SYNTHESIS, CHARACTERISATION AND ANTIMICROBIAL ACTIVITY OF MIXED LIGAND COMPLEXES OF SOME DIVALENT TRANSITION METALS CONTAINING ATETRADENTATE SCHIFF BASE (DERIVED FROM P-PHENYLENEDIAMINE AND O-ANISALDEHYDE) AND SOME AMINO ACIDS

BY

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MAY,2016

DECLARATION

I hereby, declare that this research work is the product of my sole research efforts; undertaken under the supervision of Dr. Ibrahim Tajo Siraj and has not been presented and will not be presented elsewhere for the award of a degree or certificate. All sources have duly been acknowledged.

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CERTIFICATION

This is to certify that the research work and subsequent preparation of this dissertation by MOHAMMED SULE (SPS/12/MCH/00040) were carried out under my supervision.

Dr. I. T. Siraj **Date**

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APPROVAL

This is to certify that this dissertation was undertaken by MOHAMMED SULE (SPS/12/MCH/00040) and has been read and approved by undersigned as meeting the requirements for the award of Master of Science in Inorganic Chemistry by the Department of Pure and Industrial Chemistry, Bayero University, Kano.

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DEDICATION

I dedicate this dissertation to my parents and family

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOLS

$\Psi_{g.}$	Mass magnetic moment
$\Psi_{m.}$	Molar magnetic moment
$\mu_{eff.}$	Effective magnetic moment
m·	Molar electrical conductance
Ω.	Resistance
	Frequency of vibration/stretching

ABBREVIATIONS

Ala.	D-alanine
B.M.	Bohr magneton
DMSO.	Dimethylsulfoxide
DMF.	Dimethylformamide
et al .	And others
EtOH	Ethanol
Gly.	Glycine
MeOH	Methanol
PPD.	Paraphenylenediamine

ABSTRACT

Tetradentate Schiff base N,N'-bis[o-anisaldehyde]p-phenylenediamine was synthesized by condensation of o-anisaldehyde and p-phenylenediamine in 2:1 mole ratio respectively. Mixed ligand complexes of Mn(II), Fe(II), Co(II) , Ni(II) and Zn(II) with the Schiff base and some amino acids (Glycine and D-alanine) as secondary bidentate ligands have been synthesized in 1:1:1 mole ratio by reflux in ethanol. The compounds were characterized based on melting point/decomposition temperature, solubility, molar conductance, and magnetic susceptibility measurements, infrared and gravimetric analysis. The IR spectrum of the Schiff base registered a peak at 1605cm^{-1} due to the presence of azomethine, while the spectra of the complexes showed peaks in the range of 1588 – 1680 cm^{-1}. Magnetic moment values showed that zinc complexes are diamagnetic while the remaining complexes are all paramagnetic with values in the range of 3.08 – 5.59 B.M suggesting octahedral geometry. Molar conductance of the complexes range between 66.20 to 93.01 $\Omega^{-1}cm^{-2}mol^{-1}$, suggesting they are 1:1 electrolytes. Percentages of the metals and chlorine in the complexes were determined by gravimetric method, and the values obtained were within the range of the theoretical values. The complexes were soluble in DMSO and DMF, insoluble in water, methanol, ethanol, diethyl ether and benzene. The metal complexes were screened for antibacterial and antifungal activity. They were found to be active against *Staphylococcus aureus and Eschericia coli*, but mostly inactive against *Salmonella typhi*. Antifungal screening showed that some of the complexes are active against *Aspergillus fumigatus* while some are active against *Mucor inducus.*

CHAPTER ONE

INTRODUCTION

1.0 Coordination Compounds

Coordination compounds are compounds containing central metal ion surrounded by a set of neutral molecules or ions known as ligands. Metal complexes play essential roles in biological systems, agriculture and pharmaceutical industry. The activity of the metal complexes depends on thermodynamic and kinetic properties. The nature and function of the coordination compounds depend on the metal ion, the donor atom(s) and the structure of the ligand. Metal ions do not form bonds of equal lengths with all donor atoms; similarly, a particular donor atom does not form bonds of the same strength with different metal ions (Hariprasath *et al,* 2010).

A metal complex may contain one or more ligand(s) bonded to the central metal ion. Metal complex containing only one type of ligand is referred to as binary system. If different ligands are bound to the central metal ion, mixed ligand complex is formed. Mixed ligand complexes containing two different ligands are called ternary systems, while complexes containing three different ligands are referred to as quaternary systems (Halli *et al,* 2011).

Schiff bases are well known for their ability to form stable complexes with appreciable biological activities. Mixed ligand complexes containing amino acids as secondary ligands and Schiff bases as primary ligands have been found to be very active biologically, as they tend to mimic the role of metal ions detoxification mechanism (Elmali *et al,* 2000).

1.2 Schiff base ligands

First reported by Hugo Schiff in 1864, Schiff bases are organic compounds obtained from the condensation of primary amines and carbonyl compounds (aldehydes and ketones). They are known as azomethines, or imines (Halli *et al.,* 2011). The presence of azomethine group in structures of Schiff bases confers basicity, as the lone pair of electrons on the nitrogen atom is easily donated into the partially filled d-orbitals of transition metal. Hence, Schiff bases are a significant class of ligands in coordination chemistry and find extensive applications in different fields (Ajay *et al.,* 2012). If the ligand bonds to the central metal ion through more than one donor atom, the resulting compounds are said to be metal chelates. Such ligands are called polydentate or chelating ligands (Halli*, et al., 2011*).

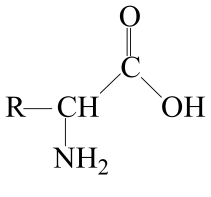
Generally, bidentate, tridentate, tetradentate and polydentate ligands are capable of forming very stable complexes with transition metals. They can only act as chelating ligands if they bear functional group(s), such as hydroxyl group, sufficiently near the site of condensation in such a way that a ring can be formed when reacting with a metal ion. The Schiff bases derived from aromatic amines and aromatic aldehydes have a wide variety of applications. Schiff bases have been noted for their applications as anti-corrosion agent and also in catalysis of many reactions like carbonylation, hydroformylation, oxidation, reduction and epoxidation. Schiff bases containing nitrogen and oxygen donor atoms and their complexes have been used as drugs and have shown good biological activities against wide range of bacterial and fungal species (Wail, 2013). Schiff bases that contain aryl substituent are substantially more stable and more readily synthesized, while those that contain an alkyl substituent are relatively unstable as they easily undergo polymerization (Cimerman *et al.,* 2000).

1.3 Amino acids

Amino acids are the structural units of proteins. They are essential constituents of all living cells and contain one or more amino and carboxylic groups and have good coordination sites for metal chelation (Naikwade *et al.,* 2013). Amino acids have been used as secondary ligands and their complexes have shown great antitumor, antifungal and antibacterial activities. Ternary complexes containing amino acid as secondary ligands are of significance as they are potential models for enzyme-metal ion substrates which play important roles in biological systems. (Anwar *et al.,* 2013).

Ten amino acids are essential; these are valine, leucine, isoleucine, threonine, methionine, lysine, arginine phenylalanine, histidine and tryptophan. Humans must obtain these ten amino acids from their diet because they either cannot synthesize them at all or cannot synthesize them in adequate amounts. The other 11 amino acids are called non-essential amino acids. They include: alanine, arginine, asparagines, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine and tyrosine. Every amino acid has a carbonyl group and amino group, and each group can exist in acidic form or a basic form, depending on the pH of the solution in which the amino acid is dissolved (Robert *et al.,* 1990).

Generally, amino acids are represented as:



$$R-\underset{NH_2}{CH}-\overset{O}{\overset{\|}{C}}-OH$$

Fig. 1.1: Amino acids

Where R = H for glycine, and for aliphatic amino acids, R represents alkyl side chains, the simplest of which is $–CH_3$ as in alanine.

1.4 p-phenylenediamine (PPD)

This is an aromatic amine also known as benzene–1, 4–diamine or 1, 4–diaminobezene, while, the ortho-isomer is known as benzene–1, 2–diamine or 1, 2–diaminobenzene.

p–phenylenediamine and related compounds have been used as antioxidant in rubber products because of their colour. p–phenylenediamine has been used for dyeing furs. It occurs as red solid or crystals that darken on exposure to air and it is slightly soluble in water (Yoshiki *et al.*, 2014).

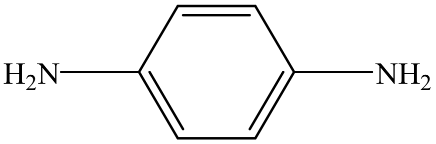
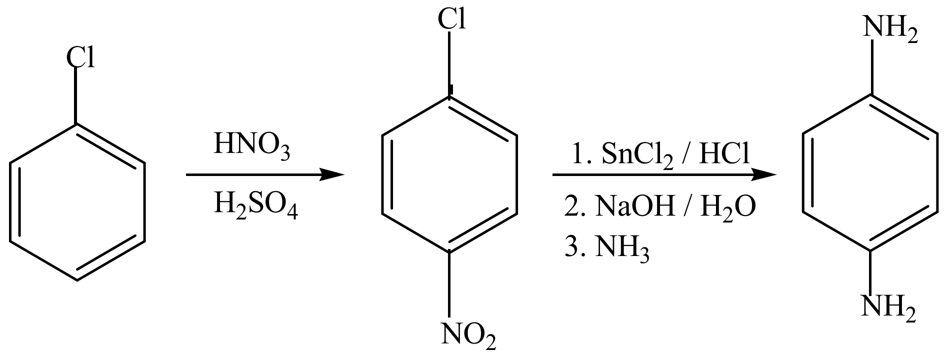


Fig 1.1: P-phenylenediamine

Aromatic amines are related to aliphatic amines in their behavior. However because of the highly conjugated benzene ring attached to them, the lone pair of electrons on the nitrogen atom is less active than the lone pair on the nitrogen atom of aliphatic amines. This is due to resonance delocalization of the lone pair of electrons in aromatic amines.

p-phenylenediamine is synthesized from chlorobenzen by nitration and subsequent reduction to form p-chloroaniline, which reacts with ammonia to form p-phenylenediamine (Ralph *et al.*, 2001).



Scheme 1.1: Synthesis of p-phenylenediamine

1.5 o–anisaldehyde

This is an aromatic compound containing an aldehyde group and a methoxy group on positions 1 and 2 of the benzene ring respectively. It is also known as 2–methoxybenzaldehyde. It is a light brown solid (flakes) at room temperature.

o–anisaldehyde may undergo condensation with amino groups of amines to form Schiff bases. The presence of the donor oxygen atom of the methoxy group at ortho position makes it suitable for synthesis of polydentate Schiff bases.

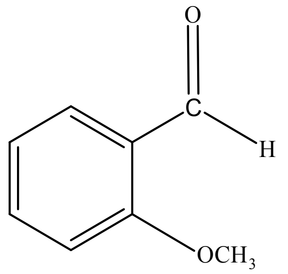
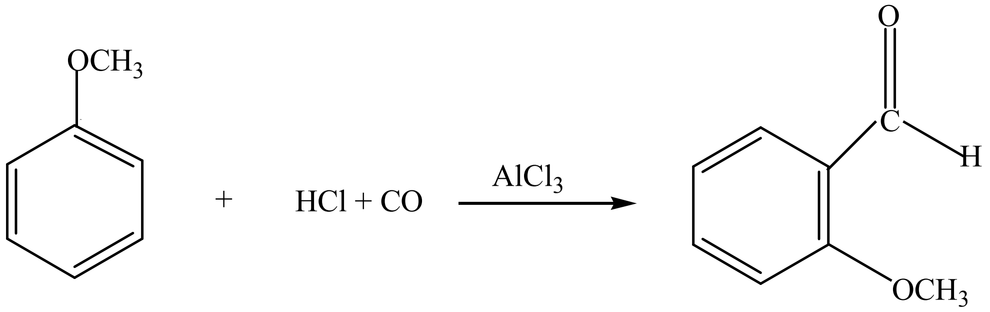


Fig 1.3: o-anisaldehyde

o- anisaldehyde is synthesized via formylation of methoxybenzene (anisole) using a mixture of hydrochloric acid and carbon(II) oxide in the presence of $AlCl_3$ as catalyst (Suresh *et al.*, 2004).



Scheme1.2: Synthesis of o-anisaldehyde

1.6 Transition metals

These are elements having partially filled d-orbitals in any common oxidation state. They are characterized by their ability to form wide range of coordination compounds due to the availability of the partially filled d-orbital which make them good lewis acids. Their ability to exhibit variable oxidation states makes it feasible for the transition metals to undergo redox reactions, and form a variety of coordination compounds in which octahedral, tetrahedral, square-planar and square-pyramidal geometries are more predominant.

The transition metals selected for this work are: Mn, Fe, Co, Ni and Zn.

1.6.1 Manganese

Manganese is the 12th most abundant element in the biosphere. It constitute about 0.098% by mass of the earth. It is widely distributed in the soil, water and biological systems. Manganese does not occur as free metal and is found in more than 100 minerals including various sulphides, oxides, carbonates, silicates, phosphates and borates. Although manganese is essential for most species of animals including humans as well as for plants, it is toxic at higher levels. In man, excess manganese affects the central nervous system with the symptoms resembling those of

Parkinson's disease. This is the reason why manganese belongs to the class of highly toxic heavy metals (Gabriela *et al.*, 2008).

Manganese exhibits oxidation states of +2, +3, +4, +6 and +7. Most of the compounds are coloured and paramagnetic. Manganese form many complexes, mostly in +2 oxidation state which are predominantly octahedral.

Manganese is roughly similar to iron in its physical and chemical properties however, manganese is harder and more brittle than iron. Manganese is an essential nutrient involved in many chemical processes in the body, including processing of cholesterol, carbohydrates and proteins. It might also be involved in bone formation (Bhupinder, 2011).

1.6.2 Iron

Iron is the most abundant transition metal in the biosphere. It occurs in combined form in different ores such as Haematite (Fe_2O_3), Magnetite (Fe_3O_4), Limonite ($Fe_2O_3.3H_2O$), Siderite ($FeCO_3$) and Iron pyrite (FeS_2). It is essential in the higher forms of life and its compounds have numerous industrial applications. The element has catalytic properties. Oxidation state ranging from +2 to +6 are known, the most common are +2 (Ferrous ion) and +3 (Ferric ion). Most common complexes are octahedral, although tetrahedral and square planar geometries are well known.

Depending on the ligand involved, high and low spin complexes can be formed. A number of iron compounds have been found to be medically useful. Ferrous gluconate, $Fe(C_6H_{11}O_7)_2.2H_2O$, and Ferric pyrophosphate $Fe_4(P_2O_7).xH_2O$ are among the compounds frequently used to treat anemia. Various ferric salts act as coagulants and myoglobin, which is iron chelate transports and stores oxygen in the blood (Bhupinder, 2011).

1.6.3 Cobalt

Cobalt exhibits various oxidation states ranging from +1 to +5. The most common oxidation states are +2 and +3. The cobalt compounds exhibit variable coordination number with tetrahedral, octahedral and square planner geometries being more prevalent. Cobalt occurs in nature in association with nickel and arsenic. Most important minerals are smaltite , $CoAs_2$ and Cobaltite, CoAsS. Only few Cobalt (III) complexes are known to have biochemical activity. Vitamin B_{12} is a cobaloxime, a cobalt complex containing a glyoxime ligand which plays a significant role in biological system. The Co^{3+} ion is unstable in water, but can be stabilized against reduction to Co^{2+} by coordination to ligands. (Eddie *et al.,* 2010).

1.6.4 Nickel

Nickel is widely distributed in the earth; there are almost 100 minerals of which it is an essential constituent. It is the 24th most abundant element in the earth's crust, comprising about 3% of the composition of the earth. Nickel is a nutritionally essential trace metal for many animal species, micro-organisms and plants. Although it can exist in different oxidation states, the prevalent oxidation state is +2. Other oxidation states (-1, +1, +3 and +4) are also encountered, though not frequently. Nickel (II) complexes exhibit mainly square-planar and octahedral geometry, which depend on the nature of solvent, concentration and temperature. Nickel also exhibit coordination number of five, in which it forms trigonal bipyramidal complex. Nickel is a micro element in biological systems and it serves as an activator for many enzymes such as arginase, acid phosphatase, decarboxylase, deoxyribonuclase and peptidase (Cempel and Nikel., 2006).

1.6.5 Zinc

Zinc makes up about 75ppm (0.0075%) of Earth's crust. It is usually found in association with other base metals such as copper and lead in ores. It exhibits oxidation states of +1 and +2. Zn(II) complexes are diamagnetic due to the filled d^{10} configuration.

The complexes of Zn(II) can have coordination numbers 4, 5 and 6. It is invariably seen that zinc forms only tetrahedral complexes with coordination number 4. However, Zn(II) complexes with coordination number 6 and octahedral geometry have been reported (Robert *et al.*, 1990).

Zinc is the second most common trace element after iron and is involved in structure and function of over 300 enzymes. Zinc is involved in the formation of enzyme which converts progesterone into testosterone. Zinc oxide (applied to the skin) is used to treat rashes and minor burns. Zinc supplementation shows beneficial effects against infectious diseases, especially diarrhea. Deficiency of Zinc in animals results in stunted growth and male sexual inmaturity (Bhupinder, 2011).

1.7 Mixed ligand complexes

Mixed ligand complexes contain more than one type of ligand bonded to the central metal ion, and they are of significance in many chemical and biological systems, like water softening, respiration, photosynthesis as well as removal of undesirable and harmful metals from the living system (Sanap and Patil, 2013).

The fact that transition metals exhibit great biological activity when associated with certain ligands mimicking metal-protein complexes has been a motivation in the study of mixed ligand complexes containing amino acids as secondary ligands . Mixed ligand complexes containing Schiff base ligands have been identified as important compounds due to their industrial and biological relevance (*Ketul et al.,* 2011). The stability of the complexes depends largely on the characteristic of the secondary ligand, the length and spatial configuration of the chelating ring and the ring size which affect the basicity of the secondary ligands. Generally, the size of the primary ligand is important, because bulky primary ligands tend to hinder secondary ligands of relatively big sizes (Suresh *et al.,* 2013).

1.8 Aim and Objectives

1.8.1 Aim of the study

This research work is aimed at the synthesis and characterization of mixed ligand complexes of a tetradentate Schiff base and some amino acids (secondary ligands) with Ni(II), Fe(II), Mn(II), Co(II),and Zn(II) ions and subsequently, study of their antimicrobial activities.

1.2 Objectives of the study

The objectives of this research work are to:

1 Synthesize a tetradentate Schiff base derived from p-phenylinediamine and o-anisaldehyde,

2 Synthesise mixed ligand complexes of Ni(II), Fe(II), Mn(II) Co(II) and Zn(II) ions with the synthesized tetradentate Schiff base and some amino acids (Glycine and D-alanine) as secondary bidentate ligands,

3 To characterize the Schiff base and its mixed ligand complexes based on:

- Solubility
- Decomposition temperature
- Magnetic susceptibility analysis
- Molar conductance measurement
- Infrared spectral analysis
- Gravimetric analysis and

4 To screen the ligand and complexes against some bacterial and fungal isolates

CHAPTER TWO

2.0 LITERATURE REVIEW

The study of coordination chemistry of biologically important metal ions with mixed ligands has been one of the important developments in the field of bioinorganic chemistry. Mixed ligand complexes containing Schiff bases and amino acids have been studied. Tetradentate Schiff bases with nitrogen and oxygen donor atoms have been widely studied for their ability to coordinate with metal ions. Their applications in catalysis, pharmacy, anti-corrosion as well as antimicrobial activity have been well documented (Wail, 2013).

Ikechukwu and Peter, (2015), synthesized complexes of Co(II), Ni(II), Zn(II) and Cu(II) with a tetradentate Schiff base derived from ethylenediamine, 2,4– dihydroxyacetophenone and 1–phenylbutane- 1, 3–dione. The complexes were characterized by elemental analysis, FTIR, UV-Visible spectroscopy and screened to establish their potentials as antibacterial agents. The analytical and spectroscopic data suggested square planar geometry for Cu(II) and Ni(II) complexes and an octahedral geometry for the Co(II) complex. The complexes were screened for their antimicrobial activity and the results showed reasonable antibacterial activities against both Gram (+) and Gram (–) bacteria.

Mixed ligand complexes of iron(III) and Au(III) with succinimide or 8–hydroxyquinoline as primary ligands and amino acids as secondary ligands were synthesized and reported by Anwar *et al.,* (2013).The complexes were prepared in solid form and characterized by elemental analysis, conductivity, magnetic moment measurement, IR analysis and NMR. The Au(III) complexes show some antibacterial activity against *Salmonella typhi, Staphylococcus aureus, and Eschericia coli* and antifungal activity against *Aspergillus niger* and *Fusarium oxysporum.*

The ligand and iron (III) complexes were found to be mostly inactive against both bacterial and fungal isolates. Electronic and other measurements confirmed their octahedral and square planar structures.

Ajay *et al.* (2012), reported the synthesis of Co(II) complexes of Schiff base (2–amino–4–nitrophenol–N–Salicylidene) with some amino acids as secondary ligands. The mixed ligand complexes were non hygroscopic and stable solids. The structural characterization of the Schiff base and its Co(II) complexes were done on the basis of their melting point, solubility, magnetic susceptibility and X–ray diffraction studies. The morphology of the complexes were studied by scanning electron microscopy (SEM).The Schiff base and complexes were also screened against some bacterial and fungal isolates; *Salmonella typhi, Staphylococcus aureus, Aspergillus niger* and *Candida albicans,*and the complexes were found to be more active against the bacterial isolates than the fungal isolates, while the ligand was inactive against both bacterial and fungal isolates.

In another report by Sanap and Patil, (2013), chiral mixed ligand (CML) metal complexes of the type [M(PMINAP) (AA). $2H_2O$], were synthesized,where M is Ni (II), PMINAP is sodium salt of p–methoxyisonitrosoacteophenone and "AA" is a chiral amino acid. The metal complexes were characterized by elemental analysis and various physico-chemical techniques. All complexes were found to be green stable solids with octahedral geometry and show varying solubility in common organic solvents. The complexes were screened against *Eschericia coli, Salmonella thypi, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* and it was found that they were active against the fungal isolates than the bacterial isolates.

Mikkat and Ihsan, (2013), have reported preparation and characterization of mixed ligand complexes of Au(III) with some amino acids and dithiocarbamates and dithiophosphates. The complexes of the type [Au(L)(L_1 or L_2)]Cl were prepared, where L = glycine, alanine, valine or methionine, L_1 = N-methylcyclohexyldithiocarbamate or benzyldithiocarbamate anions, and L_2 = O, O-dibenzydithiophosphate and O, O-dipropyldithiophosphate. The compounds were characterized by elemental analysis, molar conductivity, magnetic susceptibility and ^{1}H NMR. The complexes were coloured, square planar electrolytes of the ML type with molar conductance values in the range of 69.5 – 88.7 $cm^2\,\Omega^{-1}mol^{-1}$.

Tetradentate Schiff base N, N–bis [2–hydroxyacetoplenone] ethylenediamine by 2:1 molar condensation of 2–hydroxyl acetophenone with ethylenediamine was synthesized. Neutral bidentate ligand from benzaldehyde and o–phenylinediamine in 1:1 molar ratio in alcoholic solution was also synthesized. The two ligands were used to synthesize Ni(II), Cu(II), Co(II), Mn(II), Fe(II) and Zn(II) complexes. The compounds were characterized by elemental analysis, magnetic measurements, infrared spectral analysis, UV–Visible spectra and thermogravimetric analysis. The complexes were proposed to have octahedral geometry. The antibacterial test of the complexes shows good activity against *Bacillus substils* and *Eschericia coli* (Ketul *et al,* 2011).

Taleb, (2009), reported the synthesis and characterization of metal complexes of Cr(III), Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Ru(III), Rh(III) and Pd(II) with derivatives of 1,3,4-thiadiazole-2,5-dithiol as new ligands. The complexes were characterized based on microanalysis, UV-Visible spectroscopy, ^{1}HNMR, IR spectroscopy, atomic absorption, magnetic susceptibility continuous variation method and molar conductance technique. It was found that the complexes of Co(II), Ni(II), Cu(II), and Pd(II) were electrolytic and of the ML type with octahedral geometry. Mn(II) complex was also electrolytic but of the ML_2type, while Cr(III), and Fe(III) were the ML_3 type also with octahedral geometry. Rh(III) and Ru(III) complexes were non electrolytes.

Yuvraj *et al,* (2009), have reported the synthesis, characterization and antimicrobial activities of mixed ligand Zr(IV) complexes of the type $[M(Q)_2LNO_3xH_2O]$ using 8-hydroxyquinoline(HQ) as primary ligand and N and O donor amino acids (LNO) namely L-serine, L-alanine and glycine as secondary ligands.The complexes were non-electrolytes and thermally stable. They also show good antimicrobial activity against pathogenic bacteria *staphylococcus aureus* and *Enterococcus faeccium.* Antifungal evaluation shows that the complexes were active against *Aspergillus fumigatus, Candida albicans* and *Candida krusei.*

Synthesis, characterization and antimicrobial activities of Mn(II), Co(II), Fe(II), Zn(II) and Cd(II) mixed ligand complexes containing amino acid(L-valine) and saccharin was reported by Fayad *et al,* (2012). All the prepared complexes were characterized by molar conductivity measurement, magnetic susceptibility, infrared and elemental analysis. The study shows that the complexes have octahedral geometry. The valine acted as a bidentate ligand coordinating through the nitrogen atom of the amino group (NH_2) and the oxygen atom of the carboxylic group. The complexes also show good antimicrobial activity against*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas eruginosa.*

Bipind and Rajeam, (2013), reported a biomimicking approach to the mixed ligand complexes of bivalent transition metals. The metal complexes of the type ML_1L_2(M = Cu(II), Ni(II) and Co(II), L_1 = α, α`-diphyridyl, L_2 = hydroxybenzalidineanthranilic acid) were proposed. The resulting metal complexes have been characterized on the basis of elemental analysis, IR-spectra, electronic spectra, magnetic susceptibility and molar conductance measurements. The antifungal and antibacterial activities of the ligands and their metal complexes have been evaluated against *Aspergillus flavus* ,*Aspergillus fumigatus*, *Aspergillus niger*,*Eschericia coli* and *Staphylococcus aureus*.

Recently, Rehab and Basima, (2016), have prepared complexes of some first row transition metal(II) ions containing a tetradentate Schiff base derived from 2-benzoylbenzoic acid and ethelynediamine. The Schiff base and complexes were characterized by IR-specral analysis, ^{1}H and ^{13}C-NMR, elemental analysis, magnetic moment measurement and molar conductance. It was suggested that the Schiff base behaves as a tetradentate ligand, forming chelates with 1:1 (ligand metal) stoichiometry. *Invitro* test for antibacterial and antifungal activity showed that most of the prepared compounds displayed a good activity against *Staphylococcus aureus, Escherichia coli Bacillus subtilis* and *pseudomonas aeruginosa.*

CHAPTER THREE

3.0 Materials and Methods

All chemicals used in the preparation of the Schiff base and the mixed ligand complexes were of analytical grade.

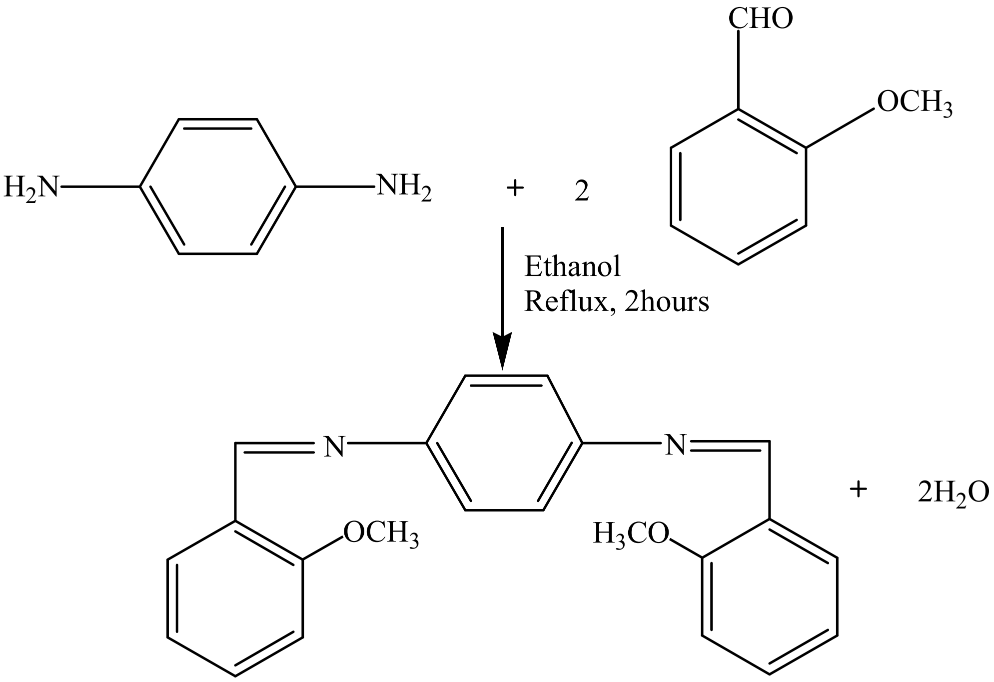
Three bacterial isolates; *Staphylococcus aureus, Eschericia coli* and *Salmonella typhi* and two fungal isolates; *Aspergillus fumigatus* and *Mucor inducus* were identified and collected from the Department of Microbiology, Bayero University Kano, Nigeria. Nutrient agar and potato dextrose agar were used as bacterial and fungal media respectively.

3.1 Apparatus

The glass wares used were washed with detergent, rinsed with distilled water and dried in oven at 110°C before use. Melting points/decomposition temperatures were determined using GallenKamp melting point apparatus. Molar conductance measurements were carried out using Jenway conductivity meter 4010 model. Magnetic susceptibility measurement was carried out using magnetic susceptibility meter MK1 model. FTIR analysis was conducted using Fourier Transform Infrared spectrophotometer, Agilent Technolgies Cary model (650-4000cm^{-1}). Biological activity studies were carried out at the Microbiology Laboratory of Bayero Univeristy Kano, Nigeria.

3.2 Preparation of the Schiff base

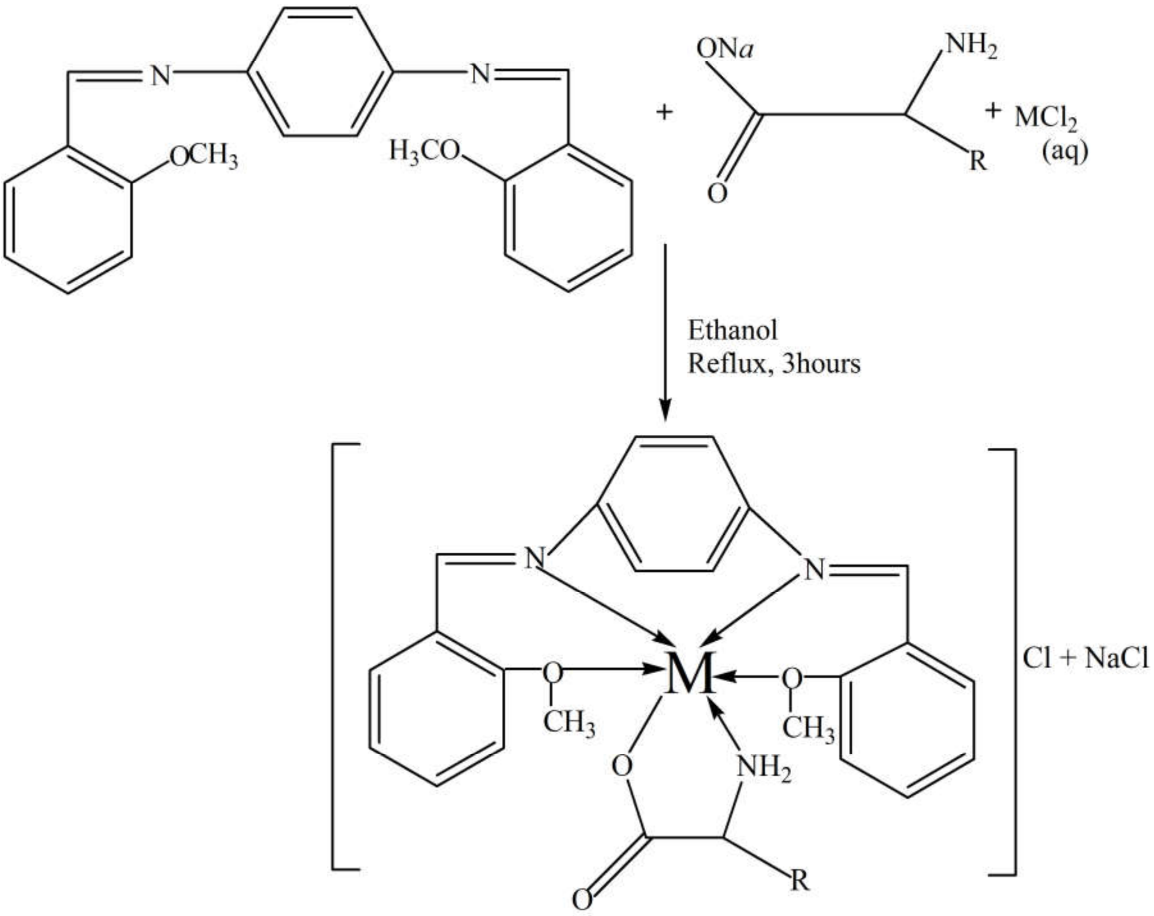
A solution of p-phenylenediamine (0.10814g, 1mmol) and o–anisaldehyde (0.272g, 2mmol) in ethanol (25ml) was refluxed for 2 hours. The reaction product was cooled.The crystalline precipitate obtained was filtered and washed with anhydrous diethylether and dried in air (Ketul *et al.,* 2011).



Scheme 3.1: Preparation of Schiff base

3.3 Preparation of mixed ligand complexes

Ethanolic solution (25ml) of the Schiff base (0.344g, 1mmol) and metal (II) chloride (1mmol) was mixed with a solution of the amino acid (1mmol) containing sodium hydroxide (0.1molar). It was refluxed for 3 hours, concentrated on water bath and the precipitate obtained was filtered and washed with absolute ethanol and then washed again with anhydrous diethylether and dried in air (Mikkat and Ihsan , 2013).



M = Mn(II), Fe(II), Co(II), Ni(II) and Zn(II)

R = H   for Glycine

R = – CH_3 for D-alanine

Scheme 3.2: Preparation of mixed lignd complexes

3.4 Melting point and decomposition temperature determination

The melting point of the Schiff base, and decomposition temperature of the complexes were determined using Gallenkamp melting point apparatus. Powdered samples were introduced into capillary tubes and inserted into the apparatus. The temperature at which the ligand/complexes melt or complexes decompose are shown in Table 4.1

3.5 Magnetic susceptibility measurement

The magnetic susceptibility of the complexes was determined using magnetic susceptibility Gouy balance model MK1. Each of the complexes was introduced into a capillary tube. The height of the sample in the capillary tube was measured and recorded. The capillary tube was inserted into the machine and the reading was taken. From the readings obtained, the mass magnetic moment of the complexes was calculated using the relation:

$$\Psi g = \frac{CL(R \quad R_{\circ})}{10^{9}M}$$

Where Ψ_g = mass magnetic moment

C = constant of the machine and it is fixed as 1

L = length of the sample in the capillary tube

R = specific magnetic moment of the sample

$R_{\circ}$ = magnetic moment of the empty capillary tube

M = w_2-w_1

$w_1$and w_2 are the weight of the empty capillary tube and capillary tube + complex respectively.

Positive value of Ψ_g indicates a paramagnetic complex, while a negative value indicates a diamagnetic complex.

Molar magnetic moment of the complexes (Ψ_m) was determined by multiplying the mass magnetic moment of each complex with its molecular weight.

The effective magnetic moment (μ_{eff}) of the complexes were obtained from the relation

$\mu_{eff} = 2.828\sqrt{}(\Psi_m \times 298)$

The results are shown on table 4.2

3.6 Conductance measurement

The conductivity of the complexes was measured using Jenway conductivity meter model 4010. 0.003 molar solutions of the complexes prepared in Dimethylsulfoxide (DMSO) were used. The conductivity was measured by immersing the electrode into the solutions and the readings were taken. After each reading the electrode was rinsed with distilled water (Geary,1971). The molar conductance of the complexes was calculated using the relation:

$$m = \frac{10^3 L}{C}$$

Where m = molar conductance

L = specific conductance of the complexes

C = concentration

The results obtained are shown in Table 4.2

3.7 FT-IR Spectroscopy

The infrared spectra of the tetradentate Schiff base and its mixed ligand complexes were obtained using Fourier Transform Infrared spectrophotometer, Agilent Technolgies Cary model (650 – 4000 cm^{-1}). Few crystals of the compounds were placed on the sample holder, the observed peaks were labeled and the values for the various functional groups were recorded as shown in Table 4.5.

3.8 Estimation of metals in the mixed ligand complexes by gravimetric method

0.2g of each of the complexes was placed in a beaker containing 25cm^3 distilled water, 5cm^3 of concentrated nitric acid was added followed by vigorous stirring with a glass rod, the mixture was then heated on a hot plate to near dryness which was removed and cooled to room temperature. The mixture was then filtered and the filtrate was collected which contains the metal ions (Vogel, 1989).

3.8.1 Determination of Manganese in Manganese (II) complexes

The filtrate was diluted to 100 cm^3 with distilled water. Dilute ammonia was added to neutralize the filtrate followed by 10g ammonium chloride, excess diammonium hydrogen phosphate $(NH_4)_2HPO_4$ and few drops of dilute hydrochloric acid. The solution was heated to 95°C followed by drop wise addition of dilute aqueous ammonia with constant stirring until a precipitate of $Mn_3(PO_4)$ began to form. The addition of ammonia was stopped immediately while the heating and stirring continued to ensure that the precipitate formed crystallized as $MnNH_4PO_4.H_2O$. Little aqueous ammonia was added with stirring until precipitation was completed.The precipitate was weighed as $[MnNH_4PO_4.H_2O]$(Vogel, 1989). The weight of the precipitate was used to calculate the percentage by weight of the manganese in the complexes by multiplying the weight of the precipitate with the gravimetric factor, where:

$$gravimetric\ factor\ of\ Mn\ in\ Mn(II)coplexes\ = \frac{\text{relative atomic mass of Manganese}}{\text{molecular weight of }[\mathrm{MnNH_4.PO_4.H_2O}]}$$

3.8.2 Determination of Iron in Iron (II) complexes

The filtrate obtained from iron (II) complexes was diluted to 100cm^3 with distilled water. Aqueous ammonia was added until precipitate began to form. The mixture was heated on steam bath, allowed to cool to room temperature, filtered, washed with 75cm^3 portion of 1% ammonium nitrate and then oven dried to constant weight and weighed as Fe_2O_3 (Vogel, 1989).

The percentage composition by weight of Fe in the complexes was calculated using gravimetric factor, where:

$$gravimetric\ factor\ of\ Iron\ in\ Fe(II)coplexes = \frac{\text{relative atomic mass of Iron}}{\text{molecular weight of } \mathrm{Fe_2O_3}}$$

3.8.3 Determination of Cobalt in Cobalt (II) Complexes

0.7g of ammoniumthiocynate was added to 80cm^3 of the filtrate and heated to boiling. 20cm^3 of pyridine was added after which heating was stopped immediately. The solution was stirred for 5 seconds and allowed to cool to room temperature. Red crystals of the precipitate were separated. The precipitate was filtered, washed, dried and weighed as dipyridinecobalt(II)thiocyanate. $[Co(C_5H_5N)_2](SCN)_2$ (Vogel, 1989).

The percentage composition by weight of cobalt in cobalt(II) complexes was calculated using gravimetric factor, where:

$$gravimetric\ factor\ of\ Co\ in\ Co(II)coplexes = \frac{\text{relative atomic mass of Cobalt}}{\text{molecular weight of } \mathrm{[Co(C_5H_5N)_2](SCN)_2}}$$

3.8.4 Determination of Nickel in Nickel (II) complexes

The filtrate of the the nickel (II) complexes was diluted with 97cm^3 of distilled water. The mixture was heated to 70-80 $^{\circ}$C and alcoholic solution of 1% dimethylglyoxime (DMG) was added in slight excess, followed by immediate dropwise addition of dilute ammonia solution.

The content was allowed to cool and the precipitate separated, washed, dried at110 °C and weighed as [Ni($C_4H_7O_2N_2$)$_2$] (Vogel, 1989).

The percentage composition by weight of Nickel was calculated using gravimetric factor of Nickel.

$$gravimetric\ factor\ of\ Ni\ in\ Ni(II) coplexes = \frac{\text{relative atomic mass of Nickel}}{\text{molecular weight of } [\mathrm{Ni(C_4H_7O_2N_2)_2}]}$$

3.8.5 Determination of Zinc in Zn(II) complexes

74cm^3 of distilled water was added to the filtrate obtained from Zn(II) complexes. 1g ammoniumthiocyanate and 1cm^3 of pyridine were added to the mixture. This was vigorously stirred until white crystals were observed. These were allowed to stand for 15 minutes before washing with a solution of 3.9g of potassiumthiocyanate and 2.7g of mercuric chloride in 100cm^3 of distilled water. The precipitate was dried at 110 °C and weighed as [Zn(C_5H_5N)$_2$](SCN)$_2$ (Vogel, 1989).

$$gravimetric\ factor\ of\ Zn\ in\ Zn(II) coplexes = \frac{\text{relative atomic mass of Zn}}{\text{molecular weight of } [\mathrm{Zn(C_5H_5N)_2](SCN)_2}}$$

3.8.6 Determination of percentage of chlorine in the complexes

0.2g each of the complexes was weighed and placed in a 250 ml beaker .150ml of water was added with stirring followed by 0.5ml of concentrated nitric acid. To the cold solution, 0.1M silver nitrate was added slowly in slight excess with stirring. The suspension was heated to boiling while stirring until the precipitate coagulated and the supernatant liquid is clear. When no more precipitate was formed with the addition of few drops of silver nitrate, the beaker was set aside in the dark and allowed to stand for about 1hr before filtration. The precipitate was filtered, collected and washed with dilute nitric acid. This was placed in a crucible and kept in an oven at 130°C for 1hr, allowed to cool in a dessicator and weighed. The percentage of chlorine in the complexes was calculated using the gravimetric factor of chlorine as follows (Vogel, 1989).

$$gravimetric\ factor\ of\ c\ \ lorine = \frac{\text{relative atomic mass of chlorine}}{\text{molecular weight of AgCl}}$$

The percentage of the Metals and Chlorine observed in the mixed ligand complexes are shown in table 4.6.

Details of calculation are shown on appendix

3.9 Antibacterial and Antifungal Test

The antibacterial and antifungal tests of the Schiff base and mixed ligand complexes were carried out by disc diffusion method. The bacterial species used in the screening were *Staphylococcus aureus, Eschericia coli* and *Salmonella typhi* and the fungal species used were *Mucor inducus* and *Aspergillus fumigatus*.

Whatman filter paper discs (6mm in diameter) were punched out with the aid of paper punch and placed in Bijour bottles.They were then sterilized by autoclaving at 121°C for 15 minutes. The discs were allowed to cool.

Three different concentrations 60µg/disc, 30µg/disc and 15µg/disc of each complex and ligand were prepared using DMSO. 0.06g of each of the complexes and the ligand was dissolved in 1ml of the solvent. 0.5ml of each solution was introduced into 50 sterile discs in the bijour bottles respectively to make 60µg/disc concentrations. 0.5ml of DMSO was added into the remaining stock solution; making 1ml. 0.5ml was taken and placed into other bottles containing 50 discs to make the 30µg/disc concentrations. 0.5 ml of DMSO was added to the stock solution and another 0.5mlwas taken and added to 50 discs in the bottles to make the 15µg/disc concentration (Yusha'u and Sadisu, 2011).

Standard innocula of the isolates were swabbed onto to the surface of the prepared and solidified nutrient Agar in separate Petri-dishes.The prepared discs of the complexes and standard antibiotic discs (Gentamycine) were placed on the surface of the inoculated media at interval. The plates were incubated at 37 $^{\circ}$C for 24 hours before observation and measurement of inhibition zone. The same procedure was used for the fungal isolates using potatodextro agar and Mancozef (antifungal drug) as control. The plates were incubated at room temperature for 48 hours (Hassan *et al*, 2006).

CHAPTER FOUR

4.0 Results and Discussion

4.1 Results

The results of the characterization and antimicrobial activity of the Schiff base and the mixed ligand complexes synthesized are presented in the following tables.

Table 4.1: Physical Properties of the Schiff Base and the Mixed Ligand Complexes.

S/N	Compound	Colour	Decomposition Temperature(°**C**)	Melting Point (°**C**)	Yield (%)
	$C_{22}H_{20}N_2O_2$	Green	–	135	83.2
2	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]\ Cl$	Grey	186	–	76.4
3	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]\ Cl$	Grey	192	–	72.5
4	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]\ Cl$	Light Brown	184	–	70.2
5	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]\ Cl$	Brown	191	–	71.7
6	$[Co(C_{22}H_{20}N_2O_2)(Gly)]\ Cl$	Dark Blue	177	–	72.2
7	$[Co(C_{22}H_{20}N_2O_2)(Ala)]\ Cl$	Dark Blue	171	–	69.7
8	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]\ Cl$	Greenish Yellow	197	–	75.2
9	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]\ Cl$	Greenish Yellow	192	–	70.4
10	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]\ Cl$	Cream	191	–	72.04
11	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]\ Cl$	Cream	186	–	68.6

Key: Gly = Glycine , Ala = D-alanine

Table 4.2: Solubility of the Schiff base and complexes

Complexes	Distilled H_2O	MeOH	EtOH	C_6H_6	Nitro benzene	Acetone	ether	CCl_4	DMF	DMSO
L	SS	S	S	IS	SS	SS	IS	SS	S	S
[MnLGly]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[MnLALa]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[FeLGly]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[FeLAla]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[CoLGly]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[CoLAla]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[NiLGly]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[[NiLAla]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[ZnLGly]	IS	IS	IS	IS	SS	SS	IS	SS	S	S
[ZnLAla]	IS	IS	IS	IS	SS	SS	IS	SS	S	S

KEY: S = Soluble, SS = slightly soluble and IS = Insoluble

L = $C_{22}H_{20}N_2O_2$, Gly = Glycine and Ala = D-alanine

Table 4.3: Molar Conductivity of the Mixed Ligand Complexes (3mmole)

S/N	Complex	Electrical Conductivity ($\Omega^{-1}cm^{-1}$) × 10^{-6}	Molar Conductance ($\Omega^{-1}cm^{2}mol^{-1}$)
1	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	69.02	69.02
2	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]$ Cl	71.06	71.06
3	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]$ Cl	86.30	86.30
4	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]$ Cl	93.01	93.01
5	$[Co(C_{22}H_{20}N_2O_2)(Gly)]$ Cl	70.50	70.50
6	$[Co(C_{22}H_{20}N_2O_2)(Ala)]$ Cl	73.80	73.80
7	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]$ Cl	84.20	84.20
8	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]$ Cl	87.07	87.07
9	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]$ Cl	66.20	66.20
10	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]$ Cl	68.70	68.70

Table 4.4: Magnetic Susceptibility of the Mixed Ligand Complexes

Complex	Xg(× 10^{-6})g^{-1}	Xm(mol^{-1})	μeff (B.M)
[Mn($C_{22}H_{20}N_2O_2$)(Gly)] Cl	25.76	0.0131	5.59
[Mn($C_{22}H_{20}N_2O_2$)(Ala)] Cl	24.45	0.0128	5.51
[Fe($C_{22}H_{20}N_2O_2$)(Gly)] Cl	19.63	0.01	4.89
[Fe($C_{22}H_{20}N_2O_2$)(Ala)] Cl	17.73	0.0093	4.72
[Co($C_{22}H_{20}N_2O_2$)(Gly)] Cl	16.78	0.0086	4.53
[Co($C_{22}H_{20}N_2O_2$)(Ala)] Cl	16.87	0.0089	4.60
[Ni($C_{22}H_{20}N_2O_2$)(Gly)] Cl	8.78	0.0045	3.26
[Ni($C_{22}H_{20}N_2O_2$)(Ala)] Cl	7.53	0.00397	3.08
[Zn($C_{22}H_{20}N_2O_2$)(Gly)] Cl	–	–	Diamagnetic
[Zn($C_{22}H_{20}N_2O_2$)(Ala)] Cl	–	–	Diamagnetic

Table 4.5: FTIR spectra of the Schiff base and mixed ligand complexes

Compound	(C=N)cm^{-1}	(NH_2)cm^{-1}	(C-O)cm^{-1}
$C_{22}H_{20}N_2O_2$	1605	-	1303
[Mn($C_{22}H_{20}N_2O_2$)(Gly)] Cl	1628	3324	1382
[Mn($C_{22}H_{20}N_2O_2$)(Ala)] Cl	1588	3073	1354
[Fe($C_{22}H_{20}N_2O_2$)(Gly)] Cl	1631	3132	1341
[Fe($C_{22}H_{20}N_2O_2$)(Ala)] Cl	1588	3075	1356
[Co($C_{22}H_{20}N_2O_2$)(Gly)] Cl	1680	3060	1389
[Co($C_{22}H_{20}N_2O_2$)(Ala)] Cl	1657	3144	1311
[Ni($C_{22}H_{20}N_2O_2$)(Gly)] Cl	1620	3263	1397
[Ni($C_{22}H_{20}N_2O_2$)(Ala)] Cl	1613	3239	1384
[Zn($C_{22}H_{20}N_2O_2$)(Gly)] Cl	1598	3268	1313
[Zn($C_{22}H_{20}N_2O_2$)(Ala)] Cl	1598	3278	1311

Table 4.6: Percentage Composition of Metals and Chlorine in the Complexes

Complex	% metal found(cal.)	%Chlorine found (cal.)
[Mn($C_{22}H_{20}N_2O_2$)(Gly)]Cl	10.94 (10.82)	7.02 (6.98)
[Mn($C_{22}H_{20}N_2O_2$)(Ala)]Cl	10.20 (10.51)	6.55 (6.78)
[Fe($C_{22}H_{20}N_2O_2$)(Gly)]Cl	10.40 (10.99)	6.92 (6.97)
[Fe($C_{22}H_{20}N_2O_2$)(Ala)]Cl	10.60 (10.68)	6.80 (6.77)
[Co($C_{22}H_{20}N_2O_2$)(Gly)]Cl	11.40 (11.51)	7.03 (6.93)
[Co($C_{22}H_{20}N_2O_2$)(Ala)]Cl	11.22 (11.18)	6.82 (6.73)
[Ni($C_{22}H_{20}N_2O_2$)(Gly)]Cl	11.30 (11.51)	7.04 (6.93)
[Ni($C_{22}H_{20}N_2O_2$)(Ala)]Cl	11.52 (11.18)	6.72 (6.73)
[Zn($C_{22}H_{20}N_2O_2$)(Gly)]Cl	12.38 (12.56)	6.87 (6.89)
[Zn($C_{22}H_{20}N_2O_2$)(Ala)]Cl	12.04 (12.18)	6.93 (6.65)

Table 4.7: Antibacterial activities of ligand and complexes

Test Organism	**Compound**	**Zone of inhibition (mm) (Conc. µg/disc)**			**Control (Gentimycine) 10mg/disc**
		60	**30**	**15**	
Staphylococcus aureus	$C_{22}H_{20}N_2O_2$	10	6	6	20
	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	10	6	6	
	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	8	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]Cl$	15	13	10	
	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	12	6	
	$[Co(C_{22}H_{20}N_2O_2)(Gly)]Cl$	15	15	12	
	$[Co(C_{22}H_{20}N_2O_2)(Ala)]Cl$	18	13	7	
	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]Cl$	18	10	13	
	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	12	10	
	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	17	12	6	
	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	17	15	15	
Eschericia Coli	$C_{22}H_{20}N_2O_2$	6	6	6	35
	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]Cl$	18	13	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]Cl$	14	13	11	
	$[Co(C_{22}H_{20}N_2O_2)(Gly)]Cl$	16	10	6	
	$[Co(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	12	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]Cl$	18	10	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	14	13	
	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	15	13	9	
	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	12	9	
Salmonella. Typhi	$C_{22}H_{20}N_2O_2$	6	6	6	30
	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Co(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Co(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	22	13	10	
	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	

Table 4.8: Antifungal activities of ligand and complexes

Test Organism	**Compound**	**Zone of inhibition (mm) Conc. mg/disc**			**Control (Mancozef) 10mg/ml**
		60	**30**	**15**	
Mucor inducus	$C_{22}H_{20}N_2O_2$	6	6	6	30
	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]Cl$	8	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	15	6	
	$[Co(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Co(C_{22}H_{20}N_2O_2)(Ala)]Cl$	15	10	10	
	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	10	6	6	
	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
Aspergillus. Fumigatus	$C_{22}H_{20}N_2O_2$	6	6	6	20
	$[Mn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Mn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Gly)]Cl$	6	6	6	
	$[Fe(C_{22}H_{20}N_2O_2)(Ala)]Cl$	6	6	6	
	$[Co(C_{22}H_{20}N_2O_2)(Gly)]Cl$	20	15	13	
	$[Co(C_{22}H_{20}N_2O_2)(Ala)]Cl$	19	17	8	
	$[Ni(C_{22}H_{20}N_2O_2)(Gly)]Cl$	13	9	6	
	$[Ni(C_{22}H_{20}N_2O_2)(Ala)]Cl$	23	18	15	
	$[Zn(C_{22}H_{20}N_2O_2)(Gly)]Cl$	16	10	8	
	$[Zn(C_{22}H_{20}N_2O_2)(Ala)]Cl$	17	10	6	

4.2 Discussion

The condensation reaction between p-phenylenediamine and o-anisaldehyde yield a green crystalline tetradentate Schiff base (Scheme 3.1) with percentage yield of 83.2%.The reaction of the synthesized Schiff base and glycine with metal (II) salts yield mixed ligand complexes of the metal (II) ions containing the Schiff base and the glycine. Similarly, the reaction between the Schiff base and D-alanine with the metal (II) salts yield the mixed ligand complexes of the metal (II) ion containing the Schiff base and D-alanine (Scheme 3.2). The tetradentate Schiff base was found to be green crystalline solid, while the mixed ligand complexes were non-hygroscopic with varied colours. Mn(II) Complexes were grey, Fe(II) Complexes were brown, Co(II) complexes were dark blue, Ni(II) complexes were greenish yellow and Zn(II) complexes were cream (Table 4.1).This was the first sign that complexation might have taken place, as the differences in the colours of the complexes and the Schiff base could be due to crystal field splitting of the d-orbitals of the transition metals due to chelation of the metal ions by the ligands and subsequently the electronic transition from t_2g to eg sub energy levels which is responsible for the absorption in the visible region of the spectrum .

The decomposition temperature of the complexes observed in the range of 184 - 197°C was found to be higher than the melting point of the Schiff base at 135°C, also suggested that complexation might have taken place forming coordination compounds that are more thermally stable than the Schiff base. The thermal stability is also consistent with the with the stability of the higher coordination number complexes as stability increase with increase in coordination number. (Table 4.1).The percentage yield of the Schiff base was found to be 83.2%, while those of the complexes were found in the range of 76.4 - 68.6% (Table 4.1). This shows that the Schiff base is obtained in better yield relative to the mixed ligand complexes, although their percentage yield could also be considered appreciable.

Result of the solubility test shows that the Schiff base is soluble in methanol, ethanol, DMSO and DMF, while the complexes are insoluble in ethanol, methanol, diethylether, benzene and distilled water, but soluble in DMSO and DMF. The result is shown in Table 4.2.

Molar conductance measurement of the complexes was carried out in 10^{-3} molar solutions of the complexes in DMSO. The complexes show conductivity in the range of 69.02 – 93.01$\Omega^{-1}cm^2mol^{-1}$(Table 4.3) this is within the range observed for 1:1 electrolytic complexes. Kailash and Sexena, (2011) have reported molar conductance values within this range for complexes of oxovadium (IV) and vanadium (III). These results indicate that the chloride ion did not act as a ligand and it is outside the coordination sphere, hence the complexes are cationic in nature.

Result of the room temperature magnetic susceptibility measurement (Table 4.4) revealed that the complexes are paramagnetic except zinc complexes which are diamagnetic. The paramagnetic Mn(II), Fe(II), Co(II) and Ni(II) have spin magnetic moment values in the range of 3.08 – 5.59 BM while Zn(II) complexes are diamagnetic this result is consistent with the number of unpaired electrons observed in the d-orbitals of these metals in the proposed octahedral geometry.

The IR spectra of the complexes register (C-O) stretching at 1311 – 1397cm^{-1}.This shows a shift from the spectra of the free Schiff base which has the band at 1303 cm^{-1} (table 4.5).The observed shift may be attributed to the participation of the oxygen atom of the methoxy group in coordination bond with the central metal ions. The spectra of the Schiff base show a sharp band at 1605 cm^{-1} which may be due to azomethine group, this band was lacking in the amine and the anisaldehyde used to form the Schiff base. Shifts were observed in the spectra of the complexes in the range of 1588 - 1628 cm^{-1} due to participation of the nitrogen atom of the azomethine in coordination bond with the metal (II) ions. Peaks in the range of 3061- 3324 cm^{-1} are assigned to NH_2 group of the amino acids. These peaks are absent in the spectra of the Schiff base. This suggests that the amino acids have participated in the chelation to the metal (II) ions.

The percentage of the metals and chlorine in the complexes were determined by gravimetric method of analysis and the results obtained were within the range of the calculated (theoretical) values. The values are shown on table 4.6.

Antibacterial activity of the Schiff base and the complexes was studied. The zone of inhibition (mm) was measured for each treatment. The metal complexes show some level of activity against the clinical isolates and in some cases no activity was observed. All complexes were active against *staphylococcus aureus* and the activity is better at higher concentrations, however, Mn(II) complexes were least active . Also same Mn(II) complexes were found to be inactive against *E.Coli*, while other complexes show some level of activity. *Salmonellathyphi* is resistant to all complexes except Zn(II) mixed ligand complex of glycine, which shows good activity. Generally, the Zn(II) complexes of glycine has relatively better activity against the bacterial isolates than the other complexes as it was active against all the three bacterial isolates used in this work. The results are shown on Table 4.7.

Studies of the antifungal activity of the complexes reveal that Co(II) and Fe(II) alanine mixed ligand complexes were the only active compounds against *Mucor inducus*, while Co(II),Ni(II) and Zn(II) complexes were active against *Aspergillus fumigatus* and the activity was better at high concentrations. The results are shown on Table 4.8.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

Mixed ligand complexes of N,N'-bis[o-anisaldehyde]p-phenylenediamine tetradentate Schiff base with Glycine and D-alanine as secondary bidentate ligands were synthesized and characterized by solubility test, molar conductance measurement, magnetic moment measurement, infrared spectral analysis and gravimetric method . Based on the data obtained, the complexes were proposed to have the formulae [MLGly]Cl and [MLAla]Cl for Glycine and D-alanine complexes respectively. Where M= Mn(II), Fe(II) ,Co(II), Ni(II) and Zn(II), L= N,N'-bis[o-anisaldehyde]p-phenylenediamine tetradentate Schiff base, Gly = glycine and Ala = D-alanine. The complexes are electrolytes with octahedral geometries and show reasonable antibacterial and antifungal activity.

Based on the results obtained, the proposed structure of the Schiff base and complexes are as follows:

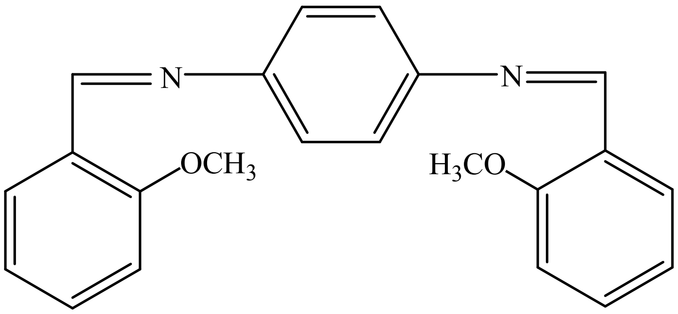


Fig. 5.1: Proposed structure of the Schiff base

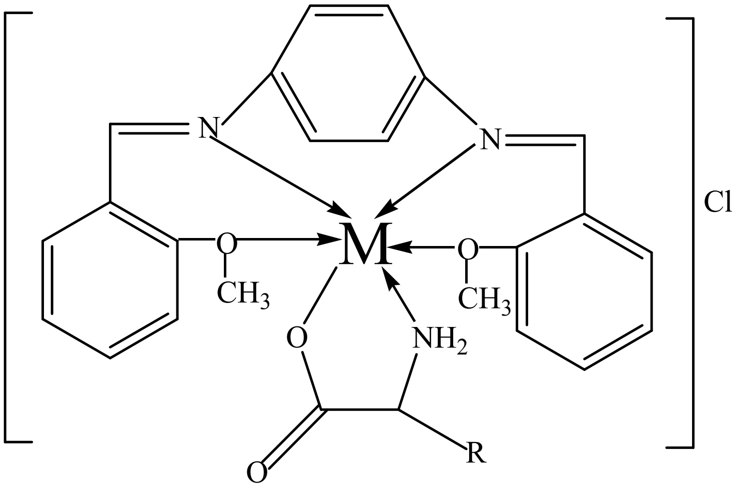


Fig.5.2: Proposed structure of the mixed ligand complexes

M = Mn(II), Fe(II), Co(II), Ni(II) and Zn(II)

R = H   for Glycine

R = – CH_3 for D-alanine

5.2 Recommendations

To fully establish the structural details of these complexes, further characterization using NMR spectra, Elemental analysis and X-ray structural analysis are recommended.

REFERENCES

Ajay R. P., Kamini J. D., Sambhaji S. P., Visgwanath R. P. and Rama S. L. (2012). Synthesis and Characterization of Co(II) mixed ligand complexes with 2-amino-4-nitrophenol salicylidene and some amino acids. *Open Access Molecules Journal,* 20: 9788-9802.

Anwar M.D., Saidul-IslamM., Asraful-Alam M.D. and Tupal S. (2013). Synthesis, Physicochemical Studies and Antimicrobial Screening of metal complexes of Fe(III) and Au(III) with amino acids. *International Journal of Scientific and Technology Research,* 2(7): 2277-8616.

Bhupinder S. (2011). Inorganic/Bioinorganics; Biological, Medical and Pharmaceutical Uses of Metals. *Journal of Pharmaceutical Education Research,* 2(1): 2355- 2617.

Bipind B.P. and Rajeem S. (2013). Bio-mimicking Approach to Mixed Ligand Complexes of bivalent Transition Metals. *International Journal of Applied Sciences and Biotechnology,* 1(1): 16-20.

Cempel M. and Nikel G. (2006). Nickel; a Review of its Sources and Environmental Toxicity. *Polish Journal of Environmental Studies* 15(3): 375-382.

Cimerman Z., Mijanic S. and Galic N. (2000). Schiff bases Derived from Aminopyridine as Spectroflourimetric Analytical Reagents. *Croatica Chemical Acta,* 73(1): 81.

Eddie L.C. Christer S. and Andre K. (2010). Cobalt Complexes as Antiviral and Antibacterial agents. *Pharmaceuticals,* (3): 1711-1728.

Elmali A., Kabak M., and Elerman Y. (2000). Synthesis and Antimicrobial Activities of Substituted Schiff bases*. Journal of Molecular Structure*, (3): 151-158.

Fayad N.K., Taghreed H.A. and Ghanim F.H. (2012). Synthesis, Characterization and Antimicrobial Activities of Mn(II), Co(II), Fe(II), Zn(II) and Cd(II) Mixed Ligand

Complexes Containing Amino Acid (L-valline) and Saccharine . *Advances in Physisc Theories and Applications*, 9: 2225-6638.

Gabriela N., Juraj L. and Ivan M. (2008). Environmental Aspect of Manganese Chemistry. *Herdu journal of Environmental Science,* 2(1) 917.

Geary W.J. (1971): The use of Conductivity Measurement in Inorganic Solvents for Characterization of Coordination Compounds. *Coordination Chemistry Reviews,* 7(1): 82-110.

Halli M.B., Vijay L., Patil B., Malikarjun K. and Sumathi R.B. (2011). Synthesis and Characteristics of Metal Complexes of Schiff bases Derived from Benzo furan. *Journal of Coordination Chemistry*, 64: 651-662.

Hariprasath K.H., Deepthi B., Sudheer B.I., Venkatesh P.S. and Soumya V. (2010). Metal Complexes in Drug Research. *Journal of Chemical and Pharmaceutical Research,* 2(4): 496-499.

Hassan S.W., Umar R.A., Lawal M., Bilkis L.S. and Muhammad B.Y., (2006). Evaluation of Antifingal Activity of *Ficussy comprus* (moraceae*): BEST Journal,* 3(2): 18-25.

Ikechuku P.E. and Peter A.A. (2015). Synthesis, Characterization and Antimicrobial Studies of metal (II) Complexes with Schiff base Derived from Phenylbutan-1- one. *Open Access Molecules Journal,* 20(97): 88-9802.

Kailash K. A. and Sexena P.N. Synthesis and Characterization of New oxovadium (IV) and vanadium (III) Complexes with aldimines on the bases of some Physicochemical Studies. *International Journal of Research in Pharmacy and Chemistry*, 1(4): 2231-2781.

Ketul P.N., Thathor Y.J. and Patel S.G. (2011): Synthesis, Characterization and Biocidal Studies of some Metal (II) Complexes with Tetradentate Schiff base and Neutral Bidentate Schiff base. *Der Chemica Sinica,* 2(1): 143-151.

Mikkat Z.H. and Ihsan A.M (2013): Mixed Ligand Complexes of Gold (III) with some Amino acids and Dithiocarbamates or Dithiophosphates. *Science Journal of Analytical Chemistry,* 1(2): 21- 27.

Naikwade S.D., Thakur S. and Mazahar F. (2013). Mixed Ligand Complexes of Cobalt (II) with Imipramine and Adenosine in Mixed Solvent System. *International Journal of Pharmtech Research,* 5(4): 974.

Ralph N.K., Cheol H.Y. and Kyung W. j. (2001). Synthesis of Secondary Amines. *Tetrahedron,*57: 7785-7811.

Rehab K.A. and Basima A.Z. (2016). Preparation and Characterization of some Transition Metal Complexes of New Tetradentate Schiff base Ligand type N_2O_2. *Scholars Academic Journal of Biosciences,* 4(1):18-26.

Robert K.M., Peter A.M., Daryl K.G. and Victor W.R (1990). *Herpers Biochemistry Prentice-Hall International inc.* Stamford 20th Edition: 21-30.

Sanap S.V. and Patil R.M. (2013). Preparation and Biological Activity of Chiral Lixed ligand Ni(II) Complexes . *Journal of Pharmaceutical Sciences,* 2(I):1-10.

Suresh M.B. and Lokanatha-Rai K.M. (2004). Synthesis of Aromatic Aldehydes via Nickel (II) reduction of oxazolines. *Tetrahedron,* 45: 7969-7970.

Suresh T.G., Aanjali S.R. and Balaheb R.A. (2013) Potentiometric Studies on Mixed Ligand Complexes Formation of Cu(II) with Enapril Maleate and some Amino acids. *Der Pharma chemical,* 5(3): 244-248.

Taleb T. Al-Nahary (2009): Synthesis and Characterization of Metal Complexes of Cr(III), Mn(II), Co(II), Ni(II), Cu(II), Ru(II), Rh(III) and Pd(II) with Derivatives of 1,3,4-thiadiazole-2,5-dithiol as New Ligand. *Journal of Saudi Chemical Society,* (13): 253-257.

Vogel T. (1989): *Practical Inorganic Chemistry, 4th edition, John Wiley Inc. England: 133-325.*

Wail Al Zoubi (2013): Biological Activities of Schif bases and their Complexes. *International Journal of Organic Chemistry,* 3:73-95.

Yoshiki I. and Musa-aki K. (2014). Determination of p-phenylenediamine and related Antioxidants in Rubber boots by High Performance Liquid Chromatography: *Journal of Health Sciences,* 46(6): 437-467.

Yusha'u M. and Salisu F. U. (2011): Inhibition Activity of Deuterium Microcarpum Extracts on some Clinical Isolates. *Biological and Environmental Science Journal for the Tropics,* 8(4): 113-117.

Yuvraj S.M., Rakesh C.P and Rajesh W.R. (2009). Synthesis, Characterization and Antimicrobial Activity of Mixed Ligand Complexes of Zr (IV). *Acta polonie pharmaceutica-drug research,* 66(1): 45-50.

APPENDICES

Appendix 1a: Calculation of the percentage yield of the Schiff base and the complexes

For the Schiff base:

$$C_6H_8N_2 + 2C_8H_8O_2 \longrightarrow C_{22}H_{20}N_2O_2 + 2H_2O$$

p-phenylenediamine + o-anisaldehylyde ⟶ schiff base + water

1 mole of $C_6H_8N_2$ will give 1 mole of $C_{22}H_{20}N_2O_2$

i.e 108.16g $C_6H_8N_2$ gives 344g $C_{22}H_{20}H_2O_2$

0.10814g $C_6H_8N_2$ will give $\frac{0.10814 \times 344}{108.14} = 0.344,$

$$\%yield = \frac{experimental\ yield}{t\ \ eoritical\ yield} \times 100$$

$experimental\ yield = 0.2816$

$t\ \ eoritical\ yield = 0.344g$

$$\therefore \%yield = \frac{0.2861}{0.344} \times 100 = 83.2\%$$

For Mn/Glycine mixed ligand complex

$$MnCl_2.\ 4H_2O \longrightarrow Mn^{2+} + 2Cl^- + 4H_2O$$

$$Mn^{2+} + L + Gly + Cl^- \longrightarrow [Mn\ L\ Gly]\ Cl$$

i.e, 197.91g $MnCl_2.\ 4H_2O$ gives 508.5g [Mn L Gly]Cl

∴ 0.19791 $MnCl_2.4H_2O$ will give $\frac{0.19791\times 508.5}{197.91} = 0.5085$

$experimental\ yield = 0.3884g$

$$\therefore \%\ yield = \frac{0.3884}{0.5085} \times 100 = 76.4\%$$

The same method was used for the remaining complexes

Appendix 2:Calculation of percentage of metals and chlorine in the complexes

2a: calculation of percentage of manganese in Mn/Glycine complexes

$$gravimetric\ factor\ of\ Mn\ in\ Mn(II) coplexes = \frac{\text{relative atomic mass of Manganese}}{\text{molecular weight of } [\mathrm{MnNH_4.PO_4.H_2O}]}$$

$$= \frac{55}{185.9564} = 0.296$$

Weight of the dried precipitate obtained = 0.074

Average weight of manganese in the dry precipitate = weight of the precipitate x G.F

= 0.075 x 0.296

= 0.02190

% composition by weight of manganese in the complexes

$$= \frac{Average\ weig\ \ t\ of\ Mn\ in\ dried\ ppt}{mass\ of\ t\ \ e\ complex\ used}$$

$$= \frac{0.02190}{0.2} \times 100 = 10.95$$

2b: calculation of % of iron in Fe/Glycine complexes

Gravimetric factor of iron $= \frac{2 \times atomic\ mass\ of\ iron}{molecular\ weight\ of\ Fe_2O_2}$

$$= \frac{112}{160} = 0.70$$

Weight of ppt obtained = 0.0297

Average weight of iron = G.F x weight of ppt

= 0.70 x 0.0297

= 0.02079

% composition weight of Fe in the complexes $= \frac{0.02079}{0.2} \times 100$

2c:calculation of percentage of cobalt in cobalt/glycine complex

G.F of cobalt $= \frac{atomic\ mass\ of\ cobalt}{molecular\ weight\ of\ ppt}$

$$= \frac{59}{333} = 0.177g$$

Weight of the ppt obtained = 0.129

Average weight of cobalt in the ppt= G.F. x weight of ppt

= 0.177 x 0.129

= 0.0228

% composition by weight of cobalt in the complex $= \frac{0.0228}{0.2} \times 100 = 11.4$

2d: calculation of percentage composition of nickel in Ni/Glycine complex

G.F of nickel $= \frac{atomic\ mass\ of\ nickel}{molecular\ weight\ of\ the\ ppt}$

$$= \frac{59}{289} = 0.204$$

Weight of ppt obtained = 0.111g

Average weight of nickel in the dried ppt

= G.F ×weight of ppt

= 0.204 × 0.111

= 0.022644

% composition by weight of nickel $= \frac{0.022644}{0.2} \times 100$

$$= 11.32$$

2e: Calculation of percentage composition of Zinc in Zn/Glycine complex

G.F of Zn $= \frac{atomic\ mass\ of\ Zinc}{molecular\ weight\ of\ ppt}$

$= \frac{65}{339} = 0.192$

Weight of ppt = 0.129g

Average weight of Zn in the ppt = G.F × weight ppt

= 0.192 × 0.129

= 0.024768

% composition by weight of Zn in the complex $= \frac{0.02478}{0.2} \times 100$

$$= 12.38\%$$

Percentage compositions of the D-alanine complexes were also determined using the same method.

Appendix 3: Calculation of percentage of chlorine in the complexes

G.F of chlorine $= \frac{atomic\ mass\ of\ chlorine}{molecular\ mass\ of\ AgCl}$

$$= \frac{355}{143} = 0.248$$

For Mn/Glycine complex

G.F chlorine = 0.248

Weight of ppt obtained = 0.0542

Average weight of chlorine in the ppt

= G.F of chlorine × weight of ppt

= 0.248 × 0.0542

= 0.0134

% of chlorine in the complex

$$= \frac{0.0134}{0.2} \times 100$$

$$= 6.7\%$$

For Iron / Glycine complex,

G.F = 0.248

Weight of ppt = 0.0451

Average weight of Cl in the ppt = G.F × weight of ppt

= 0.248 × 0.0541

= 0.01342

% of chlorine in the complex $= \frac{0.01342}{0.2} \times 100$

$= 6.71\%$

For cobalt/glycine complex

G.F = 0.248

Weight of ppt = 0.0542

Average weight of Cl in the ppt

= G.F × weight of ppt

= 0.248 × 0.0542

= 0.01344

% of chlorime in the complex $= \frac{0.01344}{0.2} \times 100$

= 6.72%

For Nickel / glycine complex

Weight of ppt = 0.0549

Average weight of Cl in the ppt = G.F × weight of ppt

= 0.248 × 0.0549

= 0.01362

% composition of Cl in the complex $= \frac{0.01362}{0.2} \times 100$

$= 6.8\%$

For Zinc / glycine complex

Weight of ppt = 0.05452

Average weight of Cl in the ppt = G.F x weight of ppt= 0.248 × 0.05452

= 0.01352

% composition of chlorine in the complexes $= \frac{0.01352}{0.2} \times 100$

$= 6.76\%$

Appendix 4 : Calculation of the effective magnetic moment of the complexes

For iron/glycine complex:

The mass magnetic moment;

$$\Psi g = \frac{CL(R \quad R_\circ)}{10^9 M}$$

C = 1, L = 2.8 cm, R = 581, $R_\circ$ = - 4, w_2 = 0.92g and w_1 = 0.85g

$$\Psi g = \frac{1 \times 2.8\ (581-(-4))}{10^9\ (0.92-0.85)}$$

$$= \frac{1638}{70 \times 10^6}$$

$= 23.4 \times 10^{-6}$ g-1

The molar magnetic moment; $\Psi m = \Psi g \times molar\ mass$

Molar mass of Fe/glycine complex = 429 $gmol^{-1}$

$\Psi m = 23.4 \times 10^{-6}$ g 1 × 429 $gmol^{-1}$

0.01mol^{-1}

Effective magnetic moment:

$$\mu_{eff} = 2.828\sqrt{(\Psi_m \times 298)}$$

$$= 2.828\sqrt{(0.01 \times 298)}$$

$$= 4.89 \text{ B.M.}$$

The effective magnetic moment of the other complexes were calculated using the same method

Appendix4: FTIR spectra of the Schiff base and the mixed ligand complexes

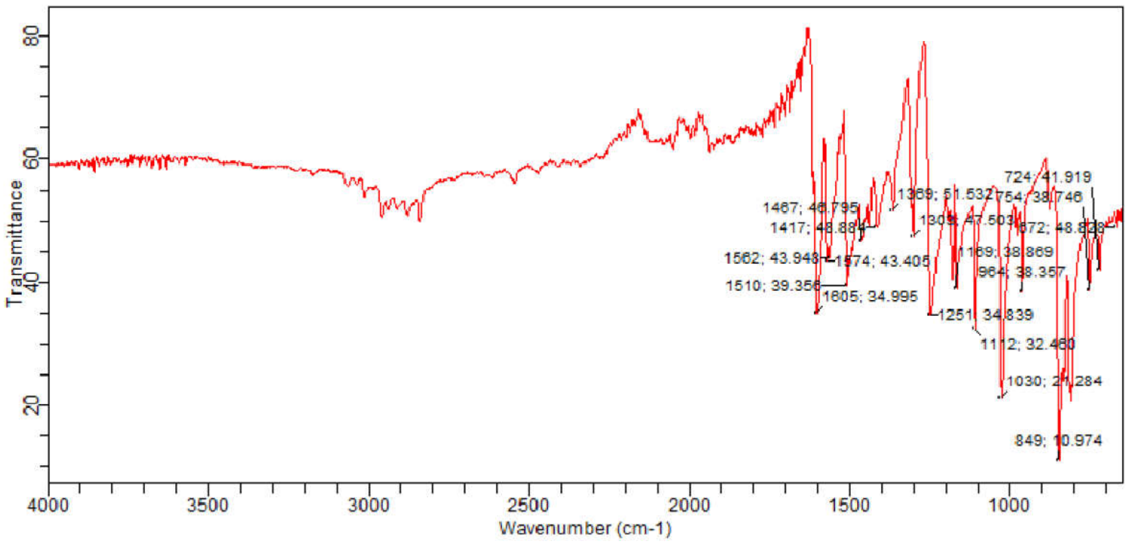


Figure1: FTIR spectrum of the Schiff base

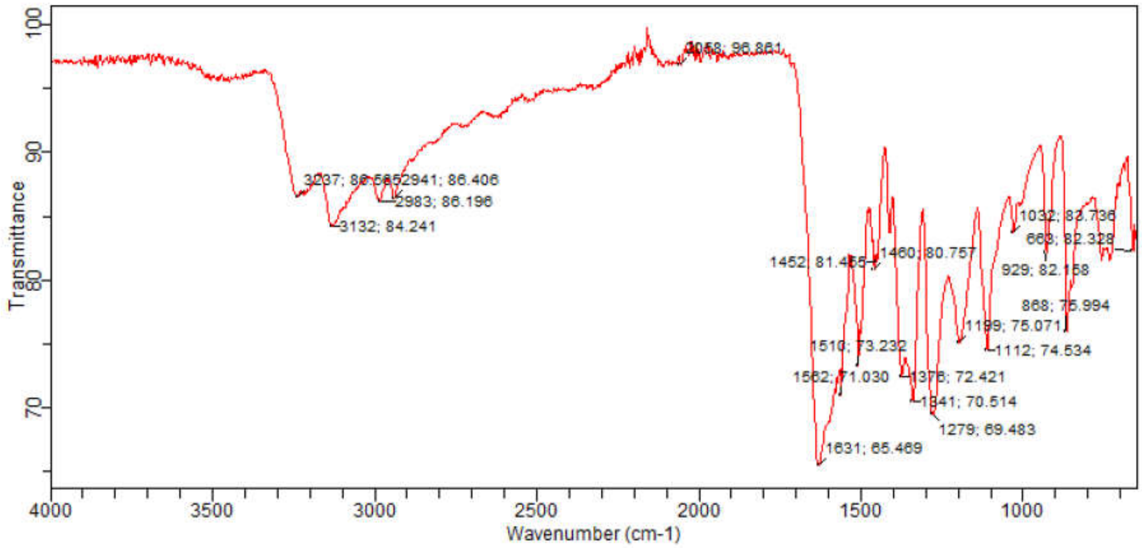


Figure 2: FTIR spectrum of Fe/glycine complex

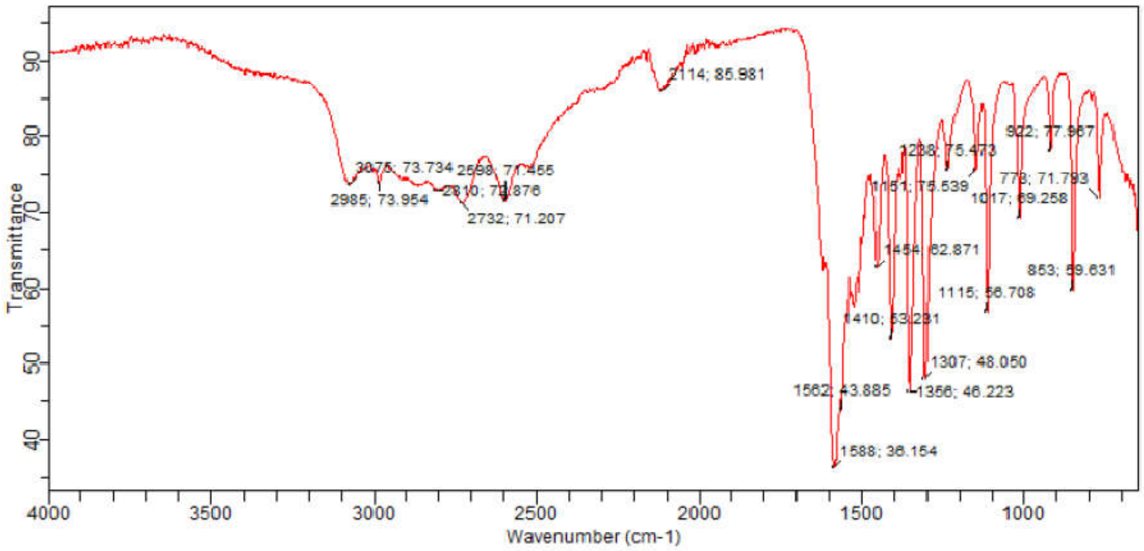


Figure3: FTIR spectrum of Fe/alanine complex

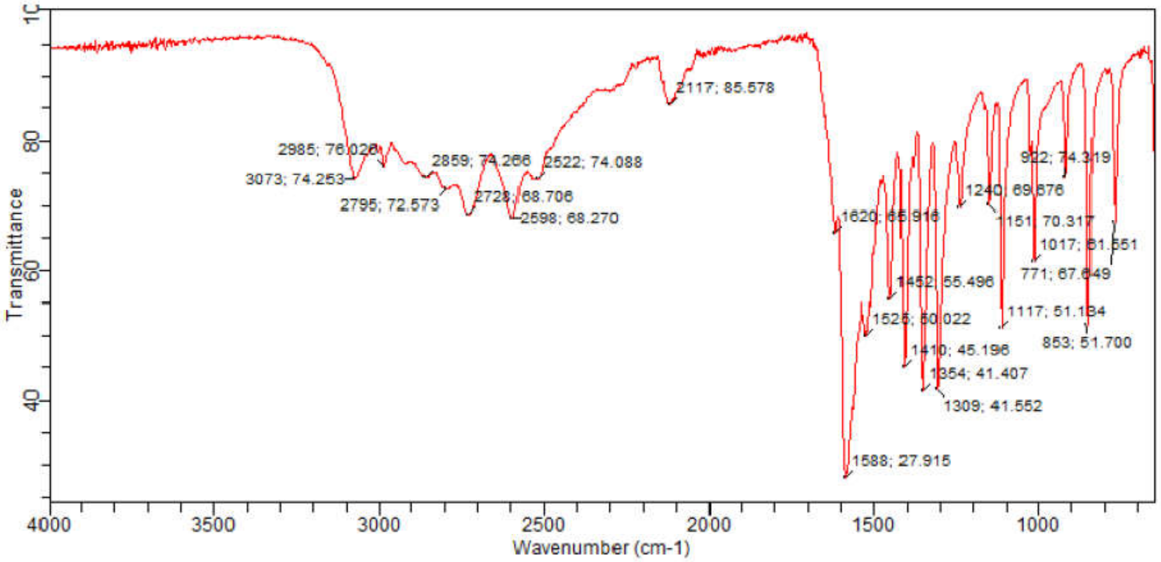


Figure 4: FTIR spectrum of Mn/alanine complex

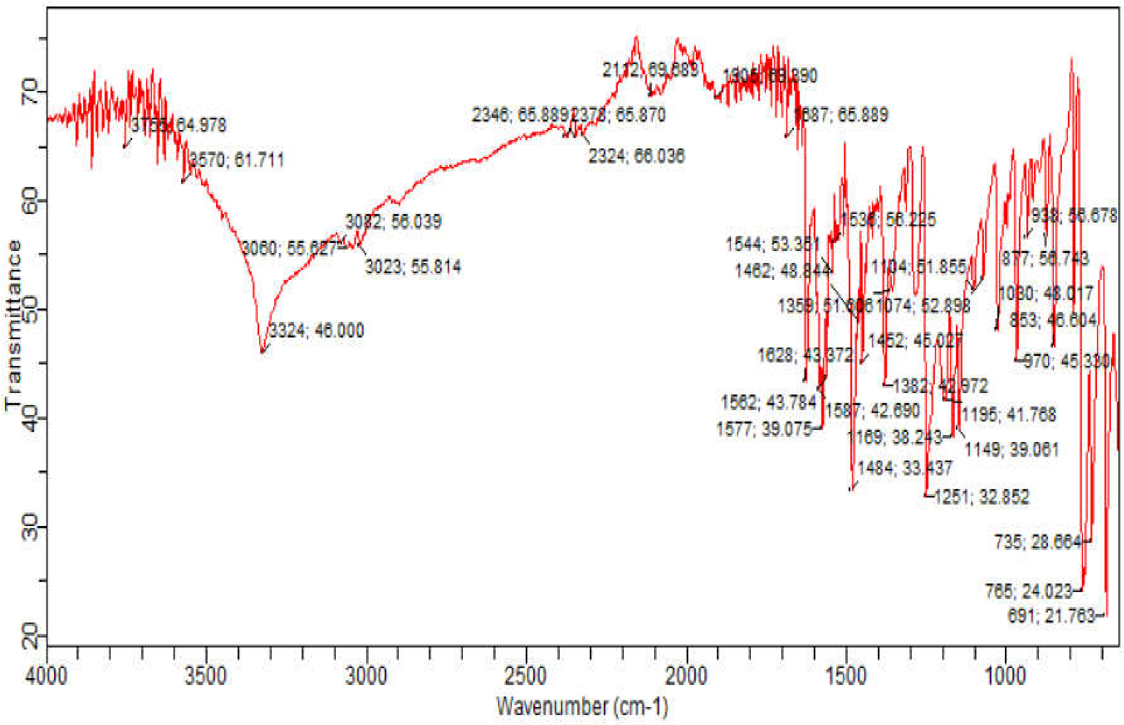


Figure5: Mn/glycine complex

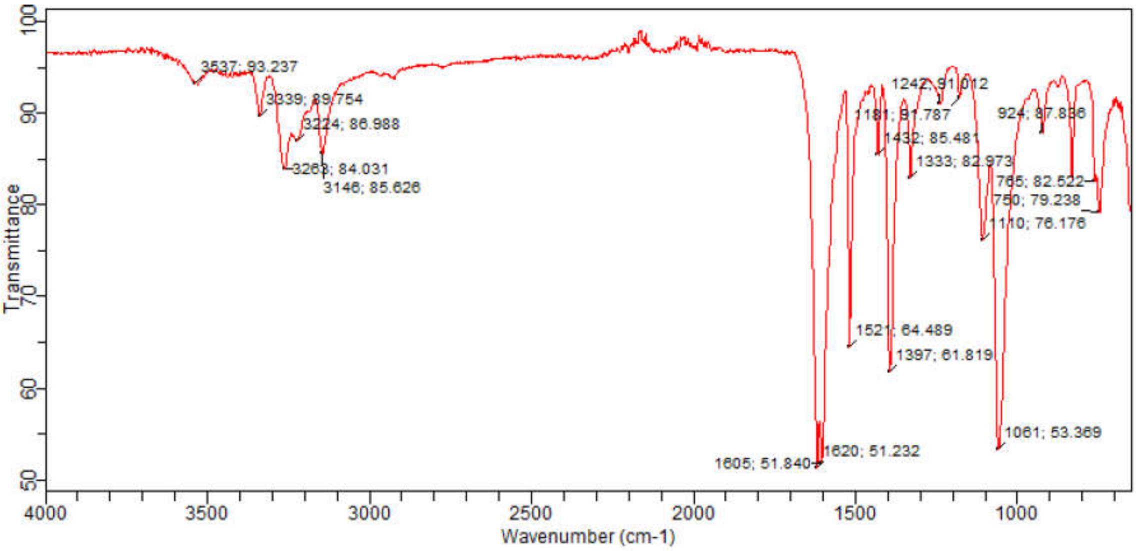


Figure 6: FTIR spectrum of Ni/glycine complex

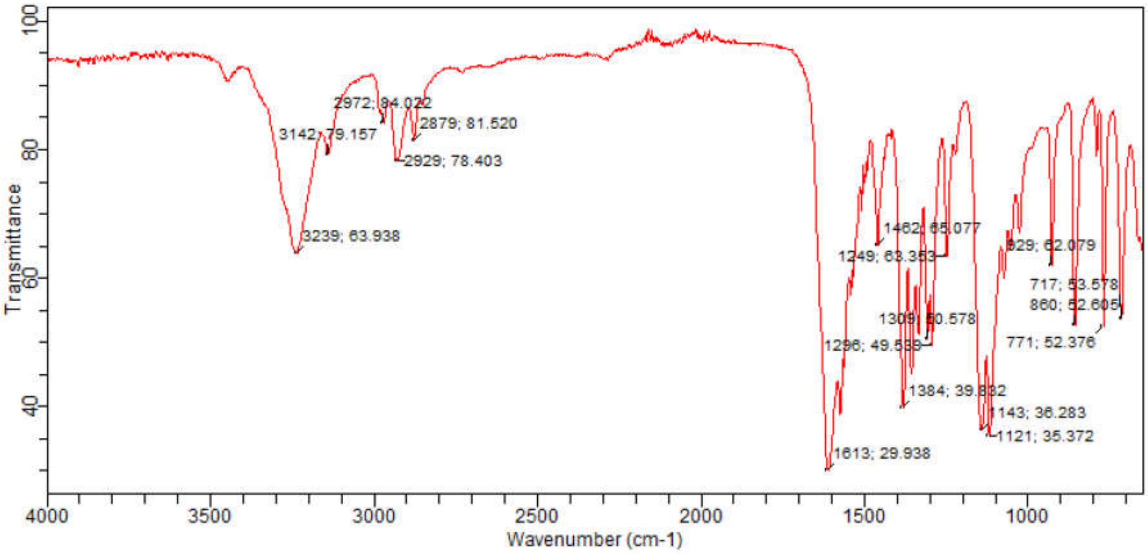


Figure 7: FTIR spectrum of Ni/alanine complex

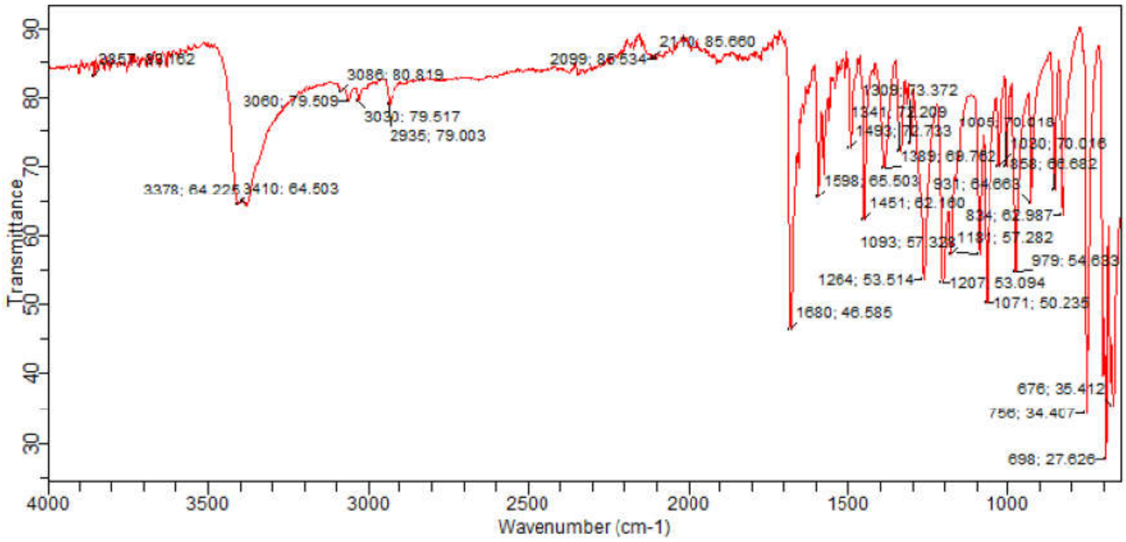


Figure 8: FTIR spectrum of Co/glycine complex

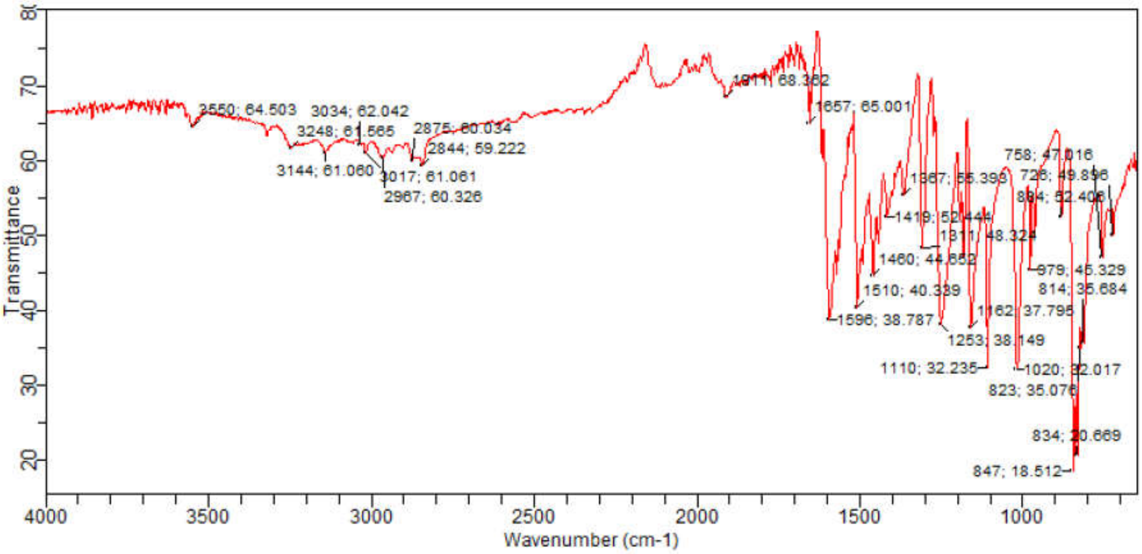


Figure 9: FTIR spectrum of Co/alanine complex

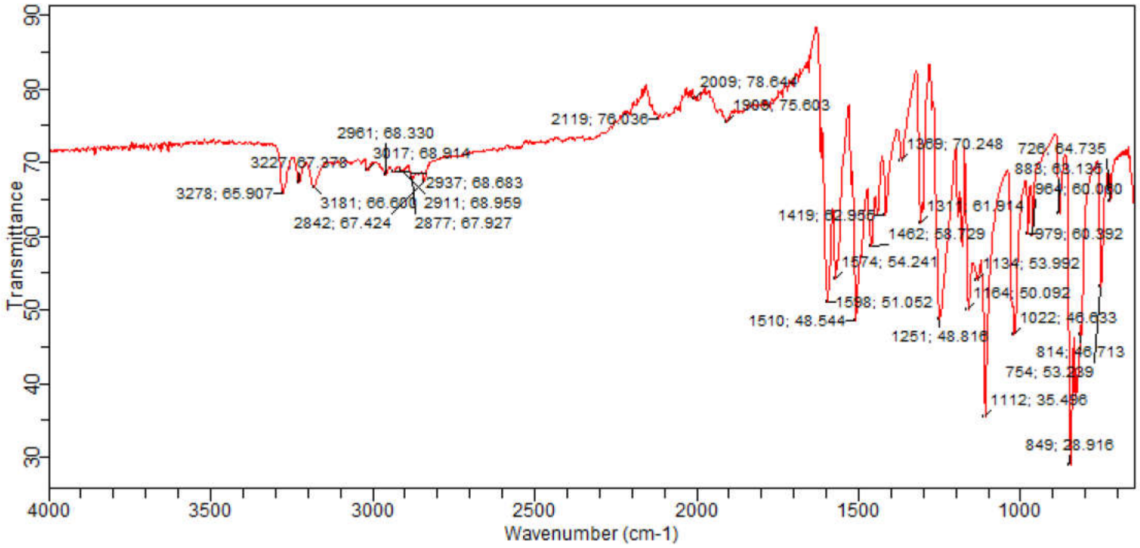


Figure 10: FTIR spectrum of Zn/alanine complex

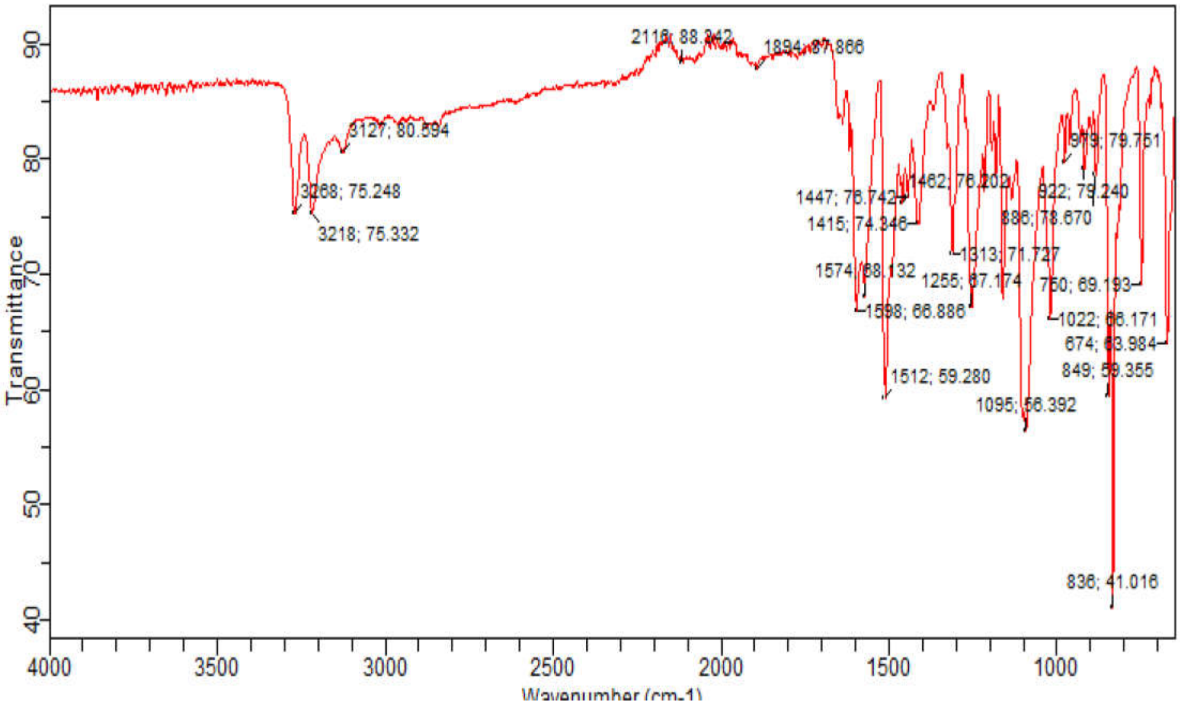


Figure 11: FTIR spectrum of Zn/glycine complex