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Ni (II) and Zn (II) COMPLEXES DERIVED from NINHYDRIN and THIOSEMICARBAZIDE in a TEMPLATE PROCEDURE—STRUCTURAL and ANTIMICROBIAL STUDIES

By: Belay Getie

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List of Abbreviations and Symbols

υ	Stretching
IR	Infrared
nm	nanometer
m .pt	melting point
Λ_m	Molar conductance
DMSO	Dimethyl sulfoxide
DMF	Dimethylformamide
TMS	Tetramethylsilane
DMSO-d6	Hexadeuterated dimethylsulfoxide
TLC	Thinlayer chromatography
UV-Vis	Ultraviolet-visible
^{1}H NMR	Proton Nuclear Magnetic resonance
^{13}C NMR	Carbon Nuclear Magnetic Resonance
PLTSC	pyridoxal thiosemicarbazone
PLITSC	pyridoxal isothiosemicarbazone

TSC	Thiosemicarbazone
PLTSC-H	Deprotonated pyridoxal thiosemicarbazone
OAC^-	Acetate ion
Me	Methyl
SALTSC	Salicylaldehyde Thiosemicarbazone
Xg	Gram magnetic Susceptibility
x_m	Molar magnetic Susceptibility
μ_{eff}	Effective magnetic moment
AAS	Atomic absorption spectroscopy
DEPT.	Distortionless Enhancement by Polarization Transfer
Redox	Reduction and oxidation
CV	Cyclic voltammetry
ppm	parts per million

Abstract

The reaction of warm EtOH solutions of ninhydrin and thiosemicarbazide with $NiCl_2.6H_2O$ (in EtOH) and $Zn(AcO)_2{\cdot}2H_2O$ yielded a tetrahedral and square planar complexes of Zn(II) and Ni(II) respectively in a 1:1:1 mole ratio using the template method of synthesis.

The Schiff base, thus formed acted as a monobasic tridentate ligand *via* ONS binding sites.

The compounds were characterized by conductometric, magnetochemical measurements, AAS, IR, NMR, and UV-Vis spectra. Besides, voltammetric study of Ni (II) complex was carried out in DMSO solution in the presence of supporting electrolyte to characterize its redox nature. The biological activities of the complexes were also investigated.

Keywords: template synthesis, ninhydrin, thiosemicarbazide, antimicrobial activity, and voltammetric studies.

1. Introduction

1.1. Complexes and Coordination Compounds

In a typical covalent bond, such as is found between carbon and hydrogen in methane, each atom is considered to contribute one electron to the two-electron, two-centre bond which is formed. However, we can envisage a second type of covalent bond in which we still have a two-centre, two-electron bond, but where both of the electrons come from one of the atoms or from a molecule. This type of bond is known variously as coordination, a dative covalent or a donor-acceptor bond. A compound containing such bonding is known as a coordination compound. The atom (or molecule) which provides the two electrons is known as the donor. The other atom (or molecule) is known as the acceptor. The term complex is used to describe a coordination compound in which the acceptor is a metal (usually, but not necessarily, a transition metal) atom or ion. The terms complex and coordination compound are now used almost interchangeably. In those coordination compounds in which the acceptor is a metal atom or ion, the donor is known as a ligand (from the Latin word ligare,which means to bind) [1].

1.2. Literature Review on Metal Complexes with Schiff Bases Derived from Thiosemicarbazide

1.2.1. Metal Complexes with Schiff-Base Ligands - Pyridoxal and Semicarbazide-Based Derivatives

Semi-, thiosemi-, and isothiosemicarbazones, (SC, TSC, ITSC, respectively) (Fig. 1), as well as their metal complexes have been the subject of great interest of many researchers for a number of years [9].

Figure 1: General structural formulas of semi- thiosemi- and isothiosemicarbazones.

The significance of these compounds, apart from their diverse chemical and structural characteristics, stems not only from their potential but also their proved application as biologically active molecules exhibiting a wide spectrum of activity. This is especially related to thiosemicarbazones and their metal complexes, for which the relationship between structure and biological activity has been covered in papers by West et al [2-8, 9].

The coordination chemistry of thiosemi-, semi-, and isothiosemicarbazones appeared to be very interesting from the point of view of both the number of metals forming complexes with them and the diversity of the ligand systems themselves i.e., their denticity, set of donor atoms, stabilization of various (less common) oxidation states of metals, etc [9].

The investigation of metal complexes with Schiff bases derived from pyridoxal (PL), e.g., 3-hydroxy-5-hydroxymethyl-2-methylpyridine-4-carboxaldehyde (one of the forms of vitamin B_6), and amines, and/or amino acids, have also been the subject of great interest of many researchers.

The primary reason for this lies in the fact that these compounds can serve as models for studying a wide range of biological reactions which are catalyzed by enzymes, in which pyridoxal phosphate (PLP), as the physiologically active form of pyridoxal, appears as an essential component. It has been shown that in the presence of metal ions, free

pyridoxal can catalyze a variety of metabolic reactions, e.g., of amino acids (transmination, decarboxylation, racemization and carbon–carbon bond cleavage), in which PLP acts as a co-enzyme [8].

In view of the fact that Schiff bases of pyridoxal are effective ligands, they are of research interest to coordination chemists. Pyridoxal thiosemi- semi-, and isothiosemicarbazones (PLTSC, PLSC, and PLITSC, respectively) (Fig. 2) form a special group of these ligands.

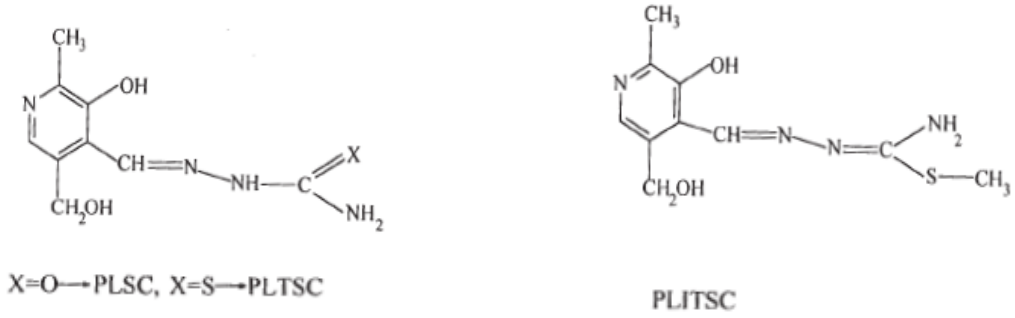


Figure-2: Structural formulas of pyridoxal semi- (PLSC), thiosemi- (PLTSC) and isothiosemicarbazone (PLITSC)

This review presents the most important results which have been obtained in the studies of these ligands and their metal complexes. Like the majority of similar Schiff bases, pyridoxal semi-, thiosemi- and isothiosemicarbazones are mainly obtained in good yield by the condensation reaction of aqueous or alcoholic solutions of pyridoxal and the corresponding semicarbazide derivative [9].

1.2.1.1a. Coordination Mode

The common coordination mode of these ligands is presented in Fig. 4. As can be seen, of seven potential ligator atoms of all three ligands, three are engaged in coordination, two of them being identical for all three ligands. Namely, the identical ligators are the phenolic oxygen and hydrazine N_1 nitrogen. In the case of PLSC and PLTSC, the third ligator atom is the oxygen or sulfur atom of the amide, i.e., thioamide group (Fig.3 a,b,c). In the case of PLITSC, the third ligator atom is the

N_4 nitrogen of the isothioamide group (Fig. 3d, e, f). In all cases, two metallocycles are formed: one six-membered (pyridoxilydene) and one five-membered (semicarbazide derivative).

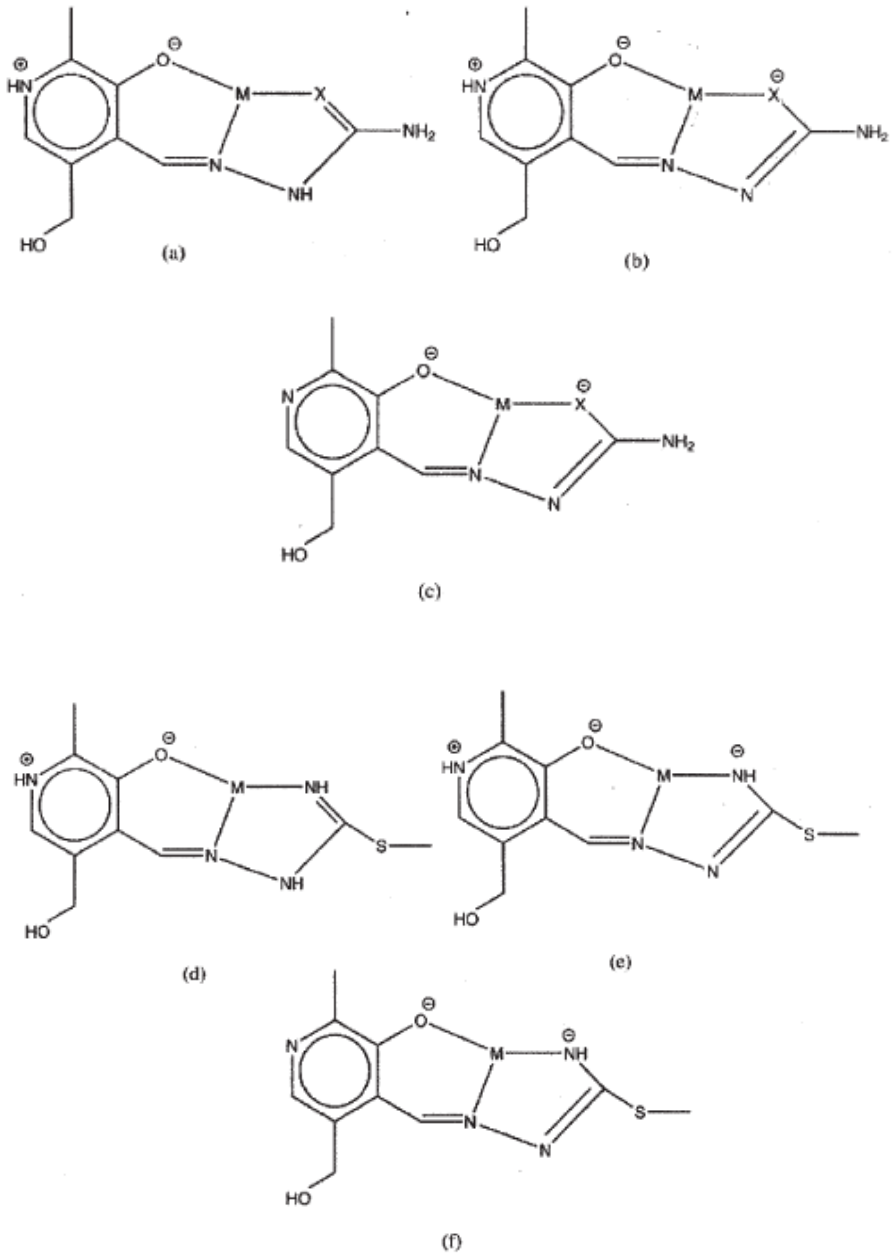


Figure-3: Coordination modes and ligand forms of PLSC, PLTSC (a, b, c) and PLITSC (d, e, f)

Furthermore all three ligands can be coordinated either in neutral, monoanionic or dianionic forms. The monoanionic forms of PLSC and PLTSC are formed by deprotonation of the enol/thiol forms (Fig. 3b),

and in the case of PLITSC, by deprotonation of the hydrazine nitrogen atom (Fig. 3e), i.e., of the imido form of the isothiosemicarbazide residue. The dianionic forms of all three ligands are formed by the additional deprotonation of the pyridine nitrogen (Fig. 3 c, f), i.e., of the zwitter ion, the existence of which is explained by the migration of the hydrogen atom from the phenolic OH group to the heterocyclic nitrogen atom. It should be pointed out that this proton transfer is characteristic not only of coordinated but also of non-coordinated pyridoxal derivatives, which in the case of PLTSC.$3H_2O$ was proved by solving its molecular structure by X-ray crystallography [9].

In view of the mentioned deprotonation sequence, the form of the coordinated ligand will depend primarily on the pH and then on the nature of the metal ion. Namely, the presence of proton acceptors (NH_3, OAc^-, Py, etc.) will facilitate the formation of complexes with the anionic forms of the ligand. Thus, for example, with Zn $(NO_3)_2$ and Ni$(NO_3)_2$, PLTSC gives [Zn(PLTSC)$(NO_3)_2$].$H_2O$and [Ni(PLTSC-H)NO_3].$2H_2O$, whereas with M$(OAc)_2$.H_2O, (M = Zn, Ni), it forms [Zn(PLTSC-H)OAc].H_2O and [Ni(PLTSC-2H)].$2H_2O$ [9].

By comparing the deprotonation of these three ligands, i.e. the deprotonation of the residue of the semicarbazide derivative, it can be concluded that thiosemicarbazide is most readily deprotonated and that deprotonation of isothiosemicarbazide is the most difficult.

Finally, it should be noticed that in the case of PLTSC, with which, by far, the largest number of complexes have been prepared compared with the other two ligands exist two exceptions from the common tridentate ONS coordination. Thus, for examaple, in the mixed-ligand Au(III) complex of the formula [Au(HDAMP)Cl(PLTSCMe)]Cl_2 , where HDAMP = 2-(N,N-dimethylamino-methyl)phenyl, and PLTSCMe = pyridoxal N4-methylthiosemicarbazone, the pyridoxal derivative is coordinated as a bidentate NIS entity, which means that the phenolic

oxygen is not included in the coordination[9]. The absence of coordinations of the phenolic oxygen has also been found with the similar tridentate ONS salicylaldehyde thiosemicarbazone (SALTSC), which in complexes of the type M $(PPh_3)_2(SALTSC\text{-}H)_2]$ (M = Ru(II), Os(II), is also coordinated as a bidentate (NS) moiety, the TSC residue in this case is not coordinted via the N1 but via the hydrazine nitrogen, N_2, thus forming a four-membered ring [38]. The same coordination mode (N2S, instead of the usual N1S) of the coordinating thiosemicarbazone residue, apart from the mentioned complexes, has also been found in the case of complexes with some other thiosemicarbazones, such as Tl (III) and Ru(II) complexes with bidentate thiosemicarbazone,derivatives of benzaldehyde and acetylferrocene [9].

Another very interesting coordination mode, characteristic not only of PLTSC but also of the other tridentate thiosemicarbazones in general, has been found in the dimeric Tl(III) complex of the formula $[TlMe_2(PLTSC\text{-}H)(H_2O)]_2$ (Fig. 5) [38]. It can be seen that PLTSC anion in its thiol form is bidentately coordinated via the enolyzed sulfur atom and, unexpectedly, via the oxygen atom of the undeprotonated phenolic hydroxyl, forming thus a nine-membered metallocycle.

Monomeric $[TIMe_2(PLTSC\text{-}H)(H_2O)]$ units are more weakly bound in the dimer via the sulfur atom. Thus, the coordination behavior of this ligand is interesting also because the PL fragment appears in the form of a zwitter ion and neither of the hydrazine nitrogen atoms participates in the coordination [9].

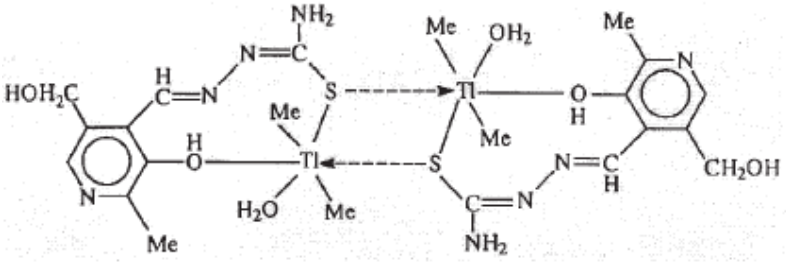


Figure-4:Structure of $[TlMe_2(PLTSC\text{-}H)(H_2O)]_2$.

1.2.1.1b. Syntheses of Complexes

The largest group of metal complexes with Schiff bases which have been synthesized and characterized up to now, represent complexes with PLTSC. The basic reason for this is the fact that the preparative coordination chemistry of the PLTSC ligand began the first (in 1986) with the paper of Italian authors [9] who have shown a continuous interest in this ligand and its derivatives and complexes.

With respect to the applied methods and synthesis conditions of metal complexes bearing these ligands, it can be said that practically all complexes were obtained by the simple non-template method, i.e., by the reaction of ready-made ligands and metal salts, mainly in warm alcoholic (less often aqueous) solution.

Some of the complexes were obtained by the template method, for example the penta coordinated monomeric and dimeric Zn complexes with PLTSC of the formulas $[Zn(PLTSC)Cl_2].2H_2O$ and $[\{Zn(PLTSC–H)Cl\}_2].2H_2O$ (Cl-bridge), which were prepared by the reaction of an EtOH (or H_2O) solution of $ZnCl_2$, PL and TSC (in the mole ratio 1:1:1 and 2:1:1, respectively).

In terms of metal complexes of the three ligands that have been investigated so far, the most numerous are copper and iron complexes. With respect to their composition, a common feature of all these complexes is that they belong to the type of mono(Schiff-base)-ligand complexes, the composition of which can be represented by the following general formulas: $Cu(L)X_2nH_2O$ [9].

1.2.1.1c.Biological Activity

One of the main reasons why thiosemicarbazones have been more extensively studied and investigated than semicarbazones and isothiosemicarbazones is the higher biological activity of the former. The biological activities of thiosemicarbazones are considered to be related to their ability to form chelates with metals [10, 11]. Since the pioneering work of Domagk et al.[12] in 1946 on the antituberculosis activity of p-acetamidobenzaldehyde thiosemicarbazone (trivial names Thiacetazone or Tibon), the number of papers concerning the pharmacological use of these compounds has dramatically increased, due to the wide spectrum of their biological activity found in recent years. Nowadays, it is known that thiosemicarbazones show antitumorous, antiviral, antifungal, antibacterial and antimalarial activities. Of course, the primary task of researches is to investigate new compounds with respect to their activity against tumors and viruses.

Of course, not only the ligands were found to be useful in pharmacological applications. It has been repeatedly shown that compared to the ligands, the thiosemicarbazone-based metal complexes (for example, Cu and Zn) are more efficient inhibitors of cancer cell growth [9, 13, 14]].

1.3. Template Method of Synthesis

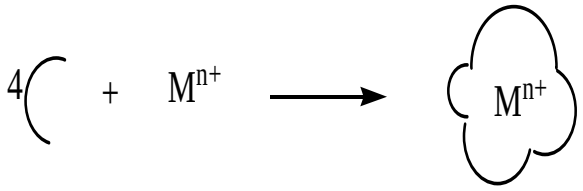
1.3.1. Types of Template Effects

The ability of metal ions to affect the course of some organic reactions has been studied at beginning of the sixties. Since then various and amazing molecular architectures have been created as a result of this effect. Metal–template reactions are ligand reactions which are

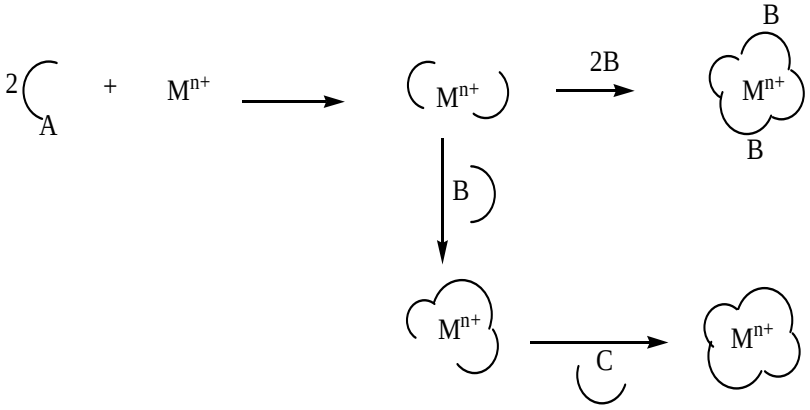
dependent on or can be significantly enhanced by a particular geometrical orientation imposed by metal coordination.

Two classes of chemical templates have been recognized: kinetic templates and thermodynamic templates. When the template effect arises from the stereochemistry imposed by metal ion coordination of some reactants, promoting a series of controlled steps, a coordination or kinetic template effect occurs. This effect provides roots to products which are not formed in the absence of the metal ion. Kinetic templates influence the mechanistic path way. Experimental data lead to the following type of kinetic metal- template reactions.

- The molecules are coordinated and assembled around a metal cation in a single step (scheme 1) [15].



Scheme-1: Molecular assembly around metal cation

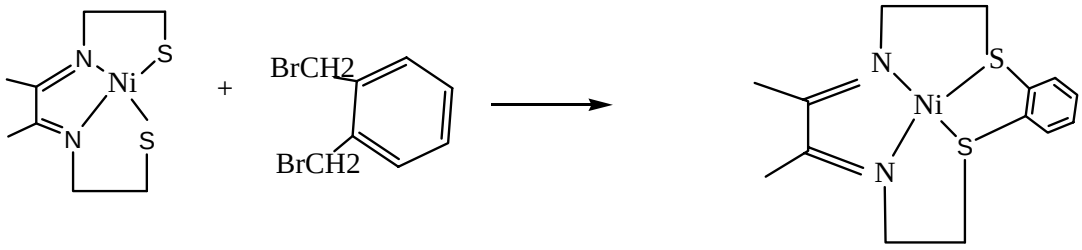


Scheme-2: Reaction of external molecules with the ligated ones.

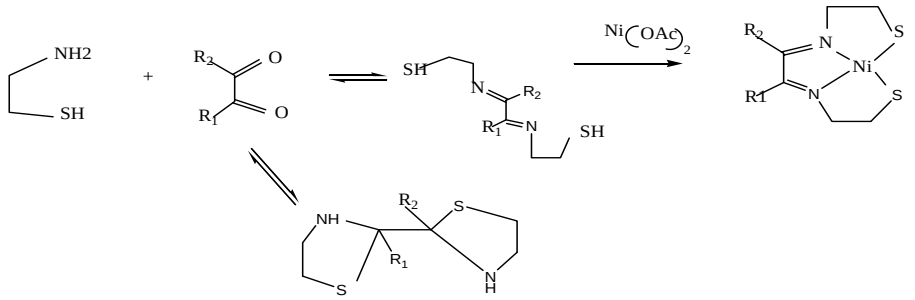
- The coordinated molecules react with an external molecule which bridges the ends of the ligated ones (scheme 2).

It can be noticed that the most frequent product are cyclic ligands. However, acyclic,linear ligands have also been obtained,mostly when the reaction is stopped along half a building stage .

Busch showed first the kinetic template effect on Studying the planar diamagnetic complex 2, 3-butane-dis (mercaptoethylimino)-nickel (II) which contains two terminal cis- mercaptide groups. It reacts with monofunctional or bifunctional alkylating agents resulting in products which contian a coordinated thioether [15].



Scheme-3: Formation of macrocyclic complex



Scheme-4: Formation of Schiff base

When the bifunctional reagent α,α'-dibromo-o-xylene is employed, a new chelate ring is formed and the product of reaction a macrocylic complex (scheme 4). The probability of ring closure is assured by the presence of the metal atom which holds the mercapto groups in cis position ,and the reaction occurs in a single rate-determining step.It is demonstrated also that this mechanism arises because the reagent α,α'-dibromo-o-xylene creates a new chelate ring *in situ*.

Thermodynamic templates refer to reactions that do proceed in the absence of the metal ion. In this case metal ion promotes formation of desired product by removing them from the equilibrium.

In other words, metal ions select and bind certain complementary structures from among an equilibrating mixture of structures. This is a "sequestration" phenomenon. The first recognized example of the thermodynamic template effect refers to the formation the Schiff base in scheme 4. In the absence of nickel (II), the reactionbetween ß-mercaptoethylamine and an α–diketone, thiazolines and mercaptals are formed in competition with the Schiff base of the corresponding type. In the presence of the metal ion, the nickel complex with schiff base as ligand is formed with a yield of 70%, which is excess. An equilibrium template effect has been identified where aspects of both effects mentioned above combine, the distinctive characteristics being the formation of different products in the metal assisted and metal free reaction [15].

1.3.2. The Template Effect as a Molecular Organizer

Molecular organization is the rule in in-vivo chemistry .The study of the simple chemical systems must be able manifest the organizational principles .The knowledge of these principles helps us to understand and exploit those in- vivo.

The template effect involves the organization of an assembly of atoms with respect to one or more geometric positions in order to achieve a particular linking of atoms .This effect recognizes thus, a molecular organization. Further, the chemical template is recognized as an evoluate and a complex molecular organizer. This is in contradiction with a primitive system where no organization does exit. The template effect involves the presence of other effects and phenomena which are

also manifestation of molecular organization process: Coordination of ligands, chelate effect, and macro cyclic effect [15].

1.3.3. Factors Affecting the Product of a Template Reaction

1.3.3.1. Coordination of Ligands

The coordination of ligands to metal ions involves electronic factors as well as geometric relation ship between the two parts Ahzland et al ,divided the acceptor metal ions in to two classes depending on whether they form more stable complexes with the smaller donor atoms as nitrogen, oxygen or fluorine or the large ones, which include sulfur , chloride or phosphorus. Regarding the formation of the complex compounds as Lewis acids and bases, Pearson elaborated the theory and principles of hard and soft acids and bases (HSAB) as it is shown in table 3. In accord with HSAB theory, hard acids react preferentially with hard bases and soft acids prefer soft bases. Although the classification is very useful, there are many metal ions which lie in a borderline region.

According to HSAB theory the preference of alkaline and alkaline earth metal ions for the oxygen donors and their template activity to form crown ethers can be understood and also the preference of the transition metal ions toward nitrogen donors.

Metal- template involves the reaction of the coordinated ligands .An effective template metal ion binds strongly to the donor atom of the precursor as well as of the result ligand and in this frame the above theories work also. The polarization induced by the coordination of the ligands to the metal ions favours the nucleophilic attack of reactants. Therefore, the terminal groups must be able to undergo a characteristic addition with the new strap forming groups and for this, special steric requirements for the pre-existing ligands are also needed. They have to occupy a conveyable cis position.

The metal ion exerts a preference for a particular kind of environment and some ligands are better able to conform to that environment than others. In addition, each ligand has its preference for a particular geometrical arrangement. The organization of metal ions with specific electronic properties within a potentially template ligand system can lead to the appropriate orientation of substrate molecules, (the pre-existing ligands in the precursor molecules) required for particular reactions [15].

Table 1: Classification of Acids and Bases according to HSAB

Acids	
Hard	Soft
H^+, Na ,Mg^{2+} , Al^{3+} , Cr^{3+} , Fe^{3+} , Co^{3+}	Cu^+ , Ag+ , Cd^{2+} , Pd^{2+}
Borderline	
Fe^{2+} , Co^{2+} , Ni^{2+}, Cu^{2+}	
Bases	
Hard	Soft
H_2O , OH^- , $CH3COO^-$, Cl^- , RO^- , RNH_2 , H_2NNH_2	RSH , RS^- , CO

1.3.3.2. The Chelate Effect

Empirical observations as well as quantitative studies have established that metal chelate complexes are more stable than those of related unidentate ligands. This is called the chelate effect. In terms of chemical thermodynamics the chelate effect is described by the following equation.

$$\log K_1(\text{polydentate}) = \log ß_n\ (\text{unidentate}) + (n-1)\log 55.5$$

where $\log K_1$ (poldentate) refers to the stability of the complex of an n-dentate polydentate ligand , $\log ß_n$ (unidentate) refers to the stabilityof the complex contiaing n unidentate analogues of the polydentate ligand,and 55.5 is the molarity of water[15].

1.3.3.3. Macrocyclic Effect

A metal template synthesis has been often used to obtain macrocyclic ligands in a simple way avoiding the organic routes which are-as a rule-a multistep task. The macrocycles thus obtained are mostly 13-to16-membered ring ligands.The most common are the polyamine ligands with four nitrogen donors situated in a plane although they can contain five or six nitrogen atoms in the ring closing a macrocycle around a labile metal center with the formation of new five– or six- membered chelate rings. Other donor atoms like oxygen, sulphur, phosphorus or arsenic can participate as donors in a macro cycle.

Macrocyclic ligands render high thermodynamic stability and exceptionally inertness of their metal complexes against ligand substitution or dissociation compared to their open- chain analogues. This is the largest known " macrocyclic effect".

Among the general factors influencing a metal-ion –controlled synthesis of marocyclic ligands, the relationship between the size of the metal ion and the opening in the middle of the ring clearly is important [15].

1.3.4. Advantages of Metal Template Reaction

Metal template reactions offer simple ways to obtain organic molecules which otherwise involve complicated organic routes, high amounts of solvents ,small yields and high costs .The organic molecules act as ligands and the high stability of the complexes allows reaction at the coordinated ligands with complex destruction .

The ligands obtained by a metal template route can be released by demetallation in a reaction with cyanide. The free ligand can then be used further to obtain complexes of other metal ions by a direct synthesis [15].

1.4. Metallo-Elements under Investigation

The chemistry and biology of the metallo-elements under study are briefly described in the following sections.

1.4.1. Zinc—its chemistry and biology

Zinc is found at the end of first transition series. Its valence shell electron configuration is $3d^{10}4S^2$. The almost invariable oxidation state of Zn is 2+ because of its complete d- shell and two additional s-electrons. Thus, Zn is not directly involved in redox processes. It has the ability to accommodate the different geometry requirements involving changes in the co-ordination number of zinc [16].

Zinc forms stable complexes with ligands containing N, S, O, halides and CN^-. Compounds of Zn (II) ions are characteristically diamagnetic and colorless. The d^{10} configuration affords no crystal field stabilization, hence Zn (II) complexes do not possess any d – d transition. Stereochemistry of a particular compound depends on the ligand size, polarizing power of the zinc(II) cation and steric requirements of the ligands. Thus zinc(II) favours four coordinated tetrahedral complexes. It generally forms planar complexes but many octahedral complexes are also known. Coordination numbers higher than six are rare.

Zinc is biologically one of the most important metals and is apparently necessary to all forms of life. The body of an adult human contains about 2 grams of zinc but its concentration is very low. Because of its d^{10} electron configuration (invariable oxidation state), Zinc tends to

form stable complexes with proteins and enzymes, it does not mediate the generation of reactive radical species like $OH\cdot$ and $O_2\cdot$ that cause cell damage [16]

Zinc plays an essential role in biological systems, because it is constituent of more than 300 enzymes. The active sites of many of Zn-containing enzymes feature a tetrahedrally coordinated zinc center that is attached to the protein backbone by three amino acid residues, with a fourth site being occupied by a water molecule (Figure 5).

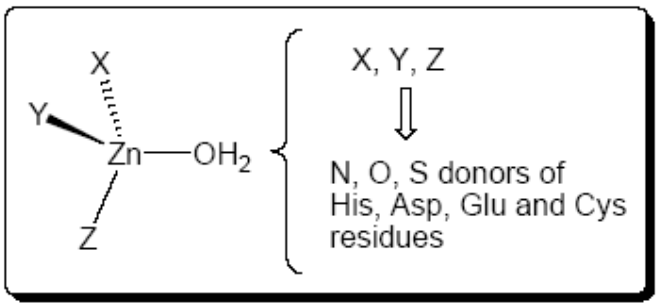


Figure-5: A common structural feature of zinc enzymes. The residues which bind zinc to protein are typically His, Glu, Asp and Cys.

Following are the properties of Zn (II) that are pertinent to its role in enzymes. First is its redox properties: Zn(II) ion is very stable with respect to oxidation and reduction and so it does not participate in redox reactions, in contrast to Cu, Fe or Mn. The second very important property of Zn(II) is its coordination geometry: the d^{10} configuration of Zn^{2+} indicates that zinc complexes are not subject to ligand field stabilization effects and so coordination number , and geometry is only dictated by ligand size and charge. In enzymes, zinc shows strong preference for tetrahedral coordination, which enhances both the Lewis acidity of the zinc center, and the Brönsted acidity of coordinated water molecules. Zinc is an element of borderline hardness. In its ligand binding properties, zinc shows its favour to bind to nitrogen, sulphur and oxygen atoms, and therefore

zinc can strongly bind to many proteins.
What is also very important is that anions, such as OH-, OR^- and SR^-, retain a nucleophilic character when coordinated to zinc[17].

1.4.2. The chemistry of Ni (II) complexes

Nickel (Ni) is a member of first transition series. It has valence shell configuration of $3d^8 4s^{2.}$ It generally exists in zero and +2 oxidation states. Nickel in zero oxidation state forms very stable complexes with tetrahedral geometry. The electronic configuration of the Ni (II) is d^8. Ni^{2+} may have a variety of co-ordination numbers and geometries, the main structural types being octahedral, tetrahedral, and square-planar [18].

Octahedral complexes: Octahedral nickel (II) complexes having $^3A_{2g}$ ground state are expected to have three spin-allowed transitions [19]: $^3A_{2g} \rightarrow {}^3T_{2g}$, $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) in the ranges of 7000-13000, 11000-20000 and 19000-27000 cm-1 respectively [20]. Two spin forbidden transitions: $^3A_{2g} \rightarrow {}^1E_g$ and $^3A_{2g} \rightarrow {}^1T_{2g}$ are also observed near the second spin-allowed transition, and between second and third spin-allowed transitions respectively.

Octahedral nickel (II) complexes have two unpaired electrons and thus possess magnetic moments ranging from 2.9 to 3.4 B.M. depending on the orbital angular momentum contribution [18].

Tetrahedral complexes: For regular or nearly regular tetrahedral complexes there are characteristic spectral and magnetic properties. Irregular geometries are less likely to meet these specifications [18].The tetrahedral nickel (II) complexes with $^3T_1$ (F) ground state generally exhibit four transitions [20].$^3T_1 \rightarrow {}^3A_2$, $^3T_1 \rightarrow {}^1E$, $^3T_1 \rightarrow {}^3T_1$ (P) and $^3T_2 \rightarrow {}^1T_1$.The band $^3T1 \rightarrow {}^3T1$ (P) is a strong band of high intensity compared with others. The transition from the ground $^3T_1$ (F) state to

the $^3T_1$ (P) state occurs in the visible region (~15,000 cm-1) and is relatively strong ($\varepsilon \approx 10^2$) compared to the corresponding $^3A_{2g} \rightarrow {}^3T_{1g}$ transition in octahedral complexes. Thus tetrahedral complexes are generally strongly colored and tend to be blue or green unless the ligands also have absorption bands in the visible region [18]. Because the ground state $^3T_1$ (F) has much inherent orbital angular momentum, the magnetic moments of truly tetrahedral Ni (II) should be about 4.2 B.M. at room temperature. However, even slight distortions reduce this value markedly (by splitting the orbital degeneracy).Thus fairly regular tetrahedral complexes have moments of 3.5 to 4.0 B.M.; for the more distorted ones the moments are 3.0 to 3.5 B.M. (i.e., in the same range as six coordinated complexes) [18].

Square planar complexes: For the vast majority of four coordinated nickel (II) complexes, square planar geometry is preferred. This is a natural consequence of the d^8 configuration, since the square planar ligand set causes one of the d orbitals (d_{x2-y2}) to be uniquely high in energy and the eight electrons can occupy the other four d orbitals but leave this strongly antibonding one vacant [18].

In tetrahedral coordination, on the otherhand, occupation of antibonding orbitals is unavoidable. Square Planar complexes of nickel (II) are thus invariably diamagnetic [21]. They are frequently red, yellow or brown owing to the presence of the absorption band of medium intensity ($\varepsilon \approx 60$) in the range 450-600 nm, but other colors do occur when additional absorption bands are present.

In square planar nickel (II) complexes, three spin allowed d-d bands corresponding to $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$, and $^1A_{1g} \rightarrow {}^1E_g$ transitions are expected. Majority of the square planar nickel (II) complexes exhibit strong absorptions in 15000-25000 and 23000-30000 cm^{-1} region.

Square planar nickel (II) complexes do not have any absorption band below 10000 cm-1, due to large crystal field splitting [18]. Hence they can be clearly distinguished from octahedral and tetrahedral complexes.

1.5. Objectives and Scope of the Present Investigation

Much attention has been given by researchers to the synthesis of metal complexes derived from thiosemicarbazide and carbonyl containing compounds like pyridoxal, salysaldehyde, etc. This is because of not only their chelating or complexation ability but also their broad spectrum of catalytic, analytical, medicinal and biological applications. However, no literature has been available on metal complexes derived from thiosemicarbazide [2-7] and ninhydrin [22-28] system. This new system has been started as a project work since 2007 in the inorganic research laboratory, AAU [29, 30]. The direct method of synthesis was applied for the synthesis and metal complexes of a cyclic ligand were obtained. However, the biological activities of these complexes were not studied**.**

The objective and scope of the present investigation was to prepare, characterize the chemical structure, and investigate the biological activity of the prepared metal complexes derived from ninhydrin and thiosemicarbazide involving template method. Analytical, spectral (IR, ^{1}H NMR, ^{13}C NMR, UV-Vis, AAS), conductance and magnetic susceptibility measurements will be applied to characterize the metal (Zn and Ni) complexes.

2. Experimental Part

2.1. Materials and Methods

2.1.1. Chemicals

Most chemicals used in this work were reagent grade (AR/Aldrich). Included are zinc acetate [$Zn(C_2H_4O_2).2H_2O$],$NiCl_2.6H_2O$, $K_4[Fe(CN)_6]$,dimethylglyoxime,$FeCl_3$,HCl,ninhydrin, thiosemicarbazide, ethanol,methanol,$AgNO_3$,DMSO,DMF,HNO_3,acetonitril,petroleumether,chloroform, distilled water, deionized water, (deuterated) DMSO, D_2O water, Nitric acid, ammonia solution, ethyl acetate, and nitromethane.

2.1.2. Instrumentation

Melting points were determined on Electro thermal IA 92000, digital melting point apparatus. The magnetic susceptibility of complexes was measured by using MSB-AUTO, Sherwood Magnetic Balance. Molar conductivities of complexes were recorded at room temperature using EC 214 (HANNA Instrument) conductivity meter. The metal estimation of the complexes was done using BUCKER MODEL SCIENTIFIC 210 VGB flame atomic absorption spectrophotometer. IR spectra of all the samples reported in this paper were recorded using Perkin-Elmer BTX FT-IR in the range of 4000-400 cm^{-1} using KBr pellet as reference material. The electronic absorption spectra were measured in the UV and Visible region (200-900nm) by SPECTRONIC GENESY'S 2PC UV-Vis spectrophotometer. NMR data were collected using BRUKER 400MHZ (^1H NMR) and 100.06 MHZ (^{13}C NMR) ultra shielded NMR spectrometer with TMS internal reference.

2.1.3. Methods

2.1.3.1. Qualitative Tests

I. Thin Layer Chromatography (TLC)

TLC was applied to evaluate the progress of a reaction in the process of synthesis. A 2x4 cm silica coated aluminum plate with ethyl acetate as mobile phase was used. TLC provides information about the purity of a substance (marking a single spot via Uv-box observation).

II.Chloride Test

A sample of the complex was digested in concentrated nitric acid. After cooling the remaining drops of the digested product, 0.1M solution of silver nitrate was added. Formation of white precipitate suggests the presence of chloride in the sample. Disappearance of the white precipitate on adding ammonia solution and regenerarion of the white precipitate on dropping few drops of concentrated nitric acid confirms the presence of chlorides.

III. Acetate Test

A sample of the complex was acidified with dilute nitric acid followed by neutralization with ammonium hydroxide. Ferric chloride solution was added to this neutral solution to observe brown-coloured solution. This solution was boiled and then cooled to get red-brown precipitate. Hydrochloric acid was added to the red brown precipitate to give yellow solution completing the test for the presence of acetates.

IV. Zn^{2+} - test

A sample of the complex was digested through refluxing in concentrated nitric acid and the test for Zn^{2+} ions was carried out.

V.Ni^{2+}- test

A sample of the complex was digested through refluxing in concentrated nitric acid and the chemical test for Ni^{2+} ions was conducted.

2.1.3.2. Quantitative Determination

Metal Estimation, molar conductance measurement, and magnetic susceptibility measurement were among the quantitative determinations carried out.

2.2. Syntheses of the Complexes

2.2.1. Template Synthesis of Nickel (II) complex

To a solution of ninhydrin (2.806 mmol, 0.5 g, Mwt=178.14 g/mol) in ethanol (2o mL), a solution of the metal salt $NiCl_2.6H_2O$ (2.806 mmol, 0.6672 g, Fw= 237.71/mol) in ethanol (25 mL) was added slowly with stirring and the mixture was refluxed for 2h until the light green colour of the mixture turned pale yellow. To this hot mixture , a solution of thiosemicarbazide (2.806 mmol, 0.2557 g, Mwt= 91.14 g/mol) in hot ethanol (40 mL) was added and further refluxed for 8 h until silvery gray precipitate was formed. Reactants were mixed in a 1:1:1 ratio. The resulting silvery gray precipitate was filtered off, washed with ethanol, and dried in vacuo in a desiccator over anhydrous calcium chloride at room temperature. Yield: 55%

2.2.2. Template Synthesis of Zinc (II) complex

Similar reaction condition was used as in section 2.2.1 where, in this case, to a solution of ninhydrin (2.806 mmol, 0.5 g, Mwt=178.14 g/mol) in ethanol (2o mL), a solution of the metal salt $Zn(AcO)_2{\cdot}2H_2O$ (2.806 mmol, 0.6159 g, Fw= 219.5 g/mol) in methanol (20 mL) was added slowly with stirring and the mixture refluxed for 2h until the pale yellow

colour of the mixture turned dark-brown. To this hot mixture, a solution of thiosemicarbazide (2.806 mmol, 0.2557 g, Mwt= 91.14g/mol) in hot ethanol (40 mL) was added and further refluxed 12 h until light brown precipitate was formed. Reactants were mixed in a 1:1:1 ratio. The resulting light brown precipitate was filtered off, washed with ethanol, and dried in vacuo in a desiccator over anhydrous calcium chloride at room temperature. Yield: 74.5%.

2.3. Antimicrobial Activity

Antimicrobial activities of the complexes and starting materials against different strains of bacteria Salmonella typhimurium (S.T), Escherichia coli (E.C), Pseudomonas aeruginosa (P.A), and Staphylococcus Aureus (S.A) were investigated by the disc diffusion method. Mueller-Hinton agar was the medium used for all bacterial strains. Samples of 25 mg/ml stock solutions in DMSO and the references (TTC and DMSO) were applied to the diffusion wells of the inoculated plates. The Mueller-Hinton agar plates were then incubated at 32-35°c for 18-24h. The diameter of the inhibition zones was measured [31].

3. Results and Discussion

3.1. Thin Layer Chromatography

A single spot was observed on a TLC plate to which solutions of each of Zn (II) and Ni (II) complexes compared to their starting materials were applied. Appearance of distinct spots suggested that the complexes were pure.

3.2. Solubility of Starting Materials and Complexes

The observed solubilities of starting materials and complexes in different solvents are described in Table 2 below.

Table 2: Solubility of ninhydrin, thiosemicarbazide, Zn (II) complex and Ni (II) complex

Solvent	Dielectric constant	Ninhydrin	Thiosemicarbazide	Zn (II) complex	Ni (II) complex
Water	80.4	ins	sol	ins	ins
DMSO	48	sol	sol	sol	sol
DMF	36.7	sol	sol	sol	sp
Acetonitrile	36.2	sol	sp	sp	sp
Methanol	32.6	sol	sp	sp	sp
Ethanol	24.3	sol	sp	sp	sp
Chloroform	4.8	_	_	sp	_

Key: ε = dielectric constant, Sp = sparingly soluble, Sol = Soluble, INS = Insoluble, and — = not checked.

3.3. Some Physical Properties of the Starting Materials and Complexes

Some of the physical properties of the starting materials and complexes are described in Table 3 below.

Table 3: some physical properties of the ligands and complexes

Compound	Molecular formula	MW (g/mol)	M.P,^{0}c (Dec. T)	Color and state	Yield %
Ninhydrin	$C_9H_6O_4$	178.14	150	pale yellow solid	
Thiosemicarbazide	CH_5N_3S	91.14	181-182	white solid	
Zn (II) complex	$[C_{12}H9O_4N_3SZn].C_2H_5OH$	401.4	230	light brown solid	74.5
Ni (II) complex	$C_{10}H_7O_3N_3SNi$	307.7	294-295	silvery gray solid	55

3.4. Analytical Studies

3.4.1. Chloride Test

The salt used for the synthesis of Ni (II) complex was $NiCl_2.6H_2O$; however, the chloride test carried out for Ni (II) complex following the procedure given in section 2.3.1.II was negative. This indicates that Ni (II) complex does not constitute chlorine in its structure.

3.4.2. Acetate Test

The salt used for the synthesis of Zn (II) complex was hydrated zinc acetate. Positive result was obtained for the acetate test applied to Zn (II) complex following the procedure given in section 2.1.3.1.II. This suggests the presence of acetate ion in the coordination (inner) sphere of the complex (as supported by the conductance data).

3.4.3. Zinc Test

The positive chemical test, according to the procedure in section 2.1.3.1.IV, supported by the AAS data, confirmed that Zn^{2+} is contained in the complex.

3.4.4. Nickel Test

The positive chemical test, according to the procedure in section 2.1.3.1.V, supported by the AAS data, confirmed that Ni^{2+} is contained in the complex.

3.4.5. AAS for Metal Estimation

18.8 mg and 17.2mg of Zn (II) and Ni (II) complexes were digested in 45 mL and 25mL, respectively, of concentrated HNO_3. Solutions of the digested product were diluted to 50 ml from which 1ml was further diluted such that sample concentration within the range of standard concentrations was registered by the atomic absorption Spectrometer.

Results obtained were 16.7% Zinc and 19.02% Nickel suggesting a 1:1 metal to ligand ratio as given in Table 7.

3.4.6. Elemental Analysis

Results obtained from the elemental analyses of Ni(II) and Zn(II) complexes, respectively, given as calculated (found) were % H 2.27(3.19] and % H 2.5(2.8). While metal contents were estimated from AAS data as % M 19.08 (19.02), and %M 16.3 (16.7). Regarding the metal content, theoretical and experimental (AAS) values are in good agreement suggesting a metal to ligand ratio of 1:1 in both complexes according to the proposed structures. However, deviations were observed between the theoretical and experimental values of hydrogen and the other elements. This might be because suitable temperature to decompose the complex was not applied or might be due to impurity of the complex.

3.4.7. Conductivity Data of the Metal Complexes

The molar conductance (Λ m) values were obtained from conductivity measurements in DMF solvent at 25°c. According to the molar conductance, Λ_M, ranges of electrolytes in DMF at 25°c as shown in Table 6 and 7 below, Ni(II) and Zn(II) complexes (of 10^{-3}M each) of Λ_M values equal to 45 and 6 mho cm^2mol^{-1}, respectively, are non-electrolytes. This verifies that outer sphere counter anions are not contained in both complexes or electrical neutrality of the complexes is maintained by the inner sphere anionic ligands.

Table 4: Molar conductance ranges and corresponding electrolyte types in DMF

Solvent (25^0c)	Range of molar conductance values and electrolyte type			
DMF	65-90	130-170	200-240	
	1:1	2:1	3:1	4:1

3.4.8. Magnetic Susceptibility

Zinc (II) is a d^{10} system of completely filled d-orbitals exhibiting diamagnetism. Nickel (II), however, can exist in different spin states and its magnetic susceptibility was measured. The gram magnetic susceptibility,Xg, obtained at 25°c for Ni(II) complex was a negative value indicating diamagnetism. Magnetic data, therefore, suggest that the geometry of Ni (II) complex is square planar.

Table 5: AAS, Analytical, molar conductance, and magnetic susceptibility data of the complexes.

Compound/ complex	%calculated(estimated from AAS data)	%calculated(found)		Λ_m (mho cm^2 mol^{-1})	μ_{eff} (BM)	M:L ratio
	Mw	M(AAS)	H			
$C_{10}H_7O_3N_3SNi$	307.7 (308.62)	19.08(19.02)	2.27(3.19)	45	–	1:1
$[C_{12}H_9O_4N_3SZn]$ $.C_2H_5OH$	401.4 (391.6]	16.3 (16.7)	3.5(2.8)	6	–	1:1

3.5. Physical Techniques of Characterizing the Complexes

3.5.1. IR Spectra (of the Complexes and Starting Materials)

The binding of starting materials (ligands) with metallo-elements was investigated by comparing the FT-IR spectra of the complexes with those of the starting materials. The disappearance, new appearance and shift in IR-absorption bands of the complexes compared to that of the starting materials indicate the occurrence of reaction and the metal to ligand binding sites [32]. In Table 6 below are the IR spectral data of Ni (II) complex and the starting materials.

Table 6: Infrared spectral data of the starting materials and Ni (II) complex, ν (cm^{-1})

compound	υ(NH_2)	υ(C=N)	υ(CS)	υ(C=O)	υ(OH)	υ(MO)	υ(MN)	υ(MS)
Thiosemicarbazide	3368.9 m 3262.9 m 3179.5 m		801.7 s					
Ninhydrin				1748.2 s 1768 w 1717.3 s				
Ni (II) complex	3350.3 m 3254.3 m	1569.8 w	703.1 s	1638 s 1607 w	3066.6 m	594.6 m	436 m	365 m

Where m = medium, s = strong, and w = weak.

The bands (in the spectrum of thiosemicarbazide) at 3368.89m and 3262.86m, attributed to primary amine N-H stretching vibration, ν(NH2), shift to 3350.36m and 3254.36m in the complex, as a consequence of the coordination of the sulfur from the C=S(NH2) group, as observed by other authors [33].

The band at 3179.53 cm–1 observed in the ligand (thiosemicarbazide) for the secondary amine N-H stretch is absent in the complex, suggesting deprotonation of –NH of the ligand via tautomerism /thioenolisation [29]. The ν (CS) at 801.76cm-1(s) in the free ligand (Appendix 1) has shifted to 703.17cm-1(s) in the complex. This band shifts 45 to 110 cm-1 to lower energy when coordination occurs with deprotonation at N (2) and formation of a single C-S bond [34].

The nature of metal–ligand bonding is confirmed by the newly formed bands assigned to M-O, M-N, and M-S. Bands at 594.65cm^{-1}, 436.19cm^{-1}, 365cm^{-1} are assignable to ν (M–O), ν (M–N), and ν (M-S) modes respectively (Appendix 4) [32].

The absence of broad band around 3400cm^{-1} and m-w number of bands in the region 900-300cm^{-1} excludes the presence of water in the complex. Absence of band in the region 2600-2500cm-1 also excludes S-H vibration supporting deprotonation. On the other hand, appearance of new bands (br) around 3000cm^{-1} suggests the presence of OH stretch in the complex.

The ν(C=O) shown for the free ninhydrin at 1748.18cm^{-1}(s) and 1717.30cm^{-1}(s) of the 1,3-dicarbonyls, and 1768cm^{-1}(w) of the middle carbonyl(which is in equilibrium with the dihydroxy species) changed to 1638.18cm-1(s), 1607.55cm-1(w), and disappearance in the complex in their respective positions.

(I) Disappearance of the band at 1768cm^{-1}(w) and appearance of new weak band at 1569 cm^{-1}(w) suggested condensation of the middle carbonyl and the formation of C=N bond which is coordinated to the metal ion center via the imine nitrogen.

(II) The coordination of one of the 1, 3-dicarbonyls was also shown by the shift in the absorption frequency of 1717 cm^{-1}(s) of free ninhydrin (Appendix 2) to 1607 cm^{-1}(w) in the complex.

Note that the IR spectral data of the Zn (II) complex were not collected due to lack of the instrument. However, analogous IR absorption frequencies to that of the Ni (II) complex are expected for the Zn (II) complex since the syntheses were conducted under similar reaction conditions.

3.5.2. UV-Vis (electronic) Spectra of the Complexes

The electronic absorption spectra are often very helpful in the evaluation of results obtained from other methods of structural investigation. The electronic spectral measurements were used for

assigning the stereochemistries of metal ions in the complexes based on the positions and number of d–d transition peaks [33]. The electronic absorption spectra of Ni (II) and Zn (II) complexes were recorded at room temperature using DMSO as solvent.

Electronic absorption spectra of Ni(II) complex (appendix 9): The appearance of bands at 388nm (25,773cm^{-1}) and 609nm (16,420cm^{-1}) (Table10) due to d-d transitions (Appendix 10) favours square planar geometry as these values lie in the absorption ranges of most square planar Ni(II) complexes which is also consistent with the magnetic datum of the complex showing diamagnetism. A diamagnetic Ni (II) complex is invariably square planar. The bands shown at 36764$cm^{-1}$and 34722cm^{-1} could be assigned to intraligand ($\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$) charge transitions (INCTs). Furthermore the absence of any band below 10,000 cm^{-1} eliminates the possibility of a tetrahedral environment in this complex [32, 33, and 41].

Electronic absorption spectra of Zn (II) complex (Appendix 11): No transitions were observed in the visible region for Zn (II) complex consistent with the d^{10} configuration of the Zn(II) ion. This complex is also found to be diamagnetic as expected for d^{10}configuration [38].

The electronic spectrum of the Zn (II) complex shows absorptions due to charge transfer transitins(INCT and LMCT). Bands at 38610cm^{-1}(s), 32679cm^{-1}(w) ,and 26809cm^{-1} (w) could be assignd to the INCT absorptions ($\pi \rightarrow \pi^*$and $n \rightarrow \pi^*$) and at 23364cm^{-1} could be due to LMCT transitions (Table 7).

Table 7: Electonic absorption spectral data of Ni (II) and Zn (II) complexes

compound	solvent	Absorption(cm^{-1})	Band assignment	geometry
Ni(II) complex	DMSO	36764 34722 25773 16420	INCT INCT $^1A_{1g} \rightarrow {}^1E_g$ $^1A_{1g} \rightarrow {}^1B_{1g}$	Square planar
Zn(II) complex	DMSO	38610 32679 26809 23364	INCT INCT INCT LMCT	

3.5.3. NMR Spectral Analysis in DMSO-d_6 of Zn (II) complex

3.5.3.1. ^{1}H NMR and D2O exchange spectral analysis

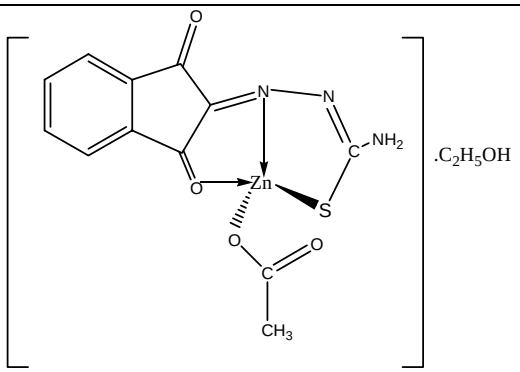
^{1}H NMR spectrum: The signals in the six different chemical shift (δ_{ppm}) regions (Appendix 5) could be assigned (from right to left) to CH_3 ($\delta_{1.07ppm}$ of alc.), CH_3 ($\delta_{1.84ppm,}$ acetyl), CH_2 ($\delta_{2.5ppm,}$ alc.), OH (δ4.4ppm, signal of alc.), CH (δ7.5-7.8ppm, multiplets of arom. H), and NH_2 (δ10.3ppm).

D2O exchange NMR spectrum: The disappearance of signals (Appendix 6) which were shown at $\delta_{4.4ppm\ and}$ $\delta_{10.3ppm}$ in the ^{1}H NMR spectrum suggests the presence of two types of acidic protons. The one at $\delta_{4.4ppm}$ could belong to the OH proton of the alcohol part while that of $\delta_{10.3ppm}$ could be assigned to the NH_2 protons. A signal which was not shown in

the ^{1}H NMR spectrum is shown at $\delta_{3.4ppm}$ which may be due to an impurity.

The data obtained from the ^{1}H NMR and D_2O exchange NMR spectra suggested the presence of ethanol (reaction medium) residued with the Zn (II) complex.

Table 8: ^{1}H NMR and D2O exchange spectral data of Zn (II) complex in DMSO-d_6

Structure of the complex	^{1}H NMR		D2O exchange
	Type of proton	δ_{ppm}	δ_{ppm}
 .C_2H_5OH	CH3 (eth.)	1.07	1.07
	CH_3 (acetyl)	1.08	1.08
	$CH_{2(eth)}$	2.5	2.5
	OH	4.4	–
	CH (arom.)	7.5-7.8	7.5-7.8
	NH_2	10.3	–
	X (may be impurity)	–	3.4

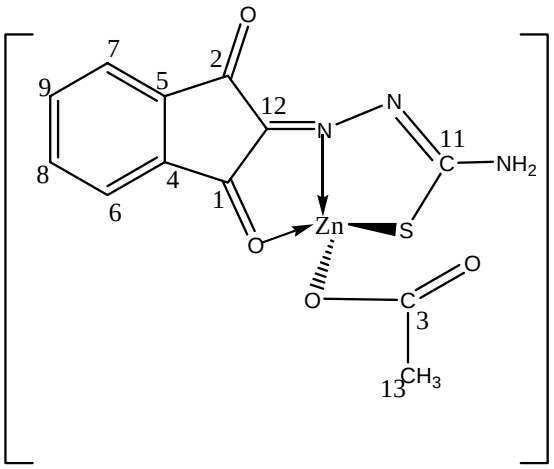
3.5.3.2. ^{13}C {H} NMR and ^{13}C DEPT Spectral Analysis

^{13}C {H} NMR spectrum: Generally, the number of signals in the ^{13}C {H} NMR spectrum corresponds to the number of magnetically non-equivalent carbon atoms in the complex. Appendix 7 shows thirteen distinct signals. A signal expected for the acetyl CH_3 is not shown. This

may due to an overlap with the signal of the solvent (DMSO) at around $\delta_{39\text{-}40ppm}$ as clearly indicated in the ^{13}C DEPT spectrum (Appendix 8).

^{13}C DEPT spectrum contains only signals arising from protonated carbons (non protonated carbons do not give signals in the ^{13}C DEPT spectrum). The signals from CH_3 and CH groups point upwards while signals from CH_2 groups point downwards (Appendix 8). Here, the signal for the acetyl CH_3 (masked or not shown in the ^{13}C {H} NMR) is shown around δ_{39ppm} adjacent to the solvent (DMSO, δ_{40ppm}). In the aromatic region, however, six signals are shown in stead of four. These extra signals shown in the ^{13}C DEPT spectrum may have impurity origin. The data obtained from ^{13}C {H} NMR spectrum and ^{13}C DEPT spectrum are shown in Table 12.

Table 9: Assignment of chemical shifts to carbons of ^{13}C {H} NMR and ^{13}C DEPT spectra of Zn (II) complex

Structure of Zn (II) complex	^{1} ^{3}C {H} NMR spectra	^{13}CDEPT spectra
 .CH_3CH_2OH 14 10	Type of C, δ_{ppm}	Type of C, δ_{ppm}
	C_1, δ_{192}	
	C_2, δ_{189}	
	C_3, δ_{170}	
	C_4, δ_{136}	
	C_5, δ_{135}	
	C_6, δ_{134}	C_6, δ 134
	C_7, δ_{129}	C_7, δ_{129}
	C_8, $\delta 1_{25}$	C_8, δ_{123}
	C_9, δ_{123}	C_9, δ120
	C_{10}, δ_{56}	C_{10}, δ_{55}
	C_{11}, δ_{80}	
	C_{12}, δ_{70}	
	C_{13}, δ—	C_{13}, δ_{39}
	C_{14}, δ18	C_{14}, δ_{18}

It can be noted that the presence of ethanol (the reaction media) with the complex is consistent in both the ^{1}H NMR and ^{13}C NMR data (Table 11 and 12). Heating the complex at a temperature below the decomposition temperature of the complex may drive the alcohol (residual) off.

Note, however, that the NMR spectrum of Ni (II) complex was not included in the report because the spectrum obtained was rather with unresolved bands than isolated signals. To get additional information about its oxidation state or redox nature and hence magnetic property, Cyclic Voltammetric study was also carried out.

3.5.4. Cyclic Voltammetric study

Cyclic voltammetry is the most versatile electroanalytical technique for the study of electroactive species [34]. The electrochemical (redox) behaviour of nickel (II) complex was studied by CV in DMSO at room temperature (appendix 12) in the presence of a supporting electrolyte $(C_2H_5)_4NBF_4)$.

Comparative cyclic voltammetric study between the free state Ni (II) ion obtained from$NiCl_2.6H_2O$ and Ni (II) complex was conducted. In the cyclic voltammogram shown (Appendix 12i), the redox process shows no potential peak for the free state Ni(II) while a small peak is shown for the Ni(II) complex which may belong to the oxidation of the ligand. On the other hand, comparative reduction peaks are shown both for the free state Ni (II) and Ni (II) complex. Appearance of comparable reduction peaks suggests the existence of nickel in (+2) oxidation state in the complex. The slight difference in their reduction peak is due to the ligand effect (Appendix 12-ii). The small reduction peak shown in the reduction curve of Ni (II) complex may also be due to reduction of the ligand [35, 36, 37, and 38].

3.6. Antimicrobial Effects

The starting materials and their metal complexes were tested against the bacterial species Salmonella typhimurium (S.T), Escherichia coli (E.C), Pseudomonas aeruginosa (P.A), and Staphylococcus Aureus (S.A). Tetracycline (TTC) as a standard antibacterial agent or reference was evaluated for their antibacterial activity and the result was compared with the free ligands and their metal complexes. Solvent effect (DMSO) was also investigated. Tests were made in duplicates and the average inhibition zones (in mm, millimeters of diameters) measured after 18h were used to compare the effects as shown Table 12 where the photographic image is shown in Appendix 12.

Table 10: Comparison of the biological activities of the synthesized compounds, starting materials and references

Compounds	Average inhibition zones (mm)			
	S.T	E.C	P.A	S.A
TTC	-	18.5	9	23.5
Ninhydrin	13.5	14	10	14.5
TSC	-	24.5	18	6.5
Ni(II) complex	16	16.5	12	13
Zn (II) complex	8	9	-	12
DMSO (solvent)	-	-	-	-

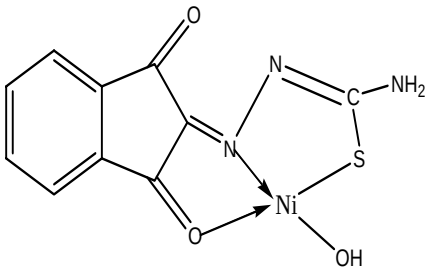
Results obtained from bacterial sensitivity test showed that Ni (II) complex has a greater effect than Zn (II) complex and one or both of the starting materials on the test microorganisms. On S.T and P.A, Ni (II) complex has even a greater effect than the standard. Zn (II) complex showed a greater effect on S.A than TSC only.

Salmonella typhimurium (S.T) is resistant to TTC (the standard) and TSC (starting material), and Pseudomonas aeruginosa (P.A) is resistant to Zn (II) complex while none of these bacterial strains was resistant to Ni (II) complex and ninhydrin (starting material).

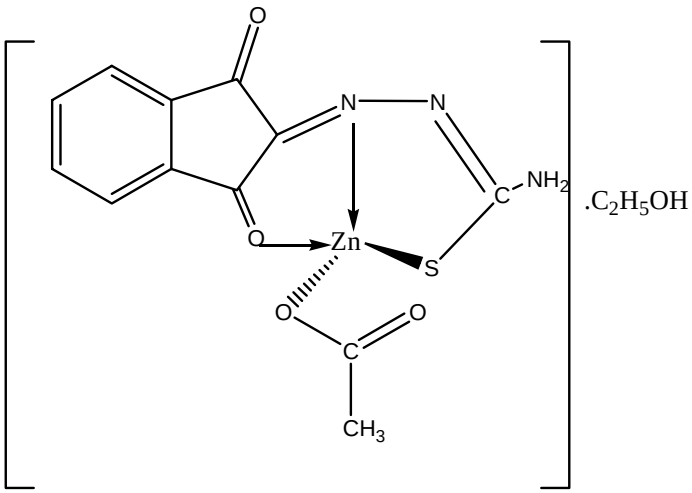
Generally, the observed enhancement on the effect of antibacterial agents (ninhydrin and thiosemicarbazide) up on complexation could be explained on the basis of chelation theory [21, 39, and 40]. Further investigation on the biological activities of these complexes *invivo* is recommended for their pharmacological and medicinal applications.

3.7 Structures of Complexes

Baesd on the magnetic, conductance, AAS, and spectral data, proposed geometries of the Ni(II) complex (square planar) and Zn(II) complex (tetrahedral) synthesized by template method are shown in figure 11(i&ii)



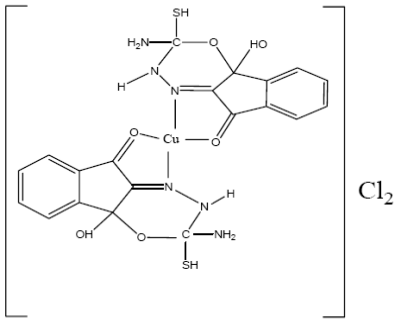
(i)Ni(II)complex (Squar planar)



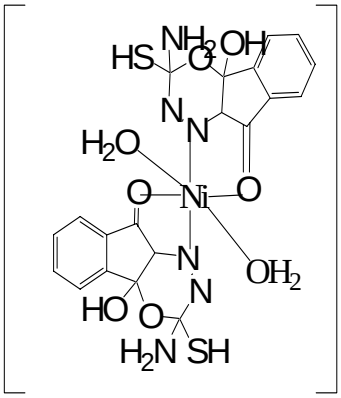
(ii) Zn (II) complex (Tetrahedral)

Figure-11(I & ii): Proposed structures of Ni (II) and Zn (II) complexes derived ninhydrin and thiosemicarbazide synthesized by template method.

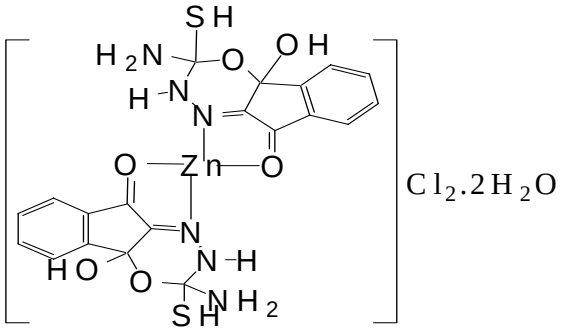
The square planar Ni (II) complex and tetrahedral Zn (II) complex obtained by template synthesis could be compared with those obtained by the direct method of synthesis from the same starting materials. For comparison, the proposed structures of Cu (II), Ni (II), and Zn (II) complexes obtained by the direct method of synthesis are shown in figure 12(I, ii, &iii) below as reported in the years July 2007 and 2008 from this same research center.



(i) Square planar



(ii) Octahedral



(iii) Tetrahedral

Figure-12: Proposed structures of M (II) complexes (I, ii& iii) of ninhydrin thiosemicarbazone synthesized by direct method, where M= $Cu^{2+,}$ Ni^{2+} & Zn^{2+}.

4. Conclusion

New Nickel (II) and Zn (II) complexes derived from ninhydrin and thiosemicarbazide were synthesized using template method. The Schiff base thus formed acted as a monobasic tridentate ligand Via ONS donor sites. Structures were elucidated based on magnetic, AAS, conductance, and Spectral data. Structures of the complexes synthesized by the template method (current investigation) and direct method (previous reports) were compared. The antimicrobial effects of these complexes were also investigated.

5. References

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Ligands. Part47. Synthesis, Physicochemical and Voltammetric Characterization of Iron (Iii) Complexes with Pyridoxal Semi-, Thiosemi- and S-Methylisothiosemicarbazones J.Serb.Chem.Soc., **68(12)**929–942

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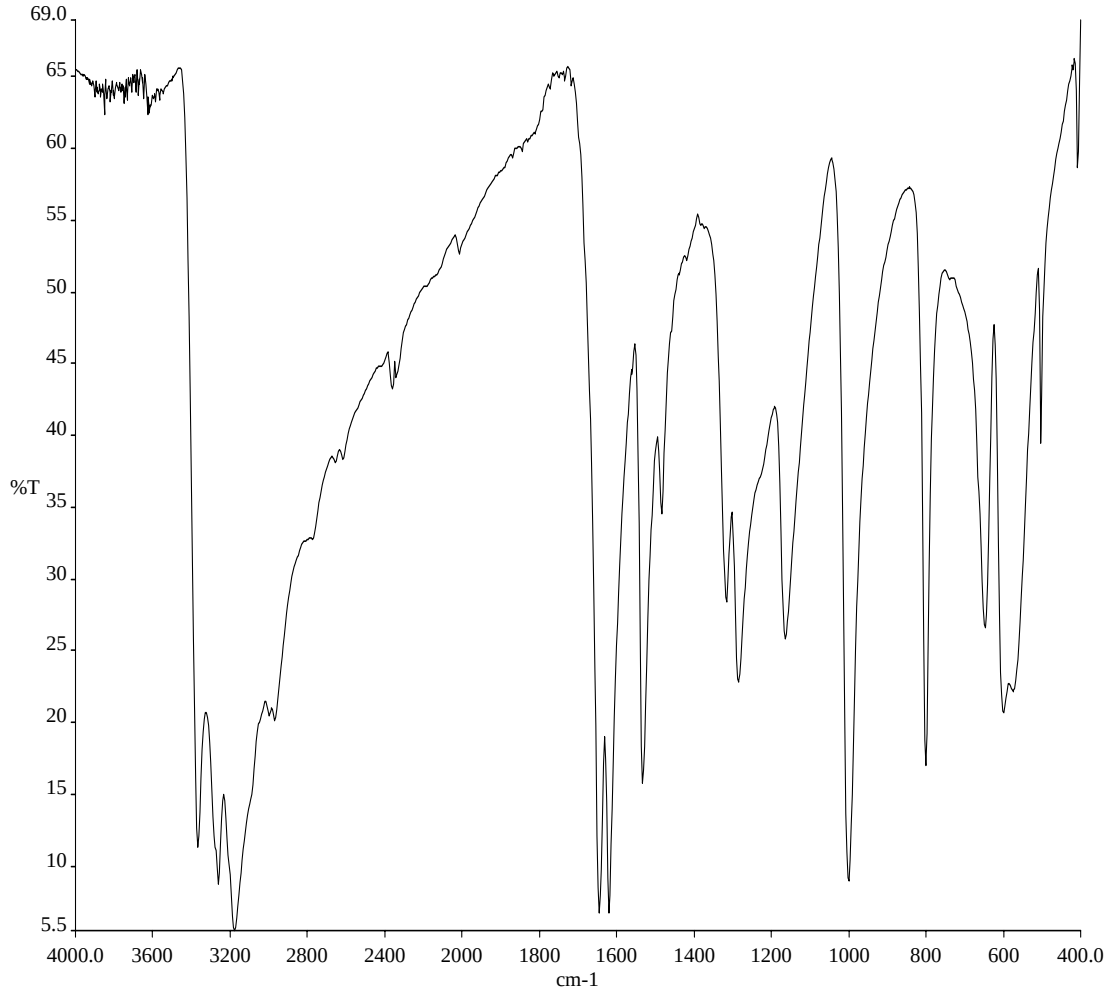
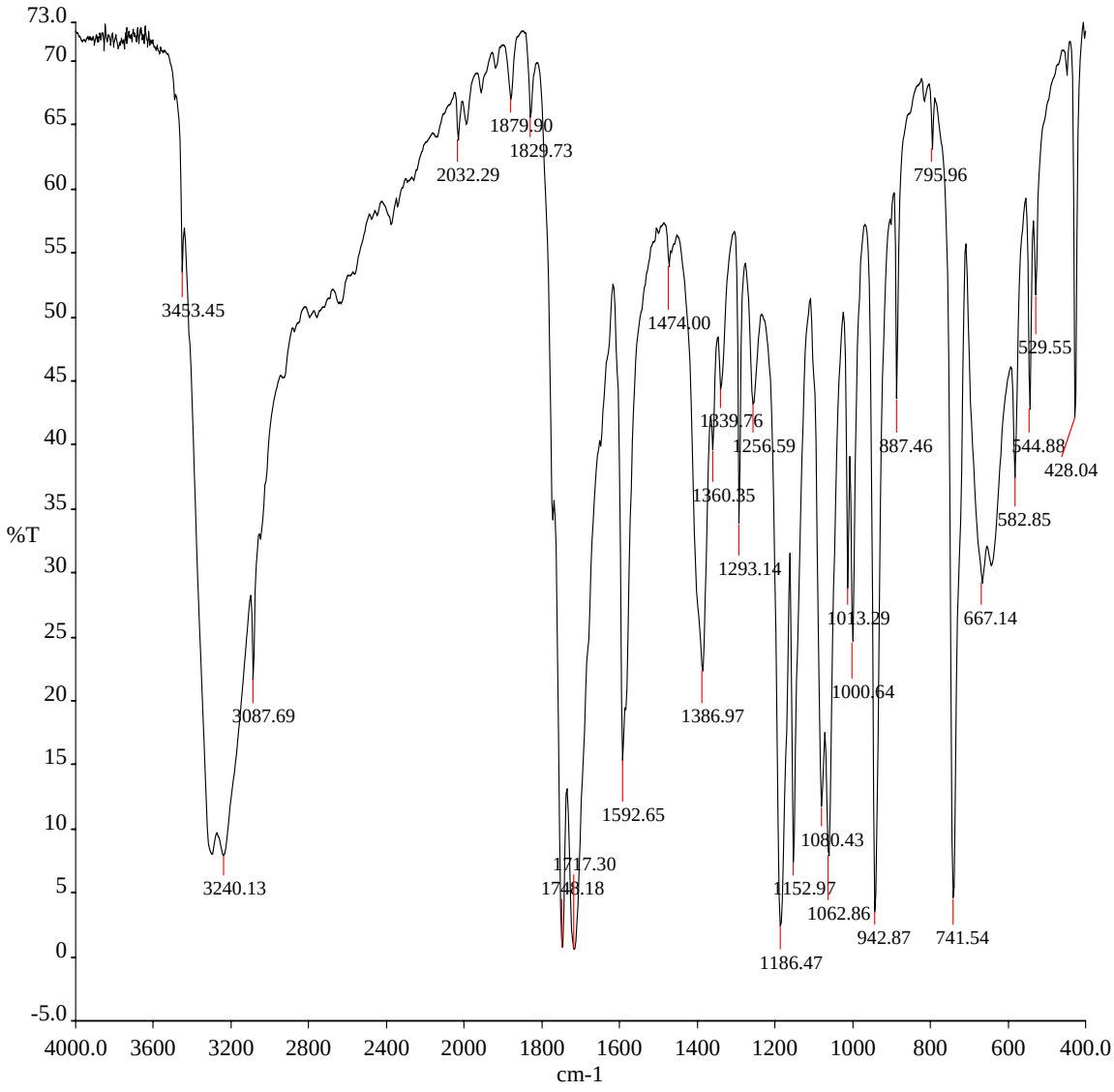
6. Appendices

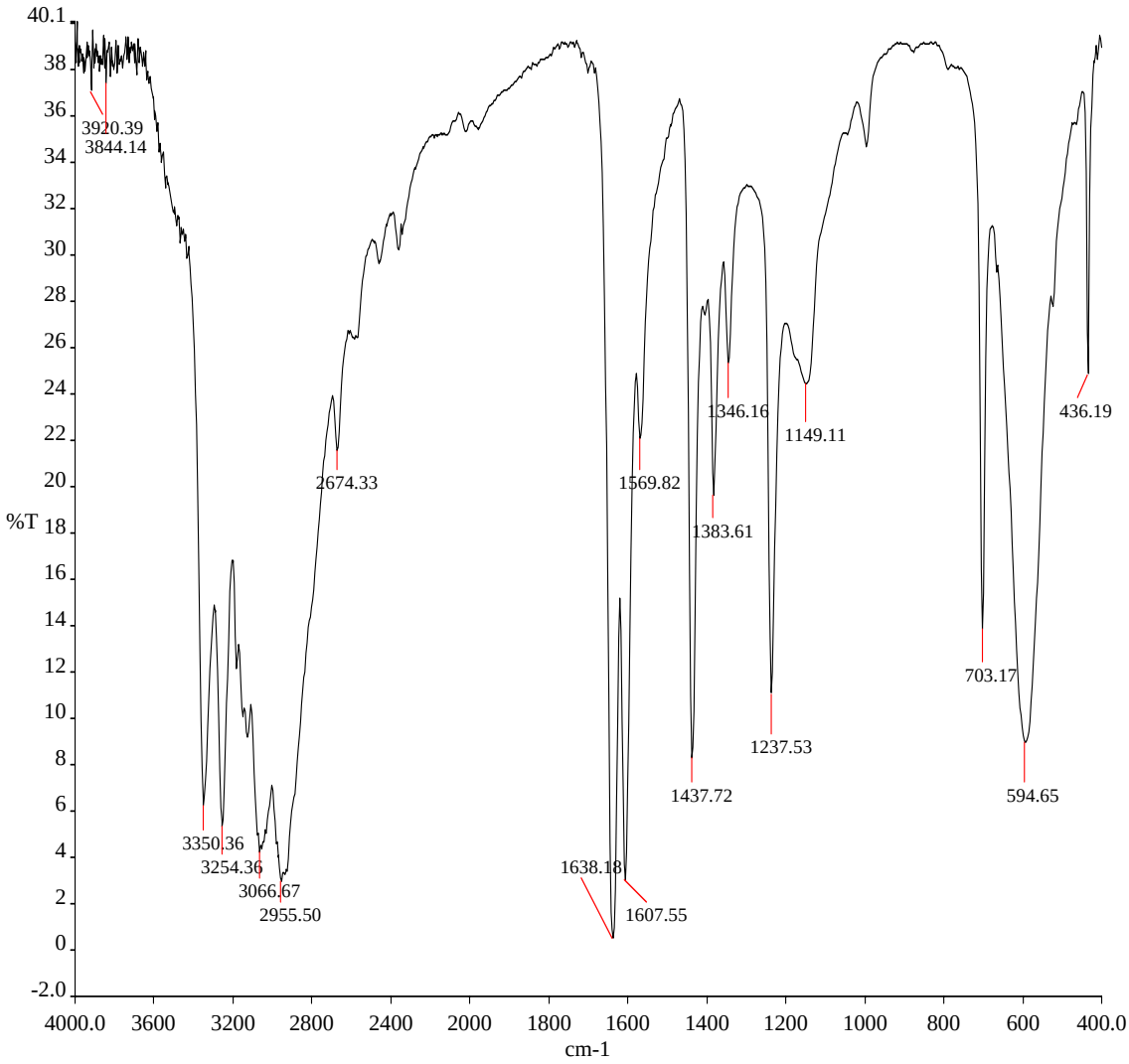
Appendix-1: IR spectrum of thiosemicarbazide



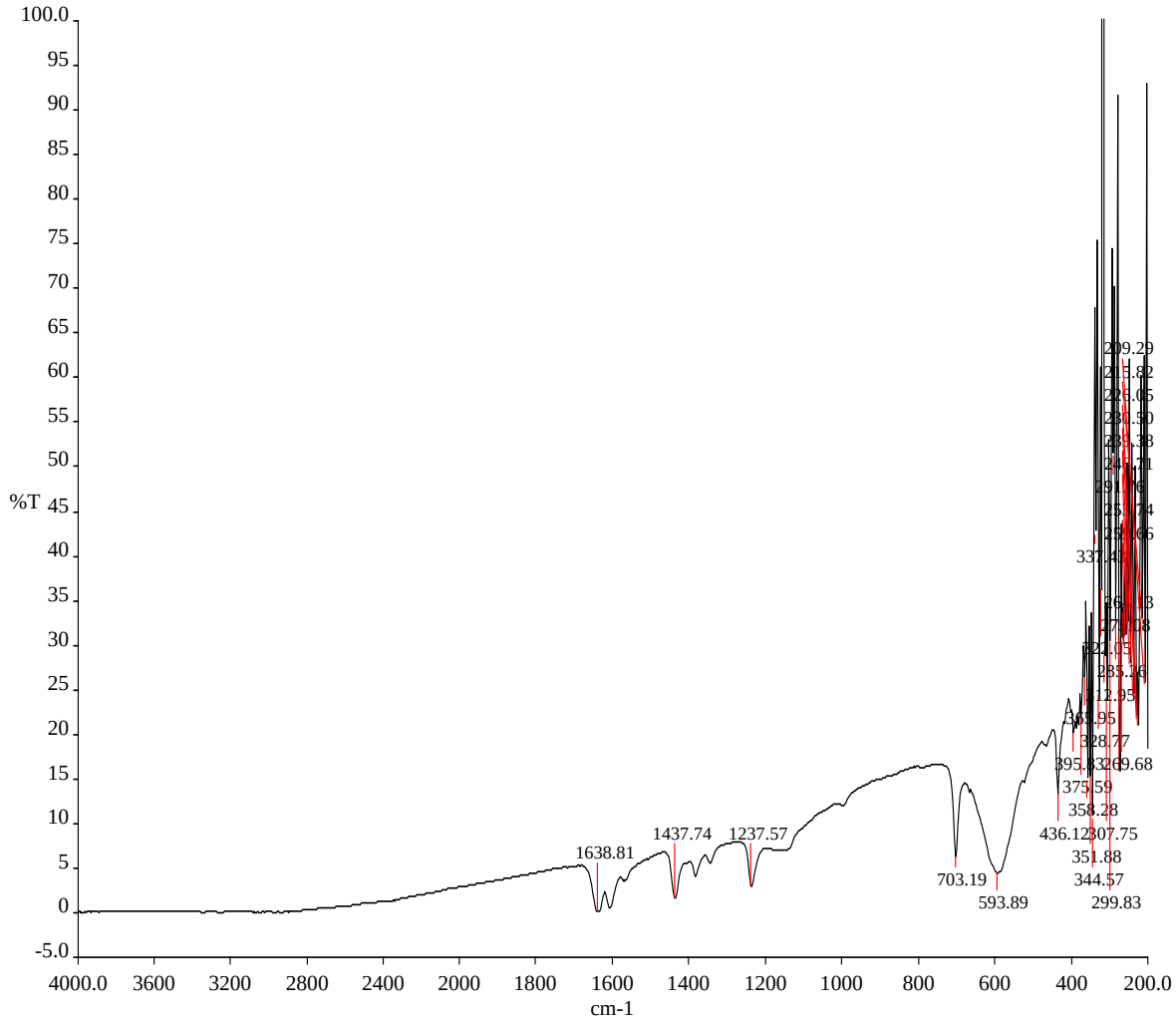
Appendix- 2: IR spectrum of Ninhydrin



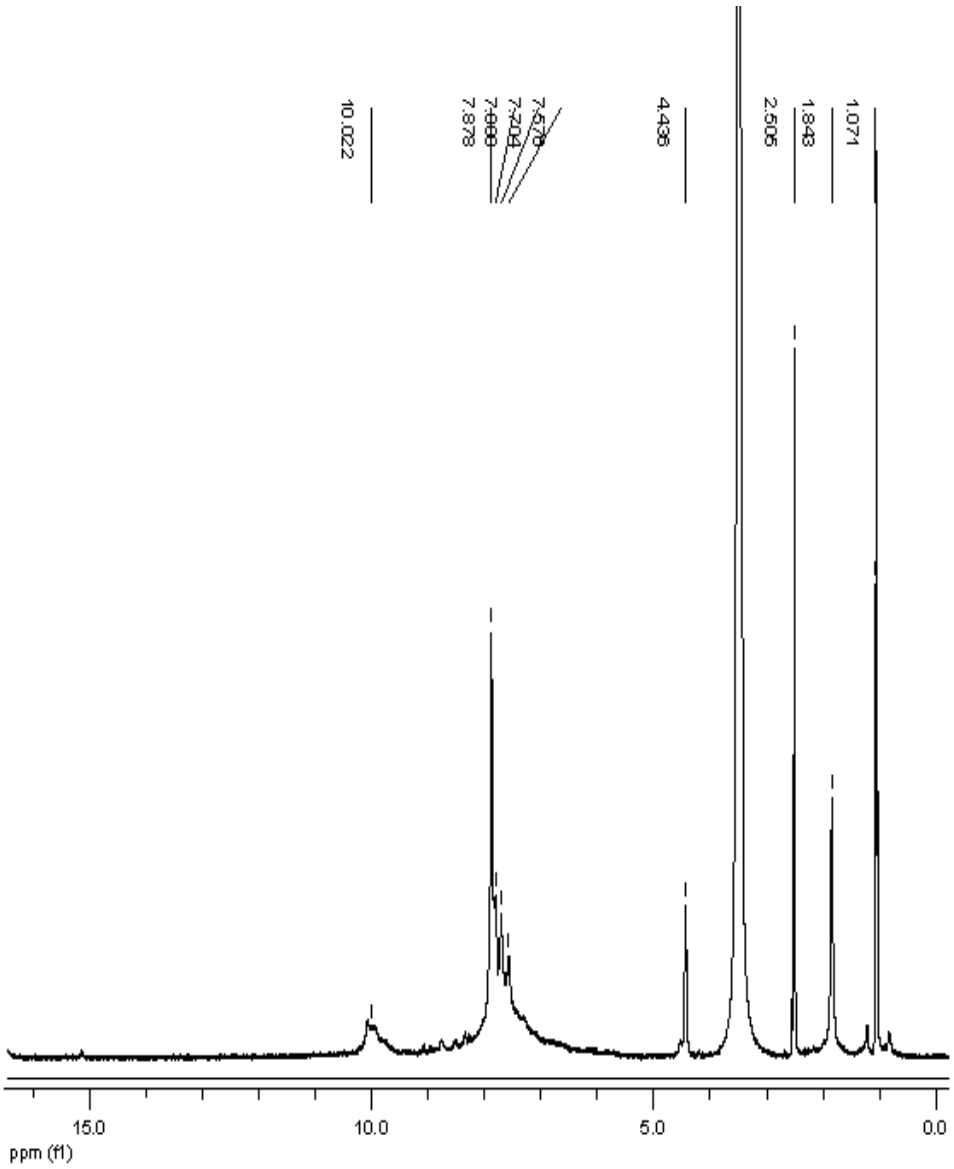
Appendix -3: IR spectrum of Ni(II) complex (4000-400cm^{-1})



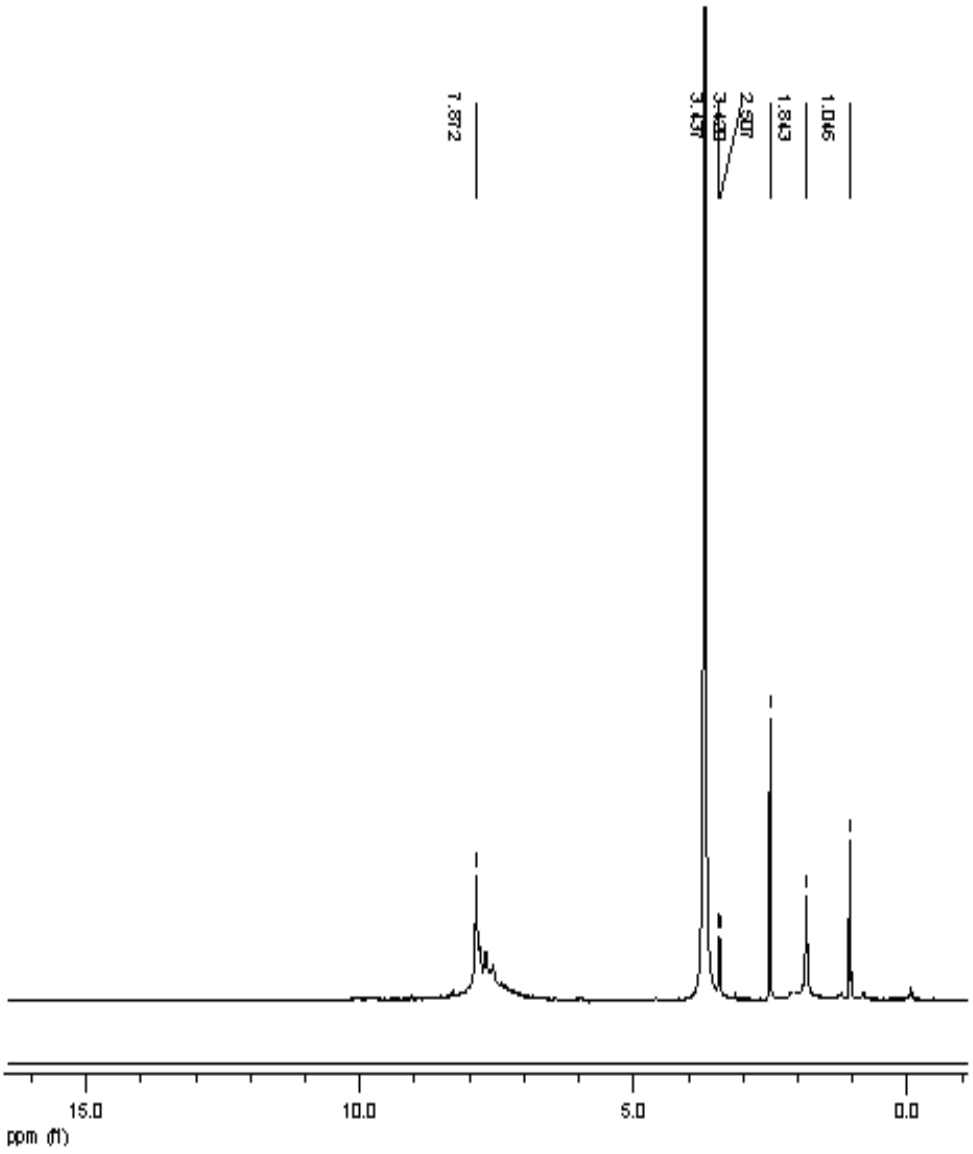
Appendix -4: IR spectrum of Ni(II) complex (4000-200cm-1)



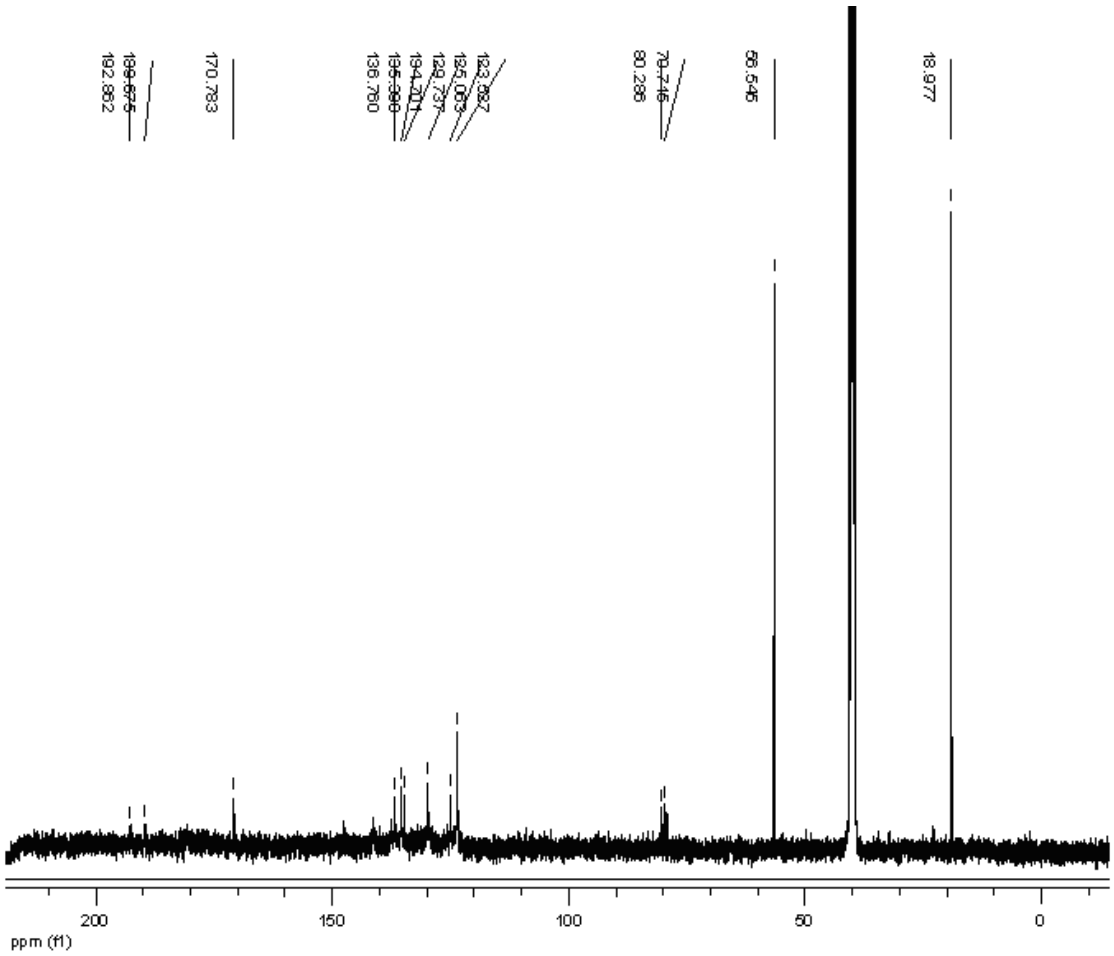
Appendix 5. ^{1}H NMR spectrum of Zn(II) complex



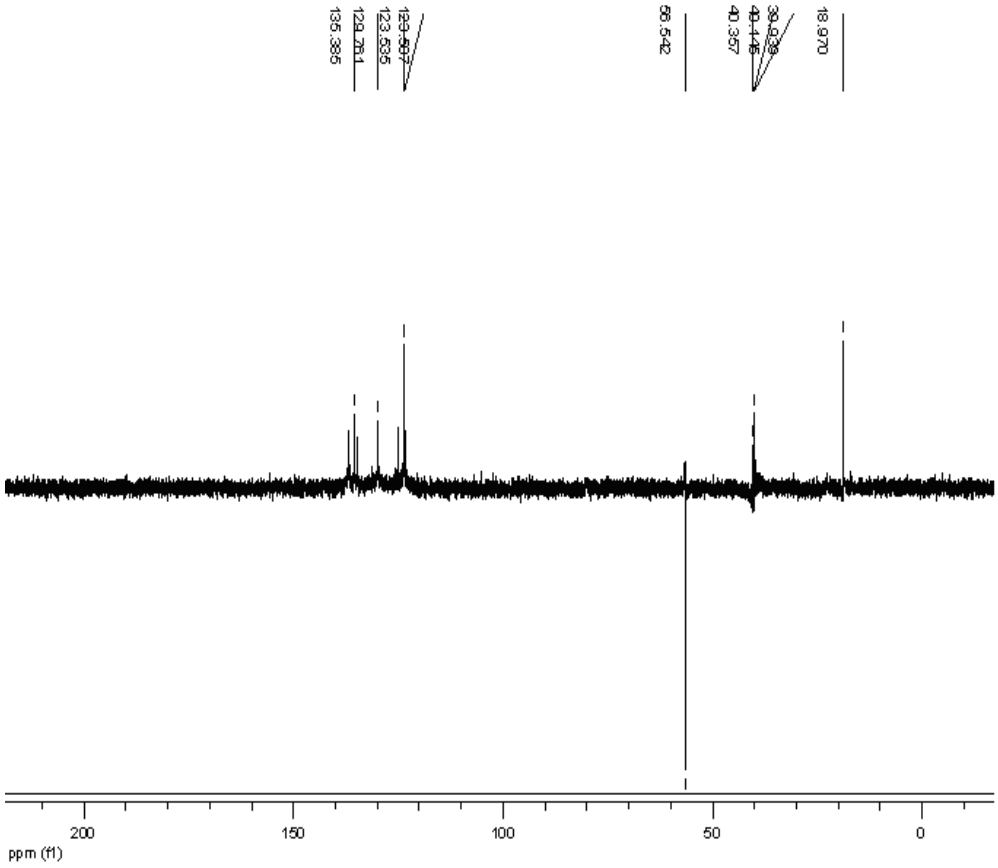
Appendix-6: D_2O exchange ^1H NMR spectrum of Zn (II) complex



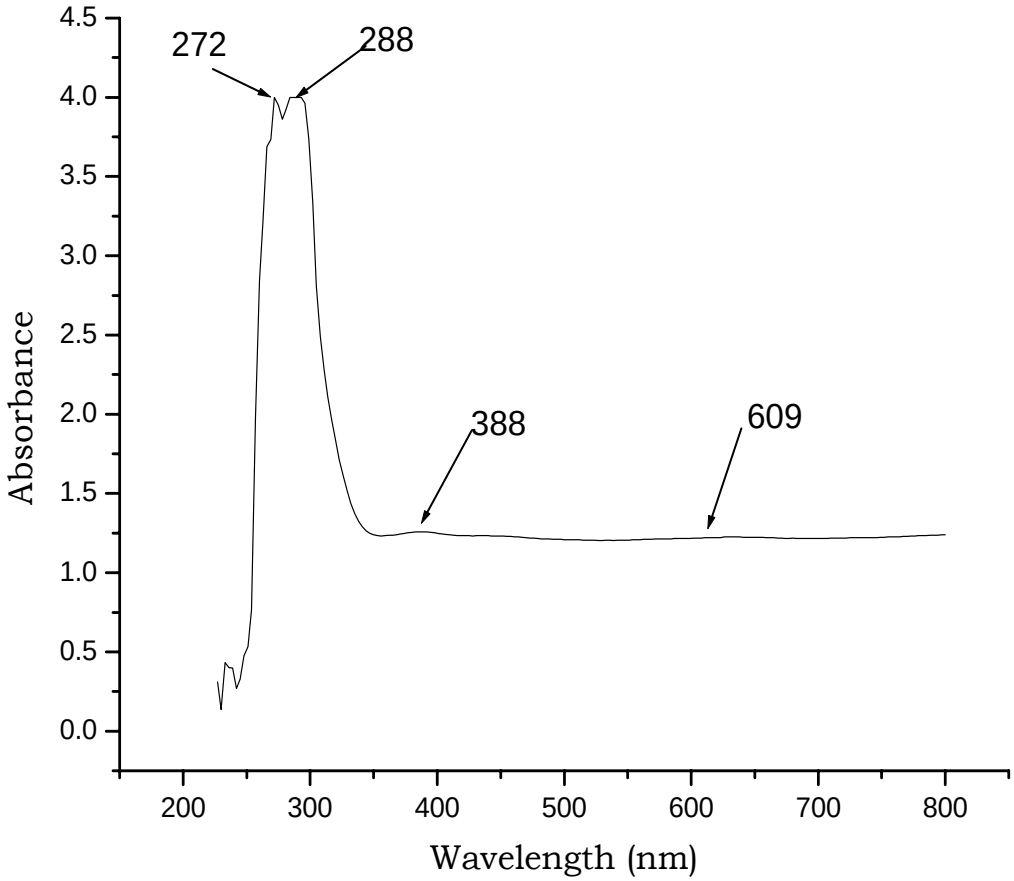
Appendix -7: ^{13}C {H} NMR spectrum of Zn (II) complex



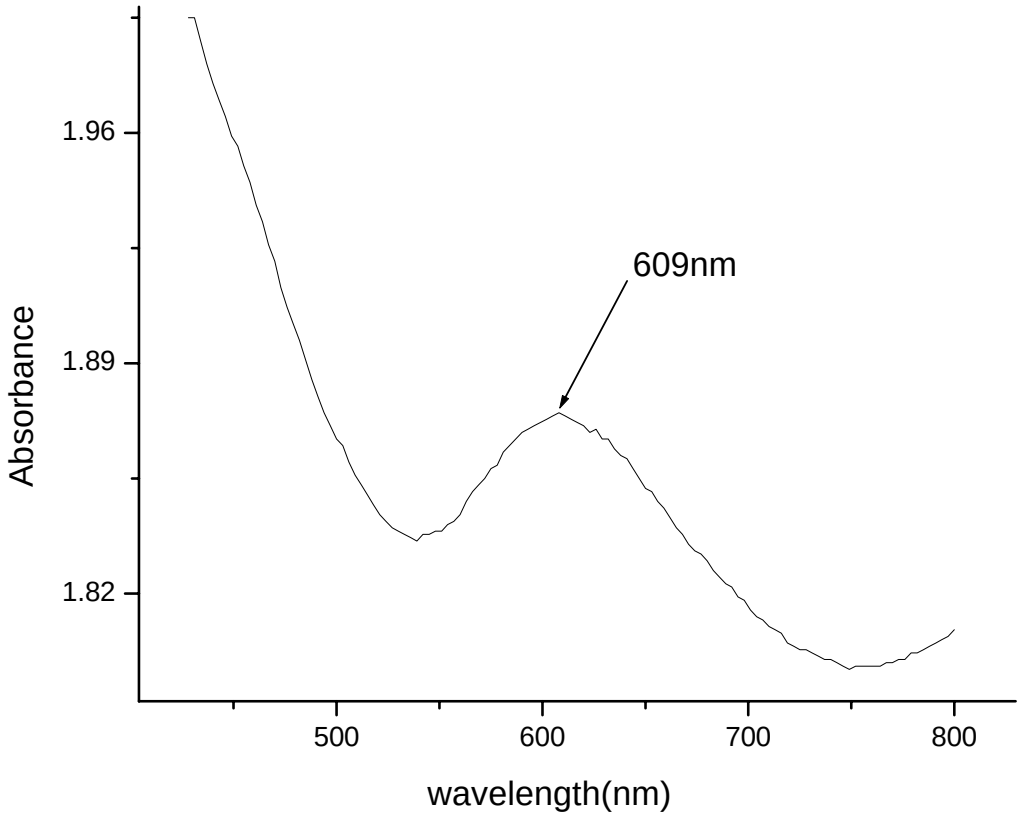
Appendix -8: DEPT spectrum of Zn (II) complex



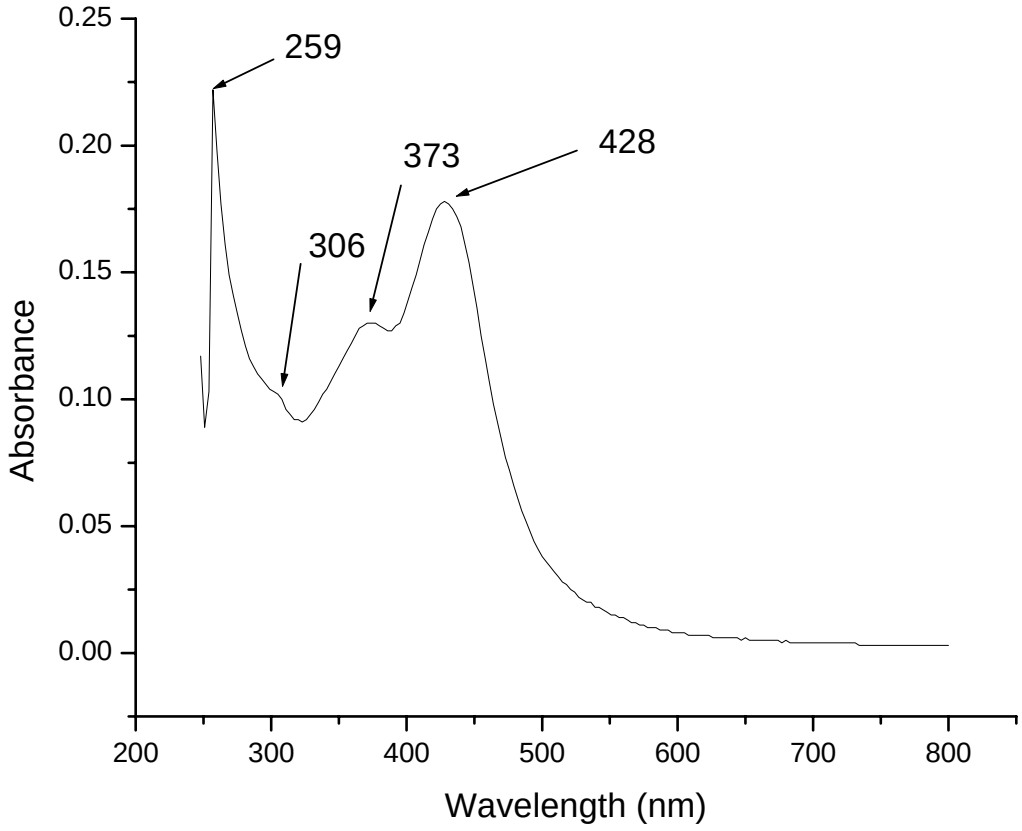
Appendix- 9: UV-Vis spectrum of Ni(II) complex



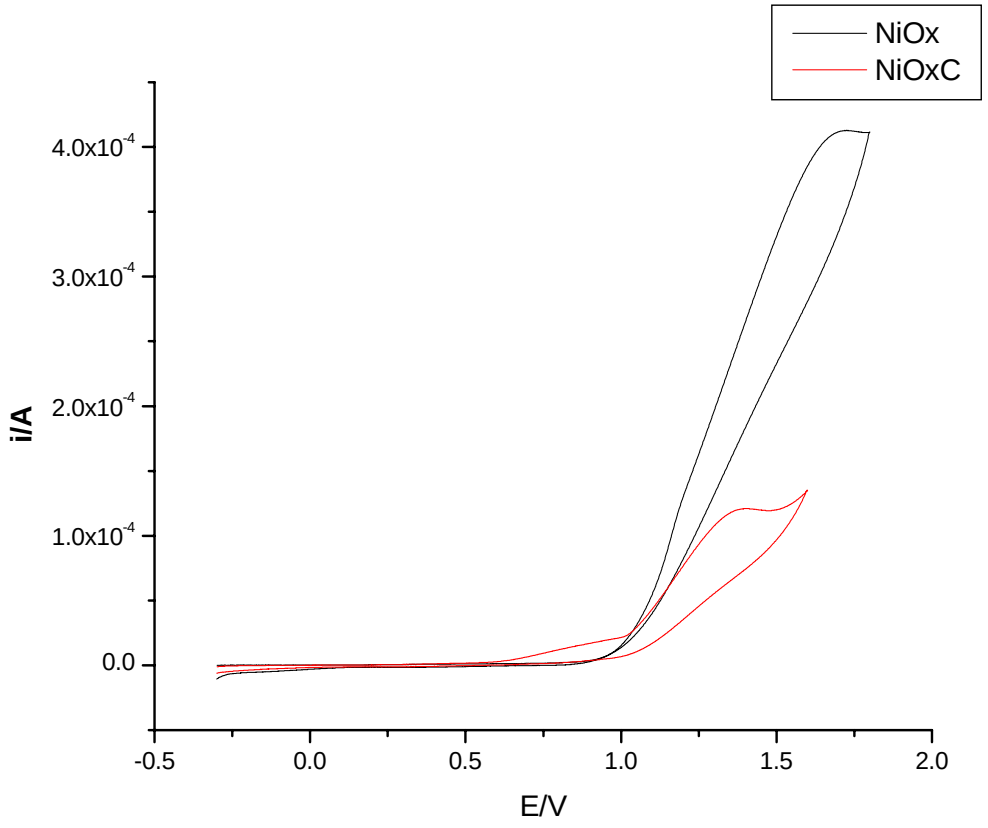
Appendix -10: UV-Vis (d-d) spectrum of Ni(II) complex



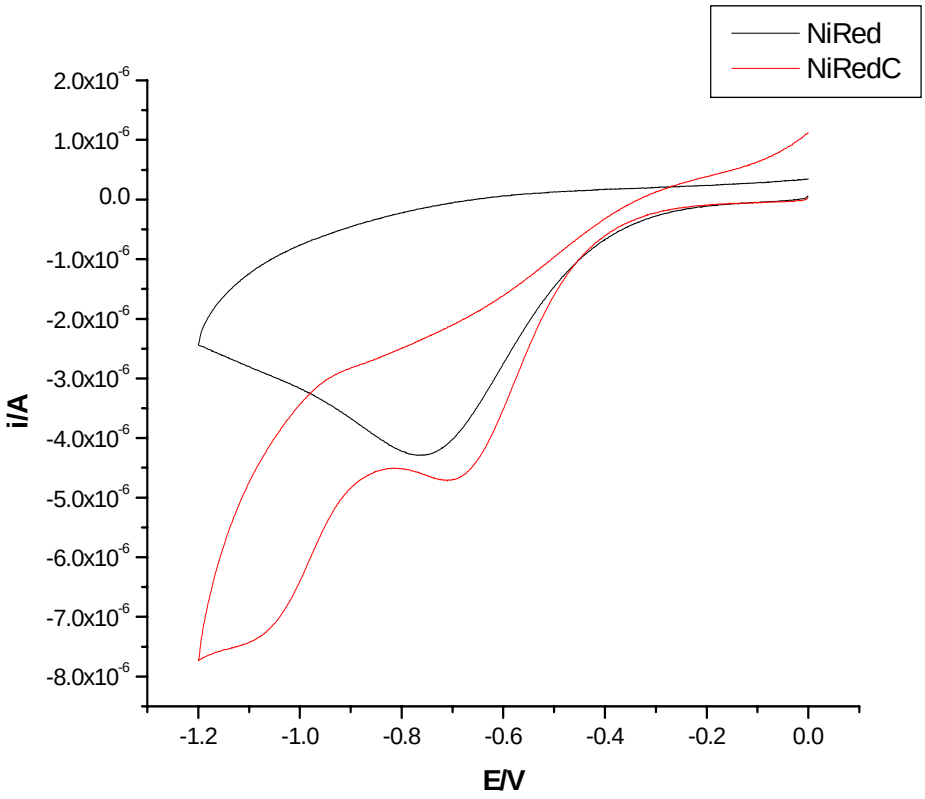
Appendix -11: UV-Vis spectrum of Zn(II) complex



Appendix-12: Cyclic voltammograms of Ni (II) complex (red line) and free state Ni(II) (dark line) in DMSO,25°c containing o.1 M $(C_2H_5)_4NBF_4$.

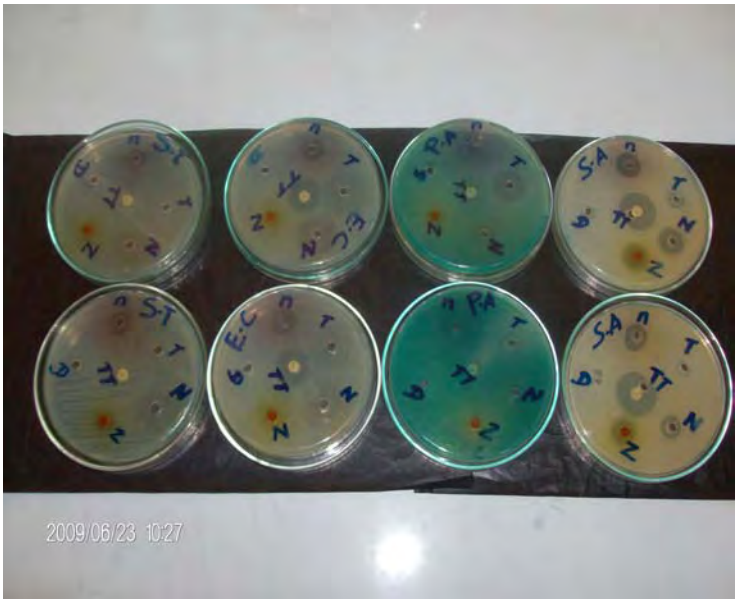


(i) Cyclic voltammogram of the oxidation process



(ii) Cyclic voltammogram of the reduction wave

Appendix-13: Photographic Image of bacterial sensitivity test by the disc diffusion method



(where samples are represented by n=ninhydrin, T=TSC, N= Ni(II) complex, Z= Zn(II) complex, TT= TTC, and D= DMSO and the strains are: S.T= salmonella typhimurium, E.C = Eschericia coli, P.A = Pseudomonas aeruginosa and S.A= Staphylococcus Aureus.

Declaration

I the undersigned confirm that the results reported in this work were obtained by research carried out by me under the supervision of my advisor in the Faculty of science, Department of Chemistry, Addis Ababa University in the academic year 2008-2009. No part of this work shall be published in scientific journals or reported in the media or presented at a conference with out the knowledge and consent of my advisor, who is the principal scientist responsible for any publication. Further more, if the work is published, the international address given should be that of the Department of Chemistry, AAU.

Name: Belay Getie Signature: _____

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

Advisor: _____

Signature: _____