

**JIMMA UNIVERSITY
COLLEGE OF NATURAL SCIENCE
SCHOOL OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY**



M. Sc THESIS ON

**SYNTHESIS, CHARACTERIZATION AND ANTI BACTERIAL ACTIVITY OF
Ni (II) SCHIFF BASE COMPLEX DERIVED FROM QUINOXALINE AND
ETHYLENEDIAMINE**

**By
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**SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NI (II)
SCHIFF BASE COMPLEX DERIVED FROM QUINOXALINE AND
ETHYLENEDIAMINE**

**ATHESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES JIMMA
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DEGREE OF MASTER OF SCIENCE IN INORGANIC CHEMISTRY**

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JIMMA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Synthesis, characterization and biological activity of Ni (II) Schiff base complex derived from quinoxaline and ethylenediamine

By Firaol Enjigu Hubata

A Thesis Submitted to School of Graduate Studies Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Inorganic Chemistry

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ABBREVIATION

DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DEPT	Distortion less Enhancement Polarization Transfer
FT-IR	Fourier Transform Infrared
HSQC	Heteronuclear single quantum correlation
χ_m	Magnetic susceptibility
χ_g	Gram Magnetic susceptibility
NMR	Nuclear Magnetic Resonance Spectroscopy
L	N, N-bis (3-quinoxaline-2-one) diamino ethane
TLC	Thin layer Chromatography
UV-Vis	Ultraviolet-Visible

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ABSTRACT

Schiff's bases ligand are flexible and synthesized from condensation of primary amines and carbonyl groups. Metal complexes of Schiff bases derived from quinoxaline derivatives have played a central role in the development of new drug for medicinal chemistry. Numerous transitional metal complex of Schiff base ligand have been investigated scientifically for antimicrobial activity but still there are anti drug resistant of anti bacterial disease. Hence, a precursor Schiff base ligand was prepared by condensation of quinoxaline and ethylenediamine. The Ni- complex was synthesized by template method by condensation of Schiff base ligand, ethylenediamine and nickel salt. The result was characterized by different physicochemical studies such as elemental analysis, magnetic susceptibility, molar conductance measurement, UV-Visible, IR, ^1H , ^{13}C -NMR spectral studies. The molar conductance measurements of the complexes indicate their non-electrolytic nature. The electronic absorption spectra and magnetic data revealed the existence of an octahedral geometry for Ni-complex. The ligand and its Ni-complex were tested for antimicrobial activity against bacteria (*E.faecalis* and *H.pylori*). The results indicated that the ligand and its Ni-complex showed good activity against bacteria. The Ni-complex was more active than standard drug and ligand against *Enterococcus faecalis* but less active against *H.pyoric* bacteria than ligand and standard drug. The new Ni-complex synthesized was a good activity for gram positive bacteria. From this point of view, it was interest to synthesize and characterize Ni-complex with quinoxaline derivatives used for antimicrobial properties.

1. INTRODUCTION

1.1 Back ground of the study

Schiff's bases ligand are flexible and synthesized by condensation of primary amines and carbonyl groups (i.e. aldehydes and ketone). Schiff's bases derived from the heterocyclic quinoxaline with ethylenediamine are also well known polydentate ligand. These compounds are very important in medicinal and pharmaceutical fields because of their wide spectrum of biological activities [1]. Most of them exhibit biological activities such as antibacterial, antifungal as well as antitumor activity. Schiff base ligand may contain a variety of substituents with different electron-donating or electron-withdrawing groups. Hence they have particular interest due to their biological activities [2].

Heterocyclic compounds are contains a ring structure and other atoms such as nitrogen, oxygen or sulfur as part of ring. It also consists of either aromatic or non-aromatic ring. The presence of hetero-atom results has significant changes in the cyclic molecular structure. This indicates, there is availability of unshared pair's electrons with different electro negativity between heteroatom and carbon. Nitrogen heterocyclic compounds displays physicochemical characteristic and reactivity from aromatic hydrocarbon. Quinoxaline are heterocyclic compounds containing benzene ring and a pyrazine ring. They have 10π electrons that are located in five bonding molecular orbital with two non-bonding orbital's that lie in the molecular plane. Each of these orbital contains an electron pair responsible for the basic properties of quinoxalines [3].

A number of synthetic strategies have been developed for the preparation of substituted quinoxaline. A quinoxaline derivative which contains azomethine compounds is Schiff base ligand and it is widely distributed in nature, such as the antibiotics, levomycin and actinomycin possess very useful biological activity [4].

Schiff base metal complexes have great role in chemistry especially for the development of inorganic chemistry, biochemistry, environment chemistry, organic synthesis, analytical reagent, metal ion catalyst and medicine. A Schiff base ligand may be attached to a metal ion by more than one donor atoms forming a heterocyclic ring (i.e, chelates ring). In such case, the ligand ligands may be termed a chelating agent and the resulting complex as metal chelates [5]

Schiff's base ligands are able to coordinate many different transition metals which stabilize them in various oxidation states [6]. Transition metal complexes of N-donor ligand (Schiff's bases) showed anti-microbial activities [7].

As various researcher stated, the metal complex of bivalent ions Ni (II) derived from direct complexation with quinoxalin-2, 3 -di one with ethylene diamines [8]. With this line, heterocyclic compounds have an important role in design and discovery of new pharmacologically active compounds. Even though, metal complex with heterocyclic quinoxaline derivative Schiff base ligand was not much synthesized.

Therefore, the aim this study was to synthesized and characterized Ni (II) Schiff base complex derived from quinoxaline and ethylenediamine and investigated its biological activity.

1.2 Statement of the Problem

The metal containing quinoxaline derivatives is an interesting field of chemistry over the last few years [9]. Heterocyclic metal complexes have attracted the interest of many research teams because of their extensive applications in wide areas from material to biological sciences [10]. Metal complexes of Schiff bases have played a central role in the development of new drug for medicinal chemistry. Indeed, the various reports were concerned highly functionalized molecules with a quinoxaline skeleton designed for biological activities hence quinoxaline derivatives are an important class of nitrogen-containing and have shown a broad spectrum of biological activities, such as antibacterial and anti-inflammatory activities [11].

The complexation of metals used to activate for Schiff base inactive compounds and the Schiff base active compounds are also made more active. Recently quinoxalines derivative and related heterocycles were introduced as prospective potential chemotherapeutic drug candidates. Besides, it gives more attention to be devoted by bioinorganic and medicinal chemistry to develop the linkage between the metal ions and Schiff base ligand [12]. Numerous transitional metal complex of Schiff base ligand have been investigated scientifically for antimicrobial activity but still there are anti drug resistant of microbial disease [13]. Based on this, the present study needs modified metal complex that contains quinoxaline derivative Schiff base ligand which has high capacity to protect anti microbial activity.

1.3 Objective of the Study

1.3.1 General objective

The aim of this work is to synthesize, characterize, and investigate antibacterial activity of Schiff base ligand and its Ni (II) complexes

1.3.2 Specific objectives

- To synthesize quinoxaline -2,3-dione from orto-phenylenediamine and oxalic acid
- To synthesize Schiff base ligand from quinoxaline-2,3-dione and ethylenediamine and characterize it.
- To synthesize and characterize Ni (II) complex of the synthesized Schiff base ligand and ethylenediamine.
- To evaluate the anti bacterial activity of Schiff base ligand and Ni (II) complex on selected bacterial strains.

1.4 Significance of the Study

Heterocyclic Schiff base ligand is very attractive class of ligand because of their ease of preparation and simple modified electronic properties. These properties are several advantages over other common ligand such as efficient electron donation, higher stability and functionality. Derivative of Schiff base have featured widely in anti-microbial, pharmacological, pesticidal, insecticidal and herbicidal studies [14].

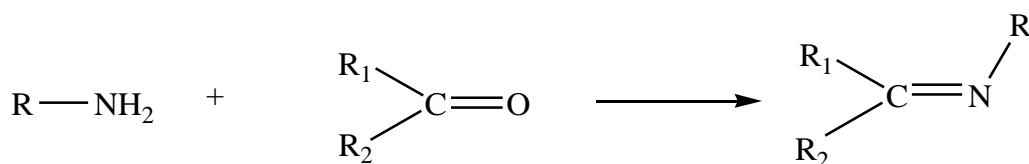
The result of this study would help to:-

- Develop a new metal complex which may has better antimicrobial activity than the other metal- complex.
- The discovery of new metal complex from derivation of quinoxaline Schiff base ligand ring, the products is also important as a means to further treat systems of infection disease.

2. REVIEW OF RELATED LITERATURE

2.1. Schiff's Bases

Schiff base was first reported by Hugo Schiff in 1864 [15]. Schiff bases can be prepared by condensing carbonyl compounds and amines in different conditions and in solvents with the elimination of water molecule. The presence of a dehydrating agent normally favors the formation of Schiff's bases [16]. The condensation of an aldehydes or ketone with primary amine leads to the formation of imines called Schiff's base [17]. The common structural feature of these compounds is the azomethine group with a general formula $R_1R_2C=NR$ where R_2 hydrogen atom or alkyl, R_1 and R are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted.



Scheme 1. Formation of Schiff's base from aldehydes or ketone in acid catalyzed dehydration

Schiff's bases are characterized by the presence of azomethine or amino ($-C=NH$) group, discovered by Von Schiff, have often been used as chelating ligand in the field of coordination chemistry and their metal complexes are of great interest for many years. It is well known that N and S atoms play a key role in the coordination of metals at the active sites of numerous metallo biomolecules [18]. Schiff's base metal complexes have been widely studied because of their industrial, antifungal, antibacterial, anticancer and herbicidal applications [19]. The development in the field of bioinorganic chemistry has witnessed the enhanced interest in Schiff's base complexes, since it has been recognized that many of these complexes may serve as models for biologically important species. Schiff's bases are typically formed by the condensation of a primary amine with aldehydes or ketone. In general, aldehydes react faster than ketone in condensation reactions, leading to the formation of Schiff's bases as the reaction centre of aldehydes are sterically less hindered than that of ketone. Furthermore, the carbon of ketone donates electron density to the azomethine carbon and thus makes the ketone less electrophilic compared to aldehydes. These Schiff's base ligands may be bidentate N,N-, N,O-,

tridentate N,O,O-, N,O,N-, N,O,S-, tetra dentate N,N,O,O-, hex dentate N,N,O,O,S,S-donor ligands [20] etc., which can be designed to yield mononuclear or binuclear complexes or one dimensional (1D) two-dimensional (2D) and three-dimensional (3D) metal-organic frameworks [21]. Schiff's base can only act as chelating ligands if they bear a functional group, usually the hydroxyl, sufficiently near the site of condensation in such a way that a five or six membered ring can be formed when reacting with a metal ion. Schiff's bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications in many fields, e.g., biological, inorganic and analytical chemistry [22].

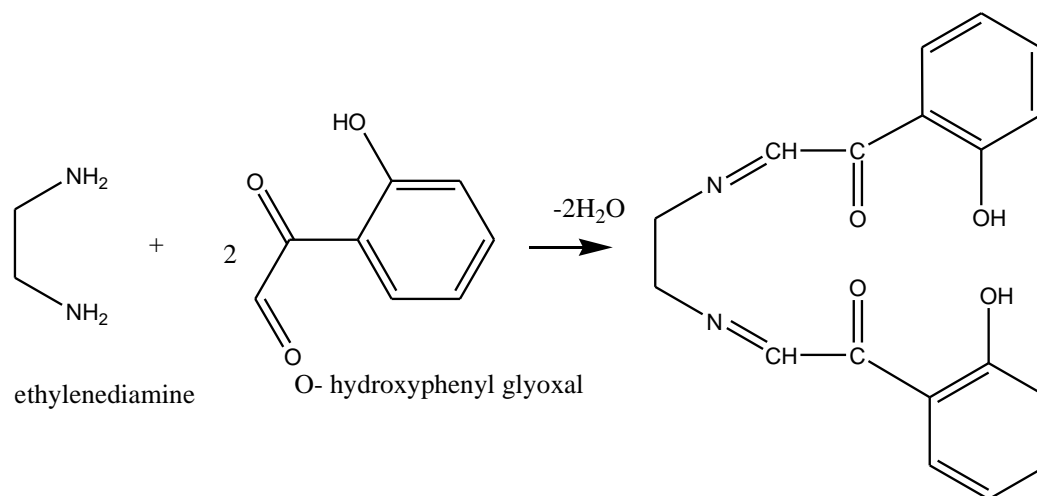
In coordination chemistry, Schiff's bases are an important class of ligand and come across widespread applications in different fields [23]. The interaction of these ligand and metal ions gives complexes of different geometries which are potentially biologically active [24]. The π -system in a Schiff's base often imposes a geometrical constriction and affects the electronic structure as well. Thermo chemical properties of Schiff's bases have attracted the attention of many researchers, in view of their ability to bind through NO or N₂O₂ donor atom sets [25]. These Schiff's base metal derivatives involving bidentate or tetra dentate bonding of ligand in biological systems, have considerable interest and also contributing to the knowledge of their structure and behavior in various activities [26].

In the different areas Schiff's base complexes do have a number of applications such as; electroluminescent materials, in non-linear optical devices, in electrochemical sensors, in medicinal chemistry etc. Many Schiff's bases are known to be medicinally important and are used to design medicinal compounds [27]. It has been seen that the biological activity of Schiff's bases is generally either increased or decreased upon chelating with metal ions [28]. Coordination compounds are important due to their role in biological and chemical systems in various ways [29]. It has been observed that metal complexes with appropriate ligand are biological active compounds [30]. Moreover, the evidences supporting the use of metal complexes in the fight against cancer, tumor, viruses and bacteria have further made this subject a matter of great research interest. There are a large number of metal complexes that are anticancer, antitumor and antibacterial [31, 32]. The complexation of metals with biologically inactive compounds renders them active and in case the compounds are already active, it makes them more active biological active compounds [33].

Moreover, the evidences supporting the use of metal complexes in the fight against cancer, tumor, viruses and bacteria have further made this subject a matter of great research interest. There are a large number of metal complexes that are anticancer, antitumor and antibacterial [34]. The complexation of metals with biologically inactive compounds renders them active and in case the compounds are already active, it makes them more active. The mechanism involved in enhancing this biological activity upon complexation is still needed to be further investigated [35, 36, 37]. Much more attention has been devoted by bioinorganic as well as by medicinal chemists to develop the relationship between the metal ions and their complexes to use as antitumor and antibacterial agents. Some synthesized ligand and metal complex by previous chemist us follows which is anti bacterial, anti fungus and anti tumor activities.

2.1.1 Synthesis of Schiff's base ligand

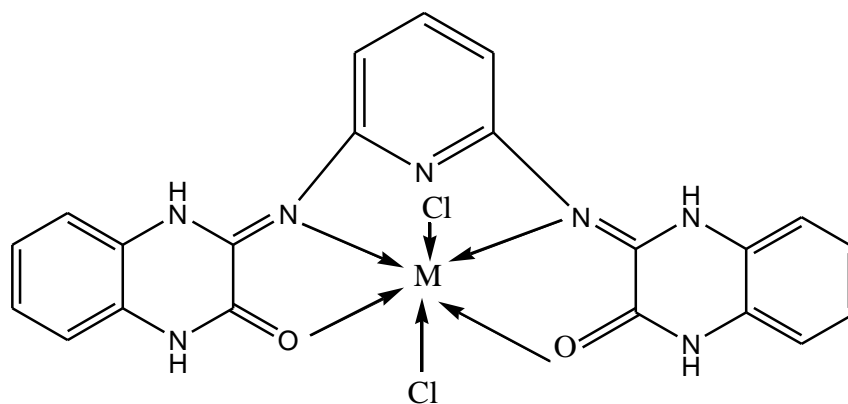
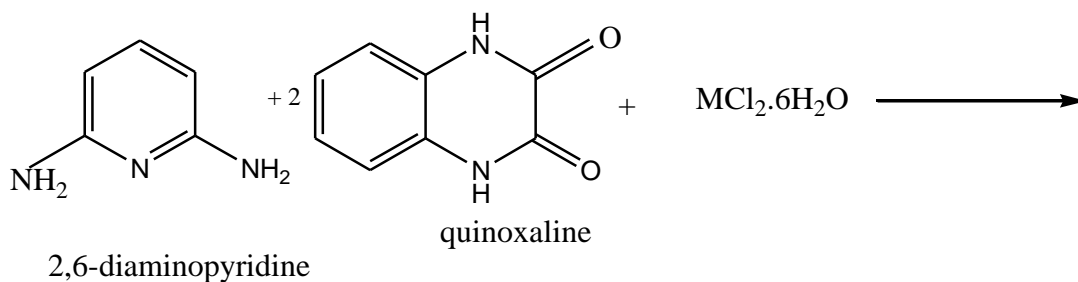
Over the past 25 years, extensive chemistry has surrounded the use of Schiff base ligand in inorganic chemistry. The Schiff base synthesized from ethylenediamine and carbonyl compound have been used for anti biological activity [38].



Scheme 2. Preparation of N,N-di (O-hydroxybenzenoylmethylene) ethylenediamine

2.1.2 Isolation of complexes

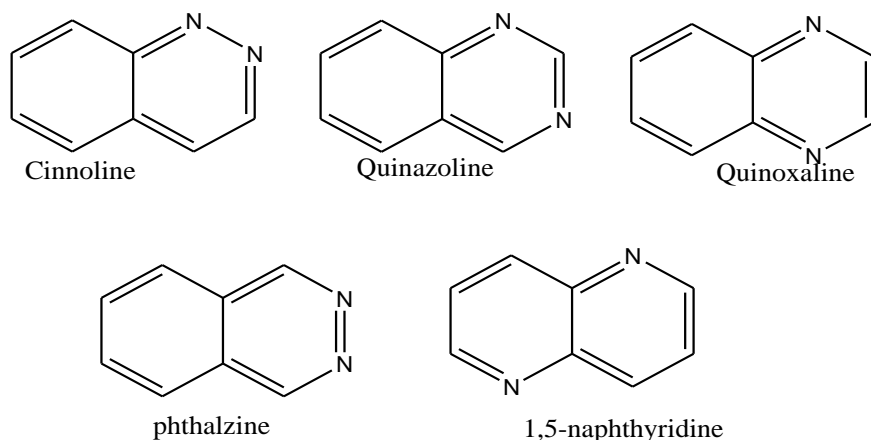
The several attempts to isolate the free macro cyclic or acyclic ligand were unsuccessful. Hence, the complexes were obtained by template synthesis more power full for anti microbial activity[39].



Scheme 3. Template synthesis of quinoxaline -2,3-dione with metal(II) complex

2.2 Chemistry of quinoxalines derivative

Quinoxalines, also called benzopyrazine, are heterocyclic compounds containing a fused ring made up of a benzene ring and a pyrazine ring, with the isomers cinnolenes, phthalazines and quinazolines. All these belong to a class of heterocyclic compounds known as diazanaphthalenes, which may have the two heteroatom in the same or different rings [40].



Scheme 4. Class of heterocyclic compounds

Quinoxaline Schiff base derivatives are an important class of benzoheterocycles which has received much attention in recent years owing to their biological importance and pharmaceutical applications. These derivatives are of particular interest since some of them show antimicrobial [41], anticancer [42], anti malarial [43], anti-inflammatory [44], anti nociceptive [45], anti tubercular [46], antihelmintic [47], anti diabetic [48] and antiepileptic [49] properties. Recent development show that quinoxaline derivative of benzimidazole moiety have antihistamine activity [50]. Certain derivatives of quinoxaline have been widely used in dyes [51] and electrical photochemical materials [52]. Quinoxaline ring moiety constitute part of the chemical structures of various antibiotics such as echinomycin, levomycin and actinoleutin [53] that are known to inhibit growth of gram positive bacteria and are active against various transplantable tumours [54].

2.3 Schiff base metal complexes

Transition metals are known to form Schiff base complexes and Schiff bases have often been used as chelating ligand in the field of coordination chemistry. Their metal complexes have been of great interest for many years. It is well known that N, O and S atoms play a key role in the coordination of metals at the active sites of numerous metallo biomolecules [55]. Schiff base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer, antiviral and herbicidal applications [56].

They serve as models for biologically important species and find applications in biomimetic catalytic reactions. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities.

There are certain metallo-elements without which the normal functioning of living organism is inconceivable. Among these metallo-elements so called, 'metals of life', four members form an island. These are Na, Mg, K and Ca, the transition elements are V, Cr, Mn, Fe, Co, Ni, Cu and Zn. These elements are present at trace and ultra trace quantities and play vital roles at the molecular level in a living system. These transition elements are known to form Schiff base complexes. Metal ions play an important role in living system both in growth and in metabolism. The active sites of the biomolecules [57] are coordination complexes comprising of one or more metal ions. The potential relation and those of synthetic coordination complexes has contributed significantly to the emergence of interdisciplinary field of bioinorganic chemistry. The bioinorganic chemistry [58] forms the molecular basis of all possible interactions between the biological molecules and metal ions which is in turn applied in the field of medicine, biology, environmental sciences, catalysis and technology.

2.4 Nickel Complex

The nickel complexes of quinoxaline derivatives show pronounced antibacterial activities against human pathogenic bacteria, fungal activities and cytotoxic activity [59]. It also plays a central role in biological redox metallo enzymes like plastocyanin, hemocyanin, azurin, galactose oxidase and others [60]. Nickel compounds are present in the active sites of urease and are used extensively in the design and construction of new magnetic materials. The nickel (II) complex have d^8 electron configuration. This configuration of nickel shows John–Teller distortion character for regular octahedral, tetrahedral and square planar symmetry, which has an effect on its stereochemistry. The magnetic moment for each geometry expected are octahedral Ni(II) gives $\mu_{\text{eff}} \cong 2.83$ B.M., tetrahedral Ni (II) gives $\mu_{\text{eff}} \cong 3.2\text{--}4.0$ B.M. and square planar Ni (II) gives $\mu_{\text{eff}} = 0$ [61].

It forms a large number of complexes, the main structural types being octahedral, tetrahedral or square planar. Octahedral nickel (II) complexes having ${}^3A_{2g}$ ground state are expected to have three allowed transitions. ${}^3A_{2g} \rightarrow {}^3T_{2g}$, ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (P) and ${}^3A_{2g} \rightarrow {}^3T_{1g}$ (F) in the range of

1428 -769 nm, 909-500 nm and 526 -370 nm respectively. Two spin-forbidden transitions are also possible; ${}^3A_{2g} \rightarrow {}^1E_g$ and ${}^3A_{2g} \rightarrow {}^1T_{2g}$. The tetrahedral nickel (II) complexes with 3T_1 ground state generally exhibit four transitions. ${}^3T_1 \rightarrow {}^3A_2$, ${}^3T_1 \rightarrow {}^1E$, ${}^3T_1 \rightarrow {}^3T_1(P)$ and ${}^3T_2 \rightarrow {}^1T_1$. The band ${}^3T_1 \rightarrow {}^3T_1(P)$ is a strong band of high intensity when compared with others. Square planar nickel (II) complexes don't have any absorption band below 10000 cm^{-1} , due to large crystal field splitting. Hence they can be clearly distinguished from octahedral and tetrahedral complexes [62].

3. MATERIALS AND METHODS

3.1 Chemicals

All the chemicals and solvents used for the synthesis were of analytical reagent grade obtained from sigma Aldrich and were used without further purification. Chemicals that were used for this study include Nickel (II) Chloride hex hydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$), orto phenyldiamine ($\text{C}_8\text{H}_8\text{N}_2$), ethylenediamine ($\text{C}_2\text{H}_8\text{N}_2$), oxalic acid ($\text{C}_2\text{H}_4\text{O}_4$), HCl, HNO_3 , AgNO_3 and other reagents with AR grade. Analytical reagent grade solvents such as ethanol ($\text{C}_2\text{H}_5\text{OH}$), methanol (CH_3OH) and acetone were used.

3.2 Apparatus and Instrument

UV chamber for detection of spots on TLC, Molar Conductance of the ligand and complex in DMSO were recorded at room temperature with JENWAY 4510 Conductometry, Elemental analysis was carried out using Flash FA 1112 Elemental analyzer, the electronic (UV-Vis) absorption spectra were measured on a Spectronic GENESY S 2PC UV-Vis spectrophotometer in the range of 200-800 nm regions in DMSO, molar magnetic susceptibilities were recorded using MSB-AUTO (Sherwood Scientific) at room temperature, IR spectra were recorded by Perkin Elmer BX between the range of 400-4000 nm using KBr discs as reference compound and NMR Spectra also used.

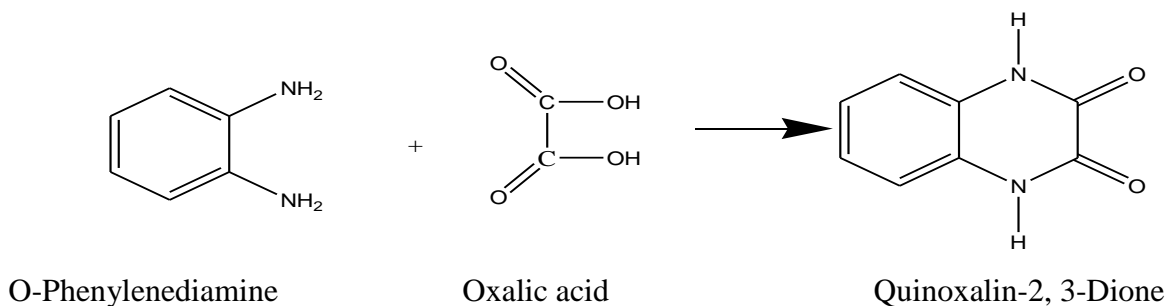
3.4. Experimental Procedure

The preparation of precursor Schiff bases carried out by the direct method. In this method, the isolation and purification of Schiff bases are carried out before complexation. One of the advantages of this method is that it is possible to perform the spectral characterization of comparing with the spectral data of starting material. In the preparation of metal complex template method was used. The template synthesis has been used to prefer assemblies that have unusual topologies, such as rotaxanes, helicates, macrocycles and catenanes [63].

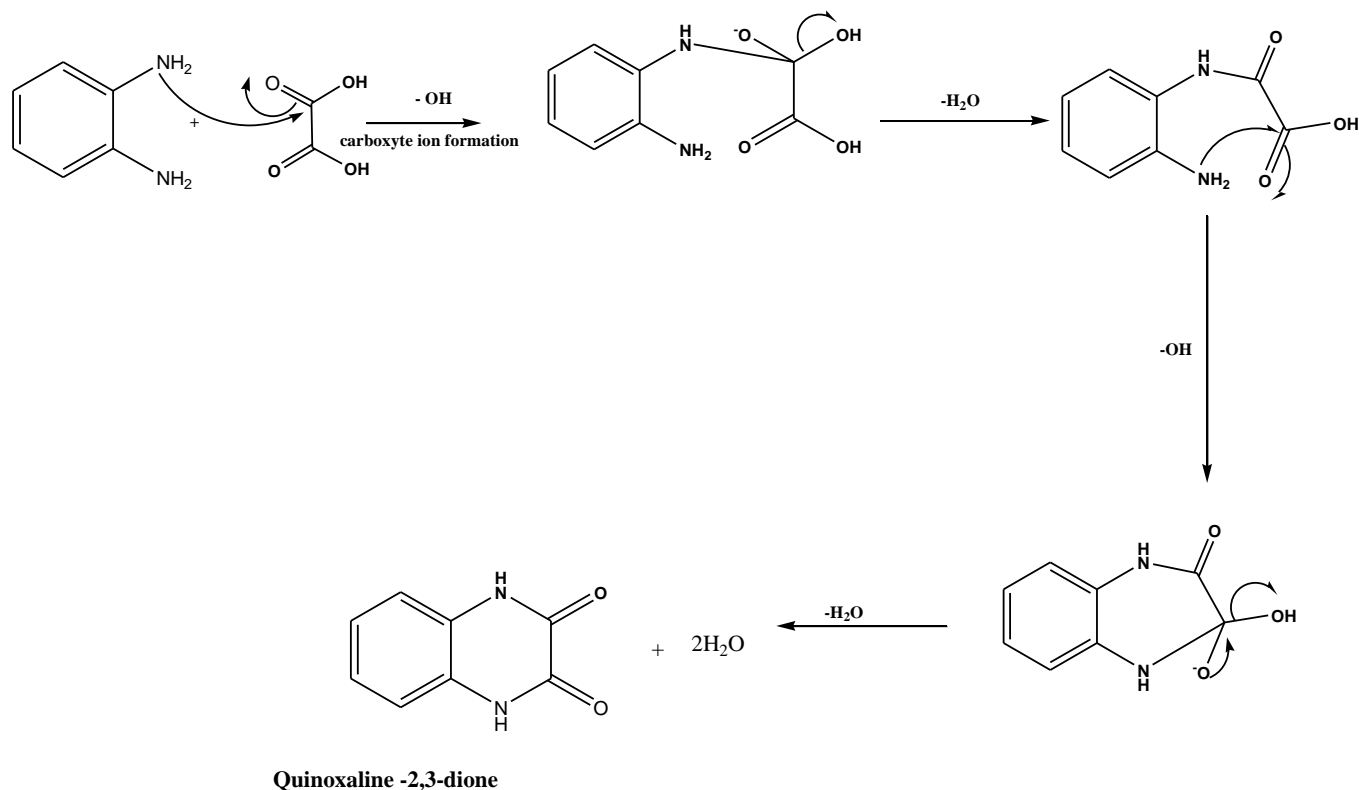
3.4.1 Synthesis of quinoxalin-2, 3-dione.

To synthesize quinoxaline-2,3-dione, 10 g of oxalic acid di-hydrated and 12.5 g ortho phenylenediamine were dissolved in 75 ml of 4N HCl and then mixed. The mixture was refluxed for 1h. The product was filtered, washed with Ethanol and dried in desiccators [64].

The reaction is shown below (**scheme 5**)



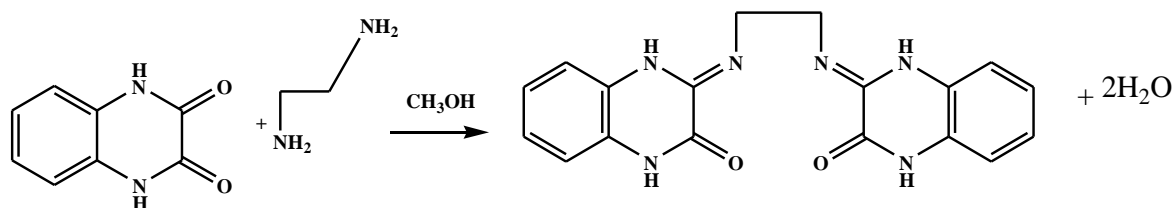
Reaction mechanism of preparation Quinoxalin-2, 3-Dione [65]



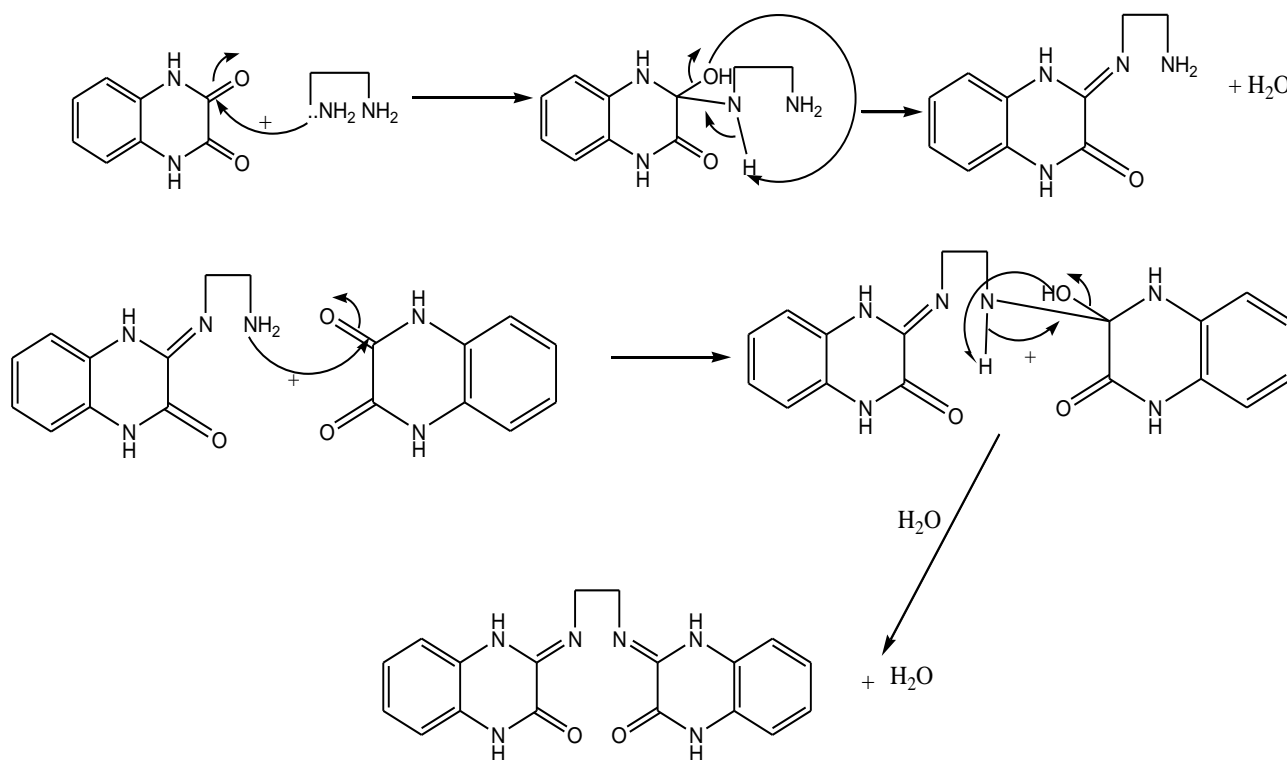
Scheme 5. Synthesis of quinoxaline -2,3-dione and its mechanism

3.4.2 Preparation of Schiff base ligand

The Schiff base ligand was prepared by adding 0.02 M of quinoxali-2, 3-dione and 0.01 M of ethylenediamine were dissolved in 30 ml of methanol and then mixed. The mixture was refluxed with stirring for 2 h. The resulting solution gave precipitated product was collected by filtration, washed several times by ethanol and recrystallized from ethanol and dried in desiccators over calcium chloride. Condensation of ethylenediamine and quinoxali-2,3-dione in methanol gives single product according to the following reaction shown in scheme.

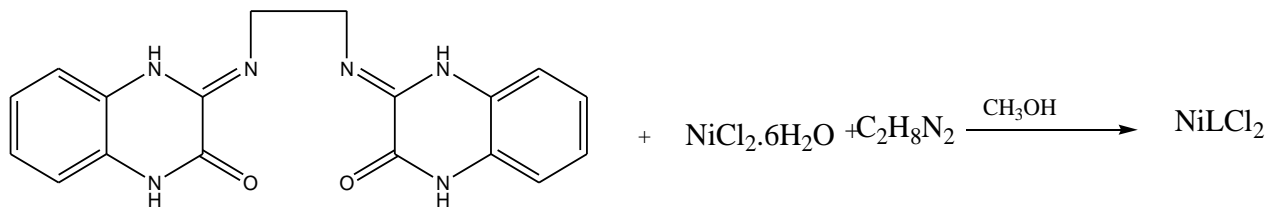


Scheme 6. Preparation of N, N-bis (3-quinoxaline-2-one) diamino ethane
Reaction Mechanism of preparation of ligand [66]



3.4.3 Synthesis of Nickel Complexes

Equimolar Schiff base ligand ($C_{18}H_{16}N_6O_2$) (0.0022 M), $NiCl_2 \cdot 6H_2O$ (0.0022 M) and Ethylenediamine (0.0022 M) were dissolved in 20 ml methanol with stirring and then mixed. The mixture was refluxed for 3 h. The product was filtered, washed several times with ethanol cooling and dried in desiccators over calcium chloride.



Where L= Ligand

Scheme 7. Synthesis of Ni- complex by template method

3.5 Antibacterial Activity Test

3.5.1 Disk diffusion method

In vitro antibacterial activity of the ligand and Ni- complex were evaluated using disc-diffusion method. Mueller- Hinton Agar was employed as microbial growth medium. The antibacterial diffusion test were carried out as described by Berghe and Vlietink [67] using a cell suspension of about 1.5×10^6 CFU/mL obtained from the McFarland turbidity standard number 0.5. Mueller –Hinton (MH) agar was poured (to a height of 5 mm) into sterile 9 cm diameter petri dishes and allowed to solidify. The solid MH agar was inoculated with bacteria strains using a platinum wire loop which had been previously sterilized by heating it red hot in a flame, cooled and then used for the application. The dishes were allowed to dry for 10 minute at 37⁰C in an incubator. Sterilized forceps were used for the application of the paper discs containing the test compounds on previously inoculated MH agar dishes. The plates were kept for 30 minute at ambient temperature to allow for pre diffusion, and then incubated at 37⁰C for 24 h. Anti microbial activity was evaluated by measuring the diameter of growth inhibition zone (IZ) in mm around the discs.

3.5.2 Test strains

The Schiff base ligand and Ni- complex were evaluated for in vitro antibacterial activities against two bacterial strains (*Enterococcus faecalis* and *Helicobacter pylori*) by disc diffusion method at Jimma University Department of Biology in microbiology laboratory. The discs having 6 mm in diameter are prepared from Whatman No.1 filter paper and sterilized by dry heat at 121 °C for 2 h. The test solution was prepared by dissolving 100 mg of Schiff base ligand and Ni- complex compound in 1 mL of dimethyl sulfoxide to achieve final stock concentration of 100 mg/mL solution of the test sample and the degrees of bactericidal activities are determined by measuring diameter of inhibition zone and comparing them with the standard drugs ciprofloxacin. After 24 h. Incubation period, zone of inhibition has carefully measured. The mean of the inhibition zone of each test sample was taken for evaluating the antibacterial activity of the Schiff base ligand and nickel complex compounds. For each bacteria strain the standard ciprofloxacin was taken as positive control and pure solvent DMSO as the negative control.

4. RESULTS AND DISCUSSION

4.1 Some physical properties of ligand and complex

The ligand was white powder soluble in common organic solvent but insoluble in water. The newly synthesized ligand Ni- complex is pale green and completely soluble in DMSO solvent but slightly soluble in water. Melting point of the complex was different from that of the Schiff base ligand, which is an evidence for complexation.

Table 4.1 Some physical properties of the ligand and its Ni- complex

Compound	Color	M	M.pt °C	Found					μ^{eff}
				(Cal)					
				%C	%H	%N	%Ni	% Cl	
Ligand (L)	White	348	380-384	52.89 (62.06)	5.20 (4.6)	23.80 (24.14)	- -		-
Ni complex	Light pale green	501.7	390-392	46.79 (47.83)	5.20 (4.0)	24.50 (22.32)	12.34 (11.89)	(14.2)	0.5 B.M

NB. M= molar mass

M.Pt = Melting point

4.2 Solubility of the ligand and its nickel complex in different solvents

Solubility test was done to identify the best suitable solvent for the next analytical and spectroscopic measurements. The observed solubility of the synthesized compounds is tabulated (**Table 4.2**).

Table 4.2 Solubility of ligand and its Ni- complex

Compounds	Solvents			
	Water	Ethanol	DMSO	Petroleum ether
Ligand	Insoluble	Soluble	Soluble	Partially soluble
Nickel complex	Insoluble	Soluble	Soluble	Partially soluble

4.3 Qualitative Test

4.3.1 TLC Test

The purity of Schiff base and its nickel complex was proved on TLC plates and the single spot was visualized under ultraviolet light. For this purpose, the compound was dissolved in ethanol. Ethanol was used as eluting agent. By this process the purity of synthesized ligand and Ni-complex was controlled. TLC examination suggested that the complete conversion of starting material to ligand and metal complex.

4.3.2 Chloride Test

A 10 mg of the nickel complex was dissolved in 5 ml concentrated nitric acid and heated until organic contents decomposed and few drops are left. To the digested solution 0.1N of AgNO_3 was added, and allowed to stand overnight, then a white precipitate AgCl was observed which confirms the presence of chlorine molecule in the Ni- complex.

4.4 Estimation of Ni metal in complex by AAS

Nickel content of the complex was determined using atomic absorption spectroscopy (AAS). Ni-complex 25 mg was placed in a clean and dry beaker, to which 10 ml portion of Conc. HNO_3 was added and the content was heated gently in hood until a few drops remains in the beaker. Then 10 ml of additional conc. HNO_3 was added in the beaker and heated slowly until a few drops remained. The latter procedure was repeated for three times until all the organic component of the complex was decomposed. Then the residue was dissolved and diluted using distilled water in a 100 ml flask. The solution was subjected to AAS study after appropriate dilution. Based on the absorbance data, the amount of Ni in the complex was calculated. The metal percentage obtained from this calculation was confirmed with the percentage theoretical part of the Ni -complex as shown in the (Table 4.1)

4.5 Elemental analysis of the ligand and Ni-complex

Elemental analysis of a compound enables to find its empirical formulae. The percent of C, H, N and M obtained from elemental analysis and atomic absorption spectroscopy are in good

agreement. Hence the general molecular formula proposed for the complexes [Ni LCl₂]. Where: whereas L= (C₁₈H₁₆N₆O₂) organic segment formed by direct route between Ethylenediamine and 1,4-dihydro-quinoxalin-2,3-dione, as shown in structure (**Scheme 6**) (**Table 4.1**).

4.6 Magnetic susceptibility (χ_m)

The magnetic susceptibility observed at room temperature and magnetic moments calculated show strikingly subnormal values. The magnetic moments of Ni- complexes is observed to be 0.5 B.M but the theoretical values of Magnetic moments for free Ni(II) ions is 2.83, so the subnormality in magnetic moments has been ascribed to anti ferromagnetism which is due to the interaction between electron spins on neighboring metal ions. It is generally accepted that the mechanism of the exchange interactions involves the mutual pairing of electron spins via orbital overlap. Anti-ferromagnetic exchange may be metal-metal interaction or super exchange [68]. Metal-metal interactions involve direct overlap of orbital's containing the unpaired electrons, which influences the magnetic behavior whereas super exchange involves the interaction of electrons with opposite spins on the two interacting ions via an intermediate diamagnetic anion(s) which results in effective pairing leading to a minimum number of unpaired spins in the ground state of the system which show octahedral geometry [69].

4.7 Infrared spectra

In order to study the binding mode of the ligand with the metal ions in the complex, the IR spectrum of the free ligand was studied to identify the functional group of the molecule in the second precursor ligand. and also the spectra of metal complexes was studied to area of metal bond with ligand and identify the functional group in the complex. The IR spectral data of the ligand showed a band at 1720 cm⁻¹, which is assigned to C=O stretching vibration. This band was shift 1676 cm⁻¹ in the complex compound that indicating the formation of condensation reaction take places involvement of band at 1676 cm⁻¹ which is assigned C=N that coordination with metal ions, resulting in the formation of the complex's [62]. The free ligand exhibits strong absorption bond at 3044-2872 cm⁻¹ due to the C-H stretching vibration and it also shift to up field 3044-2891 cm⁻¹ in the complex, which indicate the C-H stretching vibrations band. The new observed bands in the regions ~421 and ~402 cm⁻¹ corresponding to Ni-N and Ni-Cl stretching vibrations respectively that confirming the coordination of the Schiff base to the

respective metal ions. The IR spectrum of the ligand exhibited band in the regions 3327 cm^{-1} was assigned to N-H and it was shifts to higher frequency 3340 cm^{-1} in the spectra of complex. This shows the complexation of metal take places.

Table 4.3 Infrared spectral data of the ligand and its Ni- complex

Compoun d	$\nu(\text{N-H})$	$\nu(\text{C-H})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{C=C})$	$\nu(\text{C-O})$	$\nu(\text{C-N})$	$\nu(\text{Ni-N})$	$\nu(\text{Ni-Cl})$
Ligand	3320	2872- 3044	1720	1639	1601- 1511	1383	1249	-	-
Ni- Complex	3340	2891- 3044	-	1676	1514	-	1390	421	402

4. 8 NMR spectra

4.8.1 ^1H -NMR of ligand

The ^1H -NMR spectra showed δ_{H} at 2.6 ppm was indicate the proton on aliphatic carbon. It is a singlet proton which exists in the same environment that attach on N=C. This proton is shielded due to the electron donor of nitrogen to aliphatic carbon. The δ_{H} at 4.1 ppm was Shows the proton that attach on the nitrogen bonded to carbonyl group. This indicate that the nitrogen donate electron to the quaternary carbon, the electron density decrease from the proton and being de shielded. The δ_{H} at 3.1 ppm was the proton that attach to nitrogen bonded to C=N. It is more shielded than the proton on Nitrogen bonded to carbonyl group. The ^1H - NMR data shown spectra δ_{H} at 7.0-7.1 ppm was indicate that proton on benzene ring that attach at p-position is δ_{H} at 7.09 ppm less effect than the proton that attach nitrogen bonded to carbonyl group having δ_{H} at 7.1 ppm this is due to the effect of carbonyl group. The DMSO signal appeared δ_{H} at 2.50 ppm [70].(Appendix 3)

4.8.2 ^{13}C -NMR of ligand

The ^{13}C -NMR data shown spectra δ_{C} at 40.097 ppm was confirmed the aliphatic carbon which was detected under DMSO solvent peak. From ^{13}C -NMR data spectra δ_{C} at 155.8 ppm

Indicated the $C=N$. It is deshielded than the other δ_C at 153.7 ppm shows $C=O$ because of the neighboring group attached to it. Similarly the δ_C at 126.4 ppm is the carbon bonded with $C-N-C=N$ and the δ_C at 125.7 ppm was indicate the carbon bonded to $C-N-C=O$ it is shielded. The δ_H at 7.0-7.1 ppm indicate that carbon on benzene ring that attach at p- position is δ_H at 123.614 ppm more effect than the carbon that attach on pseudo p- position having δ_C at 123.27 ppm this is due to the effect of nitrogen double bond group. Nitrogen double bond has more deshielded than carbon double bond due to the neighboring group effect of long chain attached to nitrogen double bond. The carbon having δ_C at 115 ppm confirmed that the orto meta carbons on benzene ring shows one signal since they has the same environment. The DMSO signal appeared at δ_C at 38.5-39.8 ppm. [71] (**Appendix 4**).

4.8.3 DEPT-135 NMR spectra of ligand

The DEPT result from NMR data (**Appendix 5**) shows three positive signal for CH δ_C at (123.9, 124.6 and 115.7 ppm) and one negative signals for CH₂ δ_C at 40.5 ppm. This is confirmed that the ligand has contained CH and CH₂ molecule

4.8.4 ¹H-NMR spectrum of the Complex

The ¹H-NMR data spectra of complex (**Table 4.4**) shows the proton δ_H (8.3) assigned to H-N is highly deshielded compared to the protons δ_H at 7.01-7.14 ppm. is assigned to aromatic ring due to metallic effect that form complex. The proton having δ_H at 3.17 ppm. is shows to aliphatic carbon which bonded to tertiary amide group. The DMSO signal appeared δ_H at 2.50 ppm. [70]. So the spectra are indicating the signals number proton was confirmed with proposed metal complex.

4.8.5 ¹³C-NMR spectrum of the Complex

The δ_C at 155.8 ppm. is indicates from ¹³C-NMR data spectra of complex (**Table 4.4**) is the quaternary carbon which assigned at C-5a and C-7a. This confirmed that the carbons contains nitrogen double bonded that form metallic complex. The δ_C at (79.6, 115.7, 123.3 and 126.3 ppm.) is indicate the carbon assigned with (C- 6 and C-7), (C-2 and C-3), (C-1 and C-4) and

(C-4a and C-8a) is respectively. So from this it shows that the number of carbon peak is confirmed with proposed metal complex.

Table 4.4 NMR spectrum of Ni- complex in (DMSO-d6)

Position	δ_{H}	δ_{C}	HSQC
1	7.13	123.3	H-C
2	7.06	115.7	H-C
3	7.06	115.7	H-C
4	7.13	123.3	H-C
4a	-	126.3	-
5	8.3	-	H-N
5a	-	155.8	-
6	3.17	79.	2H-C
7	3.17	79.	2H-C
7a	-	155.8	-
8	8.3	-	H-N
8a	-	126.3	-

4.9 Conductivity measurement

The measurement of molar conductance is an important tool that gives valuable information about the coordination number, the structure of the complex and the nature of counter ions present in the complex. It also helps us to confirm the electrolytic nature and there by the members of ions present outside the coordination sphere.

Conductivity of the complex was measured in DMSO at 10^{-3} mol/L and room temperature the molar conductance (Λ^M) of a solution was calculated from the measured value of specific conductance;

$$\Lambda^M = 1000k.S./C$$

Where K: cell constant: Conductivity of the solution in Siemens or the resistance in ohm ($1S=1ohm^{-1}$); C: dissolution concentration of complexes in mol/L

The studies show negligible molar conductance values $0.11 S cm^2 mol^{-1}$, indicating that the complex is non-electrolyte [72, 73] and the presence of chloride ions within the coordination sphere [74] of Ni-complex.

Table 4.5 Result of Conductometric measurement of Ligand and its Ni-complex

Compound	Solvent	Λ^M $S cm^2 mol^{-1}$	Nature of complex
Ligand	DMSO	0.06	none electrolyte
Ni-Complex	DMSO	0.11	none electrolyte

4.10 Electron spectra.

Electronic spectra were used to assign stereo chemistries of metal ions in the complex based on the position and number of d-d transition peak. The electronic absorption spectra of the ligand and complex were recorded at room temperature using DMSO as a solvent (**Appendix 9 and 10**). The Uv-vis spectrum of the ligand showed two bands at 277 and 311 nm which are assigned to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$, transition respectively. Which was indicate that C=C and C=N/C=O respectively. The Uv-vis spectra of the metal complexes display different absorption spectra than that of the ligand which are shifted to higher wavelengths beside a disappearance

of the C=O bond and appearance C=N of transition which confirm the coordination through azomethine nitrogen. Also the d-d transition in this type of complexes may appear above 500 nm but it can appear at 344 nm, 402 nm and 460 nm due to the low intensity of the d-d transition [75] which is shows octahedral geometry of Ni-complex aspects.

Table 4.6 Electronic spectral data of the ligand and its Ni- complex

Compound	λ in nm	Wave number in cm^{-1}	Assignments
Ligand (L)	277	36,101	$\pi \rightarrow \pi^*$
	312	32,051	$n \rightarrow \pi^*$
[Ni (L)Cl ₂]	313	31,949	Charge transfer
	327	30,581	Charge transfer
	344	29,069	${}^3A_{2g} \rightarrow {}^3T_{1g}(F)$
	402	24876	${}^3A_{2g}(F) \rightarrow {}^3A_{1g}(P)$
	460	21739	${}^3A_{2g}(F) \rightarrow {}^3A_{1g}(F)$

4.11 Antibacterial Activity Tests

The ligand and its nickel complex were screened against two human pathogenic bacteria viz. *Helicobacter pylori* (G-) and *Enterococcus faecalis* (G+) bacteria strains to assess their potential as anti-bacterial agents by disc diffusion method. Activity was determined by measuring the diameter of the zones of inhibition (mm) (**Table 7**). The result of the antibacterial activity of ligand and nickel complex were compared with standard drug (ciprofloxacin) has indicated that a ligand and its nickel complex were more active than the standard drug against *Enterococcus faecalis* bacteria. It is found that the metal complex has higher antibacterial activity than the free ligand. Hence, it known that chelation tends to make the ligand more powerful and potent bacterial agent [76–78]

A possible explanation for this increase in the activity upon chelation is that in a chelated complex, positive charge of the metal is partially shared with donor atoms present on ligands and there is an electron delocalization over the whole chelating ring. This, in turn increases the lipid layers of the bacterial membranes. It also suggested that the chelated complexes deactivate various cellular enzymes, which play a vital role in various metabolic pathways of these microorganisms [79, 80]. Generally the qualitative antimicrobial assay results showed that Schiff base and its Ni-complex were more inhibited Gram-positive bacteria than Gram-negative bacteria [81].

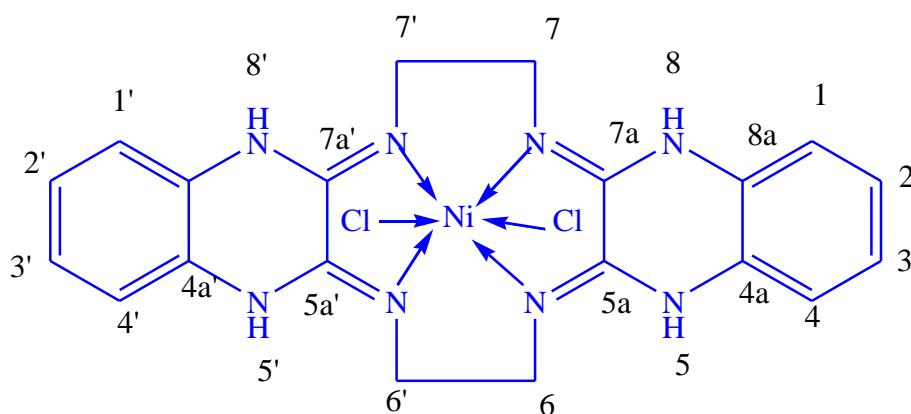
Table 4.7 Antibacterial activity's of the ligand and its Ni- complex

Bacteria strain	Gram	Zone of inhibition (mm)			
		Compound			
		Ligand	<i>Ni-complex</i>	<i>ciprofloxacin</i>)	<i>DMSO</i>
<i>E. faecalis</i>	+Ve	25	50	23	0
<i>H.pylori</i>	- Ve	20	9	33	0

5 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

A precursor Schiff base ligand has been synthesized from quinoxaline and ethylenediamine. A new Ni-complex was synthesized by template method from nickel salt, Schiff base ligand and ethylenediamine then it subjected to characterization and structural elucidation based on IR, ^1H -, ^{13}C -NMR, electronic spectra, elemental analysis, magnetic moment measurements and molar conductance result. The atomic absorption spectral data showed metal to ligand ratio to be 1:1. The conductivity of the complexes was showed non-electrolyte nature and also the complex's showed octahedral geometries. From Uv-vis spectrum a characteristic band for d-d transition is observed besides the absorbance from the ligand. The *in vitro* tests for antibacterial activities showed that most of the prepared Schiff base ligand and metal complex was shows a good antibacterial activities. But comparison of the value of ligand with its metal complex's indicates that the nickel complex exhibited higher antibacterial activities than Ligand. Compounds display a good activities on the Gram positive bacteria but less activity on the Gram negative bacteria (*H. pylori* bacteria). Based on the affirmation study structure of the newly ligand nickel complex is proposed as follow.



Scheme 8. Possible proposed structure of nickel complex

5.2 Recommendation

The Ni (II) complexes of quinoxaline derivatives are important as antibacterial agents. From this point of view, it was interest to synthesise and characterize some transitional metal with quinoxaline derivatives used for antimicrobial properties. The work done in this project is not the final but the starting point of synthesis and analysis of the ligand and their transition metal complexes.

Therefore, as far as the availability of staring materials and simplified synthetic methods are concerned, further studies and work are of immense importance for their synthesis and diverse applications.

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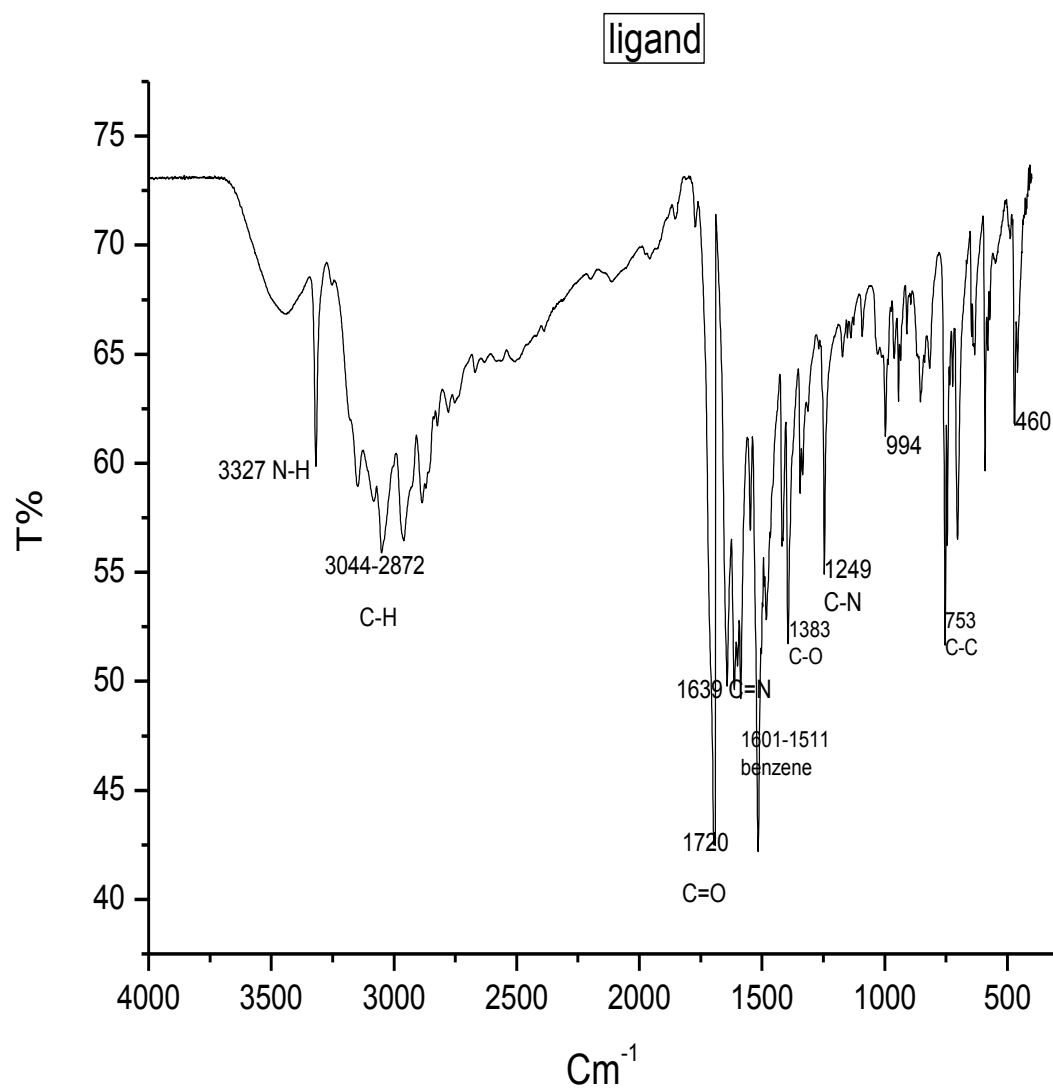
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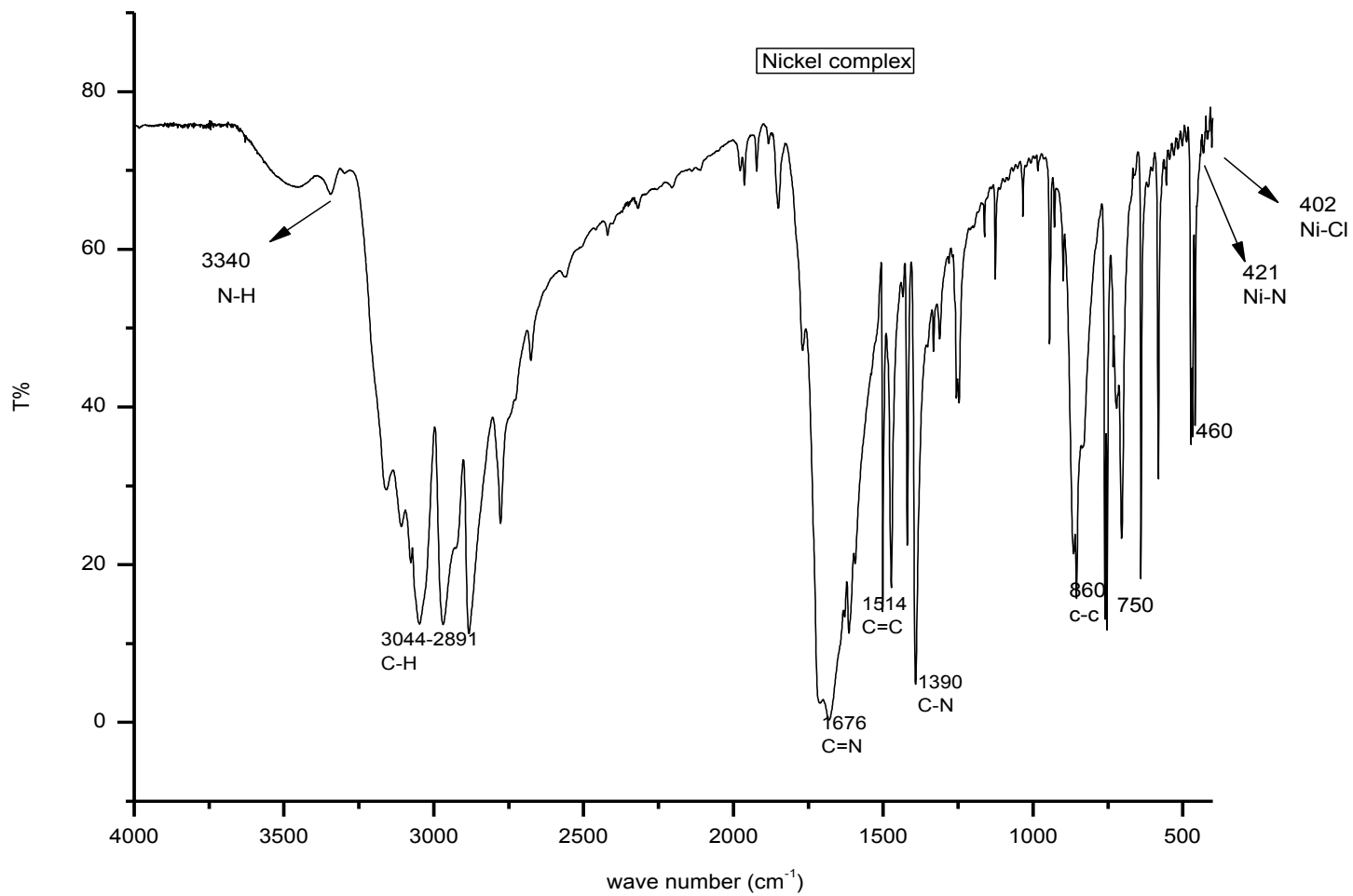
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Appendix

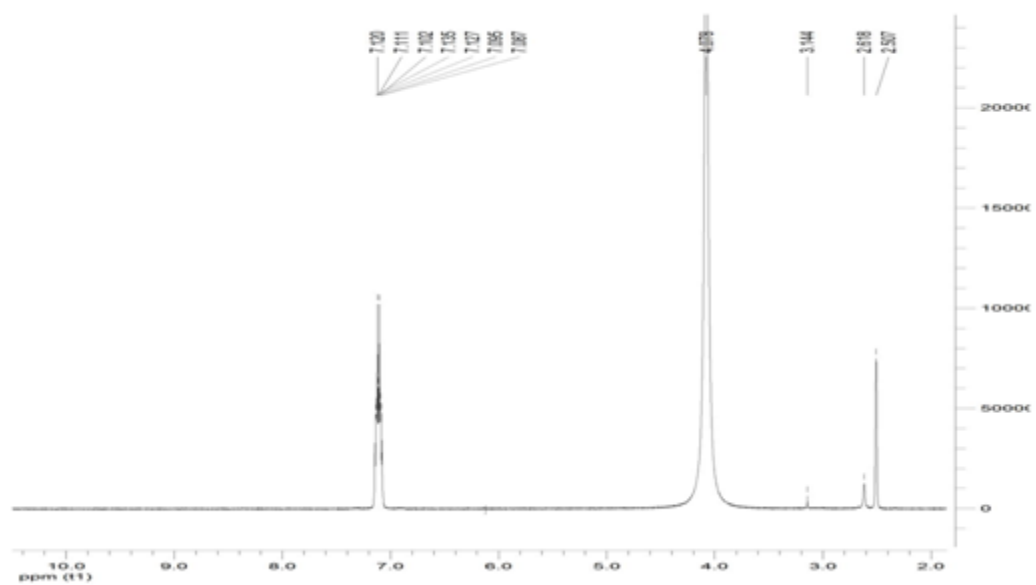
Appendix 1. IR Spectrum of Schiff base ligand



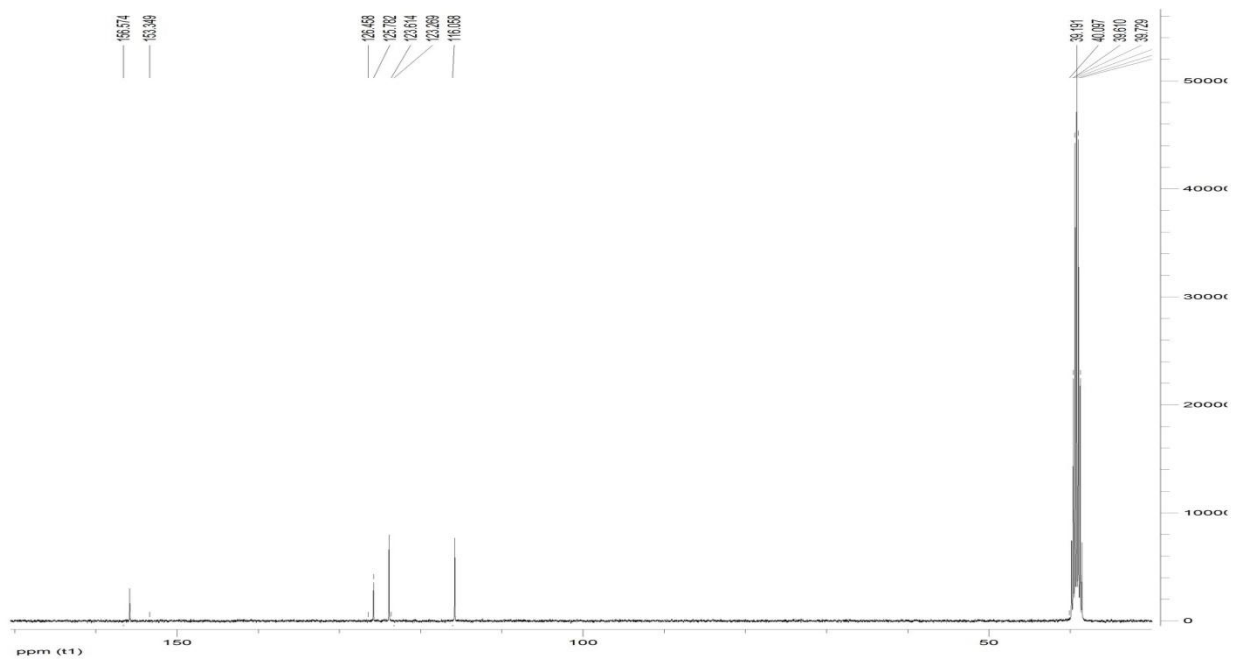
Appendix 2. IR Spectrum of Ni- complex



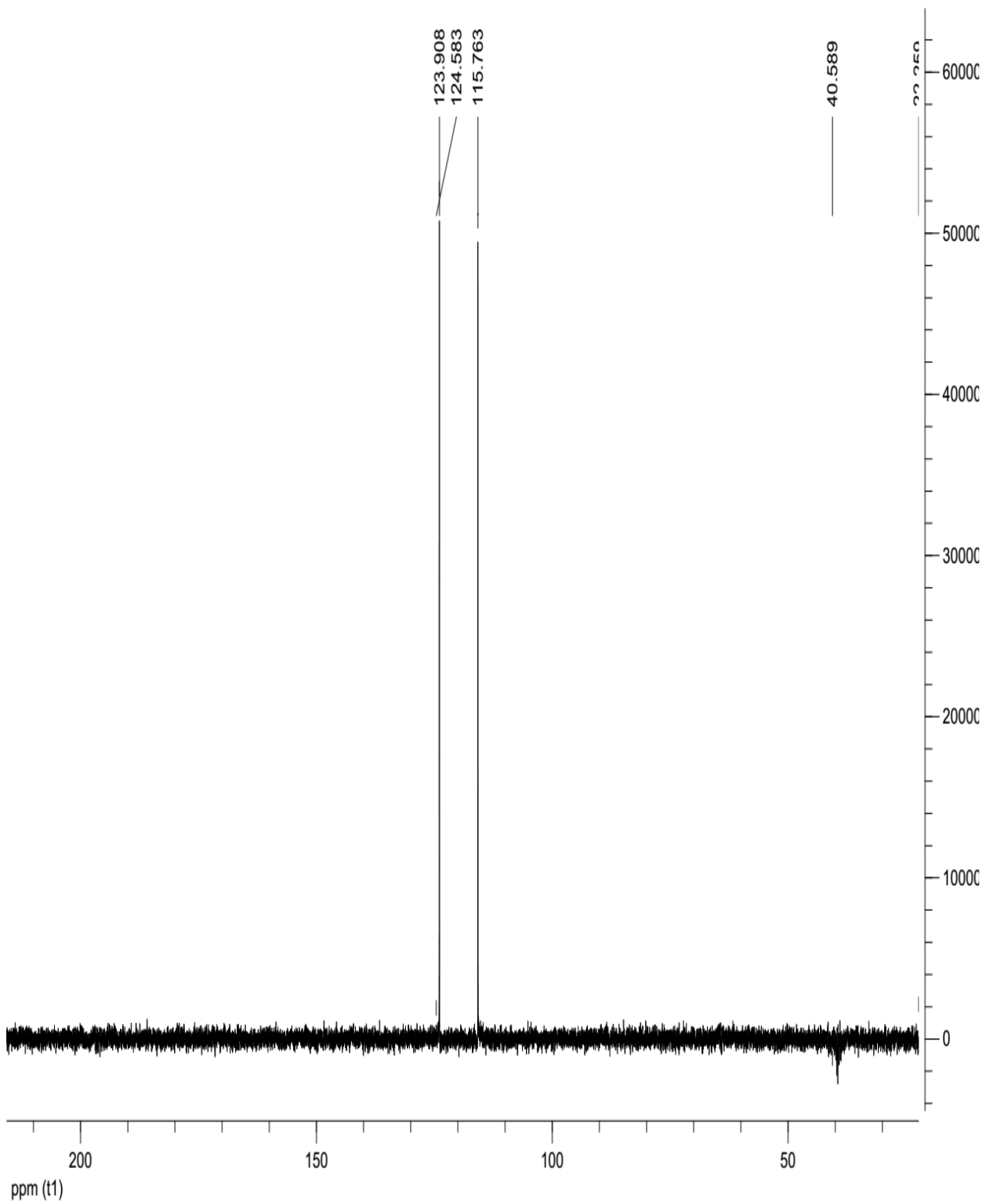
Appendix 3. ^1H - NMR Spectrum of Schiff base ligand



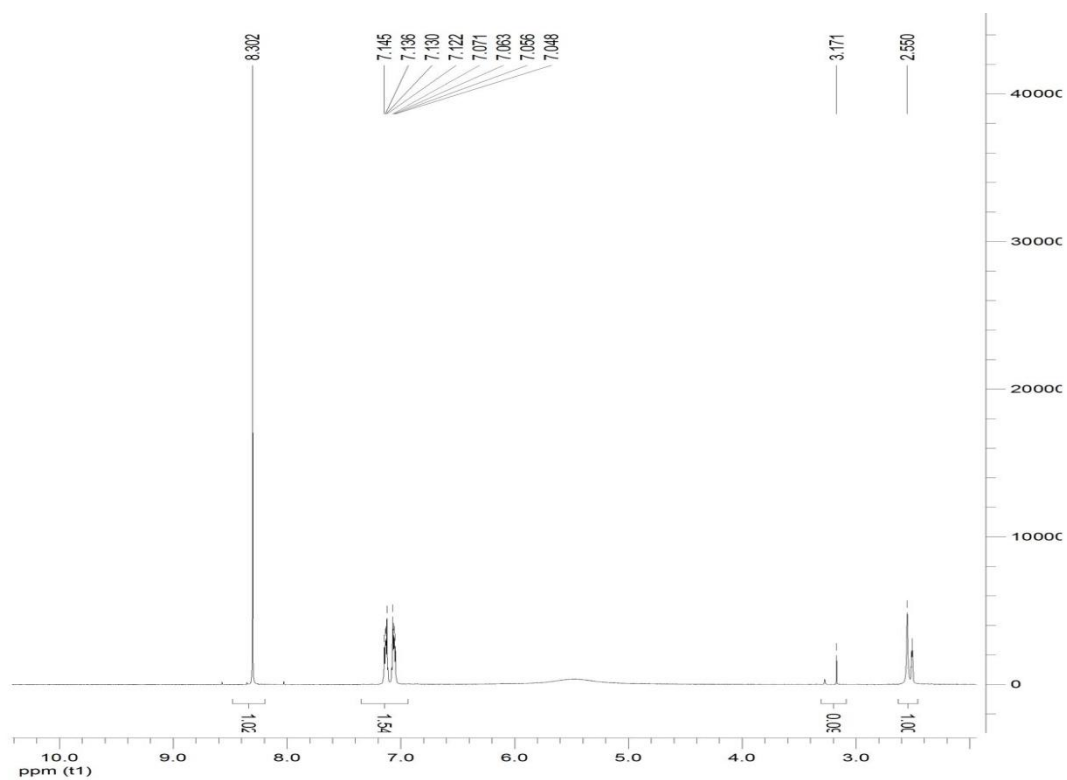
Appendix 4. ^{13}C - NMR Spectrum of Schiff base ligand



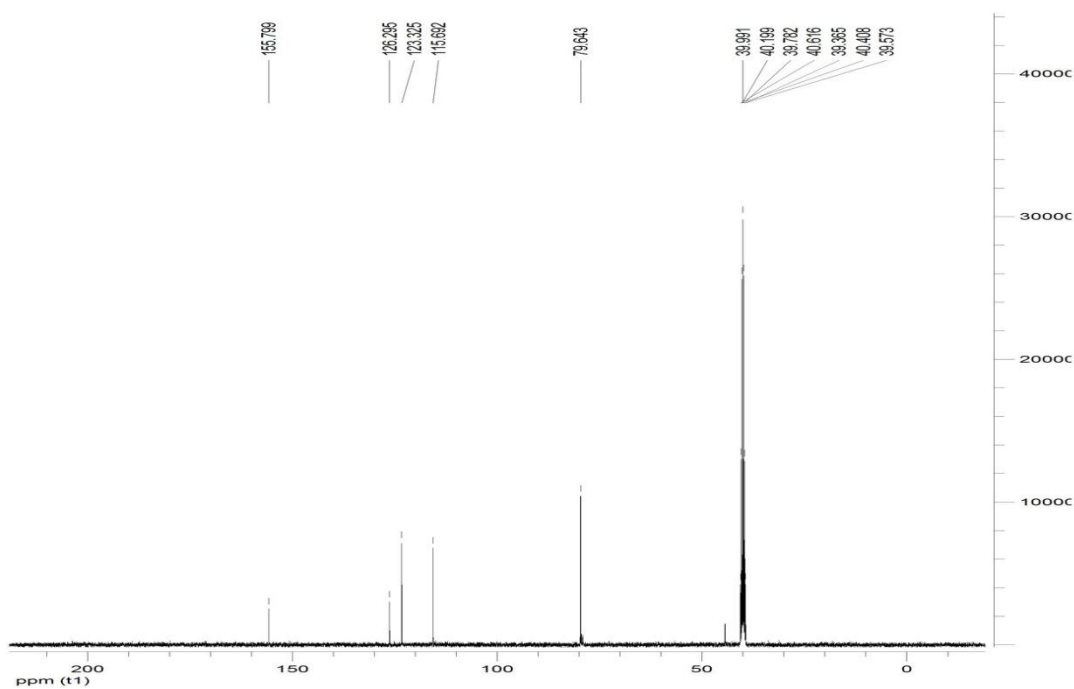
Appendix 5.DEPT-135 Spectrum of Schiff base ligand



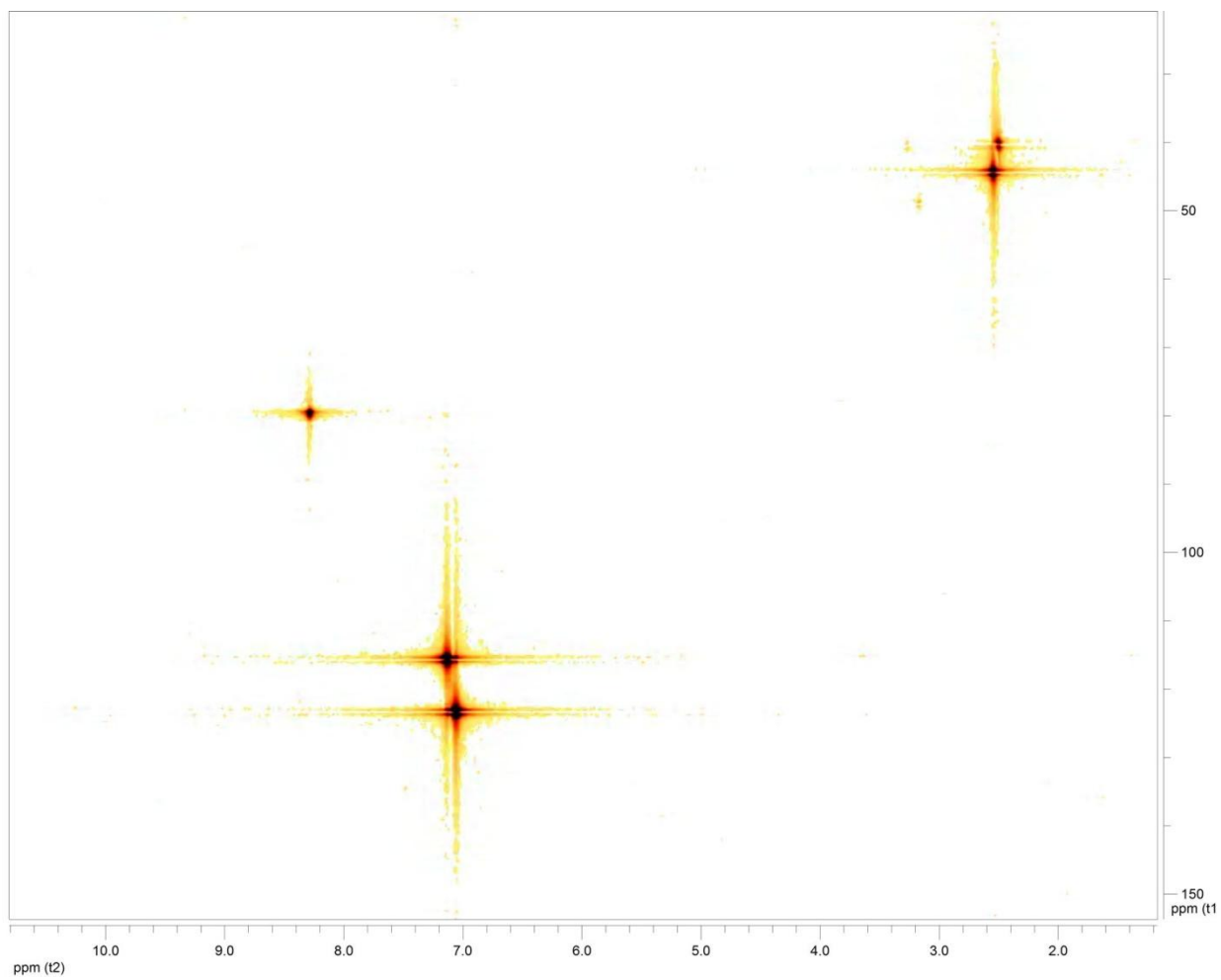
Appendix 6. ^1H NMR Spectrum of Ni- complex



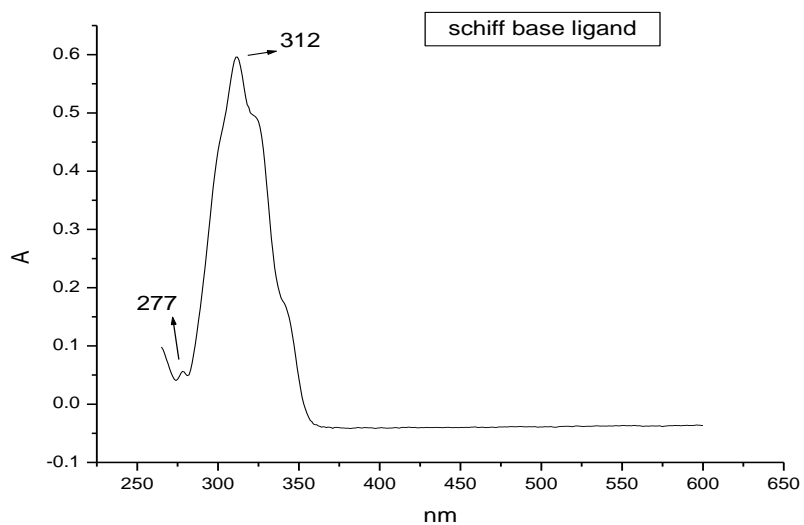
Appendix 7. ^{13}C - NMR Spectrum of Ni- complex



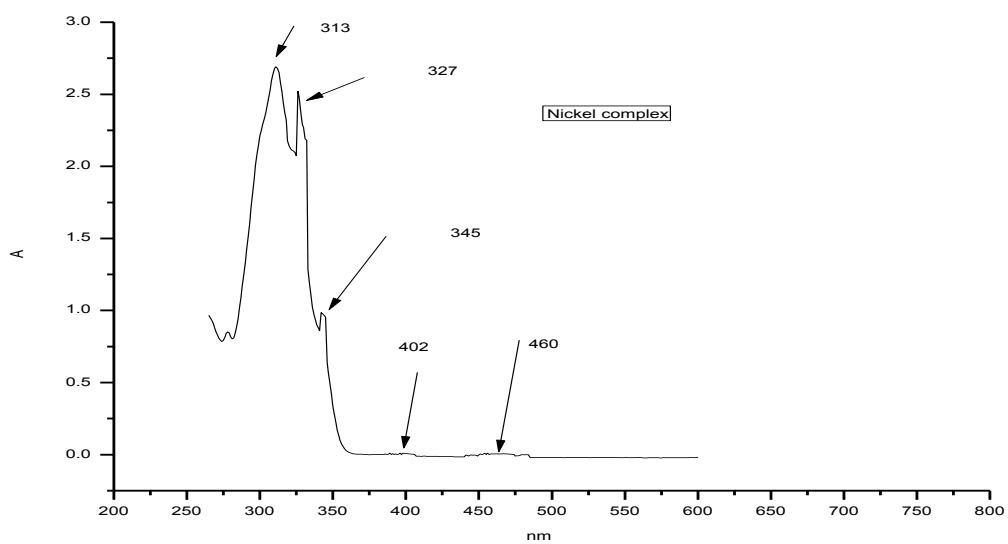
Appendix 8. HSQC spectra of Ni-complex



Appendix 9. UV-VIS of the Schiff base ligand



Appendix 10. UV-VIS of the Ni- complex



Appendix 11 . Antibacterial activity of ligand and its Ni- complex

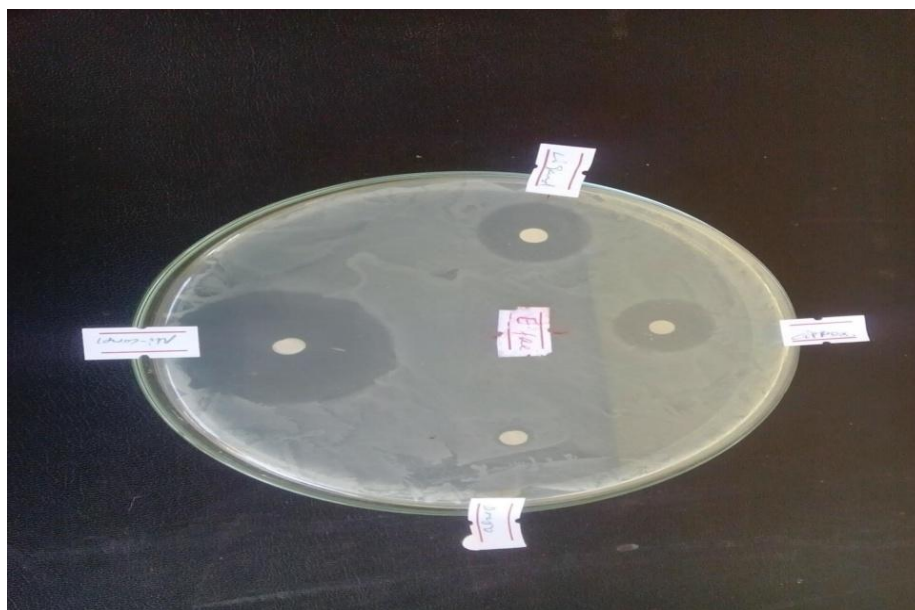


Figure 1. Effects of ligands and their complexes on *Enterococcus faecalis*

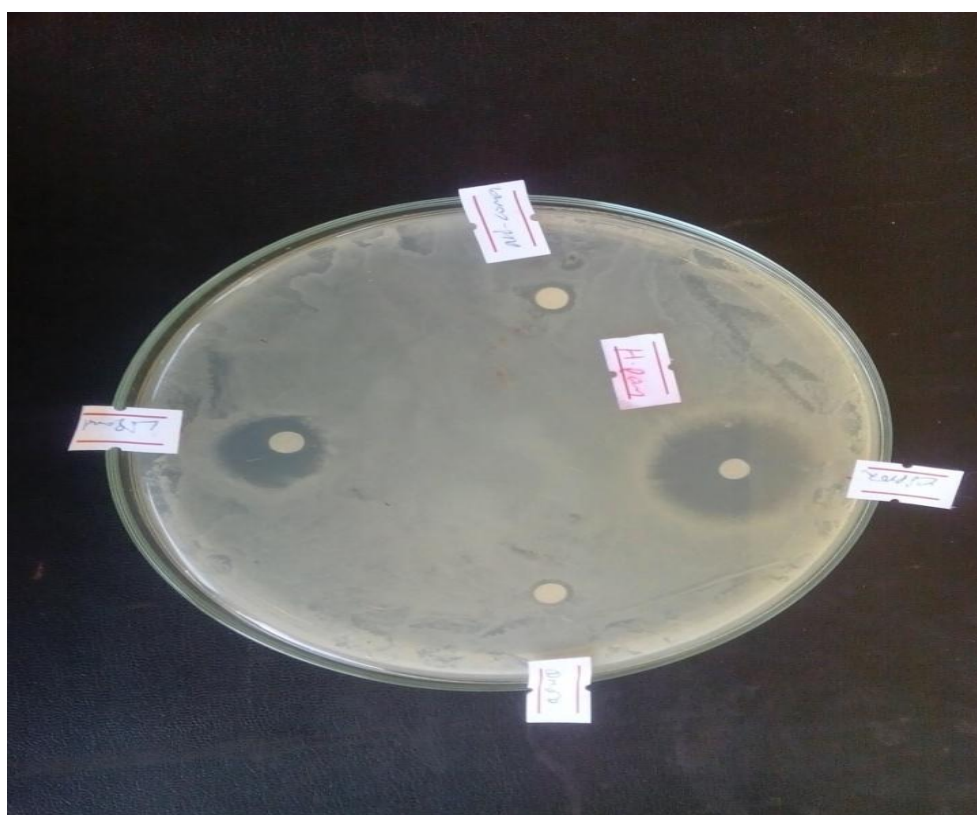


Figure 2. Effects of ligands and their complexes on *Helicobacter pylori*