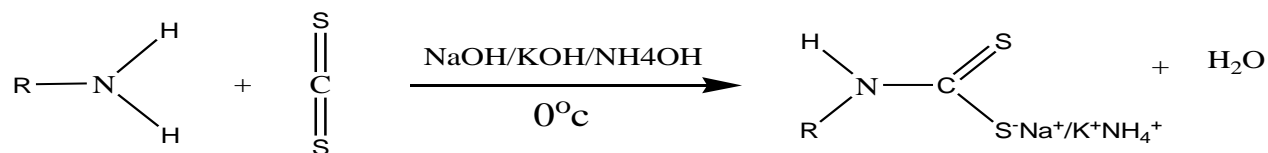


Figure 2.3 General synthesis routine of dithiocarbamate ligands (R= Alkyl or Aryl group)

Dithiocarbamate ligands are soft bases and they had been found to act almost as uninegative bidentate ligands coordinating through sulfur atoms and both tetra and hexa co-ordinate complexes of many transition metal ions have been isolated [34]. Due to wide applicability of sulfur bearing ligand in biological and industrial field, the dithiocarbamate complexes with various aliphatic and aromatic have been reported [35,36].

2.1.2 Dithiocarbamate Complexes

Dithiocarbamate ligands form complexes with most main group and transition metals [37]. The ability to form complexes with metals is related to the presence of sulfur atoms and the delocalization of positive charge from the metal to the circumference of the complex [38-39]. Transition metal dithiocarbamate complexes were first reported in the 1900 and since these complexes have been widely studied in a variety of ways. Transition metal dithiocarbamate can be synthesized using different methods such as direct addition and one pot synthesis . The most commonly used method is the direct addition of dithiocarbamate ligand to the metal salts. The typical example of the direct addition reaction of dithiocarbamate ligands and metal salts to form metal complexes is shown.

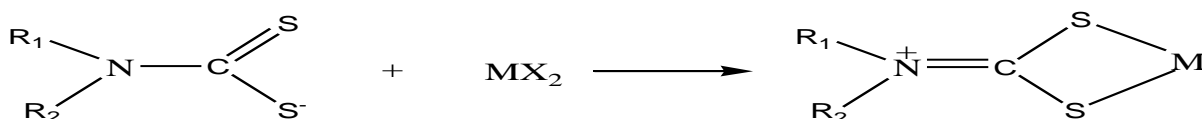


Figure 2.4 Preparation of metal dithiocarbamate complexes by direct addition (R₁ and R₂ alkyl or phenyl groups and MX₂ is metal salt)

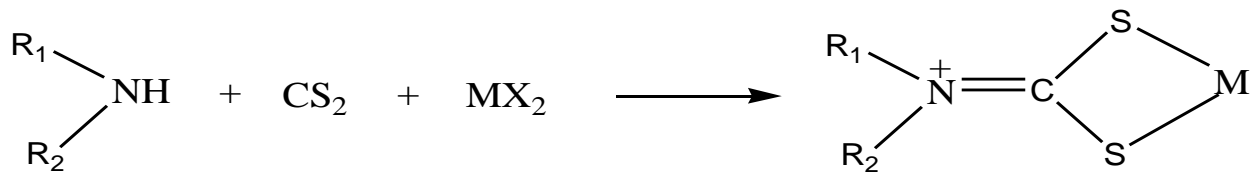


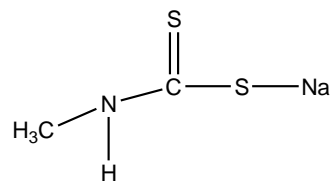
Figure 2.5 Preparation of metal dithiocarbamate complexes by one pot synthesis (R_1 and R_2 alkyl or phenyl groups and MX_2 is metal salt).

In one pot synthesis method the dithiocarbamate complex is prepared in a single step. The amine and the carbon disulfide in suitable solvents are allowed reacting for a short period of time followed by addition of the aqueous solution of metal salt [40].

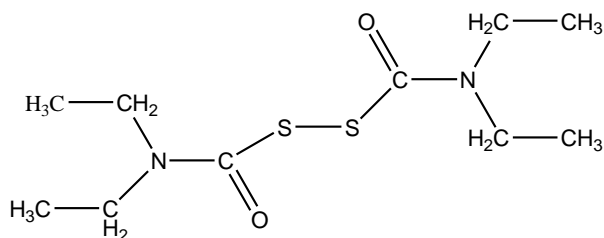
2.1.3 Dithiocarbamate complexes as fungicides

Fungicides are chemical compounds or biological organisms used to kill or inhibit fungi or fungal spores. Fungi can cause serious damage in agricultural resulting in critical losses of yield, quality, and profit and fungicides are used both in agriculture and to fight fungal infection in animals[45].

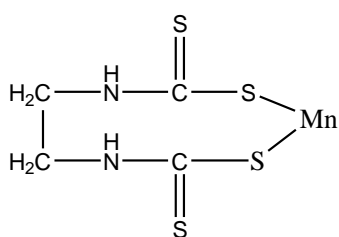
Dithiocarbamate compounds are widely used fungicides in agriculture and some are commercially available. Dithiocarbamates such as Metam, Maneb , Mancozeb and Ferbam are widely used as standard fungicides which used in variety of plant diseases [42]. They were introduced 50 years ago, dithiocarbamates fungicides still represent an important class of plant protection product that are still widely used today [43,44]. They act as multisite contact fungicides that work by protecting the plant surface to prevent infection. In general, dithiocarbamates are considered chemicals of low toxicity for humans. The effect of Maneb in humans are diverse with some causing problems in human via absorption through the skin and respiratory system[45]. Ziram and Zineb are effective dithiocarbamate fungicides and it should be mixed with water before use because its toxic doses may result in nerve damage, but Ziram has been proven to be more toxic than Ferbam [46]. Mancozeb has low toxicity to animals and fishes [47]. These are some of the most widely used dithiocarbamates in agricultural sector.



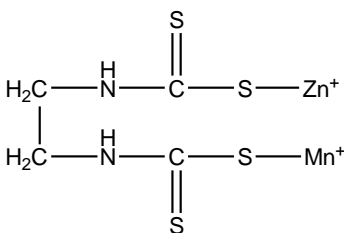
Matam Sodium(nemotocide)



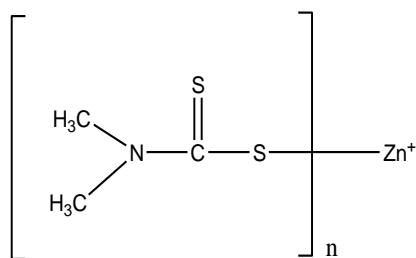
Tetra ethyl thiuram disulphide
Disulfram(drug)



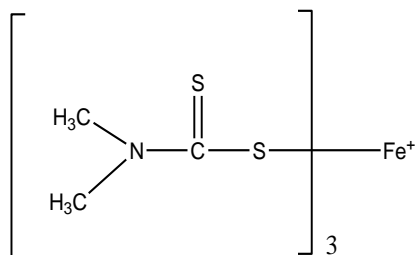
Maneb(fungicides)



Ethylene bis dithiocarbamate
Mancozab (fungicide)



Zinc dimethyl dithiocarbamate
Ziram(fungicide)



Iron dimethyl dithiocarbamate
Ferbam (fungicide)

Figure.2.6 Structure of some of the widely used fungicide dithiocarbamate metal complexes and trade name

2.2. Nickel(II) and Zinc(II) compounds and their applications

2.2.1 Coordination Chemistry of Nickel and Zinc

The +2 oxidation state is the most common oxidation state for nickel. The absence of any other oxidation state of comparable stability for nickel implies that compounds of Ni(II) are normally participate in coordination chemistry. The coordination number of Ni(II) rarely exceeds six and its principal stereochemistries are octahedral, tetrahedral, square planar, square pyramidal and trigonal bipyramidal. Octahedral nickel(II) complexes have two unpaired electron and the magnetic moment range from 2.9-3.4 BM. In octahedral field, three spin allowed transitions are expected because of the splitting of the free ion ground 3F , 3P term. For tetrahedral complexes, the magnetic moment value are in the range of 3.0-3.5 BM and three transitions are observed in the electronic spectra of these of these complexes which is more intense than octahedral one [54].

A considerable number of both trigonal bipyramidal and square pyramidal complexes occur in high and low spin complexes of each geometry are known. Among four coordinate complexes of Ni(II), square planar complexes are the most common. They are diamagnetic specially with strong field ligands.

The +2 oxidation state is the most common oxidation state for Zinc and it does not show variable valency since it has a d^{10} configuration it can't produce d-d spectra and many of its compounds are white. When zinc forms complexes four, five or six coordination number but four coordination number tetrahedral geometry is very common which resulted due to sp^3 hybridization[54].

2.2.2 Nickel and Zinc Complexes with biological activities

Many biologically active compounds used as drugs possess modified pharmaceutical and toxicological potentials when administered in the form of metal based compounds. Various metal ions potentially and commonly used are cobalt, nickel, copper and zinc due to forming low molecular weight complexes and there for, prove to be more beneficial against several diseases [20].

The Co(II), Ni(II), Cu(II) and Zn(II) complexes of N-phenyl and p-chloro phenyl dithiocarbamate ligands have been synthesized in 2016. The in-vitro antimicrobial activities of metal complexes were evaluated against four bacteria strains using agar method. The result revealed that all the complexes showed strong potency against gram negative bacteria even much stronger than the potency exhibited

by tetracycline (common antibiotic). Also, the complexes of Nickel and Zinc exhibited strong antibacterial action on gram positive bacteria strains. Other complexes recorded low activity[48].

The antibiotic activity of metal complexes of 2,4 -dichloro-N-phenyldithiocarbamate against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* were investigated in 2015. Square planar Cu(II) complex and tetrahedral Zn(II) complex showed good potency on both bacteria strains where as octahedral Fe(III) , Co(II) and Ni(II) showed strong effect only on gram negative bacteria[56].

The antibiotic activity of divalent transition metal (Co , Ni , Cu , Zn and Hg) complexes of 4-nitro-N- imidazole dithiocarbamate were investigated in 2013 against four bacteria strains. The obtained result showed that all the complexes exhibited strong antibacterial activity. But Hg (II) complex showed inhibitory effect on selected types of bacteria [20].

Mixed complexes of N-imidazole dithiocarbamate and 1,10 phenanthroline of Co(II) ,Ni(II) ,Cu(II) and Zn(II) were synthesized and characterized in 2012. The complexes adopt a distorted octahedral geometry around the metal ion. Among the synthesized complexes, copper and cobalt complexes showed remarkable antibacterial activities [32].

The antibiotic activity of divalent transition metals (Ni and Cu) complexes of 2-amino pyridine dithiocarbamate have been done against three bacteria strains (*Escherichia coli* , *Staphylococcus aureus* and *Bacillus subtilis*) in 2013. The screening results showed that Cu(II) complex showed remarkable activities against *Staphylococcus aureus* and *Bacillus subtilis* while Co(II) complex showed potency only in *Staphylococcus aureus* [58].

Mixed Ligand Divalent Metal Complexes of N-Phenyl Dithiocarbamate with Alanine - Schiff Base of Mn(II) , Fe(II) , Co(II) , Ni(II) ,Cu(II), Zn(II), Cd(II) and Pb(II) have been synthesized in 2016. The ligands and metal complexes were screened in-vitro for antibacterial activity against *Escherichia coli*, *Pseudomonas* , *Staphylococcus aureus* and *Bacillus subtilis* . The results obtain showed that all the complexes have remarkable activity on both *Escherichia coli* and *Pseudomonas* but low activity were recorded against *Staphylococcus aureus* and *Bacillus subtilis* [59].

3. MATERIALS AND METHODS

3.1 Materials, Instruments and Methods

3.1.1. Reagents and chemicals

All chemicals and reagents were purchased and used without further purification. Aniline (Nice Lab reagent Ltd) and 4-methylaniline (Blulux Laboratory(p) Ltd) were used without purification. Hexa hydrated Nickel Chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) and Zinc chloride (ZnCl_2) were purchased from Nice Lab reagent Ltd, Carbon disulfide (CS_2) were purchased from BDH Chemicals Ltd Poole England, other chemicals such as diethyl ether, absolute methanol, ethanol and concentrated aqueous ammonia were purchased from LOBA Chemie Ltd Company.

3.1.2. Instruments

The characterization work such as AAS, FT-IR, Elemental analysis and magnetic susceptibility measurements were done in AAU. While ^1H and ^{13}C NMR were done in Germany. UV-Vis spectroscopy, melting point determination and conductivity measurement were done at Jimma university. Moreover, the microbial activities were done at Jimma university microbiology laboratory.

3.2. Experimental Procedures

3.2.1. Synthesis of Dithiocarbamate Ligands

(i) Synthesis of N- Phenyl Dithiocarbamate Ligand (L_1)

Aniline (0.1 mol, 9.13 mL) was transferred in a clear beaker placed in ice cage and cold solution (0.1 mol, 15 mL) of concentrated aqueous ammonia was added to it. Then carbon disulfide (0.1 mol, 6 mL) was added in to the mixture in drop wise from separatory funnel with constant stirring. The resulting solution was cooled and stirred in ice at 2°C for eight hours. The white product was filtered off using Buchner filter paper, and then washed with diethyl ether and dried at room temperature. yield: 59.5% M.P. ($^\circ\text{C}$): 94 - 96

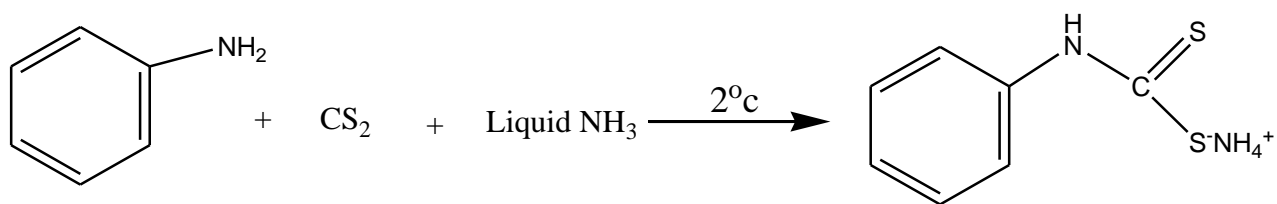


Figure 3.1 Reaction equation for the synthesis of ammonium N-Phenyl Dithiocarbamate $\text{NH}_4[\text{C}_6\text{H}_5\text{NHCS}_2]$.

(ii) Synthesis of 4-Methyl-N-Phenyl Dithiocarbamate Ligand (L_2)

A 0.1 mol (10.72 g) of p-toluidine was dissolved in 30 mL of ethanol in a clear beaker which was placed in ice cage and cold solution (0.1 mol , 15mL) of concentrated aqueous ammonia was added to it. Then carbon disulfide (0.1 mol, 6 mL) was added in to the mixture in drop wise from separatory funnel with constant stirring. The resulting solution was cooled in ice at 2°C for six hours. The white product was filtered off using Buchner filter paper, and then washed with diethyl ether and dried at room temperature. Yield: 65% M.P.($^\circ\text{C}$): 83-85

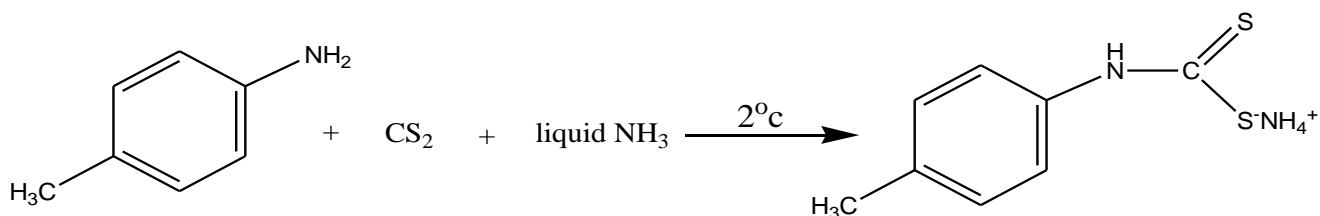


Figure 3.2 Reaction equation for the synthesis of ammonium 4-Methyl-N- Phenyl Dithiocarbamate $\text{NH}_4[(\text{CH}_3)\text{C}_6\text{H}_5\text{NHCS}_2]$.

3.2.2 Synthesis of Metal Dithiocarbamate Complexes $[\text{ML}_1\text{L}_2]$ where (M= Ni and Zn)

(i) Synthesis of Ni(II) Complex , $[\text{NiL}_1\text{L}_2]$

A 0.02 mol (4.7538 g) of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ was dissolved in 20 mL deionized water and was added drop wise in 250 mL of beaker which contain the mixture of 25 mL of 0.02 mol (3.7242 g) N-phenyl dithiocarbamate ammonium salt (L_1) and 25 mL of 0.02 mol (4.0044 g) 4-methyl-N-Phenyl dithiocarbamate ammonium salt (L_2) which were dissolved in separated beakers using deionized water. The mixture was stirring using magnetic stirrer for two hours at room temperature.

The light yellow orange Ni(II) complex was filtered and washed several times with de ionized water and finally the resulting complex was dried at room temperature.

Yield: 81% M. P.(⁰C): 202-205

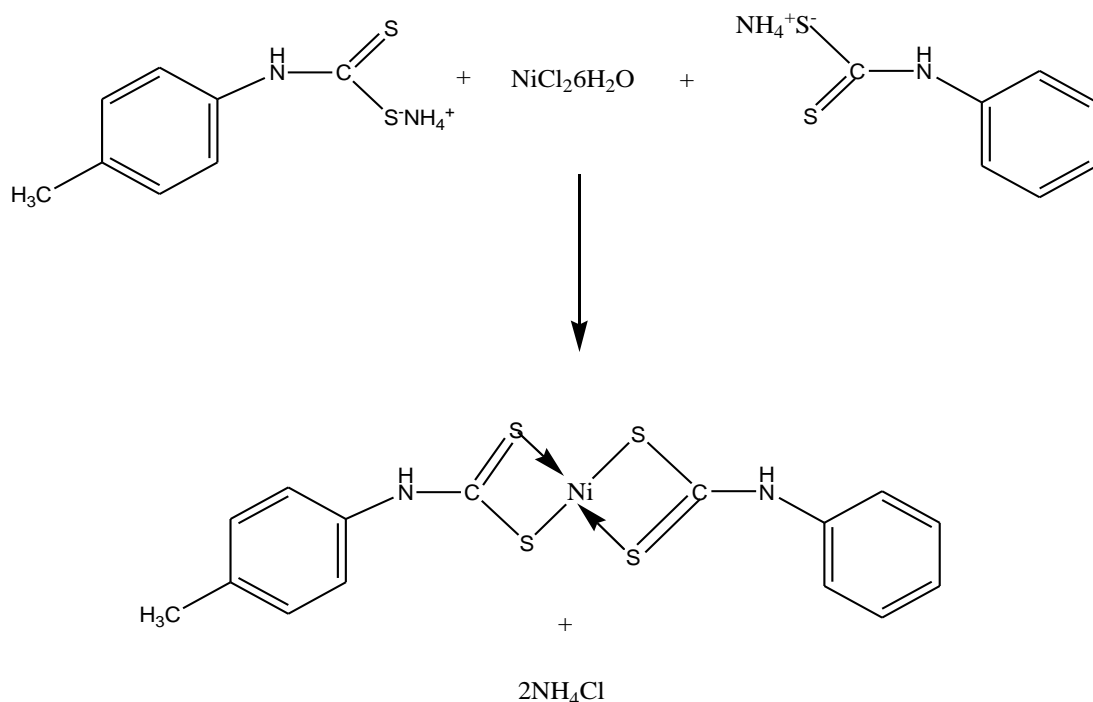


Figure 3.3 Reaction equation for synthesis of Nickel(II) complex , [NiL₁L₂]

(ii) Synthesis of Zn(II) Complex , [ZnL₁L₂]

A 0.02 mol (2.7263 g) of ZnCl₂ was dissolved in 25 mL deionized water and was added drop wise in 250 mL of beaker which contain the mixture of 25 mL of 0.02 mol (3.7242 g) N-phenyl dithiocarbamate ammonium salt (L₁) and 25 mL of 0.02 mol (4.0044 g) 4-Methyl-N-Phenyl dithiocarbamate ammonium salt (L₂) which were dissolved in separated beakers using deionized water. The mixture was stirring using magnetic stirrer for three hours at room temperature.

The white Zn(II) complex was filtered and washed several times with deionized water and finally the resulting complex was dried at room temperature.

Yield: 74% M. P.(⁰C): 190-193

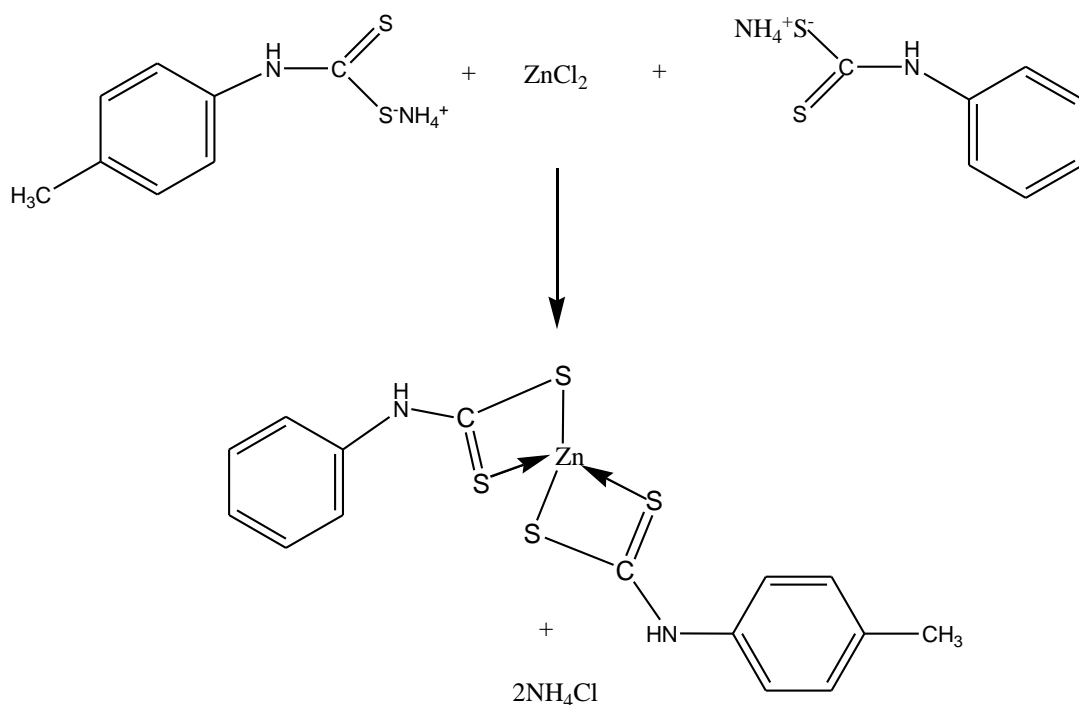


Figure 3.4 Reaction equation for the synthesis of Zinc (II) complex [ZnL₁L₂]

3.3. Test of the Purity of the Products

The purity of the sample was checked by using thin layer chromatography (TLC) conducted on silica coated aluminum plates as stationary phase and ethanol as a mobile phase. A single spot in all samples indicates that the complexes are pure.

3.4. Chloride Analysis

To the solution of each complex (0.02 g) in methanol, aqueous solution of AgNO₃ was added. In the complexes there was no precipitate. In order to know the presence of coordinated chlorine in the complexes, 0.05 g of each complex was decomposed with concentrated HNO₃ and diluted and to the resultant solutions aqueous solution of AgNO₃ was added. In each case, no cruddy white precipitate was observed indicates the absence of chlorine in the coordination zone of the metals.

3.5. Physico - Chemical Methods of Characterization

Fourier Transform Infrared (FT-IR) spectra recorded in [4000-400] cm^{-1} were identified the presence of functional groups on dithiocarbamate ligands and metal complexes as KBr discs on a perkin Elmer paragon 2000 spectrophotometer. Nuclear Magnetic Resonance (NMR) spectra were run on a Bruker EMX 400 MHz spectrometer for ^1H and 100Mz for ^{13}C ; the chemical shift values were reported in parts per million (ppm) relative to TMS as internal standard. Chemical shifts were also reported with respect to DMSO- d_6 δ_{C} at 40 and DMSO- d_6 δ_{H} at 2.23.

The percentage composition of C, H, N and S on ligands and complexes were run on EA1112 Flash CHNS/O-analyzer at condition of carrier gas flow rate of 120mL/min, reference flow rate 100mL/min ,oxygen flow rate 250 mL/min , furnace temperature of 900 $^{\circ}\text{C}$ and oven temperature of 75 $^{\circ}\text{C}$.

The electronic spectra of the ligands and complexes in solution were run in the range of [200-800] nm on 6705 UV/Vis spectrophotometer (jenway). The samples were placed in quartz cuvettes of 1cm path length. The solvent used depending on the solubility of the ligands and complexes, in this particular cause analytical grade absolute alcohol (ethanol) was used for making solution of both ligands and complexes for measure the absorbance of samples.

The atomic absorption of spectrum of Nickel and Zinc atoms on the dithiocarbamate complexes were recorded on ZEF nit 700p (anaytikjenu)-Flame AAS using Nickel and Zinc cathode lamp after that the concentration of metal ions were calculated by Beer-Lambart Law and the percentage of metal atom in complexes calculated. The gram susceptibility of Nickel complex was recorded at 20 $^{\circ}\text{C}$ using balance magnetic apparatus.

In melting point determination, solid melts when it undergoes a phase change from solid to liquid. It can be used not only to identify a compound but also in qualitative assess of purity of a compound [55]. Melting point of synthesized ligands and complexes were determined using Griffin melting point apparatus by inserting sample in capillary tube with 2cm height in temperature increment 2 $^{\circ}\text{C}$ per min. Characterization works such as FT-IR, AAS, elemental analysis and magnetic susceptibility measurement were done in AAU while ^1H and ^{13}C NMR was done at Germany. UV-Vis and conductivity measurements were done in JU.

3.6. Antimicrobial activity tests

(i) Antibacterial tests

Antibacterial activity using Disc Diffusion Test (DDT) was carried out to determine antibacterial behavior of synthesized dithiocarbamate ligands , Nickel(II) and Zinc(II) complexes against selected bacterial strain by measuring its inhibition zone (Two types of bacterial strains were used; Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) were used in this test.

The media used was prepared by dissolving 4 g of the Mueller Hinton agar powder in 100 mL of deionized water and was sterilized in an autoclave at 121⁰C for 15 minutes and then stored overnight in a refrigerator after cooling [25].

20 mg each sample was dissolved in 5 mL of dimethyl sulfoxide to obtain final concentration of 4 mg/mL for the compounds. Dimethyl sulfoxide was also used as the negative control. The positive controls was Gentamicin commercial antibiotics manufactured by *Auronext pharma pvt .Ltd (India)*. There were four petri dishes used. Each petri dish represented different bacteria. Bacterial layer was fully swabbed onto nutrient agar to make sure cultivation of bacteria is uniform. six paper discs which soaked contain various samples were put in one petri dish. Each disc was put far from one another to avoid overlap of inhibition zone. All of complete petri dishes were incubated in the inverted fashion for 24 hours at 37⁰C [29]. In the final procedure, diameter of inhibition zone was measured using ruler in mm unit and was compared with positive and negative control

(ii) Antifungal tests

Antifungal activity using Disc Diffusion Test was carried out to determine antifungal behavior of synthesized dithiocarbamate ligands , Ni(II) and Zn(II) complexes against selected fungal strain by measuring its inhibition zone and four fungi strains, *Aspergillus(spp)*, *Penicillium(spp)* ,*Fusarium(spp)* and *Trichoderma(spp)* were used in the test. The media was prepared used by dissolving 4 g of the Mueller Hinton agar powder in 100 mL of deionized water and was sterilized in an autoclave at 121⁰C for 15 minutes and then stored overnight in a refrigerator after cooling [10].

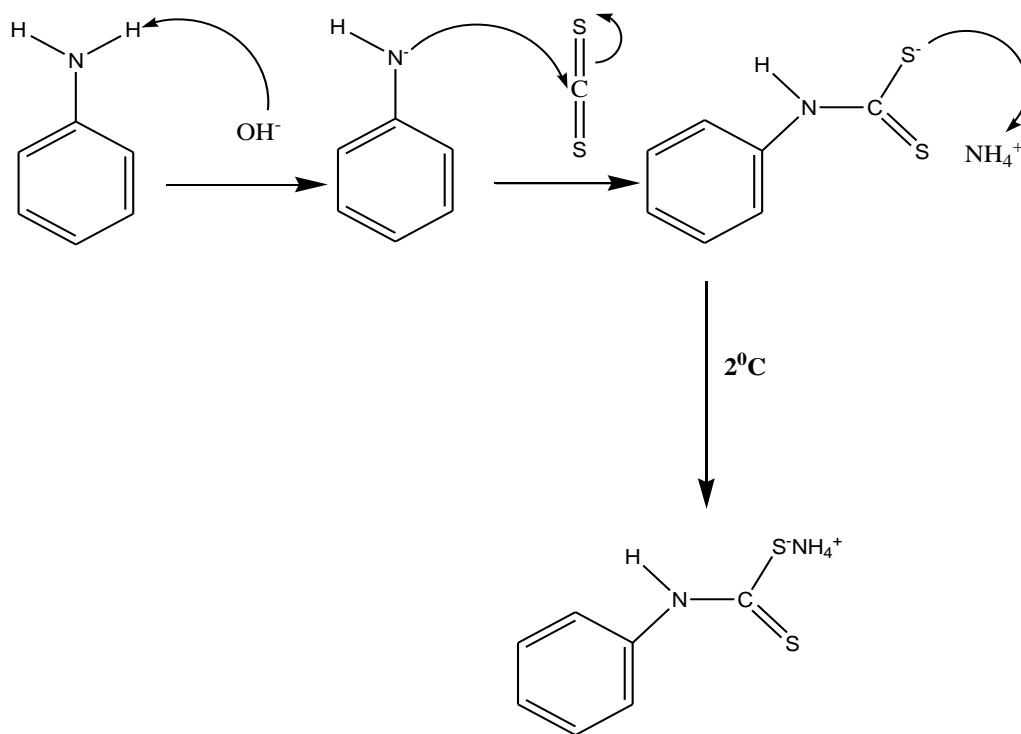
10 mg each sample was dissolved in 5 mL of dimethyl sulfoxide to obtain final concentration of 2 mg/mL for the compounds. Dimethyl sulfoxide was also used as the negative control. The positive controls was Mancozeb 80% which is commercial antifungal reagent manufactured by *Coromardel*

International pvt .Ltd (India). There were four petri dishes used. Each petri dish represented different fungi. Fungal layer was fully swabbed onto nutrient agar to make sure cultivation of fungi is uniform. six paper discs which contained various samples were put in one petri dish. Each disc was put far from one another to avoid overlap of inhibition zone. All of complete petri dishes were incubated for 72 hours at 27⁰C [10]. In the final procedure, diameter of inhibition zone was measured using ruler in mm unit and was compared with positive and negative control.

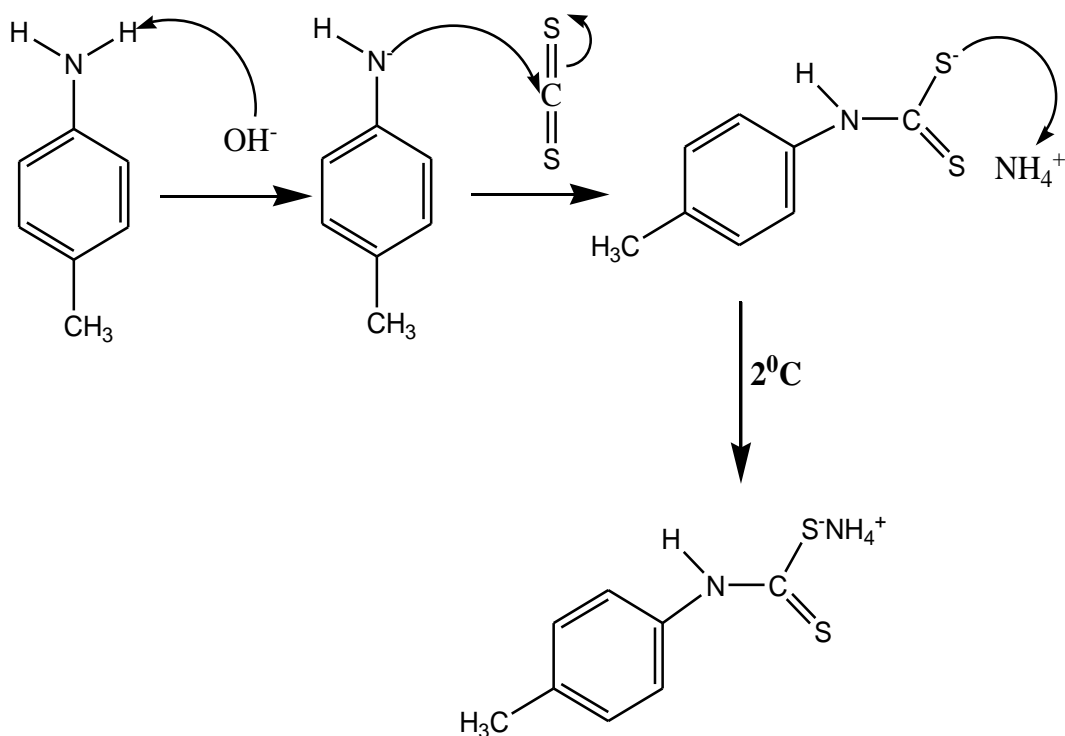
4. RESULTS AND DISCUSSION

4.1 Synthesized Ligands and Complexes

Since carbondisulfide is highly volatile species and dithiocarbamate salts most often decompose at high temperature, they are usually prepared at very low temperature and this method can give high yield [20]. Therefore, this procedure was used in this study. The synthesis of dithiocarbamate ligands were carried out at a temperature about 2°C . The mechanisms of formation of the ligands [28] are shown in figure 4.2



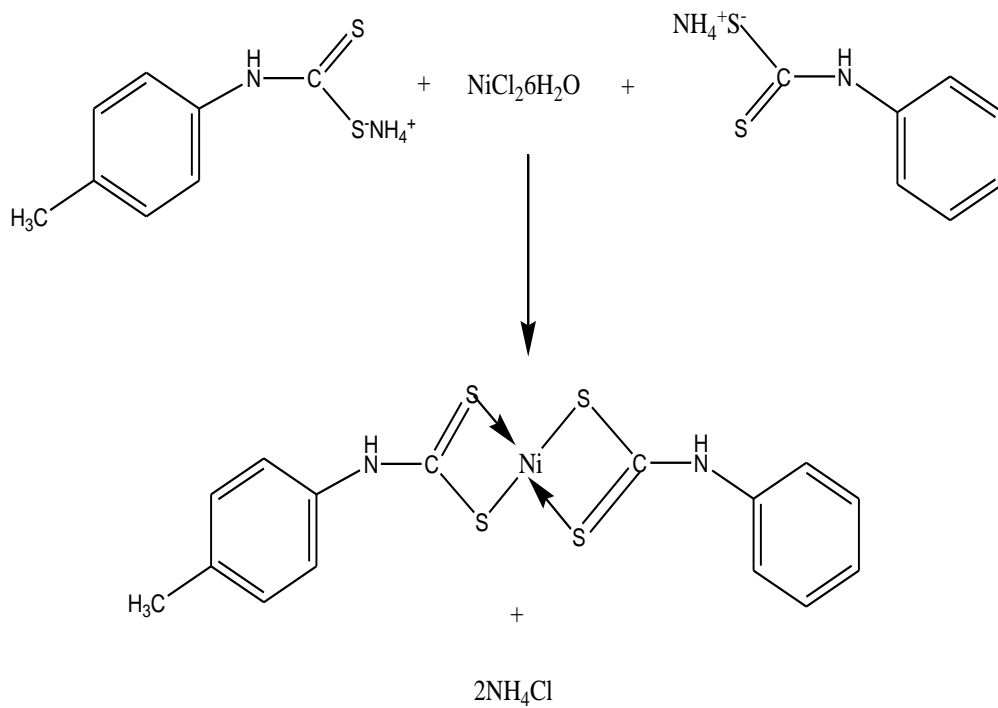
a) Mechanism of formation for N- Phenyl Dithiocarbamate (L₁)



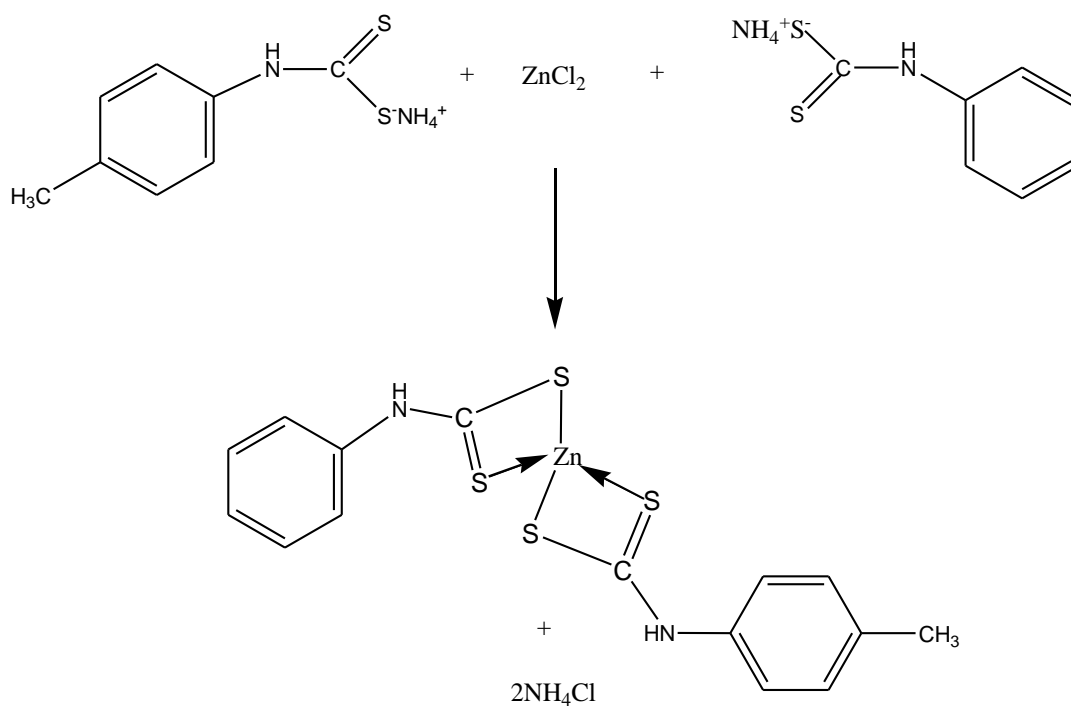
b) Mechanism of formation for 4- Methyl-N- Phenyl dithiocarbamate (L_2)

Figure 4.2 Mechanism of formation of dithiocarbamate ligands.

The reaction of the respective amines with carbon disulfide and ammonium hydroxide gives ammonium dithiocarbamate salts which stable at room temperature. For the synthesis of metal dithiocarbamate complexes the direct method was employed. Two different dithiocarbamate ligands (L_1 and L_2) and their respective metal salts solutions were mixed in 1:1:1 mole ratio. The complexes were isolated in good yield and stable at room temperature. The general reaction for the synthesis of complexes is represented in figure 4.3 and the analytical data for the ligands and complexes are presented in table 4.3.



a) Formation of Nickel (II) complex



b) Mechanism of formation of and Zinc(II) complex

Figure 4.3 Mechanism of formation of dithiocarbamate complexes

4.2 Elemental Analysis (CHNS)

Elemental analysis has been done to all of the ligands and complexes. CHNS elemental analysis is done to determine the weight percentage of carbon (C), hydrogen (H), nitrogen (N) and sulfur (S) in sample. This analysis also is useful to check purity of the complex [12]. All the values obtained actual weight percentage are in the range of accepted theoretical values. However deviation observed on sulfur in L₂ due to impurity or instrumental error.

Table 4.1 Analytical data for the synthesized ligands and complexes.

					Elemental Analysis (%)			
Cpds Code	Color	M.p (°C)	Yield (%)	Molecular formula (Molecular Mass)	C	H	N	S
L ₁	White	94-96	59.5	C ₇ H ₁₀ N ₂ S ₂ (186.21)	(45.15) 44.2	(5.37) 5.11	(15.04) 13.24	(34.4) 32.4
L ₂	White	83-85	65	C ₈ H ₁₂ N ₂ S ₂ (200.22)	(48) 46.2	(5.9) 5.80	(13.9) 11.5	(32) 26.34
NiL ₁ L ₂	yellow orange	202-205	81	C ₁₅ H ₁₄ S ₄ N ₂ Ni (409.11)	(44.03) 44.7	(3.42) 3.51	(6.84) 7.13	(31.34) 33.7
ZnL ₁ L ₂	White	190-193	74	C ₁₅ H ₁₄ S ₄ N ₂ Zn (415.78)	(43.3) 42.7	(3.36) 3.31	(6.74) 7.42	(30.8) 32.2

*In bracket: Theoretical Value.

4.3 Physical Measurements of Ligands and Complexes.

4.3.1 Solubility tests

Different solvents were used to test the solubility of ligands and complexes. All ligands were soluble in water, methanol, ethanol and dimethyl sulfoxide. Among these, water was used to synthesize the complexes. The solubility of the metal complexes were tested using various solvents like water, methanol, ethanol, dimethyl sulfoxide and non-polar solvents like diethyl ether, chloroform and hexane.

All complexes did not dissolved in water because of the bulkiness and there are many hydrocarbon group attached to those complexes but complexes were soluble in most of the solvents such as, dimethylsulfoxide, methanol and ethanol. Consequently, those solvents were mostly used in experiment to dissolve the complexes for further analysis. The solubility test of two ligands and two complexes were conducted in different solvents and presented in table 4.2

Table 4.2 Solubility of ligands and complexes in various solvents

Solvents	Water	Methanol	Ethanol	Chloroform	Diethyl ether	Dimethyl sulfoxide
L ₁	Soluble	Soluble	Soluble	Partially Soluble	Insoluble	Soluble
L ₂	Soluble	Soluble	Soluble	Partially Soluble	Insoluble	Soluble
NiL ₁ L ₂	Insoluble	Soluble	Soluble	Soluble	Partially Soluble	Soluble
ZnL ₁ L ₂	Insoluble	Soluble	Soluble	Soluble	Partially Soluble	Soluble

4.3.2 Conductivity Measurements of Ligands and Complexes

Conductivity measurements provide a method of testing the degree of ionization of a given compounds in solution. The higher the number of mobile ions a compound gives in solution , the higher will be its conductance. The conductivity measurements were carried out at 25⁰c by dissolving 0.5 g of each compound in 100 mL of DMSO. For all the ligands and complexes DMSO was used as solvents and the molar conductance value was measured.

The observed molar conductance value for the complexes shows that they are non-electrolytes in solution and thus confirm the formation of the complex. However, in ligands the observed conductance value showed that the ligands are electrolyte in solution and confirmed the existence of freely migrating ions.

Table 4.3 Molar conductance of ligands and complexes

Compounds	Molar conductance ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	Electrolytic nature
L ₁	599	Electrolyte
L ₂	646	Electrolyte
NiL ₁ L ₂	7.56	Non electrolyte
ZnL ₁ L ₂	10.07	Non electrolyte

4.3.3 Melting Point Determination of Ligands and Complexes

Melting point is a temperature when a solid phase compound changes into liquid phase. This is a method used to check the purity of a compound. A compound can be assumed as pure if it has melting point range less than 3⁰C [58]. From the result, all dithiocarbamate ligands and complexes has melting point found in this range. They can be assumed pure.

4.4 Infrared Spectra Studies of the Ligands and Complexes

Three important bands are expected in the IR spectra of dithiocarbamate compounds. In the range 1550-1450 cm⁻¹ corresponding to $\nu(\text{C-N})$ stretching vibrations; in the range of 1250-940 cm⁻¹ associated with $\nu(\text{C-S})$ and between 300-250 cm⁻¹ associated with the $\nu(\text{M-S})$ vibration [28]. The infrared spectra of the ligands and their corresponding complexes were compared and carefully assigned and the observed results were agreed with the expected bands. Relevant FTIR data presented in table 4.6 and the graph presented in appendix 1

Table : 4.4 Selected FT-IR region of dithiocarbamate ligands and their complexes.

Name of compounds	ν (N--H) cm^{-1}	ν (C--H) cm^{-1}	ν (N--C) cm^{-1}	ν asym (C-S) cm^{-1}	ν sym(C--S) cm^{-1}
N-Phenyl Dithiocarbamate (L_1)	3427	3124	1491	1163	999
4-Methyl-N-Phenyl Dithiocarbamate (L_2)	3438	3149	1513	1143	998
Nickel(II) Complex (NiL_1L_2)	3433	3169	1533	----	1000
Zinc(II) Complex (ZnL_1L_2)	3435	3170	1524	----	1009

In Infrared Spectra of N-Phenyl dithiocarbamate , Nickel(II) and Zinc(II) complexes ; the broad band in the spectrum of free ligand at 3427 cm^{-1} due to ν (N-H) which remained unaffected after complexation ,This implies that nitrogen in free ligand was not involved in coordination with metal. The band had been observed at 3124 cm^{-1} in the ligand spectrum due to ν (C-H) in aromatic ring. This bands also observed around 3170 cm^{-1} in both complexes. The ligand shows its characteristics ν (C-N) band at 1491 cm^{-1} .The position of this band shifted to higher frequencies in the prepared Nickel(II) and Zinc(II) complexes at 1533cm^{-1} and 1524cm^{-1} respectively. This observation indicated that the dominant contribution of the thioureide ($\text{N}^+=\text{C}$) resonance form of the dithiocarbamate. The spectrum of free ligand shows two absorption bands at 1163 cm^{-1} and 990 cm^{-1} due to asymmetric and symmetric stretching of ν (C-S) respectively; these band changed in a sharp single band appeared at 1000 cm^{-1} and 1009 cm^{-1} in Nickel(II) and Zinc(II) complexes respectively due to ν (C-S), This implies symmetrical coordination of the ligand through both sulfur atoms and giving bidentate nature of dithiocarbamate ligand in prepared complexes.

In Infrared spectra 4-Methyl -N-Phenyl dithiocarbamate, Nickel(II) and Zinc(II) complexes; the broad band in the spectrum of free ligand at 3438 cm^{-1} is due to ν (N-H) which no significant change in complexes. This indicated that nitrogen on the ligand was not involved in coordination with metals. In ligand , the peak at 3149 cm^{-1} is due to C-H stretching vibration in aromatic ring. Comparing the spectra of the ligand and that of the complexes the ν (C-N) stretching vibration was observed at 1513 cm^{-1} in the ligand and this band shifted in to 1533 cm^{-1} and 1524 cm^{-1} in Nickel (II) and Zinc (II) complexes respectively; and this blue shift ascribed the double bond character of thioureide bond due to the lone pair electron of nitrogen for the formation of complexes. The C-S symmetrical and asymmetrical stretching were observed in the ligand at 998 cm^{-1} and 1143 cm^{-1} respectively and the graph shown in appendix 1. In the Nickel (II) and Zinc (II) complexes only one peak was observed at 1000 cm^{-1} and 1009 cm^{-1} respectively due to symmetrical stretching of ν (C-S) which shows that the ligand is bidentately bonded to both Nickel (II) and Zinc (II) complexes through two sulfur atoms. Since IR spectrum was done on the range of $4000\text{-}400\text{ cm}^{-1}$, in both complexes M-S stretching vibration not observed and which usually found around $300\text{-}250\text{ cm}^{-1}$ [59]

4.5 Electronic Spectra of Ligands and Complexes

In general, dithiocarbamates electronic spectra , show three principal bands which arises from thioureide bond , electron pair of sulfur and charge transfer (CT) [32]. However , the d-d transition band may or may not be occur , which rely on the stereochemistry of complex. In this work all the electronic transitions were presented in agreement with previous published literatures for dithiocarbamate ligands and complexes [32, 35]. And the electronic spectra measurement were carried out at 25°C by dissolving 0.50 gram of each compounds in 100 mL of absolute ethanol. For all the ligands and complexes absolute ethanol was used as solvent.

Table: 4.5 Electronic spectra data of the dithiocarbamate ligands and their metal complexes

Compounds	λ_{max} (nm)	Possible assignment
N-Phenyl Dithiocarbamate (L ₁)	323	$\pi \rightarrow \pi^*$
4-Methyl-N- Phenyl Dithiocarbamate (L ₂)	319	$\pi \rightarrow \pi^*$
	365	$n \rightarrow \pi^*$
Nickel(II) Complex (NiL ₁ L ₂)	307	$\pi \rightarrow \pi^*$
	428	LMCT
Zinc(II) Complex (ZnL ₁ L ₂)	302	$\pi \rightarrow \pi^*$

4.5.1 Electronic Spectra N- Phenyl Dithiocarbamate(L₁), Nickel(II) and Zinc(II) Complexes

All the electronic transitions were presented in agreement with previous published literatures for dithiocarbamate ligands and complexes [32,35]. The ligand showed two bands that assigned to $\pi \rightarrow \pi^*$ attributed to (N⁺=C--S) group at 323 nm and the $n \rightarrow \pi^*$ transition which is due to the lone pairs of electrons on the sulfur atom at 372 nm which shown in appendix 2a. The Nickel(II) complex showed a band at 307 nm which was assigned $\pi \rightarrow \pi^*$ in thioureide (N⁺=C) bond which observed at shorter wavelength compared with free ligands ,This implies that the dominant of thiouride resonance in complexation due to the involvement of lone pair electron from nitrogen. Moreover since the ligands with relatively high- energy lone pairs on sulfur atoms, the band observed at 428 nm in Nickel(II) complex due to ligand to metal charge transfer. The obtained gram susceptibility value showed diamagnetic nature for Nickel(II) complex confirm that square planar geometry and dsp^2 hybridization of this complex ,this implies d-d transition not observed in the spectrum of Nickel(II) complex. Only one band was observed at 302 nm in Zinc(II) complex which was assigned $\pi \rightarrow \pi^*$ in thioureide (N⁺=C) bond observed at shorter wavelength compared with free ligands due to increase double bond character in thioureide bond due to the involvement nitrogen electrons.

The electronic spectrum of Zinc(II) complex was not showed d-d transition and the magnetic moment for this complex to be zero and electronic configuration $d^{10} (t_{2g}^6 e_g^4)$ to be diamagnetic nature and tetrahedral geometry and sp^3 hybridization of this complex.

4.5.2 Electronic Spectra of 4-Methyl-N-Phenyl Dithiocarbamate(L_2), Nickel(II) and Zinc(II) Complexes

The ligand showed two bands that assigned to $\pi \rightarrow \pi^*$ attributed to ($N^+=C-S$) group at 319 nm and the $n \rightarrow \pi^*$ transition which is due to the lone pairs of electrons on the sulfur atom at 365nm which shown in appendix 2b. The Nickel(II) complex showed a band at 307 nm which was assigned $\pi \rightarrow \pi^*$ in thioureide ($N^+=C$) bond which observed at shorter wavelength compared with free ligands ,This implies that the dominant of thioureide resonance in complexation due to the involvement of nitrogen lone pair electron nitrogen. Moreover since the ligands with relatively high- energy lone pairs on sulfur atoms, the band observed at 428 nm in Nickel(II) complex due to ligand to metal charge transfer (LMCT). The obtained gram susceptibility value showed diamagnetic nature for Nickel(II) complex which confirm square planar geometry and dsp^2 hybridization of this complex. In addition , d-d transition not observed in the spectrum of Nickel(II) complex. Only one band was observed at 302 nm in white color Zinc(II) complex which was assigned $\pi \rightarrow \pi^*$ in thioureide ($N^+=C$) bond observed at shorter wavelength compared with free ligands due to double bond character in thioureide which indicated that nitrogen lone pair electrons were involved .The electronic spectrum of Zinc(II) complex did not showed d-d transition and the magnetic moment for this complex to be zero and electronic configuration $d^{10} (t_{2g}^6 e_g^4)$ to be diamagnetic character and tetrahedral geometry and sp^3 hybridization.

4.6 1H NMR and ^{13}C NMR Spectra

4.6.1 1H NMR Spectra of Nickel (II) Complex

1H NMR experiment was used to characterize the dithiocarbamates . In the NMR spectra of complex shown in Appendix 3a all the chemical shifts were found in the expected regions. The 1H NMR of Nickel(II) complex showed peaks at different region of spectrum in which peaks appearing at the range (7.55--7.16) ppm due to three different protons environment in which protons at ortho position gave doublet peaks at 7.55 ppm and protons at meta position gave doublet of doublet peaks at 7.20

ppm where proton at para position gave multiple peaks at 7.41 due to coupling with adjacent protons found in phenyl ring. The highly intense peak appearing at 2.29 ppm is due to deuterated dimethylsulfoxide which was the solvent used. The single peak observed at 2.50 ppm assigned to the methyl hydrogen ($-\text{CH}_3$) which attached at para position on phenyl ring [15]. The peaks appeared at 11.75 and 11.67 ppm assigned to the protons attached to nitrogen ($-\text{NH}$) which observed in high deshielded area due to the cumulative effect of the aromatic ring and electro withdrawing effect of nitrogen on it. Since deuterated dimethylsulfoxide has synthesized by replacing protons with D_2O several times, commercial available DMSO-d_6 contain residue of water[58]. The peak observed 3.40 ppm is due to the residue water found in solvent.

4.6.2 ^1H NMR Spectra of Zinc(II) Complex

^1H -NMR experiment was used to characterize the dithiocarbamate complexes. In the NMR spectra of complex shown in Appendix 3b all the chemical shifts were found in the expected regions. The ^1H NMR of zinc (II) complex showed peaks at different region of spectrum in which multiple peaks appearing at the range of (7.47--7.20) ppm due to coupling of each protons found in phenyl ring [24]. The highly intense peak appearing at 2.28 ppm is due to deuterated dimethylsulfoxide (DMSO-d_6) which was the solvent used. The single peak observed at 2.50 ppm assigned to the methyl hydrogen ($-\text{CH}_3$) which attached at para position on phenyl ring. The broad peak appeared deshielded region at 12.29 assigned to the proton attached to nitrogen ($-\text{NH}$) due to the cumulative effect of the aromatic ring and electro withdrawing effect of nitrogen on it. Since deuterated dimethylsulfoxide has synthesized by replacing protons with D_2O several times, commercial available DMSO-d_6 contain residue of water[58]. And the peak observed 3.40 ppm is due to the residue water found in solvent.

4.6.3 ^{13}C NMR Spectra of Nickel (II) Complex

In the ^{13}C NMR spectra of complexes shown in Appendix 4a all the chemical shifts were found in the expected regions. The ^{13}C NMR of nickel(II) complex showed peaks at different region of spectrum respect to TMS. The ^{13}C NMR spectrum the peaks observed at 201.08 ppm which assigned to two ^{13}C of NCS_2 moiety due to more deshielding effect experienced through the nuclei of the carbon chain of aromatic rings. The eight signals observed at (145.97-114.50) ppm were assigned to the $\text{C}=\text{C}$ bond in the aromatic ring which arranged with respect amine functional group attached on

phenyl ring in which the Ipso carbon is the most deshielded while meta carbon is the least one (Ipso> ortho> para > meta) [28].

The peaks observed around 40 ppm is due to ^{13}C of deuterated dimethylsulfoxide (DMSO- d_6) which was solvent used. The peak appeared at 20.66 ppm in the spectrum was assigned methyl carbon ($-\text{CH}_3$) which attached to the para position in phenyl ring.

4.6.4 ^{13}C NMR Spectra of Zinc (II) Complex

In the ^{13}C NMR spectra of complexes listed in Appendix 4b all the chemical shifts were found in the expected regions. The ^{13}C NMR of zinc (II) complex showed peaks at different region of spectrum respect to TMS. The ^{13}C NMR spectrum the peaks observed at 206.41 ppm which assigned to two ^{13}C of NCS_2 moiety due to more deshielding effect experienced through the nuclei of the carbon chain on aromatic ring. The twelve signals observed at (140.61--123.43) ppm were assigned to the $\text{C}=\text{C}$ bond in the aromatic ring which arranged with respect amine functional group attached on phenyl ring in which the Ipso carbon is the most deshielded while ortho carbon is the least one (Ipso> ortho> para> meta) [28]. The peaks observed around 40 ppm is due to ^{13}C of deuterated dimethylsulfoxide (DMSO- d_6) which was solvent used. The peak appeared at 20.60 ppm in the spectrum was assigned methyl carbon ($-\text{CH}_3$) which attached to the para position in phenyl ring.

4.7 Magnetic Susceptibility Measurements

The magnetic properties of complexes in terms of unpaired electrons and their magnetic or spin properties are useful in determining structural features in transition metal compounds. Complexes that contain unpaired electrons are paramagnetic and are attracted in to magnetic fields. Diamagnetic compounds are those with no unpaired electrons are repelled by a magnetic field. The number of unpaired electrons can be determined by the magnitude of the interaction of the metal compound with magnetic field. Magnetic susceptibility balance measuring the force that a magnet exerts on a sample; the opposite force that the sample exerts on a suspended permanent magnet is measured and can be expressed using gram susceptibility in which the gram susceptibility is positive if the substance is paramagnetic and negative if the substance is diamagnetic [50].

Magnetic Susceptibility Measurements on complex was done at 20°C and the observed Gram Susceptibility (χ_g) was (-0.277×10^{-6}) which confined that Nickel (II) complex is diamagnetic nature

and it has square planar geometry. Since all electrons are paired , Zinc(II) complex is also diamagnetic nature and it has tetrahedral geometry.

Table: 4.6 Magnetic susceptibility value of Nickel (II) complex

Sample Code	Gram Susceptibility (χ_g)	Remark
NiL ₁ L ₂	-0.277 x 10 ⁻⁶	Diamagnetic

4.8 Atomic Absorption Spectroscopy

The absorption of spectrum of Nickel and Zinc atoms on the dithiocarbamate complexes were recorded on ZEF nit 700 p (anaytikjenu) using Nickel and Zinc cathode lamp. After that the concentration of metal ions was calculated by Beer-Lambart Law and the percentage of metal atom in complexes calculated and compared with theoretical value. Four calibration points for every component and samples were run in triplicate and the average values have been taken. For Nickel(II) complex , 0.2 gram taken and 0.1 gram of Zinc (II) complex taken for analysis and 50 mL, volume of solution, used for both complexes.

Based on Beer-Lambart Law , using the obtained absorbance values ; the concentration of nickel and zinc were calculated and become 488 ppm and 233.4 ppm respectively.

The concentration and absorbance due to standards and complexes of Nickel and Zinc shown in table 4.7 and 4.8 and the graph of standard Nickel and Zinc presented in appendix 5.

Table 4.7 AAS analysis Results Nickel Standard and Nickel(II) complex

<i>Concentration (ppm)</i>	<i>Absorbance</i>
2	0.01071
2.5	0.01226
5	0.02516
7.5	0.0363
<i>Nickel complex</i>	1.953

Table 4.8 AAS analysis Results Zinc Standard and Zinc(II) complex

<i>Concentration (ppm)</i>	<i>Absorbance</i>
1	0.01804
2.5	0.03605
5	0.05806
7.5	0.07618
Zinc complex	1.8782

Based on the concentration data the percentage metal in the complex can be calculated based on the following relation[60].

$$\% \text{ Ni} = \frac{\text{Concentration(ppm)} \times \text{Volume diluted(mL)} \times 100}{\text{Mass of sample taken (mg)} \times 1000} = \frac{488 \text{ mg/L} \times 50 \text{ mL} \times 100}{200 \text{ mg} \times 1000} = 12.2$$

$$\% \text{ Zn} = \frac{\text{Concentration(ppm)} \times \text{Volume diluted(mL)} \times 100}{\text{Mass of sample taken (mg)} \times 1000} = \frac{233.4 \text{ mg/L} \times 50 \text{ mL} \times 100}{100 \text{ mg} \times 1000} = 11.67$$

Table 4.9 Percent composition metal in complexes

Metal in complex	Percentage due to theoretical value	Percentage due to AAS analysis
Nickel (Ni)	14.34%	12.2 %
Zinc (Zn)	15.7 %	11.67 %

From the result, all the values obtained are in the range of accepted theoretical values. However small deviation due to impurity or instrumental error.

4.9. Proposed Structure of dithiocarbamate ligands and their metal Complexes

Based upon the characterization results, the following structures are proposed

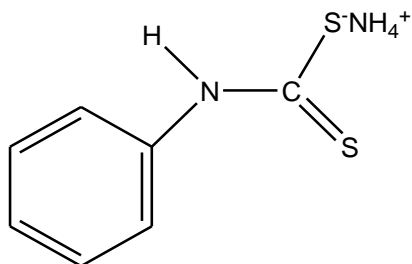


Figure 1. Proposed structure of L₁

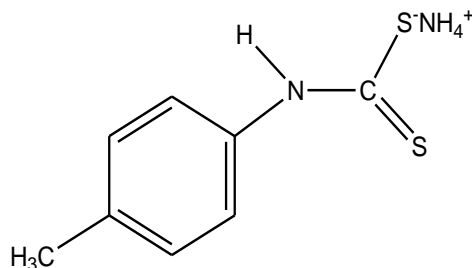


Figure 2. Proposed structure of L₂

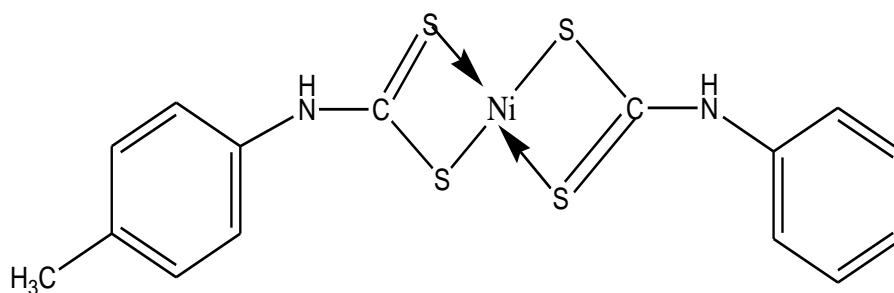


Figure 3. Proposed structure Ni(II) complex

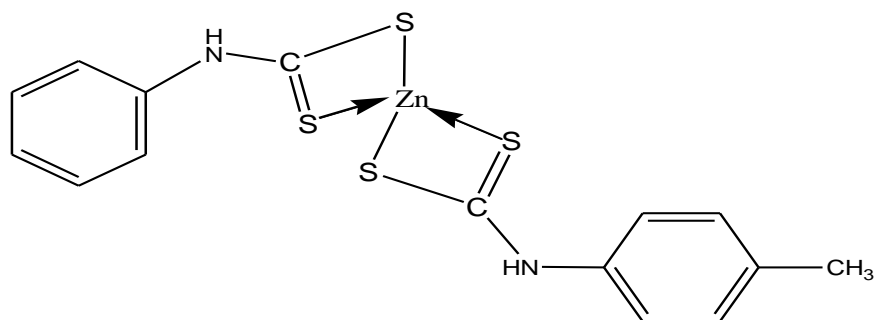


Figure 4. Proposed structure Zn(II) complex

4.10. Antimicrobial studies of dithiocarbamate ligands and metal complexes

4.10.1 Antibacterial Test

The antibacterial activity results were given in the table 4.10. According to the data collected, Zinc(II) complex showed strong inhibition zone against three bacterial strains *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. and even better than standard drug and moderate activity in one bacterium strain, *Enterococcus faecalis*. Nickel complex showed reduced activity for all selected bacteria strains which is may be due to its high viscous nature. On chelation the polarity of the metal ion reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups. It was further noted that the delocalization of electrons over the whole chelate ring enhanced the lipophilicity of the complexes. This increased lipophilicity enhanced the penetration of the complexes into lipid membrane of microorganism thus retarding the normal cell processes [58].

Table:4.10 antibacterial activities of ligands and metal complexes with each of concentration 4 mg/mL and zone of inhibition in mm

Compounds	Gram Positive Bacteria		Gram Negative Bacteria	
	<i>Staphylococcus aureus</i>	<i>Enterococcus faecalis</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>
L ₁	8	7	8	8
L ₂	7	7	7	8
NiL ₁ L ₂	--	8	7	7
ZnL ₁ L ₂	22	10	18	20
Gentamicin	16	19	12	12
DMSO	--	--	--	--

4.10.2 Antifungal Test

The antifungal activity results were given in the table 4.11. According to the result observed Zinc(II) complex showed strong inhibition zone against two fungi strains, *Penicillium(spp)* and *Trichoderma(spp)* even better than standard drug. Since zinc complexes can reacting with sulfhydryl groups of amino acids of enzymes of fungal cell , resulting disruption of lipid metabolism , respiration and production of ATP thus restrain the normal cell processes of fungi [10]. In two fungi strains, *Aspergillus(spp)* and *Fusarium(spp)*, Mancozeb showed better clear zone. Two ligands showed moderate activities on *Penicillium(spp)*, *Trichoderma(spp)* and *Fusarium(spp)* but in *Aspergillus(spp)* showed less activity. Nickel (II)complex showed reduced activity in three fungi strains but moderate activity in on fungi strain, *Trichoderma(spp)*.

Table:4.11 antifungal activities of ligands and metal complexes with each concentration of 2 mg/mL and zone of inhibition in mm

Compounds	<i>Penicillium(spp)</i>	<i>Trichoderma(spp)</i>	<i>Aspergillus(spp)</i>	<i>Fusarium(spp)</i>
L ₁	8	10	8	10
L ₂	10	11	7	10
NiL ₁ L ₂	7	12	7	7
ZnL ₁ L ₂	22	23	9	12
Mancozeb	16	18	24	25
DMSO	---	---	---	---

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Two dithiocarbamate ligands were synthesized from aniline and 4-methyl aniline. Nickel (II) and Zinc (II) dithiocarbamate complexes were successfully synthesized from these ligands. The ligands and complexes were all characterized by Elemental analysis, Infrared spectroscopy (FT-IR) and UV-Vis spectroscopy, NMR spectroscopy, Atomic absorption spectroscopy and Magnetic susceptibility measurements. By using all the above mentioned analytical data concluded that ligands are symmetric bidentate through two sulfur atoms during complexation. And also confirmed the proposed stereochemistry of the complexes as four coordinate in which the suggested geometry for Nickel (II) complex is square planar and tetrahedral geometry for Zinc (II) complex.

In the antimicrobial study, It was revealed that Zinc (II) complex has a much enhanced activity relative to synthesized ligands against all selected bacteria strains but Nickel (II) complex showed a reduced activity against *Staphylococcus aureus*. Moreover, In antifungal study Zinc (II) complex showed better zone of inhibition against *Penicillium(spp)* and *Trichoderma(spp)* relative to standard drug. With the support of works of other scholars who in their various studies have established that dithiocarbamate complexes synthesized from zinc revealed that strong to moderate antifungal activity against various fungal species.

5.2 Recommendation

From the study result it has been concluded that the dithiocarbamate ligands exhibit bidentate nature and form stable complexes with promising antimicrobial activity results. And I recommend the synthesize of other d-block transition metal complexes. In addition to this, further tests are needed to evaluate broad spectrum activities especially on zinc complex against other (additional) microbial species to explore all possibilities to evaluate the potential of zinc complex in the development of pharmaceutical drugs. On the other side, fungicidal compounds may have effects both on the host and the environment. Thus, additional works are required in this perspective.

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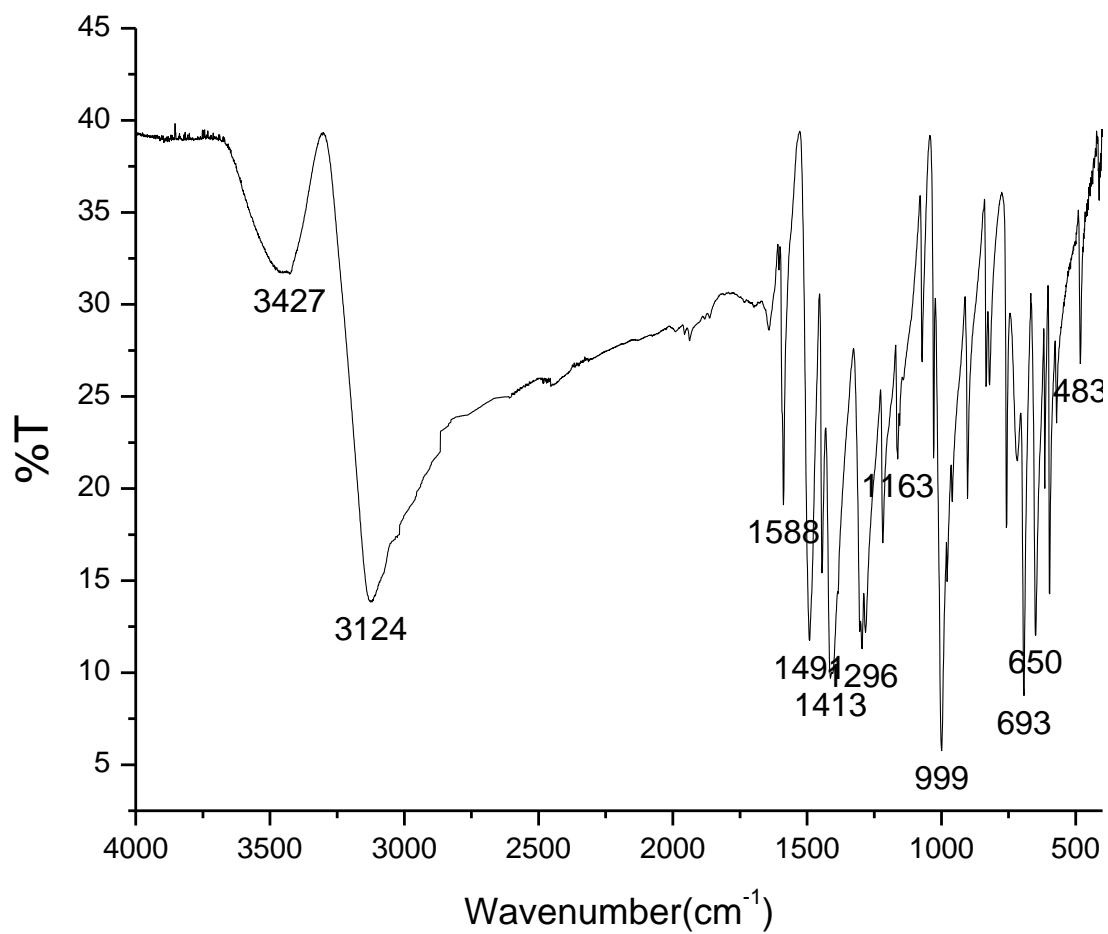
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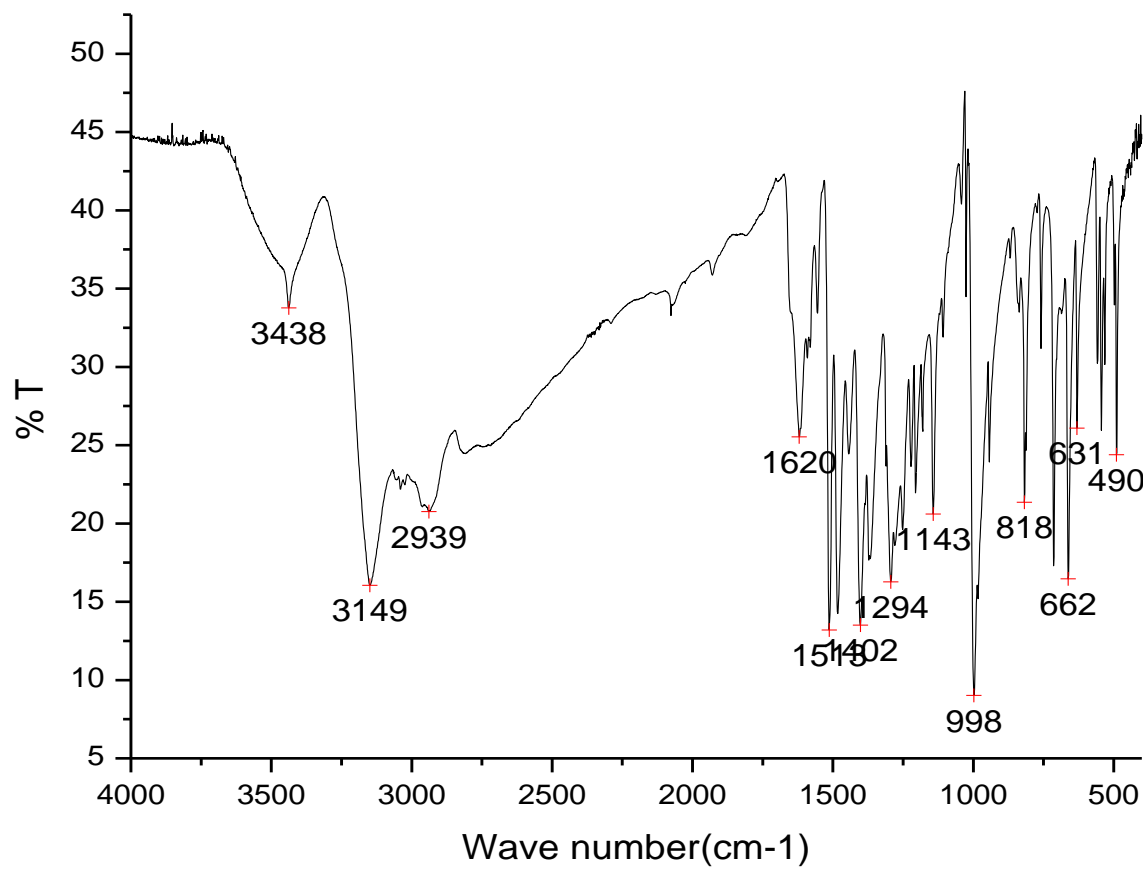
APPENDICES

Appendix 1 IR spectrum

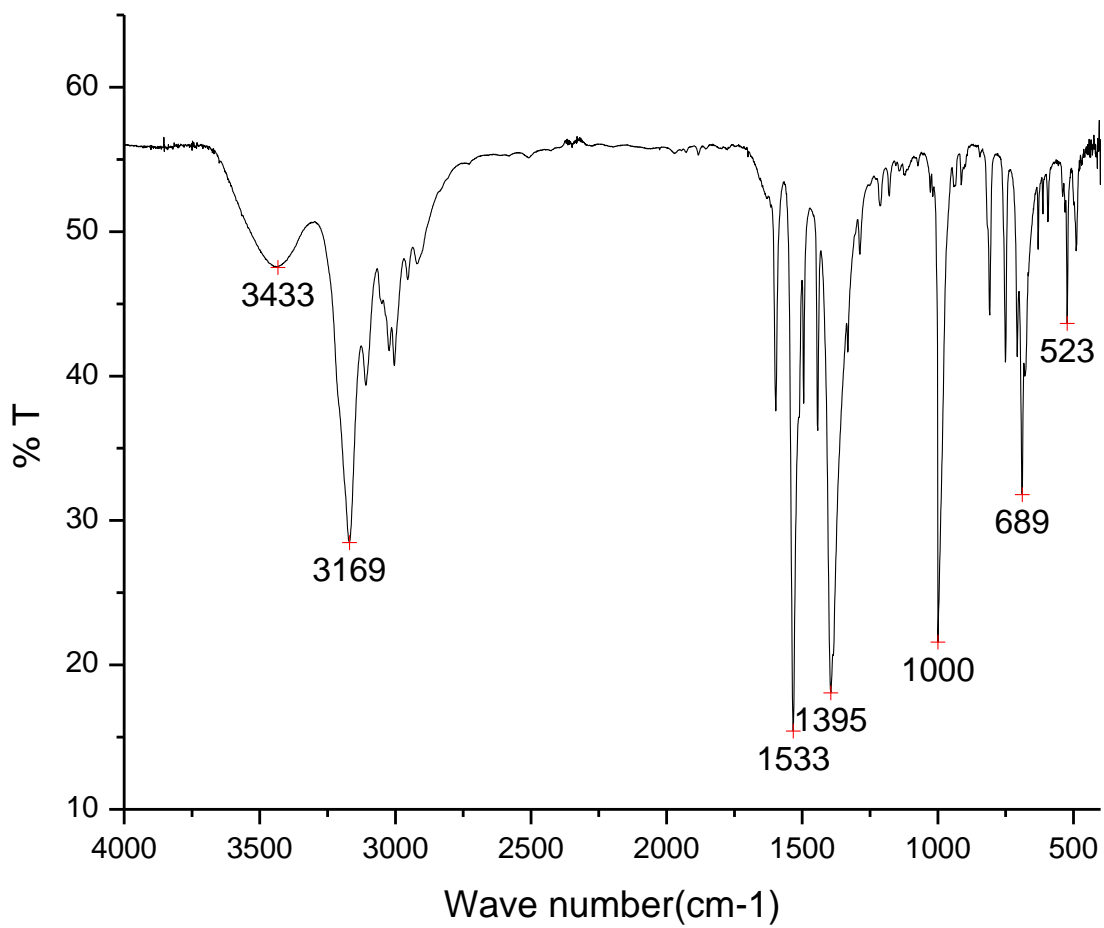
a) N- Phenyl Dithiocarbamate Ligand (L₁)



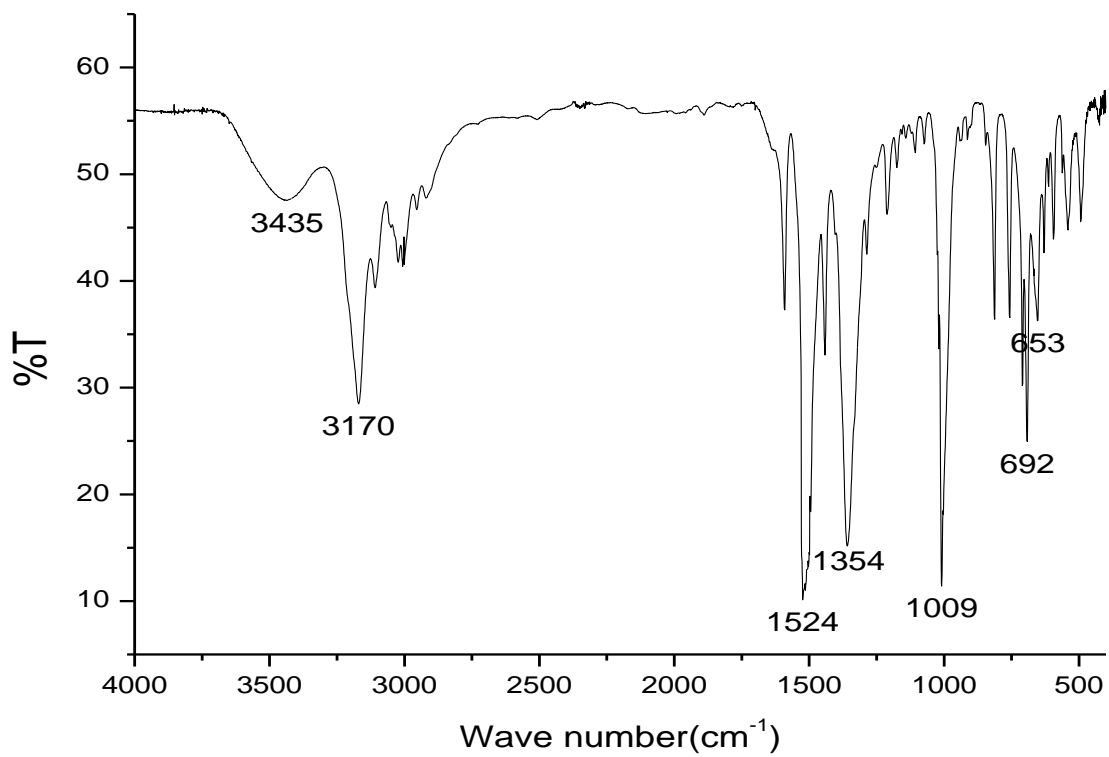
b) 4-Methyl-N-Phenyl Dithiocarbamate Ligand (L₂)



c) Nickel(II) Complex (NiL₁L₂)

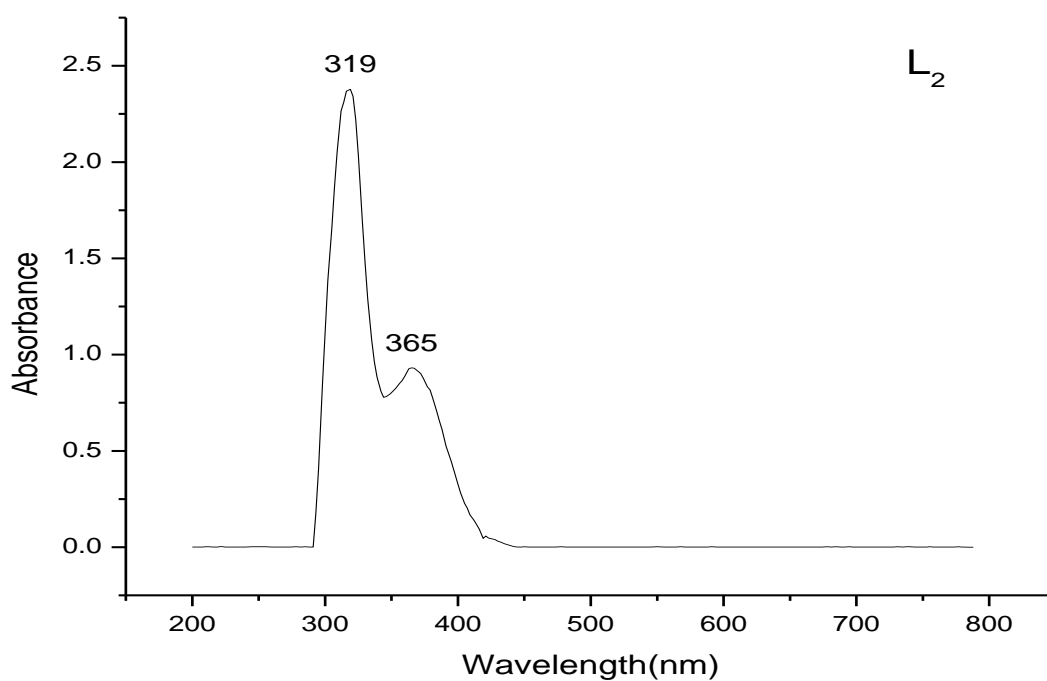
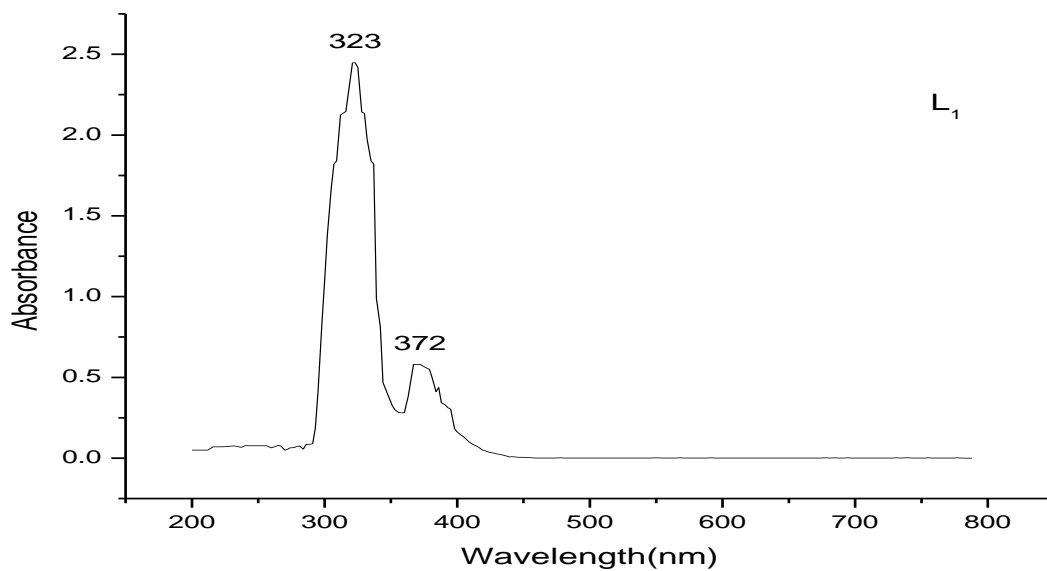


d) Zinc(II) Complex [ZnL₁L₂]

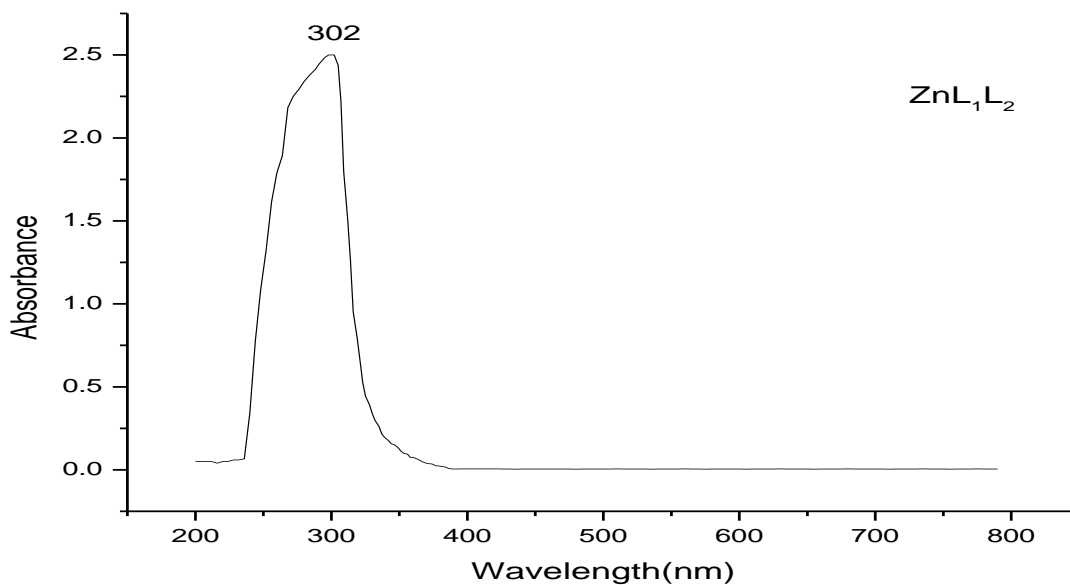
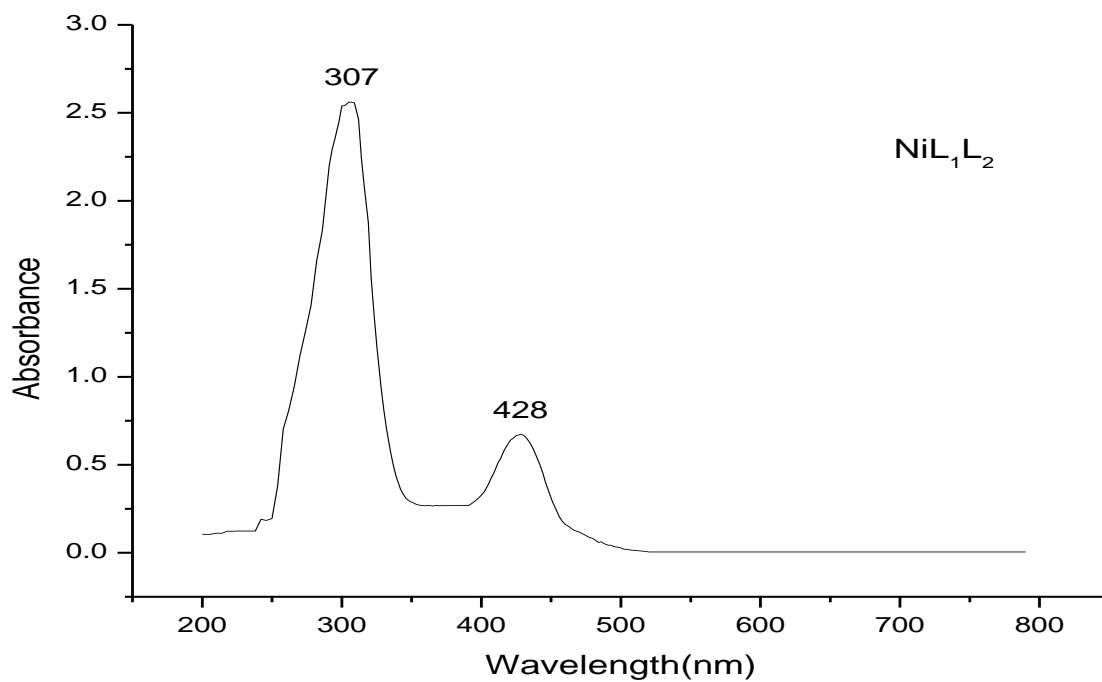


Appendix 2 Electronic spectrum

a) N- Phenyl Dithiocarbamate (L_1) and 4-Methyl-N- Phenyl Dithiocarbamate (L_2) ligands

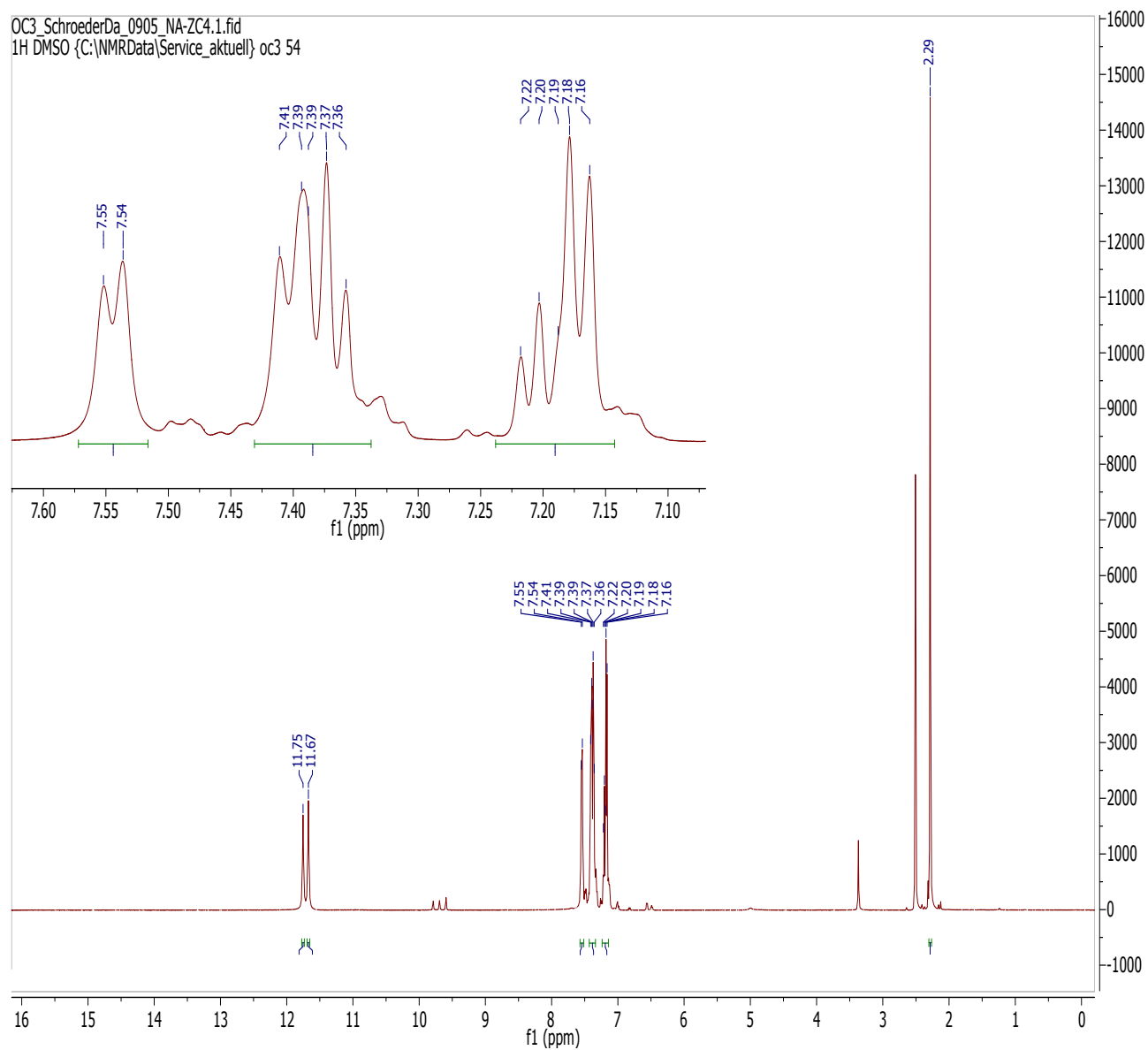


b) Nickel(II) [NiL₁L₂] and Zinc(II) [ZnL₁L₂] Complexes

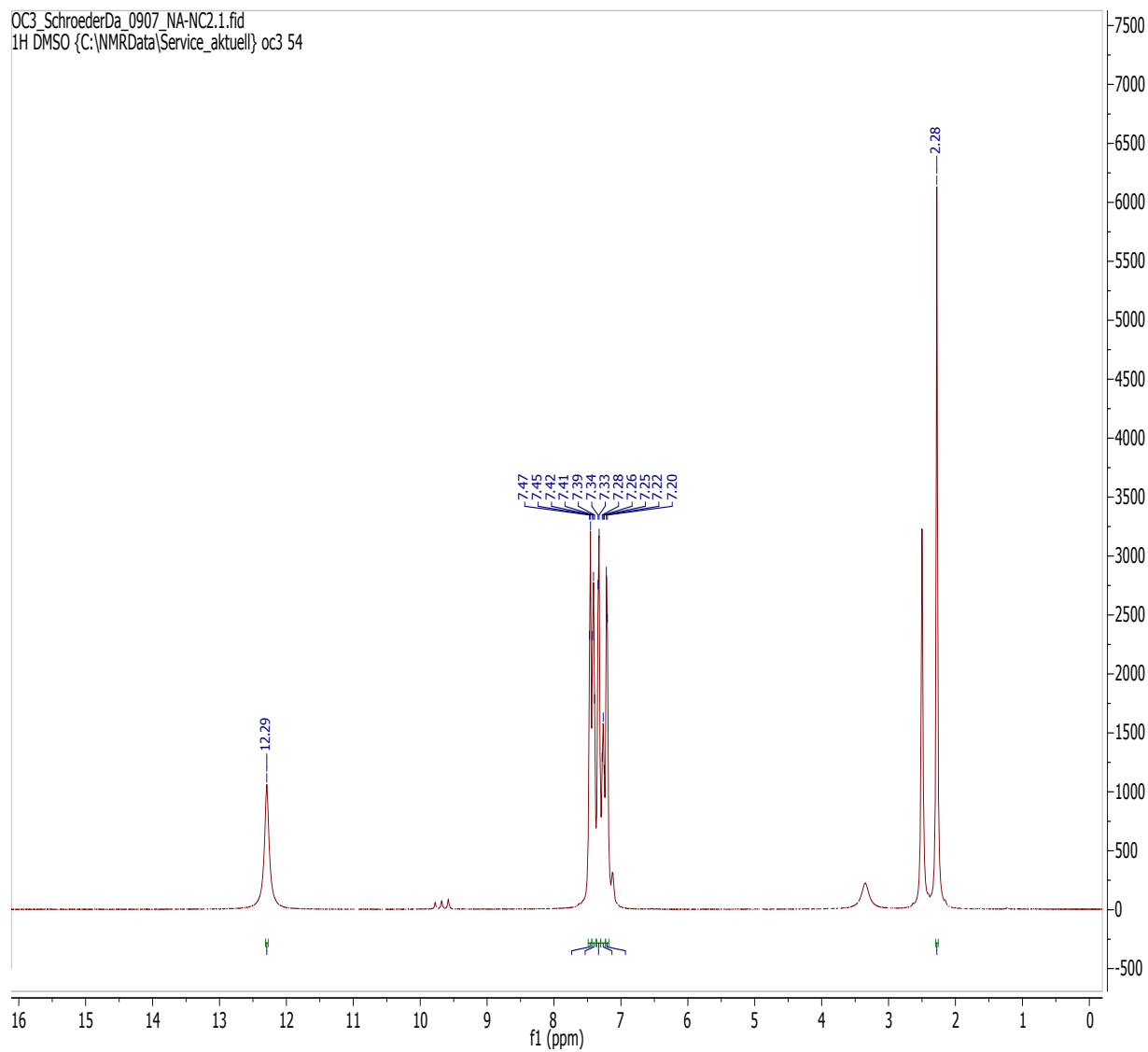


Appendix 3 ^1H -NMR

a) Nickel(II) Complex $[\text{NiL}_1\text{L}_2]$

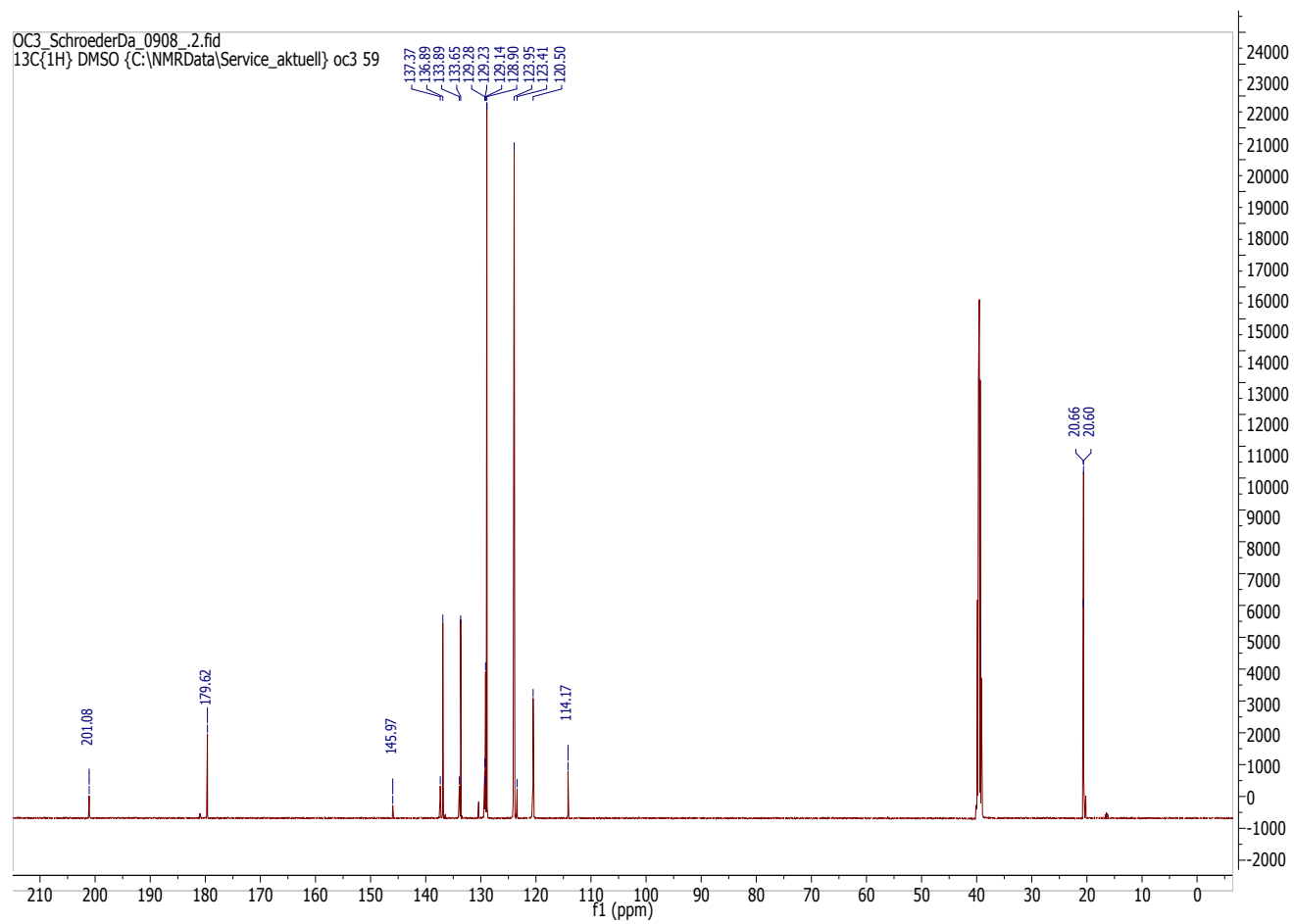


b) Zinc(II) Complex [ZnL₁L₂]

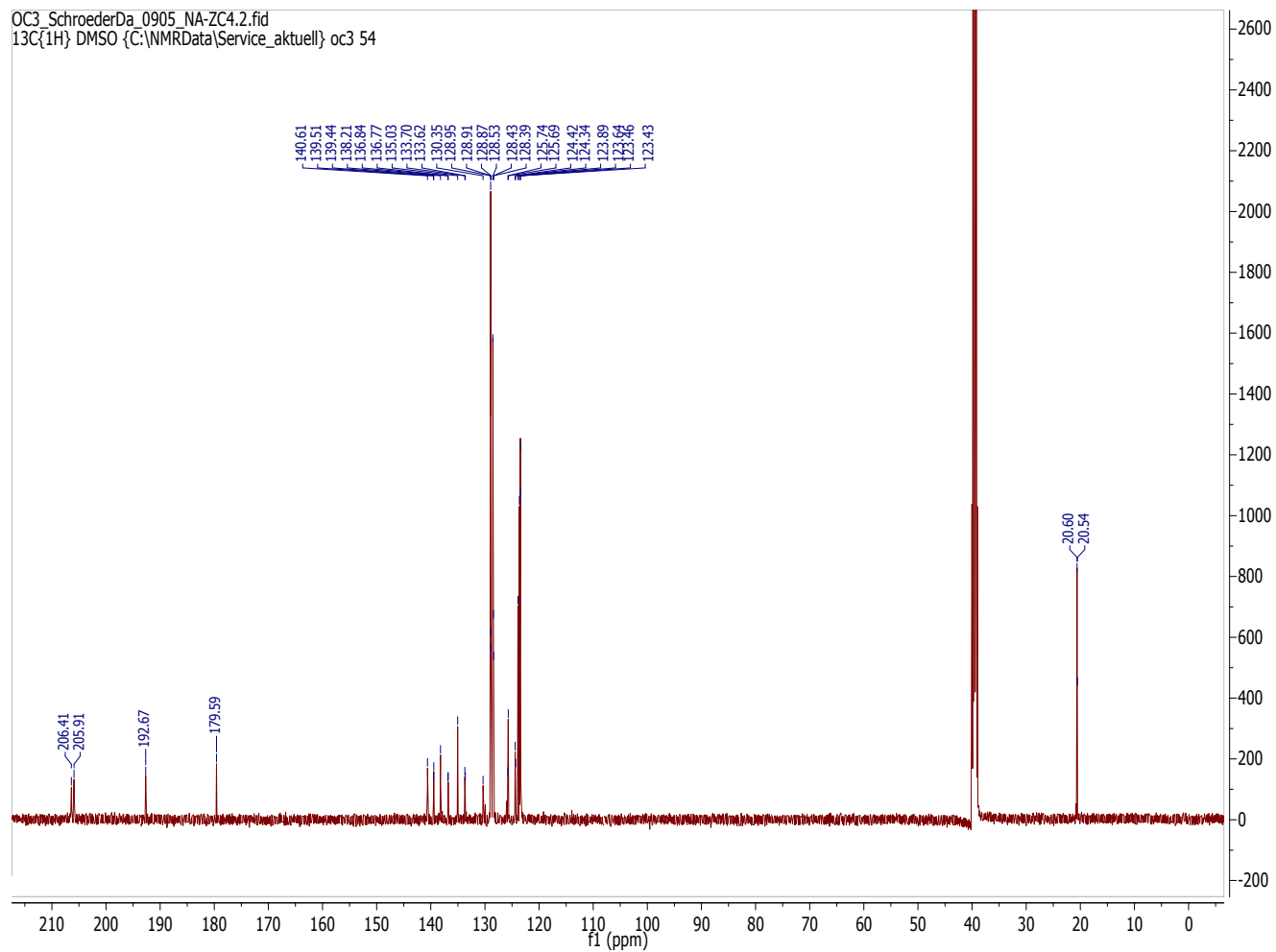


Appendix 4 ^{13}C -NMR

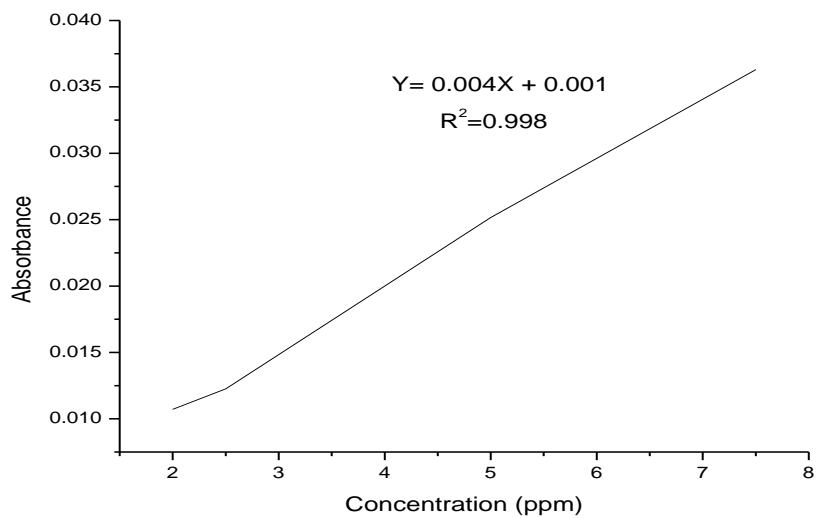
a) Nickel(II) Complex $[\text{NiL}_1\text{L}_2]$



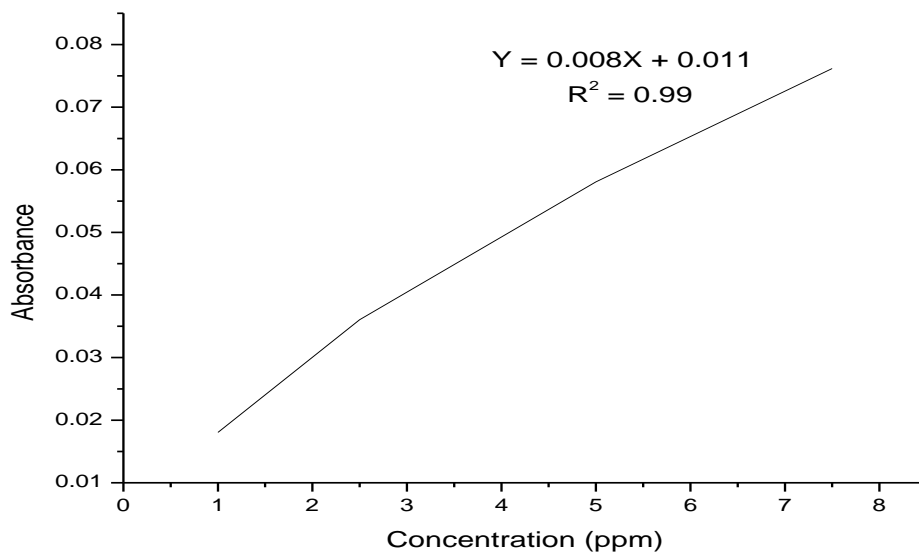
b) Zinc(II) Complex [ZnL₁L₂]



Appendix 5 Concentration Versus Absorbance Graph of Standard Nickel and Zinc Metals

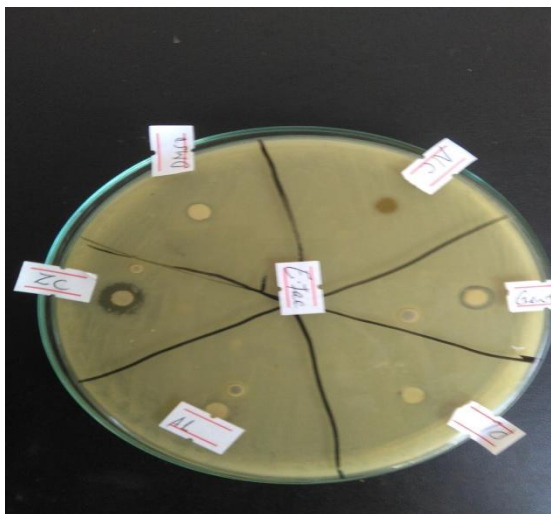


Nickel Standard

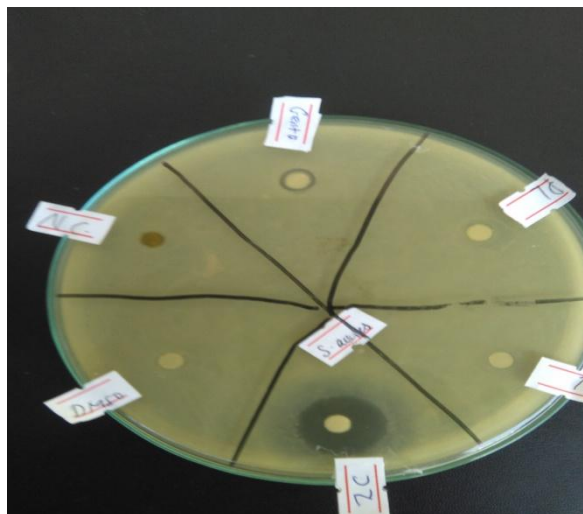


Zinc Standard

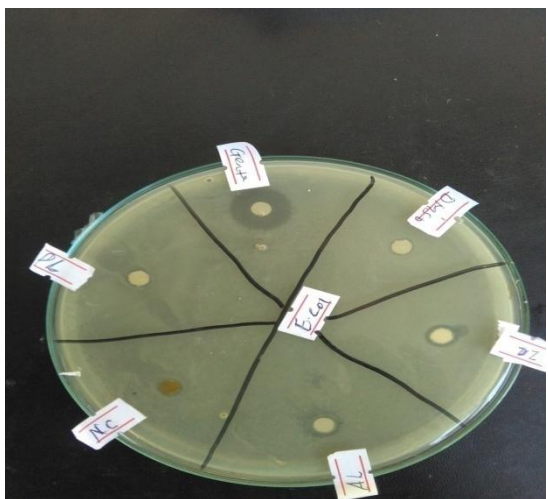
Appendix 6 Antibacterial Activities of Synthesized Ligands and Metal Complexes in mm



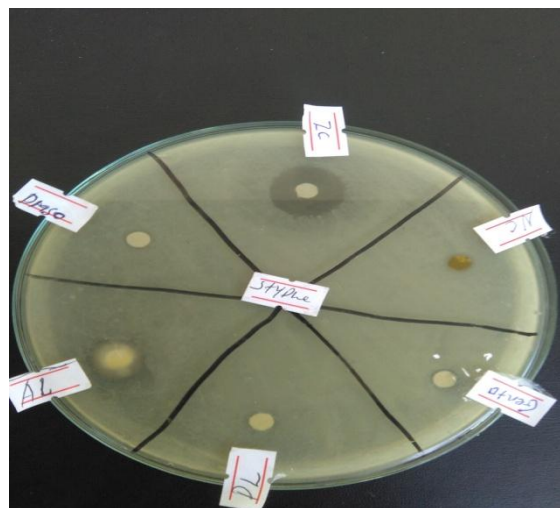
Enterococcus faecalis



Staphylococcus aureus

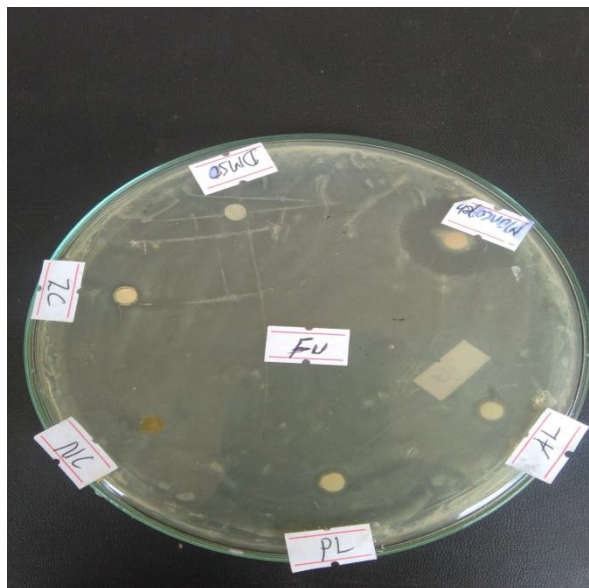


Escherichia coli

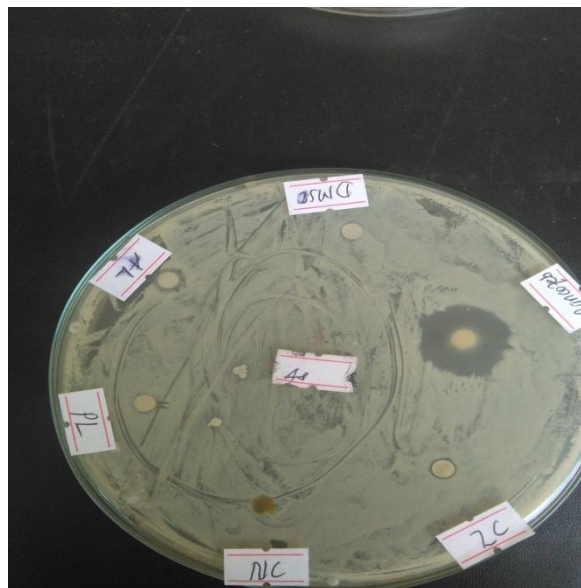


Salmonella typhi

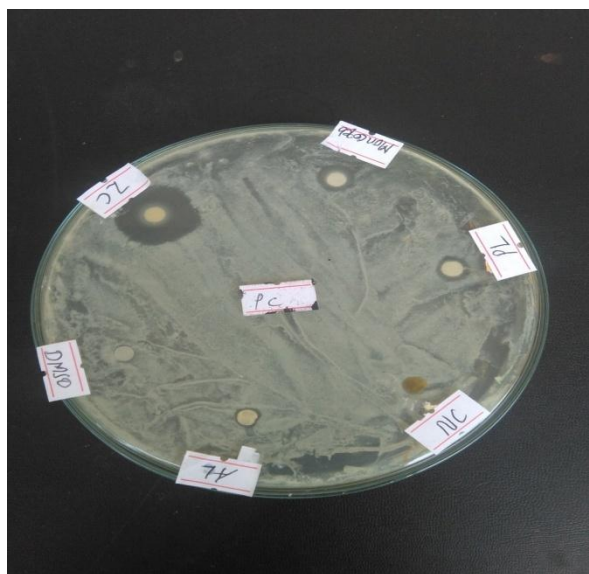
Appendix 7 Antifungal Activity of Synthesized Ligands and Metal Complexes in mm



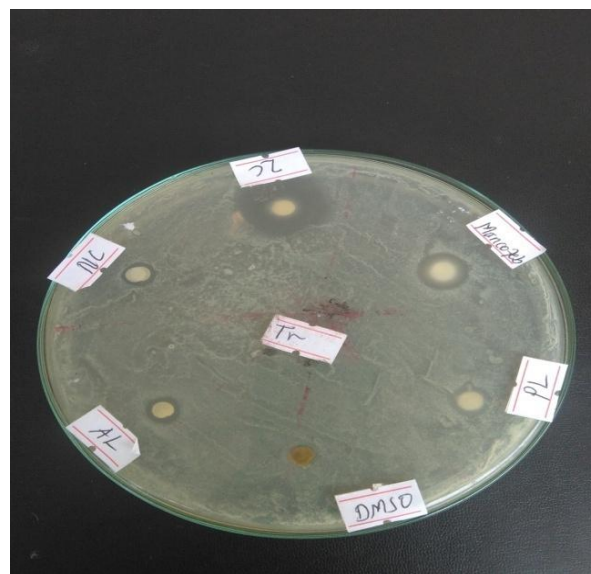
Fusarium(spp)



Aspergillus(spp)



Pencillium(spp)



Trichoderma(spp)