SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME METAL (II) COMPLEXES OF L-METHIONINE AND L-LEUCINE

BY

MICHAEL AONDONA IORHEMBA

DEPARTMENT OF CHEMISTRY
FACULTY OF PHYSICAL SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JULY, 2018

SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME METAL(II) COMPLEXES OF L-METHIONINE AND L-LEUCINE

BY

Michael Aondona IORHEMBA, B.Sc. Chemistry (UAM) 2014
P15SCCH8032

A DISSERTATONSUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN INORGANIC CHEMISTRY

DEPARTMENT OF CHEMISTRY
FACULTY OF PHYSICAL SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

JULY, 2018

Declaration

I hereby declare that the work in this dissertation entitled "Synthesis, characterization and evaluation of biological activity of some metal(II) complexes of l-methionine and l-leucine" has been carried out by me in the Department of Chemistry of Ahmadu Bello University Zaria under the supervisionof Prof. S. O. Idris and Dr. G. A. Shallangwa. To the best of my knowledge, no part of this workhas been previously presented for the award of another degree or diploma at this or any other institution. All information obtained from literature has been duly acknowledged in the text and a list of references has been provided.

Michael Aondona IORHEMBA _____ _____

Signature Date

Certification

This dissertation entitled "SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME METAL(II) COMPLEXES OF L-METHIONINE AND L-LEUCINE" by Michael Aondona IORHEMBA, meets the regulations governing the award of the degree of Master of Science in Inorganic Chemistry of Ahmadu Bello University Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. S. O. Idris_____ _____
Chairman, Supervisory Committee Signature Date

Dr. G. A. Shallangwa_____ _____
Member, Supervisory Committee Signature Date

Prof. A. O. Oyewale _____
_____
Head of Department Signature Date

Prof. S. Z. Abubakar _____
_____
Dean, School of Postgraduate Studies Signature Date

Dedication

This research work is dedicated to my brother and friend, Mr. Steven Liamnger Batur, a great humanitarian who believes in pragmatic approach to making the society a better place.

Acknowledgment

My profound gratitude goes to God Almighty, for His provision, guidance and protection. Having kept me in a sound mind throughout the course of this research, I give Him all the glory.

The efforts of my esteemed supervisors,Prof. S. O. Idris and Dr. G. A. Shallangwa in terms of supervision, tolerance and mentorship can never be overemphasized. Their invaluable suggestions counseling and intellectual contributions were the driving force for the success of this work. I am most grateful for their encouragement, guidance and coaching.

My appreciationis also extended tothe Head of Department, Prof. A. O. Oyewale for providing a convenient avenue for this research to take place and all the lecturers of the Department for their support. The assistance rendered by the technical staffof the Department is also acknowledged. In addition, I am indeed grateful to the technical staff of Chemistry Department, Federal College of Education, Zaria, for providing me with furnace and electronic balance for this work.

My sincere appreciation goes to my Dad, Mr. Fredrick I. Iorcher, my foster Mother, Dr. L. A. Nnamonu, my sister, Awashima Ijirbo, my Aunties, Mrs. Grace Joko, Mrs. Juliet Yansha, my fiancée, Linda Anvah and the entire members of my family. I honestly appreciate them for among others, their moral, spiritual and financial support.

I wish to deeplyappreciate the financial support of my brother, Mr. Steven Batur, His Excellency, Engr. Benson Abouno, Prof. R. Sha'Ato, Prof. A. D. Garkida, Dr. S. Ande, Dr. A. D. Onu, Dr. E. N. Iornumbe, Dr. John Anyam, Mrs. Patience Iorungwa, Dr. I. S. Eneji, and Dr. S. G. Yiase. I am very grateful for your contributions to the success of this project.

My gratitude also goes tomy research mates, Musa Shuaibu, Jibrin Hamza, Nkole Ikechukwu and Osunkwo Chinedu. Our laboratory experience together was indeed an inspiring moment.

Finally, I wish to appreciate my friends, Oladunni Nathaniel, Aliyu Yakubu, Abdullahi Usman, Solomon Igbetar, Iorshenge Matthew, Nyikwagh Eric, Adakole Veronica, Akerityo Doom, Abele James, Igomu Ephraim, Asar Samuel, Eche and John Sunday. In addition, to Ejeh Steven, Iorhemen Raymond, Iwuala Beatrice, Tyohemba Samuel, Akaakase Timothy and Sule Blessing, I am most grateful for your financial and other many contributions to the success of this work.

Table of Contents

Contents Page

Cover page i

Fly leaf ii

Title page iii

Declaration iv

Certification v

Dedication vi

Acknowledgement vii

Table of Content viii

List of Tables xiii

List of Figures xiv

List of Appendices xv

Abbreviations xvi

Abstractxviii

CHAPTER ONE1

1.0 INTRODUCTION 1

1.1 Transition Metals 1

1.2Methionine 2

1.3 Leucine 2

1.4 Transition Metal Complexes **3**

1.5 Statement of Research Problem **3**

1.6 Justification of the Research **4**

1.7 Aim and Objectives of the Research **5**

CHAPTER TWO **6**

2.0 LITERATURE REVIEW **6**

2.1 Biological Importance of Cobalt **6**

2.2 Biological Importance of Copper **6**

2.3 Biological Importance of Zinc **7**

2.4 Biological Importance of Nickel **8**

2.5 Amino Acids **8**

2.5.1 Essential amino acids 9

2.5.2 Non-essential amino acids 10

2.5.3 Biological importance of amino acids 10

2.6 Transition Metal Complexes with Amino Acids11

2.7 Biological Importance of Coordination Complexes of Amino Acids **11**

CHAPTER THREE17

3.0 MATERIALS AND METHODS 17

3.1 Materials 17

3.2 Methods18

3.2.1 Synthesis of metal(II) methionine complexes 18

3.2.2 Synthesis of metal(II) leucine complexes 18

3.3 Characterization 19

3.3.1 Melting point analysis 19

3.3.2 Solubility test 19

3.3.3 Molar conductivity 20

3.3.4 Magnetic susceptibility 20

3.3.5 Determination of water of hydration in the complex compounds 21

3.3.6 Metal content analysis 21

3.3.7 Infra-red spectroscopy 22

3.3.8 UV-Vis spectroscopy 22

3.3.9 Powder x-ray diffraction 22

3.4 Antimicrobial Screening 23

3.4.1 Minimum inhibition concentration 24

3.4.2 Minimum bactericidal concentration/minimum fungicidal concentration 25

CHAPTER FOUR 26

4.0 RESULTS 26

4.1 Physical Properties 26

4.2 Solubility Studies **26**

4.3 Spectroscopic Analyses **26**

4.4 Magnetic and Conductivity Measurements **31**

4.5 Gravimetric Analyses **31**

4.6 X-ray Diffraction Studies **31**

4.7 Antimicrobial Studies **31**

4.7.1 Zone of inhibition 31

4.7.2 Minimum inhibition concentration 39

4.7.3 Minimum bactericidal concentration/minimum fungicidal concentration 39

CHAPTER FIVE46

5.0DISCUSSION46

5.1 Physical Properties of Metal(II) Complexes **46**

5.2 Solubility **47**

5.3 Infra-red Spectroscopic Analysis **47**

5.4 UV-Vis Spectroscopy **50**

5.5 Magnetic Susceptibility Measurements **51**

5.6 Molar Conductivity Measurement **53**

5.7 Metal Content Analysis **54**

5.8 Hydration Water Analysis **54**

5.9 Powder X-ray Diffraction Studies **54**

5.10 Antimicrobial Screening **60**

CHAPTER SIX64

6.0SUMMARY, CONCLUSION AND RECOMMENDATION64

6.1 Summary and Conclusion64

6.2 Recommendation 65

REFERENCES 66

APPENDICES **77**

List of TablesPage

4.1 Physical properties of metal(II) complexes 27

4.2 Solubility of complexes in some selected solvents 28

4.3 IR frequencies of the L and $L^1$and their metal complexes 29

4.4 UV-Visible spectra data ofL and $L^1$and their metal complexes30

4.5 Room temperature magnetic susceptibility and Molar Conductivityof the M(II) complexes 32

4.6 Metal content analysis 33

4.7 Hydration water analysis 34

4.8 Powder X-ray diffraction analysis 35

4.9 Results of zone of antimicrobial inhibition for ligand and complexes on Gram-positive bacteria 36

4.10 Results of zone of antimicrobial inhibition for ligand and complexes on Gram-negative bacteria 37

4.11 Results of zone of antifungal inhibition for ligand and complexes 38

4.12 Results of minimum inhibition concentrationfor ligand and complexes on Gram-positive bacteria 40

4.13 Results of minimum inhibition concentration for ligand and complexes on Gram-negative bacteria 41

4.14 Results of minimum inhibition concentration for ligand and complexes on some fungi strains 42

4.15 Result of minimum bactericidal concentration for ligand and complexes on Gram-positive bacteria 43

4.16 Result of minimum bactericidal concentration for ligand and complexes on Gram-negative bacteria 44

4.17 Result of minimum fungicidal concentration for ligand and complexes on some fungi strains 45

5.1 Proposed molecular geometry and crystal class of metal(II) complexes 56

List of Figures

1.1	Structures of Methionine and Leucine	3
5.1	Proposed structure of Cu(II) and Zn(II) complexes	57
5.2	Proposed structure of Co(II) complexes	58
5.4	Proposed structure of Ni(II) complexes	59

List of Appendices

1.	Stacked IR spectra of L and ML complexes respectively	77
2.	Superimposed IR spectra of L with ML complexes respectively	78
3.	Stacked IR spectra of L^1 and ML^1 complexes respectively	79
4.	Superimposed IR spectra of L^1 with ML^1 complexes respectively	80
5.	XRD pattern of Cu-Met complex	81
6.	XRD pattern of Cu-Leu complex	82
7.	XRD pattern of Co-Met complex	83
8.	XRD pattern of Co-Leu complex	84
9.	XRD pattern of Zn-Met complex	85
10	XRD pattern of Zn-Leu complex	86

List of Abbreviations

Abbreviation	Name
BDH	British Drug House
BM	Bohr Magnetons
C	Ciprofloxacin
DMSO	Dimethyl sulphoxide
HL	L-methionine
HL^1	L-leucine
IR	Infrared
Leu^-	leucinato ion
Leu	Leucine
M & B	May and Baker
MBC	Minimum bactericidal concentration
Met^-	methioninato ion
Met	methionine
MFC	Minimum fungicidal concentration
MIC	Minimum inhibition concentration
MRSA	Methicilin resistant staphylococcus aureus
MSB	Magnetic Susceptibility Balance
PDF	Powder diffraction files
Q	Fulcin
UV – visible	Ultra Violet – visible
VRE	Vancomycin resistant enterococci
XRD	X-ray diffraction

λ_{max}	Maximum wavelength

Abstract

Eight metal(II) amino acids complexes in aqueous basic solution, $[CuL_2]$, $[CuL^1{}_2]$, $[CoL_2]$, $[CoL^1]$, $[ZnL_2]$, $[ZnL^1{}_2]$, $[NiL_2]$, and $[NiL^1{}_2]$ with L=L-methionine and L^1=L-leucine were synthesized and analyzed by gravimetric analyses, molar conductivity measurements, magnetic susceptibility measurements, powder X-ray diffraction analysis, UV-Vis and IR spectroscopies.Molar conductivity measurements showed that the complexes are non-electrolytic, suggesting a metal-ligand ratio of 1:2 for the complexes, with respect to the amino acids. Solubility studies indicated DMSO as the most suitable solvent for dissolving the compounds;hence, all solvent bound analyses were carried out in DMSO. The IR spectra showed that the amino acids acted as bidentate ligands with coordination involving the carboxyl oxygen and the nitrogen of the amino group. Electronic spectra and magnetic susceptibility measurements suggested a square planar geometry for Cu(II), tetrahedral for both Zn(II) and Ni(II) complexes, and octahedral for those of Co(II). Magnetic susceptibility studies also revealed that the complexes of Cu, Co and Ni are paramagnetic while those of Zn are diamagnetic. The result also suggested L-methionine and L-leucine to be weak field ligands as they formed high spin complexes with Co(II) ion ([CoL] = 6.0 BM, $[CoL^1]$ = 5.10 BM) and tetrahedral complexes with Ni(II) ion.Gravimetric analyses showed that the complexes of Cu, Co and Zn contain one mole of water of crystallization while those of Ni contain two moles.Powder XRD studies suggested that the amino acid complexes are crystalline in nature and that they possibly crystallized in monoclinic fashion. The agar diffusion antimicrobial studies revealed that the amino acids are biologically active with their metal complexes showing significantly enhanced activity against the studied microbial strains in comparison to the free ligands.

CHAPTER ONE

1.0 INTRODUCTION

Metals perform quite a number of functions in human body. Hemoglobin carries oxygen to vital areas of the body by binding it to the iron atom contained within it. Metal ions such as zinc provides the structural framework for the zinc fingers that regulate the function of genes in the nuclei of cells (O'Shea, 2004). Minerals containing calcium are the basis of bones which are the framework of the human body. Metals such as zinc, copper, iron and manganese are incorporated into catalytic processeswhich facilitate a number of chemical reactions needed for life (Da Silva and Williams, 1991).

The ability of metals to lose electrons to form positively charged ions allow them to play important roles in biological systems. Whereas metal ions are electrophilic, most biological molecules such as protein and DNA are electron rich. The attraction of these opposing forces lead to interaction between metal ions and biological molecules (O'Shea, 2004). Metal ions have been reported to improve, or induce the activity of biologically important moieties (Sadler and Guo, 1998) and that of some known drugs such as paracetamol, sulfamethoxazole and aspirin (Agbaje *et al.*, 2014; Osowale, 2017).

1.1 Transition Metals

A transition metal isone thatcontains a partially filled *d*or *f*sub-shellin elemental state or one of its important oxidation states (Petrucci *et al.*, 2002; Rayner-Canham and Overtone, 2006). Several transition elements are important to the chemistry of living systems, the most familiar examples being iron, cobalt, copper, zinc, nickel, and molybdenum. Iron is by far the most widespread and important transition metal that has a function in living

systems; proteins containing iron participate in two main processes, these are; oxygen transport and electron transfer (oxidation–reduction) reactions (Sundberg and Martin, 1974; Vallee and Auld, 1993).

The chemistry of transition metal complexes is well known. However, the evaluation of their antimicrobial activities has continued to attract more and more attention (Pierpont and Lange, 2007; Patil *et al.*, 2011; Pllana-Zeqiri *et al.*, 2012). This is because bacteria can cause foodborne diseases and other infections that affect our lives; therefore, there has been a growing effort towards synthesizing new antimicrobial agents. Coordination complexes of transition metals have been widely studied for their antimicrobial activities (Faundez *et al.*, 2004).Efforts to understand the function of transition metals in biological systems have led to the growth of the field of bioinorganic chemistry (Farrel *et al*., 2003).

1.2 Methionine

Methionine, with molecular formula $C_5H_{11}NO_2S$ (Fig.1.1a)is one of the amino acids containing sulphur (Nelson *et al.*, 2005).It helps to prevent disorders of the hair, skin and nails, in lowering the cholesterol levels by increasing the liver's production of lecithin and reduces fat build-up in the liver and body (Cao *et al.*, 2000). It is known that methionine presents antioxidant properties in several models of oxidative stress. It has been shown that it acts as a powerful endogenous antioxidant agent, leading to the reduction of lipid peroxidation in membranes (Gill and Tuteje, 2010).

1.3 Leucine

Leucine, an amino acid with molecular formula $C_6H_{13}NO_2$ (Fig. 1.1b) is a major component of the subunits in ferritin, astacin, and other "buffer" proteins (Nelson *et al.*, 2005). It is used in the liver, adipose tissue, and muscle tissue. Adipose and muscle tissue use leucine in the formation of sterols. Combined leucine use in these two tissues is seven times greater than in the liver (Rosenthal and Farkas, 1974). Leucine is rapidly taken up into the brain where astrocytes convert it into α-ketoisocaproate via transamination of α-ketoglutarate to glutamate (Yudkoff, 2005).

Figure 1.1: Structures of methionine (a) and leucine(b)

1.4 Transition Metal Complexes

These consist of a central transition metal atom or ion, called the coordination center, and a surrounding array of bound molecules or ions known as ligands or complexing agents. Transition metal complexes play very important roles in biological systems (Rayner-Canham and Overtone, 2006; Noro *et al*., 2009).

1.5Statement of the Problem

Many of the current antimicrobial agents that are used in therapy are either becoming resistant or have adverse side effects (Agbaje *et al*., 2014). This hasled to continued search for new antimicrobial compounds, including coordination complexes of biologically

important molecules (Farrell *et al.*,2003).The medicinal uses and applications of metals and metal complexes are of increasing clinical and commercial importance. The field of inorganic chemistry in medicine has been divided into two main categories: firstly, ligands as drugs which target metal ions in some form, whether free or protein-bound; and secondly, metal-based drugs and imaging agents where the central metal ion is usually the key feature of the mechanism of action (Reynold and Martidale, 1996; Farrel *et al.*, 2003).The use of chelating agents in the treatment of Wilson's disease is a good example of how medical problems due to free metal ion toxicity beenarrested by chelating agents (Sarkar, 1999).Continued research aimed at developing metal-based drugs which perhaps work by different mode of action and therefore arrest the challenge of resistance, is therefore, a way forward (Agbaje *et al.*, 2014).

1.6Justification of the Research

There have been some reports of antimicrobial activity of certain metal complexes of amino acids. However, there is little information on coordination compounds of methionine. Literature studies showed that there is scanty information on the structure of M(II) coordination compounds of methionine and leucine in solid and aqueous phase as those reported have often been poorly characterized with such analytical tools as X-ray diffraction, molar conductivity, magnetic susceptibility and metal content analysis often ignored (Asemave *et al.*, 2015; Vilhena *et al.*,2017). The biological activity of some bio-important moieties have been reportedly induced or elated on coordination with transition metal ions (Sadlers *et al.*, 2007; Agbaje *et al.*, 2014; Osowale, 2017).

In spite of the interesting biological features exhibited by methionine and leucine, their coordination complexes with transition metals have not been well studied (Hakimi and Aliabadi, 2012) and this has ledtothe neglect in exploring their potential antimicrobial activity.It will rather be a step forward to synthesize and study the biological activities of complexes of such ligands with other similar biologically important metal ions as well in ordertoadd to the already existing body of knowledge available on such complexes. The reported widespread applications of amino acids and their coordination compounds, therefore, informed our interest in the synthesis ofneutral metal(II) complexes of L-methionine and L-leucine with the aim of obtaining alternative antimicrobial therapeutic agents to arrest the challenge of drug resistance.The current study is thus focused on synthesizing amino acid complexes of some metal(II) and characterizing them using XRD, magnetic susceptibility and molar conductivity in addition to other already used methods, and evaluating their biological potency against some pathogenic bacteria and fungi.

1.7 Aim and Objectives of the Research

The aim of this work is to synthesize and characterizeamino acid complexes of Cu(II), Co(II), Zn(II) and Ni(II), and evaluate their microbial potential.

To achieve this aim, the following objectives were set:

(a) To synthesize Cu(II), Co(II) Zn(II) and Ni(II) complexes of L-methionine and L-leucine.

(b) To characterize the amino acid complexes through spectrophotometric (UV, FT-IR and X Ray Diffraction) and classical methods(magnetic susceptibility measurements, molar conductivity and gravimetric analyses).

(c) To investigate the antibacterial and antifungal potential of the synthesize complexes on some fungi and bacteria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biological importance of cobalt

Cobalt is a transition element with symbol Co, atomic number 27 and electronic configuration [Ar] $3d^7$ $4s^2$ (Lee, 2013). It is essential to the metabolism of all animals and a key constituent of cobalamin, also known as vitamin B_{12}, the primary biological reservoir of cobalt as an altratrace element. Vitamin B_{12} is an organocobalt complex essential to human diet. Deficiency of B_{12} in diet has proven to be the cause of pernicious anemia (Sigel *et al.,* 2010). Bacteria in the guts of ruminant animals convert cobalt salts into vitamin B_{12}, a compound that can only be produced by bacteria (Schwarz *et al*., 2000). The minimum presence of cobalt in soils therefore markedly improves the health of grazing animals, and an uptake of 0.20 mg/kg a day is recommended because they have no other source of vitamin B_{12} (Schwarz *et al.,* 2000).

2.2 Biological importance of copper

Copper is a transition element with symbol Cu, atomic number 29 and electronic configuration [Ar] $3d^{10}$ $4s^1$ (Lee, 2013). In biological systems, copper is primarily a catalytic metal. The scarcity of copper in the biological systems put it in the category of a micronutrient, or more specifically a trace metal. The most primary of its actions is to allow the bio system to deal with iron. Other studies have linked copper in animals and humans with the synthesis of neurotransmitters, pituitary hormones, bio-pigments, and the

establishment of a firm connective tissue network, functions that are manifested through copper-dependent enzymes like catalase. Cu deficiency can occur after prolonged administration of a diet based solely on cows' milk which has a lower Cu content than human milk (Walravens, 1980).

Copper is required in the body for growth, keratinization, pigmentation, bone formation among other functions. These roles of copper are based upon the requirement of coordinated copper at the active site of copper-dependent enzymes: ceruloplasmin, tyrosinase, lysyl oxidase, ceramide galactosyl transferase, etc. The use of copper coil as an intrauterine device to regulate fertility has been common for many years (Harris, 1983).

2.3 Biological importance of zinc

Zinc, Zn is a *d* block element with the atomic number of 30 and electronic configuration [Ar] $3d^{10}$ $4s^{2}$ (Housecroft and Sharpe, 2005). It is an essential trace element, or a micronutrient, essential for the normal growth and the reproduction of all higher plants and animals, and of humans. In addition, it plays a key role during physiological growth and fulfills an immune function (Frassinatti *et al.,* 2006). It is vital for the functionality of more than 300 enzymes, for the stabilization of DNA, and for gene expression. Zinc is also needed for maintaining special senses such as smell and taste.

During pregnancy, infancy, and childhood the body needs zinc forproper growth and development. Zinc takes part in the catalytic function of many metalloenzymes (Frassinatti *et al.,* 2006). Zinc is an important trace mineral that people need to stay healthy. This element is second only to iron in its concentration in the body. Zinc is found in cells throughout the body, and is needed for the body's defensive (immune) system to work

properly. It plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates (Roohani *et al.,* 2011). Zinc is a common element in human and natural environments and plays an important part in many biological processes (Whitney and Rolfes, 2005). Copper and zinc are critical trace minerals for production and reproduction in farm animals. The inorganic form of trace minerals is not sufficiently absorbed and retained. However, the organic form of these minerals are absorbed efficiently and retained in the tissue to enhance performance, improve immunity, health and reproduction (Pal *et al.,* 2010).

2.4 Biological importance of nickel

NickelNi, a transition element with atomic number 28, electronic configuration [Ar] $3d^{10}$ $4s^{2}$ (Housecroft and Sharpe, 2005) is usually found in the 0 or +2 oxidation state, but oxidation states +1, +3, and even +4 have been demonstrated. However, these latter ion species are usually not stable in aqueous solutions. The element is ubiquitous but not distributed equally.For instance, it is a natural constituent of plant tissues and normal concentrations range from 0.05 to 10 μg per g of dry matter, but up to 1 mg nickel per g can be found in some "hyperaccumulators" plant species (Küpper and Kroneck, 2007) by contrast, nickel concentrations in human serum are in the nanomolar range.

Several enzymes and one cofactor have recently been shown to contain nickel. For example, urease of jack beans has been found to be a nickel protein. Nickel is very tightly bound to the protein probably via the amino acid functional groups (Diekert *et al.*, 1980; Maroney, 1999).

2.5 Amino Acids

Amino acids are biologically important organic compounds containing amine and carboxylic functional groups, along with a side-chain (R group) which is specific to each amino acid. They can be classified according to the core structural functional groups' locations as alpha- (α-), beta- (β-) or gamma- (γ-) amino acids (Muller *et al*., 1999). Other categories relate to polarity, pH level and side-chain group type. Amino acids are the structural units (monomers) that make up proteins. They join to form short polymer chains called peptides or longer chains called polypeptides or proteins. These polymers are linear and unbranched, with each amino acid within the chain attached to two neighboring amino acids via peptide bonds (Rodnina *et al.,* 2007).

Twenty natural amino acids comprise the building blocks of proteins, which are chemical species indispensable to perform a large number of biological functions (Muller *et al.*, 1999). From these 20 amino acids, nine are essential and cannot be produced by the human body. Methionine and leucine are two of these essential amino acids. Complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes such as oxygen conveyor, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions (Kumar *et al.,* 2014; Chandraleka *et al.,* 2014).

2.5.1 Essential amino acids

Essential amino acids, or indispensable amino acids, are amino acids that cannot be synthesized by the organism, and thus must be supplied in its diet. These are nine in number and cannot be synthesize by the human system. They include phenylalanine, valine,

threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine (Furst and Stehle, 2004). Six other amino acids are considered conditionally essential in the human diet, meaning that their synthesis can be limited under special pathophysiological conditions, such as prematurity in infant or individuals in severe catabolic distress. These six are arginine, cysteine, glycine, glutamine, proline and tyrosine (Wagner and Musso, 1983; Furst and Stehle, 2004).

2.5.2 Non-essential amino acids

Non-essential amino acids are those that can be synthesized by the body. The term 'non-essential' does not infer that those amino acids are any less important however. The body is simply capable of creating them on its own; therefore, it is not necessary for it to obtain them from external source. These non-essential amino acids serve many functions to create optimal health. For instance, during exercise, muscle tissue breaks down and toxins are released (Dougherty, 2000; Fuller, 2012). Alanine works to remove these toxins so the liver is able to metabolize them and eliminate them from the body. Alanine may also help to keep cholesterol levels in check, while asparagine helps the nervous system maintain its equilibrium. It also acts as a detoxifier in the system and regulates metabolism. Five amino acids are dispensable in humans, meaning they can be synthesized in the body. These are alanine, aspartic acid, asparagine, glutamic acid and serine (Metcoff, 1988; Wilson and Wilson, 2006).

2.5.3 Biological importance of amino acids

Because of their biological significance, amino acids are important in nutrition and are commonly used in nutrition supplements, fertilizers, and food technology. Industrial uses

include the production of drugs, biodegradable plastics, and chiral catalysts.In the form of proteins, amino acids comprise the second-largest component after water, of human muscles, cells and other tissues. Outside proteins, amino acids perform critical roles in processes such as neurotransmitter transport and biosynthesis (Latham, 1997).

The ability of amino acids to exist as zwitterions under biological conditions makes them good ligands to suitable metal centers in coordination chemistry. In this form, the carboxylic end is deprotonated to form the carboxylato anion, $-COO^-$, which can bind to a metal center under suitable conditions, while the amine end is protonated to form the analogic ammonium group. If the pH of the medium is made alkaline, hydroxyl ions will dominate protons in solution and hence convert them to water. This would make the $–NH_2$ end unprotonated and so its electron pair on nitrogen atom become readily available for coordinate bonding with suitable metal centers. Thus, in a typical metal-amino acid complex one would expect bonds formed with metal by the ligands via –N, –O atoms, or both. This fact was established by Saxena *et al.*, 1996 for complexes of copper(II), nickel(II), cobalt(II) and zinc(II) with histidine and glycine or alanine.

2.6Transition Metal Complexes with Amino Acids

Metal complexes with amino acids are of great interest considering their use as models for metalloproteins. Copper(II) complexes are even of particular interest as they do not only serve as metalloprotein models but as transport forms of copper in living organisms (Garcia *et al.*, 2006). Many reports have been devoted to understanding the coordination character of such complexes especially copper(II) complexes with histidine, as well as model

complexes with histidine-containing oligopeptides (Sovago *et al.,* 2012; Bukharov *et al.,* 2014).

2.7Biological Importance of Coordination Complexes of Amino Acids

Metal ion complexes are playing an increasing role in the development of antimicrobial agents (Chang *et al.,* 2010). Coordination complexes of transition metals have been widely studied for their antimicrobial activities (Pllana-Zeqiri *et al.,* 2012).Mixed ligand complexes of Zn(II) and Co(II) with cysteine, histidine, cysteinemethylester, and histidinemethylester have been synthesized. Histidine and histidinemethylester acted as bidentate ligands involving amino and imidazole nitrogen in the complexes. In addition, the cysteine and cysteinemethylester acted as bidentate ligands coordinating via thiol sulphur and amino nitrogen. Tetrahedral geometry was proposed for Zn(II) and Co(II) complexes (Reddy *et al.,* 2005).Promising antimicrobial activities of some amino acids chelates have been reported, including those of histidine arginine and glutamic acid (Legler *et al.,* 2001; Saha *et al.,* 2009). However, there is dearthof information on coordination compounds of methionine and leucine, and their antimicrobial potential.

In a recent study, copper- and iron-leucine complexes were observed to have relatively same antibacterial activities but higher than their respective ligands (Asemave *et al.,* 2015). The study emphasized the use of Cu-Leu complex as new alternative antibacterial therapeutic agents.It has been reported that the antibacterial activity of a complex is influenced by its stability. The lower the stability of the amino acid complex, the greater its antibacterial activity. This is may be attributed to the fact that they have more free ions in the solution, which can enhance the cooperative interaction between the metal ions and the

ligands (Stanila *et al.,* 2007). Another feature linked to the biological activities of transition complex is that, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there is π-electron delocalization over the whole chelate ring. This in turn increases the lipophilic character of the metal chelate and favours its permeation through the lipid layers of the bacterial membranes (Faundez *et al.,* 2004).

Compounds containing –N and–O as donor atoms play an important role as ligands in coordination chemistry. The amino acid, histidine, is the most frequently occurring ligand of zinc in the active center of zinc containing enzymes. Both catalytic and structural zinc in enzymes are always coordinated to at least one cysteine and one histidine donor. Among allthe metal ligating amino acids bearing N, O and S containing donors in their side chains, cysteine and histidine are the most prominent ones for zinc (Rombach *et al.,* 2002).Most biological molecules contain –N, –O or –S as donor atoms and are therefore electrons rich. It has been shown from previous reports (Sadlers *et al.,* 2007; Mohamed *et al.,* 2009; Agbaje *et al.,* 2014; Osowale, 2017) that coordination of metal ions to biologically active agents may improve their efficiency and accelerates their bioactivity. Furthermore, earlier studies have also shown that some coordination compounds of coumarin derivatives and those of amino acids exhibited promising antimicrobial and cytotoxic activities on chelation (Chohan *et al.,* 2006).Promising antimicrobial activities of some amino acids chelates have also been reported (Nomiya *et al.,* 2000; Legler *et al.,* 2001; Saha *et al.,* 2010), including those of histidine, arginine and glutamic acid. It was further concluded that chelation may enhance greatly the bioavailability of coordination compounds as therapeutic agents (Aiyelabola *et al.,* 2012a, Aiyelabola *et al.,* 2016). Coordination compounds of glycine and phenylalanine metal-ligand ratio 1:2 have been reported with marginal antimicrobial

activities. It was suggested therefore, based on the chelate theory, that increasing the number of chelate rings may improve the antimicrobial activity of these complexes (Chang *et al.,* 2010).Copper(II) complexes of amino acids and peptides containing the chelating bis (imidazolyl) residues have been reviewed by Sovago *et al.,* 2003. The results reveal that bis (imidazolyl) analogues of these biomolecules are very effective ligands for metal binding. The nitrogen donor atoms of the chelating agent are the major metal binding sites under acidic conditions. In the presence of terminal amino group, the multidentate character of the ligands results in the formation of various polynuclear complexes including the ligand and the imidazole bridged dimeric species. The most intriguing feature of the coordination chemistry of these ligands is that the deprotonation of the coordinated imidazole-N (1) H groups results in the appearance of a new chelating site in the molecules. It leads to the formation of stable trinuclear complexes via negatively charged imidazolato bridges (Sovago *et al.,* 2003).

In recent years, Co(II)and Cu(II) amino acids complexes have received much attention because they proved to be useful antibacterial agents against *Staphylococcus aureus* and*Escherichia coli* (Stanila *et al.,* 2011). In addition, complexes of transition metals with amino acids in proteins and peptides are utilized in numerous biological processes, such as oxygen conveyor, electron transfer and oxidation. In these processes, the enzymatic active site, which is very specific, forms complexes with divalent metal ions (Vemrlinov *et al.,* 2006).

Recently, the copper amino acids complexes in aqueous solution: $[Cu(L1)_2]\cdot H_2O$ (L1=D-methionine), $[Cu(L2)_2]\cdot H_2O$ (L2=D-phenylalanine) and $[Cu(L3)_2]$ (L3=D-triptophan) were synthesized and analyzed by means of elemental analysis, thermogravimetric differential

analysis, atomic absorption, IR, UV–Vis and ESR studies (Stanila *et al.,* 2007). Metal–ligand ratio in Cu(II)- amino acid complexes have beenreported to be 1:2 as the amino acids are shown to act as bidentate ligands with coordination involving the carboxyl oxygen and the nitrogen atom of amino group. The electronic and ESR spectra have been employed to confirm square-planar local symmetry for the copper ion in these complexes (Stanila *et al.,* 2007). It is however, important to prioritize L-isomers of the amino acids for the synthesis since they are the most important class of the compounds, and to evaluate the biological activities of the synthesized metal(II) complexes of L-amino acids.Also, employing such techniques as XRD, magnetic measurements, molar conductivity and metal content analyses, for structural characterization in addition to those used by Stanila and coworkers (2007) is apparently an open area of research.

Yokota *et al.*,(2016) studied the crystallization behavior of Cu(II) complexes of branched chain amino acids (BCAAs). In the report, mixing of Cu(II) and L-leucine in solution caused immediate precipitation of Cu(II)-leucine complex, while no precipitates were obtained when L-valine or L-isoleucine were used as ligands. The results were discussed based on structure differences among the precipitates. Questions may arise as to why the use of a base was ignored in the synthesis of the Cu(II) complexes of BCAAs since amino acids exist as zwitterions and may become resistant to bidentate coordination considering their ionization complications. The use of a strong base in the synthesis of similar complexes have been indicated as a reliable way of arresting this challenge (Aiyelabola *et al.,* 2012a).

Although it is not possible to elucidate or confirm the structures of complex species by pH measurements, they can be speculated upon based on the literature reports and chemical

knowledge. In aqueous solutions, metal ions are coordinated by six water molecules. Amino acids replace water molecules and form metal–amino acid complexes (Chen *et al.*, 2000a,b; Damaj *et al.*, 2009; Patil *et al.*, 2009). Depending on the nature of the ligands and metal ions and based on the basic chemical knowledge tentative structures of complexes are proposed. Carboxyl oxygen and amino nitrogen of L-valine are bonded to the metal ions though nitrogen can associate with hydrogen ions in physiological pH ranges. Hence, there is often significant competition between hydrogen and metal ion for this donor site and it results in the formation of protonated species. The Cu(II) ion forms distorted octahedral or square planar complexes due to the Jahn–Teller effect (Grigoreva and Dusheiko, 1970).

A biomimetic study of metal ion complexes with L-valine in acetonitrile–water mixtures indicated that the complexes are protonated in acidic pH medium (Bhushanavathi and Rao, 2013). An investigation was also made into the experimental conditions for the formation of poorly soluble complexes of the divalent Zn(II) combined with the amino acids: tyrosine, tryptophan, cysteine, histidine, and alanine, in the form of suspensions for intravenous administration. The number of Zn(II)-binding sites in the amino acid (n) as well as the amino acid affinity to Zn(II) (Ka), were determined by Dolinska, (2001). The study focused rather on the stability of such complexes formed.

It becomes apparent from literature reports that the amino acid complexes of copper(II) have been prioritized against other metal(II) species of similar biological importance. However, there is hardly any clear justification for such neglect. Biological studies for the reported copper(II) amino acid complexes have been limited to a few strains of bacteria, mostly,*Escherichia coli* and *Staphylococcus aureus* (Stanila *et al.*, 2011). Investigation of the biological activity of the metal amino acid complexes on a wide range of bacteria, and

some fungi strains is still an open area of research. In addition, characterization for the reported complexes has also not adoptedsuch techniques as magnetic susceptibility, molar conductivity and X-Ray Diffraction (XRD). Questions whether such complexes are crystalline or amorphous, and the like remain to be answered; this could be achieved by carrying out powder XRD analysis (Munoz-Espi *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

The following apparatus and equipment were used: water bath (improvised), oven (heating and drying oven DGH-9053), furnace (Nabertherm programmable furnace, Model GmbH), digital weighing balance (FINLAB Electronic Balance Model ESJ200-4), melting point apparatus (Stuart Automatic Model SMP40) and magnetic stirring hot plate (BIBBY STERILIN Model HC500). Others werescientific magnetic susceptibility balance (Sherwood AUTO scientific MSB), conductivity meter (HACH conductivity meter:

Sension5), UV-visible spectrophotometer (Agilent Cary Series), IR spectrophotometer (Agilent Cary 360) and XRD machine (Xpert-Pro XRD coupled with PDF-2 software).

The chemicals used includeL-methionine, L-leucine (Sigma-Aldrich Chemicals), copper(II) chloride dihydrate, cobalt(II) chloride hexahydrate, zinc(II) sulphate heptahydrate, nickel(II) chloride hexahydrate, sodium hydroxide, dimethyl sulphoxide, methanol, ethyl acetate, ethanol (BDH products) and distilled water.The chemicals and solvents were of analytical grade and were used without further purification. Themicrobes (bacteria and fungi) were clinical strains obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. The standard drugs used wereciprofloxacin and fulcin (May & Baker Pharmaceutical Company).

3.2 Methods

3.2.1 Synthesis of metal(II) methionine complexes

The complexes were synthesized using a procedure adopted from Aiyelabola *et al.*, 2012bwith slight modification as follows:

Stoichiometric amounts of2.130 g methionine (0.014 mol), 1.530 g methionine (0.010 mol), 1.240 g methionine (0.008 mol) and 1.521 g methionine (0.010 mol) were weighed and dissolved separately in 40 mL of distilled water. Appropriate amounts of 0.560 g (0.014 mol), 0.420 g (0.011 mol), 0.310 g (0.008 mol) and 0.410 g (0.010 mol) sodium hydroxide were then weighed and dissolved in 10 mL of distilled water and added to the respective

methionine solutions for deprotonation of the amino acid.Aqueous solutions of the metal salts containing 1.210 g $CuCl_2.2H_2O$(0.007 mol), 1.230 g of $CoCl_2.6H_2O$ (0.005 mol), 1.210 g of $ZnSO_4.7H_2O$ (0.004 mol) and 1.210 g of $NiCl_2.6H_2O$ (0.005 mol) were prepared using 40 mL distilled water. Thesewere added to the deprotonated amino acid solutions respectively, and the resultant mixture heated on a water bath with constant stirring for about 2 hrs. Precipitates obtained were filtered off, washed with water several times and dried in an oven at 60^{o}C.

3.2.2. Synthesis of metal(II) leucine complexes

The complexes were also synthesized using a procedure adopted from Aiyelabola *et al.*, 2012b with slight modification as follows:

Stoichiometric amounts of 1.880 g leucine (0.014 mol), 1.340 leucine (0.010 mol), 1.100 g leucine (0.008 mol) and 1.340 g leucine (0.010 mol) were weighed and separately dissolved in 40 mL of distilled water. Appropriate amounts of 0.580 g (0.015 mol), 0.420 g (0.011 mol), 0.330 g (0.008 mol) and 0.260 g (0.007 mol) sodium hydroxide were thenweighed and dissolved in 10 mL of distilled water and added to the respective leucine solutions for deprotonation of the amino acid.Aqueous solutions ofthe metal salts containing 1.210 g $CuCl_2.2H_2O$(0.007 mol), 1.230 g of $CoCl_2.6H_2O$ (0.005 mol), 1.200 g of $ZnSO_4.7H_2O$ (0.004 mol) or 1.210 g of $NiCl_2.6H_2O$ (0.005 mol) were prepared using 40 mL distilled water. These were added to the deprotonated amino acid solutions respectively, and the resultant mixture heated on a water bath with constant stirring for about 2 hrs. Precipitates obtained were filtered off, washed with distilled water several times and dried in an oven at 60^{o}C.

3.3. Characterization

The synthesized complexes were subjected to spectrophotometric and classical analyses for structuralelucidation.

3.3.1. Melting point analysis

The temperature of decomposition (d) of the complexes was measured using open capillary tubes on a Stuart automatic melting point apparatus model SMP40, with starting temperature of 40^{o}C at a temperature ramp of 15^{o}Cmin^{-1}.

3.3.2. Solubility test

Solubility test for the complexes was done at room temperature using the cold solvents, water, methanol, ethanol, ethyl acetate and DMSO. The test was carried out by placing a small amount of the sample in different test tubes each containing different solvent and the mixture was vigorously hand-shaken to check whether the complex dissolved or not.

3.3.3. Molar conductivity

Molar conductivity measurements were carried out at room temperature using a HACH conductivity meter model Sension5. The analysis was done using a procedure adopted from Jamil *et al.,* (2015). A 0.02 g of the sample was placed in a 10 cm^3 of 0.001M dimethyl sulphoxide, DMSO solvent, in a test tube. The resultant solution was vigorously shaken to ensure complete dissolution of the sample. The electrode was then dipped into the test tube containing the dissolved sample and the readings from the conductivity meter was obtained.

3.3.4. Magnetic susceptibility

The magnetic susceptibility measurement for the synthesized complexes were obtained at room temperature using an AUTO (Sherwood scientific) magnetic susceptibility balance (MSB). This was done by first taking the magnetic measurement of an empty capillary tube (a negative value, since glass is diamagnetic) recorded as R_o then that of capillary tube with pre-weighed amount of sample as R. The length of the sample in the column was taken as L, the proportionality constant (C) given to be 1.0, mass (M) of the sample alone weighed from the digital weighing balance was also recorded. The gram susceptibility (X_g) of the complexes was thus evaluated using the relationship in equation 3.1 (Lotz, 2008; Sharwood-Scientific, 2017).

$$X_g = \frac{C \times L\,(R-Ro)}{10^9 \times M} \qquad 3.1$$

A negative value of X_g indicates that the complex is diamagnetic while a positive value is indicative of a paramagnetic behaviour.

The molar susceptibility (Xm) was then obtained using equation 3.2.

$$Xm = Xg \times MM \qquad 3.2$$

where MM is the molecular mass of the complex.Hence,effective magnetic moments (U_{eff}) for the complexes was evaluated using the expression in equation 3.3.

$$U_{eff} = 2.83\sqrt{Xm \times T} \qquad 3.3$$

Where T is the room temperature in Kelvin, and $U_{eff}$is the effective magnetic momentsin Bohr magnetons (BM).

3.3.5. Determination of hydrationwater in the complex compounds

This was done by following a procedure reported by Aliyu and Abdullahi, (2009). Specific weight of the complexes (0.1000 g) in a crucible was placed in an oven at 110^{o}C for 1 hour. This was removed and reweighed after allowing it to cool in a desiccator. The procedure was repeated until constant weight was obtained. The lost in weight was recorded and the percentage composition of water of hydration in the complex determined using equation 3.4 (Vogel, 1966; Aliyu and Abdullahi, 2009).

$$\frac{loss\ in\ weig\,ht(g)}{Weig\,ht\ of\ sample\ \ taken\ (g)} \times 100\% \qquad 3.4$$

3.3.6. Metal content analysis

The metal content was determined gravimetrically by converting the complexes into their corresponding oxides. Weighted amount of the complexes (in a crucible) were ignited using a Nabertherm programmable furnace (Model GmbH) at a temperature ramp of 20^{o}C min^{-1}**,** and held at a plateau temperature of 800^{o}C for three hours (Refat *et al.,* 2013a). The weights of the resultant residue (corresponding metal oxides) were recorded, and percentage of metal content determined using equation 3.5.

$$\frac{weig\,ht\ of\ metal\ \ oxide\ \times Gravimetric\ \ \ factor}{Weig\,ht\ of\ complex\ \ \ taken} \times 100\% \qquad 3.5$$

3.3.7. Infra-red spectroscopy

IR spectra were recorded on Agilent Cary 630 FTIR Spectrophotometer within wavenumber range of 4000–650 cm^{-1}. Information about metal(II) ions' coordination was obtained by comparing the IR frequencies of the ligands with those of their respective metal complexes (Stanila *et al.,* 2007).

3.3.8. UV-Vis spectroscopy

The UV–visible spectra were determined in the DMSO solvent with concentration (1.0×10^{-3} M) for the free ligands and their metal complexes using Cary series UV-vis Spectrophotometer with 1cm quartz cell, in the range 200–800 nm (Refat *et al.*, 2013a).

3.3.9. Powder X-ray Diffraction

The X-ray powder diffraction analysis was carried out using Xpert-Pro X-ray diffractometer with Cu Kα1 radiation with λ=1.54056Å. The scans were made at a temperature of 25°C within 2θ range of 5.00-80.00° at scan rate of 8.00°min^{-1}. Unit cell parameters were found by employing trial and error method using the Powder Diffraction File software (PDF-2). The average crystallite sizes for all complexes were calculated using Debye Scherrer's formula, equation 3.6.

$$ד_{xrd} = \frac{0.9 \times \lambda}{\beta\ Cos\theta} \qquad 3.6$$

where ד is the average crystallite size, λ is the wavelength of Cu Kα radiation (1.54056 Å), β is the full width at half maximum (FWHM in radians) and θ is the Braggs reflection angle. 0.9 is the machine constant K. Inter-planer separations (*d*-spacing) were obtained using the Bragg's law, equation 3.7 (Kavitha *et al.*, 2013; Bunaciu *et al.*, 2015).

$$d = \frac{\lambda}{2\ Sin\theta} \qquad 3.7$$

3.4 Antimicrobial screening

The antimicrobial activities of the ligands and the complexes were determined using some clinical strains ofmicrobes obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.The *in vitro* antibacterial activity studies were therefore carried out against three Gram positive (*Staphylococcus aureus, Streptococcus pyogenes*and*Bacillus cereus*) and three Gram negative(*Escherichia coli, Salmonella typhi*and*Shigella dysentriae*).In addition, antifungal potential of four fungal strains(*Fusarium oxysporum, Aspergillus niger, Candida albicans*and *Aspergillus flavus*) was also investigated. The agar diffusion method was adopted for screening the compounds.The zone of inhibition (ZI), minimum inhibition concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were all carried out using the procedure reported by CLSI, (2016).

A mass of 0.002 g of the compound in each case was weighed and dissolved in 10 mL of DMSO to obtain a concentration of 200µgmL^{-1}.This was the initial concentration of the compounds used to determine their antimicrobial activities using the Mueller Hinton and Sabouraud dextrose agars as media for the growth of the microbes. The media was prepared according to the manufacturer's instructions, sterilized at 121^{o}C for 15 min, poured into sterile petri dishes separately and the media allowed to cool and solidify. Mueller Hinton agar was seeded with the test bacteria while the Sabouraud dextrose agar was seeded with the fungi. The inoculum of the test microbes in the medium was spread evenly over the surface of the medium by the use of a sterile swab. A well was cut at the center of each inoculated medium using a standard cork borer of 6 mm diameter. 0.1 mL of the solution of the extract was then introduced into the well on the inoculated media. Incubation followed at 37^{o}C for 24 hours for the bacteria, and at 30^{o}C for 7 days for the fungi after which the

media plates were observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result recorded in millimeters (CLSI, 2016).

3.4.1 Minimum inhibition concentration

The minimum inhibition concentration of the compounds was determined using the broth dilution method. MuellerHinton and Sabouraud dextrose broth were prepared by dispensing 10 mL of the broth into test tubes and were sterilized at 121^{o}C for15 min, then allowed to cool. McFarland's turbidity standard scale number 0.5 was prepared to give a turbid solution. Normal saline was prepared by dispensing 10 mL of NaCl into sterile test tubes and the test microbes inoculated and incubated at 37^{o}C for 6 hours. Dilution of the test microbes was done in the normal saline until the turbidity matched that of the McFarland's scale by visual comparison at which point the test pathogens had a concentration of 1.5×10^{8}colony forming units ($CFUL^{-1}$). Two-fold serial dilution of the compounds in DMSO was made to obtain the concentrations of 200µgmL^{-1}, 100µgmL^{-1}, 50µgmL^{-1}, 25µgmL^{-1} and 12.5µgmL^{-1}. The initial concentration was obtained by dissolving 0.002 g of the compounds in 10 mL of the DMSO. Having obtained the different concentrations of the compound in the sterile broth, 0.1 mL of the test strains in the normal saline was then introduced into the different concentrations with the bacteria introduced into the Mueller Hinton broth and the fungi in Sabouraud dextrose broth. Incubation followed at 37^{o}C for 24 hrs for bacteria, and at 30^{o}C for 7 days for the fungi after which the test tubes of the broth were observed for turbidity (growth). The lowest concentration of the compounds in the broth which showed no turbidity was recorded as the MIC (CLSI, 2016).

3.4.2 Minimum bactericidal concentration/minimum fungicidal concentration

MBC/MFC was carried out to determine whether the test organisms were killed or only their growth was inhibited. Mueller Hinton and Sabouraud dextrose agars were prepared, sterilized at 121^{o}C for 15 min, poured into sterile petri dishes and allowed to cool and solidify. The content of the MIC in the serial dilutions were then sub-cultured onto the prepared media with the bacteria sub-cultured onto the Mueller Hinton ager and incubated at 37^{o}C for 24 hrs, and the fungi sub-cultured onto the Sabouraud dextrose agar at 30^{o}C for 7 days, after which the plates of the media were observed for colony growth. The MBC/MFC was the plates with lowest concentration of the compound without colony growth (CLSI, 2016).

CHAPTER FOUR

4.0 RESULTS

4.1 Physical Properties

The physical properties of the synthesized complexes and their percentage yields are shown in Table 4.1. The data obtained proved the formation of the complexes. Their observed colours were in agreement with those expected, grey-blue for Cu complexes, pink for Co, white for zinc and pale-blue for those of Ni (Stanila *et al*., 2007; Refat *et al*., 2013b).

4.2 Solubility Studies

The results of solubility analysis of the complexes were performed in some selected solvents and the results are presented in Table 4.2. Dimethyl sulphoxide (DMSO) was found to be the best solvent for the complexes. Thus, studies of electronic spectroscopy and conductivity measurements were carried out by dissolving the complexes in DMSO Solvent.

4.3 Spectroscopic Analyses

The infrared spectroscopy analysis is reported in Table 4.3. Both bathochromic and hypsochromic shifts were observed and assigned appropriately. Most significant were the shifts observed in the $–NH_2$ and –COO functional group domain. Also, the O-H group, missing in the spectra of the ligands but appeared in that of the complex compounds.

Electronic spectra data recorded in Table 4.4 shows transition which were unique for each complex compound formed. All the complexes showed *d-d* transition in the visible region except those of Zn(II) which only showed transition in the UV- region mainly characteristics of n-π*and $\pi - \pi$* transition.

Table 4.1: Physical properties of metal(II) complexes

Complex	**Formula weight ($gmol^{-1}$)**	**Decomposition temperature (^oC)**	**Colour**	**Yield (%)**
$[Cu(\mathbf{L})_2].H_2O$**(1)**	377.97	267-269	Grey-blue	50.74
$[Cu(\mathbf{L^1})_2].H_2O$**(2)**	341.91	254-257	Light-blue	53.06
$[Co(\mathbf{L})_2(H_2O)_2].H_2O$**(3)**	409.35	277-280	Pink	69.10

$[Co(\mathbf{L}^{1})_2(H_2O)_2].H_2O$**(4)**	373.29	276*	Pink	55.80
$[Zn(\mathbf{L})_2].H_2O$**(5)**	379.80	285-288	White	57.59
$[Zn(\mathbf{L}^{1})_2].H_2O$**(6)**	343.74	287-290	White	57.90
$[Ni(\mathbf{L})_2].2H_2O$**(7)**	391.11	233-237	Pale-blue	51.70
$[Ni(\mathbf{L}^{1})_2].2H_2O$**(8)**	355.05	269-270	Pale-blue	51.80

*=decomposition temperature, L = L-methionine and L^1 = L-leucine

Table 4.2: Solubility of complexes in selected solvents

Complex	Water	Methanol	Ethanol	Ethyl acetate	DMSO
[Cu**L**]**(1)**	IS	IS	IS	IS	S
[Cu$\mathbf{L}^{1}$] **(2)**	IS	SS	SS	IS	S
[Co**L**]**(3)**	IS	SS	IS	IS	S

[Co**L**^{1}] **(4)**	IS	IS	IS	IS	S
[Zn**L**]**(5)**	IS	SS	IS	IS	S
[Zn**L**^{1}] **(6)**	IS	SS	SS	PS	S
[Ni**L**]**(7)**	IS	IS	IS	IS	S
[Ni**L**^{1}] **(8)**	IS	SS	SS	IS	S

IS=Insoluble, SS=sparingly soluble, S=Soluble, DMSO=dimethyl sulphoxide, L = L-methionine and L^1 = L-leucine

Table 4.3: IR frequencies ofL and L^{1}and their metal complexes

Compound	*v*(OH;H_2O)	*v*(N-H)	*v*(C=O)	*v*(C-N)	*v*(COO)	*v*(C-O)	*v*(CH_3-S-CH_2)
L	-	2944	1651	1333	1408	1159	2914
L^{1}	-	2955	1572	1312	1405	1133	-

[Cu**L**]**(1)**	3287	3131	1610	1330	1427	1151	2914
[Cu$\mathbf{L^1}$] **(2)**	3317	3242	1613	1394	1472	1192	-
[Co**L**]**(3)**	3347	3272	1565	1345	1453	1151	2914
[Co$\mathbf{L^1}$] **(4)**	3365	3280	1587	1304	1472	1189	-
[Zn**L**]**(5)**	3295	3254	1595	1386	1423	1159	2914
[Zn$\mathbf{L^1}$] **(6)**	3324	3268	1606	1244	1468	1196	-
[Ni**L**]**(7)**	3339	3280	1654	1341	1420	1185	2914
[Ni$\mathbf{L^1}$] **(8)**	3369	3276	1587	1397	1472	1189	-

L = L-methionine and L^1 = L-leucine, frequency in cm^{-1}

Table 4.4:UV-Visible spectra data ofL and $\mathbf{L^1}$and their metal complexes

Compound	λ_{max}(nm)	λ_{max} (cm^{-1})	Assignment of transitions
L	270	37037	n-π*
	264	37879	π-π*

L^1	270	37037	n-π*
	261	38314	π-π*
[Cu**L**]**(1)**	761	13141	$^2B_{1g} \rightarrow {}^2E_{1g}$
[CuL^1] **(2)**	771	12970	$^2B_{1g} \rightarrow {}^2E_{1g}$
[Co**L**]**(3)**	514	19455	$^4T_{1g} \rightarrow {}^4A_{2g}$
	354	28249	n-π*
[CoL^1] **(4)**	510	19608	$^4T_{1g} \rightarrow {}^4A_{2g}$
[Zn**L**]**(5)**	268	37313	π-π*
[ZnL^1] **(6)**	271	36900	n-π*
[Ni**L**]**(7)**	645	15504	$^3T_{2g} \rightarrow {}^3A_{2g}$
	385	25974	n-π*
[NiL^1] **(8)**	731	13680	$^3T_{2g} \rightarrow {}^3A_{2g}$
	392	25510	n-π*

L = L-methionine and L^1 = L-leucine

4.4 Magnetic and Conductivity Measurements

Table 4.5 presents the result of magnetic susceptibility and molar conductivity studies for the synthesized complexes. Magnetic moments obtained showed that Cu(II) Co(II) and Ni(II) complexes were paramagnetic, while those of Zn(II) were diamagnetic. Conductivity

measurements showed that the complexes were non-electrolytic which is expected for neutral complexes (Refat *et al*., 2013b).

4.5 Gravimetric Analyses

Results of metal contents and water of hydration analyses are recorded in Tables 4.6 and 4.7 respectively.The result showed in both cases that the calculated values were in close agreement with the observed ones thus corroborating the molecular formula presented in Table 4.1.

4.6 X-ray Diffraction Studies

Data of powder XRD analysis are presented in Table 4.8. The average crystal size obtained by use of the Scherer equation proved the crystallinity of the complexes, and similarity in bonding modes for all the complexes studied.

4.7 Antimicrobial Studies

4.7.1 Zone of inhibition

The zone of antimicrobial inhibition for the ligands and the complexes are presented inTables 4.9–4.11. The CLSI (2016) standard zones of inhibition for the standard drugs were used as basis for establishing activity. Most of the complexes proved to inhibit the growth of

Table 4.5:Room temperature magnetic susceptibility and Molar Conductivity of the M(II)complexes

Complex	μ_{eff} (B.M)	Temperature (K)	$\Lambda(\Omega^{-1} mol^{-1} cm^2)$
[Cu**L**]**(1)**	1.97	299.50	18.47

[Cu**L**^{1}] **(2)**	2.04	299.50	18.99
[Co**L**]**(3)**	6.00	299.50	43.80
[Co**L**^{1}] **(4)**	5.10	299.50	24.90
[Zn**L**]**(5)**	0.00	300.00	25.10
[Zn**L**^{1}] **(6)**	0.00	300.00	18.63
[Ni**L**]**(7)**	2.26	300.00	29.90
[Ni**L**^{1}] **(8)**	4.21	300.00	30.80

μ_{eff} = Effective magnetic moment, Λ= Molar conductivity, B.M=Bohr magnetons, K=Kelvin, molar conductivity in 1×10^{-3} DMSO solution

Table 4.6: Metal content analysis

Complex	Weight of complex (g)	Weight of oxide (g)	Gravimetric factor	Percentage(%) of composition of metal	Percentage(%) of composition of metal

				(Calculated)	(Observed)
[Cu**L**]**(1)**	0.1500	0.0305	0.7987	16.81	16.24
[Cu$\mathbf{L^1}$] **(2)**	0.1500	0.0358	0.7987	18.59	19.07
[Co**L**]**(3)**	0.1500	0.0281	0.7865	14.40	14.73
[Co$\mathbf{L^1}$] **(4)**	0.1500	0.0290	0.7865	15.79	15.21
[Zn**L**]**(5)**	0.1500	0.0329	0.8034	17.21	17.62
[Zn$\mathbf{L^1}$] **(6)**	0.1500	0.0352	0.8034	19.02	18.85
[Ni**L**]**(7)**	0.1500	0.0289	0.7858	15.01	15.14
[Ni$\mathbf{L^1}$] **(8)**	0.1500	0.0299	0.7858	16.53	15.66

Table 4.7: Water of hydration analysis

Complex	Initial weight of complex (g)	Final weight of complex (g)	Loss in weight (g)	Percentage(%)of water of hydration (Calculated)	Percentage(%)of water of hydration (Observed)

[Cu**L**]**(1)**	0.1000	0.0955	0.0045	4.76	4.50
[Cu$\mathbf{L}^{1}$] **(2)**	0.1000	0.0949	0.0051	5.26	5.10
[Co**L**]**(3)**	0.1000	0.0953	0.0047	4.40	4.70
[Co$\mathbf{L}^{1}$] **(4)**	0.1000	0.0947	0.0053	4.82	5.30
[Zn**L**]**(5)**	0.1000	0.0954	0.0046	4.74	4.60
[Zn$\mathbf{L}^{1}$] **(6)**	0.1000	0.0948	0.0052	5.24	5.20
[Ni**L**]**(7)**	0.1000	0.0911	0.0089	9.20	8.90
[Ni$\mathbf{L}^{1}$] **(8)**	0.1000	0.0902	0.0098	10.14	9.80

Table 4.8: Powder X-ray diffraction analysis

Complex / Parameters	Cu-Met	Cu-Leu	Co-Met	Co-Leu	Zn-Met	Zn-Leu
a (Å)	12.26	9.59	14.98	4.90	16.19	24.65
b (Å)	10.99	7.15	2.81	2.81	5.11	24.65
c (Å)	6.40	8.54	4.90	9.86	9.99	24.65
α (°)	90.00	90.00	90.00	90.00	90.00	90.00
β (°)	90.50	90.50	95.91	93.40	95.50	90.00
γ (°)	90.00	90.00	90.00	90.00	90.00	90.00
τ_{xrd} (nm)	63.68	55.30	74.69	55.79	84.26	91.42
V $(Å)^3$	862.86	581.46	205.25	410.49	822.37	3744.47

τ_{xrd}= Average crystal size and V = cell volume

Table 4.9: Results of zone of antimicrobial inhibition(mm)for ligand and complexes on Gram-positive bacteria

	S. aureus	*S. pyogenes*	*B. cereus*
L	18	0	0
L^1	0	23	20
[Cu**L**]**(1)**	21	0	0
[CuL^1] **(2)**	0	27	24
[Co**L**]**(3)**	25	27	24
[CoL^1] **(4)**	0	26	25
[Zn**L**]**(5)**	25	0	26
[ZnL^1] **(6)**	27	0	24
[Ni**L**]**(7)**	25	27	0
[NiL^1] **(8)**	29	26	0
C	29	0	0

C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.10: Results of zone of antimicrobial inhibition(mm)for ligand and complexes onGram-negative bacteria

	E. coli	*S. typhi*	*S. dysentriae*
L	24	0	0
L^1	20	0	0
[Cu**L**]**(1)**	26	0	0
[CuL^1] **(2)**	25	0	0
[Co**L**]**(3)**	0	0	25
[CoL^1] **(4)**	24	0	0
[Zn**L**]**(5)**	27	0	0
[ZnL^1] **(6)**	23	0	0
[Ni**L**]**(7)**	24	0	0
[NiL^1] **(8)**	0	0	26
C	28	21	27

C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.11: Results of zone of antifungal inhibition(mm) for ligand and complexes

	C. albicans	*A. niger*	*A. flavus*	*F. oxyparum*
L	20	0	23	0
L^1	21	0	18	0
[Cu**L**]**(1)**	25	0	26	0
[CuL^1] **(2)**	25	0	24	0
[Co**L**]**(3)**	23	27	0	0
[CoL^1] **(4)**	0	24	0	23
[Zn**L**]**(5)**	26	0	24	23
[ZnL^1] **(6)**	24	0	23	25
[Ni**L**]**(7)**	0	0	0	25
[NiL^1] **(8)**	25	0	0	0
Q	30	26	30	32

Q = fulcin, L = L-methionine and L^1 = L-leucine

clinical strains of bacteria and fungi used in this study, with activity of the ligands increasing on complexation. The Gram-positive bacteria largely proved more susceptible to the complex compounds than their Gram-negative counterparts (Tables 4.9 and 4.8 respectively).

4.7.2 Minimum inhibition concentration

The MIC for the ligands, complexes and standard drugs were determined against the test organisms (Tables 4.12-4.14). The result proved that the standard drugs have the lowest MIC against most of the pathogens studied except for a few microbes.

4.7.3 Minimum bactericidal concentration/minimum fungicidal concentration

The MBC and MFC was evaluated to ascertain whether the test microbes were only inhibited or killed (Tables 4.15-4.17). All the active complexes showed both bactericidal and fungicidal potency with minimum bactericidal concentrations and minimum fungicidal concentrations ranging between 50-200 μgmL^{-1}.

Table 4.12: Results of minimum inhibition concentration(μgmL^{-1})for ligand and complexes on Gram-positive bacteria

	S. aureus	*S. pyogenes*	*B. cereus*
L	50	-	-
L^1	100	50	50
[Cu**L**]**(1)**	50	-	-
[Cu**L^1**] **(2)**	-	25	50
[Co**L**]**(3)**	50	25	50
[Co**L^1**] **(4)**	-	50	50
[Zn**L**]**(5)**	50	-	25
[Zn**L^1**] **(6)**	25	-	50
[Ni**L**]**(7)**	50	25	-
[Ni**L^1**] **(8)**	25	25	-
C	25	-	-

– = no activity, C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.13: Results of minimum inhibition concentration(μgmL^{-1})for ligand and complexes onGram-negative bacteria

	E. coli	*S. typhi*	*S. dysentriae*
L	50	-	-
L^1	50	-	-
[Cu**L**]**(1)**	25	-	-
[Cu**L^1**] **(2)**	50	-	-
[Co**L**]**(3)**	-	-	50
[Co**L^1**] **(4)**	50	-	-
[Zn**L**]**(5)**	25	-	-
[Zn**L^1**] **(6)**	50	-	-
[Ni**L**]**(7)**	50	-	-
[Ni**L^1**] **(8)**	-	-	25
C	25	12.5	25

− = no activity, C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.14: Results of minimum inhibition concentration(μgmL^{-1})for ligand and complexes onsome fungi strains

	C. albicans	*A. niger*	*A. flavus*	*F. oxyparum*
L	50	-	50	-
L^1	50	-	100	-
[Cu**L**]**(1)**	50	-	50	-
[Cu**L^1**] **(2)**	50	-	50	-
[Co**L**]**(3)**	50	25	-	-
[Co**L^1**] **(4)**	-	50	-	50
[Zn**L**]**(5)**	25	-	50	50
[Zn**L^1**] **(6)**	50	-	50	50
[Ni**L**]**(7)**	-	-	-	50
[Ni**L^1**] **(8)**	50	-	-	-
Q	25	50	25	25

− = no activity, Q = fulcin,L = L-methionine and L^1 = L-leucine

Table 4.15: Results of minimum bactericidal concentration(μgmL^{-1})for ligand and complexes on Gram-positive bacteria

	S. aureus	*S. pyogenes*	*B. cereus*
L	200	-	-
L^1	-	100	200
[Cu**L**]**(1)**	200	-	-
[Cu**L^1**] **(2)**	-	50	100
[Co**L**]**(3)**	100	50	-
[Co**L^1**] **(4)**	-	100	100
[Zn**L**]**(5)**	100	-	100
[Zn**L^1**] **(6)**	50	-	100
[Ni**L**]**(7)**	100	50	-
[Ni**L^1**] **(8)**	50	50	-
C	100	-	-

−= no bactericidal activity, C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.16: Results of minimum bactericidal concentration(μgmL^{-1}) for ligand and complexes on Gram-negative bacteria

	E. coli	*S. typhi*	*S. dysentriae*
L	100	-	-
L^1	200	-	-
[Cu**L**]**(1)**	100	-	-
[Cu**L^1**] **(2)**	100	-	-
[Co**L**]**(3)**	-	-	100
[Co**L^1**] **(4)**	100	-	-
[Zn**L**]**(5)**	50	-	-
[Zn**L^1**] **(6)**	100	-	-
[Ni**L**]**(7)**	100	-	-
[Ni**L^1**] **(8)**	-	-	100
C	100	50	50

− = no bactericidal activity, C = ciprofloxacin, L = L-methionine and L^1 = L-leucine

Table 4.17:Results of minimum fungicidal concentration(μgmL^{-1})for ligand and complexes on some fungi strains

	C. albicans	*A. niger*	*A. flavus*	*F. oxyparum*
L	200	-	100	-
L^{1}	200	-	200	-
[Cu**L]** **(1)**	100	-	100	-
[Cu**L^{1}**] **(2)**	100	-	100	-
[Co**L]** **(3)**	100	50	-	-
[Co**L^{1}**] **(4)**	-	100	-	100
[Zn**L]** **(5)**	100	-	100	100
[Zn**L^{1}**] **(6)**	100	-	100	100
[Ni**L]** **(7)**	-	-	-	100
[Ni**L^{1}**] **(8)**	100	-	-	-
Q	100	200	200	100

− = no fungicidal activity, Q = fulcin,L = L-methionine andL^{1} = L-leucine

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physical Properties of the Metal(II) Complexes

The reaction of L-methionine (HL) and L-leucine (HL1) with the metal(II) chlorides (Cu, Co and Ni), and Zn(II) sulphate under alkaline pH gave coloured complexes except for Zn complexes which are white (Table 4.1). Good yields were obtained for the complexes (50-70 %), with the highest yield recorded for Co-methionine complex (69.10 %), while Cu-methionine complex gave the least percentage yield (50.74 %). The complexes were formed according to the equations 5.1-5.8.

$$CuCl_2$$

where, HL= L-methionine and HL^1 = L-leucine.

The decomposition temperature of the complexes was measured using a Stuart automatic melting point apparatus model SMP40. The range of values observed for the complexes were reasonably fair since they are in agreement with previous reports for similar compounds, with differences at about 2.0-3.0^{o}C. This shows that the melting points are fairly sharp, inferring that the complexes under study were isolated considerably in their pure form. The result of melting point obtained herein is akin to that obtained by Stanila and his coworkers in 2007 for similar complex compounds (Stanila *et al.*, 2007).

Formation of the metal(II) complexes was confirmed by percentage metal content, distinct colour, and solubility, infrared and electronic spectroscopies. The discrepancy in the biological activity of the ligands and their resultant metal complexes also support complex formation.

5.2 Solubility

The solubility of the metal complexes was tested in water, methanol, ethanol, ethyl acetate and dimethylsulphoxide (DMSO). However, the complexes were only soluble in DMSO (Table 4.2). Consequently, the molar conductivity measurements and electronic spectra studies for the complexes were carried out in DMSO. The result is in concomitance with the findings of Corbi and co-workers in 2000 for analogous compounds, nickel(II) complexes with methionine sulfoxide (Corbi *et al.*, 2000).

5.3 Infrared Spectroscopic Analysis

The infrared spectra analyses of the ligands, **L** (L-methionine) and $\mathbf{L^1}$ (L-leucine), and their respective complexes were carried out and the relevant peaks are given in Table 4.3.

Information about metal(II) ions coordination was obtained by comparing the IR frequencies of the ligands with those of their respective complexes.

In the spectra of the ligands, **L** and $\mathbf{L^1}$, the *v*(N-H) stretching vibrations appear at 2944 cm^{-1} and 2955 cm^{-1} respectively. These bands appear to be shifted towards higher wavenumbers in the spectra of the complexes with 3131 cm^{-1} for Cu-Met (**1**), 3272 cm^{-1} for Co-Met (**3**), 3254 cm^{-1} for Zn-Met (**5**) and 3280 cm^{-1} for Ni-Met (**7**). Also, 3242 cm^{-1} for Cu-Leu (**2**), 3280 cm^{-1} for Co-Leu (**4**), 3268 cm^{-1} for Zn-Leu (**6**) and 3276 cm^{-1} for Ni-Leu (**8**). This provides an evidence for the involvement of the $-NH_2$ group in complex formation (Batiu *et al.,* 2005; Kumar, 2011). A further supporting evidence for the involvement of the $–NH_2$ group in metal-ligand bonding is obtained from the bands in the spectra of **L** and $\mathbf{L^1}$ at 1333 cm^{-1} and 1312 cm^{-1} respectively, which are assigned to *v*(C-N) stretching vibrations. This band at 1333 cm^{-1} in the spectra of **L** appear to be shifted to 1330 cm^{-1}, 1345 cm^{-1}, 1386 cm^{-1} and 1341 cm^{-1} for **1**, **3**, **5**, and **7** respectively. Similarly, the band at 1312 cm^{-1} in $\mathbf{L^1}$ appear to be shifted to 1394 cm^{-1}, 1304 cm^{-1}, 1244 cm^{-1} and 1397 cm^{-1} for **2**, **4**, **6** and **8** respectively (Batiu *et al.,* 2005).

The ligands showed strong vibrations at 1651 cm^{-1} for **L** and 1572 cm^{-1} for $\mathbf{L^1}$ which are attributed to *v*(C=O) stretching. These bands appear to be shifted reasonably in the spectra of the corresponding complexes (Table 4.3) with the bands appearing at 1610 cm^{-1} (**1**), 1565 cm^{-1} (**3**), 1595 cm^{-1} (**5**) and 1654 cm^{-1} (**7**). Also, 1613 cm^{-1} (**2**), 1587 cm^{-1} (**4**), 1606 cm^{-1} (**6**) and 1587 cm^{-1} (**8**). Such shifts in band positions provide an insight into the bonding modes of the metal(II)-amino acid complexes. The fact that such peaks appear to have shifted bathochromically or hypsochromically in the spectra of their respective complexes can be ascribed to the involvement of the carboxylic oxygen of the amino acid

in the metal-ligand coordinate bonding (Stanila *et al.,* 2007). The bands at 1159 cm^{-1} and 1133 cm^{-1} in the spectra of **L** and $\mathbf{L^1}$ respectively are assignable to the *ν*(C-O) vibration. These bands appear to be shifted to lower wavenumbers or higher in the spectra of the corresponding metal(II) complexes providing further supporting evidence that the carboxyl oxygen of the ligand is involved in coordinate bonding with the metal(II) centers (Aiyelabola *et al.,* 2012a). The bands at 1408 cm^{-1} and 1405 cm^{-1} in the spectra of **L** and $\mathbf{L^1}$ respectively, assigned to *ν*(COO) vibrations are bathochromically shifted in the spectra of the corresponding metal complexes. This fact further supports the assertion that this group is involved in coordinate bond formation with the metal centers (Kulkarni *et al.,* 2014; Aiyelabola *et al.,* 2016).

The *ν*(O-H) stretching vibration does not appear in the spectra of **L** and $\mathbf{L^1}$ but does in those of their metal complexes with **1**, **3**, **5** and **7** at 3287 cm^{-1}, 3347 cm^{-1}, 3295 cm^{-1} and 3339 cm^{-1} respectively. Also, **2**, **4**, **6**, **8** at 3317 cm^{-1}, 3365 cm^{-1}, 3324 cm^{-1}, 3369 cm^{-1} respectively. This suggests the presence of water of crystallization and, or water of coordination in these complexes (Stanila *et al.,* 2011). This finding is in close agreement with that of Stanila and his coworkers in 2007 for complex **1**. The band at 2914 cm^{-1} in the spectra of the complexes **1**, **3**, **5** and **7** are attributed to the CH_3-S-CH_2 symmetric and asymmetric vibrations and are not shifted when compared to that of **L** (2914 cm^{-1}). This implies that the sulphur atom of this group is not involved in coordination with the metal(II) centers (Wokaun, 1995).

A combined spectrum of the ligands and their respective metal(II) complexes formed from them are shown in Appendices 1 and 3 respectively. The spectra of the complexes in each case appear distinct from those of their respective ligands with major variations occurring

at the $–NH_2$ and –COO domain. Also, the absence of O-H vibrational bands in the ligands become an exception rather than the rule for the complexes as new resolved peaks emerged for all the complexes at > 3300 cm^{-1} which suggest the presence of water of crystalization in the complex compounds. When the spectra of the complexes were superimposed on those of their respective ligands from which they were formed (Appendices 2 and 4), two observations were made. First, the spectra of the complexes show peaks that do not match with those of their respective ligands. This infers that a reaction has taken place. Secondly, the spectra of the complexes show peaks deviating from those of their respective ligands at similar positions. Consequently, the spectra of the complexes appear somewhat similar. This fact supports the hypothesis that the bonding modes in these complexes are the same.

5.4 UV-Visible Spectroscopy

The electronic spectra of the two ligands and their complexes in DMSO were obtained and spectra data are presented in Table 4.4. The free ligands show maximum wavelengths at 270 cm^{-1}**L** and $\mathbf{L^1}$ assignable to n-π* transition within the ligands. The bands at 264 nm (**L**) and 261 nm ($\mathbf{L^1}$) are assigned to π-π* transitions. Electronic spectra provide valuable information about the arrangement of the ligands around the metal ions (Singh *et al.,* 2012). According to Liu *et al.,* 2007, Cu(II) complexes exhibiting bands at about 690 nm due to $^2E_g \rightarrow {}^2T_{2g}$ transition are a feature of Cu(II) ion in an octahedral environment. In the visible spectra of Cu(II) complexes however, such bands are absent. However, well resolved bands emerged at 761 nm (**1**) and 771 nm (**2**) which are assignable to $^2B_{1g} \rightarrow {}^2E_{1g}$ transitions. This may rule out the possibility of an octahedral local symmetry around Cu(II) ion but suggests a four coordinate environment (Liu *et al.,* 2007; Singh *et al.,* 2012). Ni(II) complexes exhibited bands at 645 nm (**7**) and 731 nm (**8**) respectively, assigned to $^3T_{2g} \rightarrow {}^3A_{2g}$

transitions which may be ascribed to a four coordinate local symmetry around Ni(II) ion. Consequently, the *d-d* bands observed for the respective Cu(II) and Ni(II) complexes are a feature that is expected for a square planar MO_2N_2 chromophore (Stanila *et al.,* 2007; Stanila *et al.,* 2009).

The visible spectra of cobalt complexes exhibited *d-d* transitions whose maxima were at 514 nm (**3**) and 510 nm (**4**) which are assigned to $^4T_{1g}$ (F)$\rightarrow$$^4A_{2g}$ (F), characteristic of an octahedral geometry (Patil *et al.,* 2011). The result is in concomitance with the findings of Stanila and his co-workers for similar compounds, Co(II) complexes with phenylalanine, tryptophan and methionine (Stanila *et al.,* 2009).

The complexes of Zn(II) are diamagnetic and do not possess any *d-d* transitions as no bands are observed in the visible domain of their spectra. This is due to the fully filled 3*d* orbital of Zn(II) ion. Their magnetic moments of zero corroborated this further (Banerjee *et al.,* 2008).

5.5 Magnetic Susceptibility Measurement

Magnetic moments for the complexes are shown in Table 4.5 with the results revealing paramagnetic character for the complexes of Cu(II), Co(II), and Ni(II). The complexes of copper, **1** (1.97 BM) and **2** (2.04 BM) showed values of magnetic moments which are in concomitance with a single spin state (n=1) (Canpolat *et al.,* 2002). Values in the range 5.10-6.00 BM were observed for cobalt complexes, suggesting their existence in a high spin state. This implies that the amino acid ligands are so weak that they create only a weak ligand field around the Co(II) center, causing a low energy e_g-t_{2g}*d*-splitting in an octahedral environment. Such an observation is valuable and important to assist in suggesting that the

amino acids, L-methionine and L-leucine are weak field ligands, and that Co(II) ion is in an octahedral environment in the complexes formed from these ligands. The later assertion being supported by *d-d* transitions in the visible spectra of Co(II) complexes observed at 514nm and 510 nm for **3** and **4** respectively, which are characteristic of an octahedral local symmetry around Co ion (Ozkan *et al.*, 2005; El-Tabl, 2008). The observed moments of 6.0 BM (**3**) and 5.10 BM (**4**) is thus supportive of octahedral geometry since room temperature magnetic moments in the range of 4.6-6.0 BM are reported for octahedral Co(II) complexes as validated by Housecroft and Sharpe, (2005).

The complexes of nickel also showed paramagnetic behaviour with magnetic moments between 2.21-4.11 BM. This obviously rules out the possibility of a square planar local symmetry around nickel in these complexes as square planar complexes of d^8electronic systems are usually diamagnetic. Consequently, tetrahedral structure appear more reasonable for the amino acid complexes of Ni(II) under investigation (Lee, 2013). This also supports the hypothesis that the amino acids, L-methionine and L-leucine are weak field ligands.The magnetic moments of the paramagnetic complexes appeared to be greater than the theoretically expected values of 1.73 BM for Cu^{2+}, 3.87 BM for Co^{2+} and 2.83 BM for Ni^{2+} ions. This is because for the theoretical calculations, only the spin moments (spin only magnetic moments) are considered, but the observed moments sums both spin and orbital contributions (Lee, 2013).

The complexes of zinc are diamagnetic with magnetic moments of zero indicating that there are nounpaired electrons in the degenerate 3*d* orbitals of the metal ion, such is expected for a d^{10} electronic system. The absence of *d-d* bands in the visible spectra of the complexes supports this further and is due to the filled 3*d*-orbital of the Zn(II) ion. The

result is in accord with what was obtained by previous workers on Zn(II) complexes (Miessler and Tarr, 1996; Aiyelabola *et al.*, 2012b).

5.6 Molar Conductivity Measurements

The molar conductivity values for the amino acid complexes in 10^{-3} moldm^{-3} DMSO are presented in Table 4.5. The values are in the range of 18-44 Ω^{-1} mol^{-1}cm^{2}suggesting these complex compounds to be non-electrolytes. In cases of presence of ions outside the coordination sphere (electrolytes), higher conductivity measurements are observed in the electrolytic range which is 106-311Ω^{-1} mol^{-1}cm^2(Refat *et al.*, 2013b). Conductivity measurements have frequently been used in structure elucidation of metal complexes (mode of coordination) within the limits of their solubility. They provide a method of probing the degree of ionization of the complexes, the molar ions a complex liberates in solution (Refat, 2007).

It becomes clear from the conductivity data that the complexes under study are most likely to be non-electrolytes. This would only infer that the complexes all form in a 1:2 metal-ligand ratio since charge neutrality can only be maintained when 1 mole of M^{2+} combine with 2 moles of Met^- or Leu^-. This fact is important in establishing that there is the absence of such ions as Na^+ in the outer coordination sphere of these complexes which eliminates the possibility of a 1:3 metal-ligand ratio. Similarly, the possibility of a metal-ligand ratio of 1:1 is untenable as the non-electrolytic nature of these complexes rationalizes the absence of Cl^- or SO_4^{2-} ions in their outer coordination sphere (Mohamed *et al.*, 2009; Abou-Hussein and Linert, 2015). This fact was strongly supported by simple qualitative analysis where Cl^- or SO_4^{2-} ions are not precipitated on addition of aqueous solutions of

silver nitrate or barium chloride respectively to a solution of the complexes (Refat, 2007; Keypour *et al.,* 2015). This proved a good match with the fact that the filtrates (by-products) obtained during isolation of complexes gave precipitates instantly on addition of aqueous solutions of silver nitrate or barium chloride respectively (Refat, 2010; Refat *et al.,* 2013a).

5.7 Metal Content Analysis

Table 4.6 shows the results of metal content analysis. It can be observed therein that the calculated values of percentage metal in the complexes are in close agreement with the experimental. This corroborated further the molecular formula.

5.8 Hydration Water Analysis

The results of infrared spectroscopy reveal the presence of coordination, and or hydration water in the metal(II) complexes (Table 4.3). Consequently, percentage hydration water in the complex compounds was determined (Table 4.7). The experimental values of percentage water present in the complexes are in close agreement with the calculated values. This corroborated the molecular formula as chronicled in Table 4.1.

5.9 Powder X-ray Diffraction Studies

Powder XRD patterns of the synthesized metal(II) complexes were recorded. All complexes were characterized at room temperature, 25^{o}C (298 K). The observed cell parameters and average particle sizes are given in Table 4.8. Clear resolved peaks were observed for all the complexes indicating that the complexes so formed are crystalline in nature (Sharma *et al.,* 2014). The powder XRD patterns of the complexes appear to be

similar with the most prominent peak in each case appearing between 2θ values of 5-20^{o} (Appendices 5 − 10). The average crystallite sizes of the complexes were observed to be within the range of 63.68-91.42 nm. This suggests that all the complexes possess similar bonding modes (Xiao-Quan *et al.,* 2005). This fact further corroborated with the results of vibrational spectroscopy. All complexes except Zn-Leu are proposed to be monoclinic based on comparison with standard reference patterns (Gomez-Ruiz *et al.,* 2010; Kavitha *et al.,* 2013). The complex, Zn-Leu however, showed referenced unit cell parameters that suggest it to have crystallized in a cubic fashion with a=b=c, and α=β=γ. The average crystallite sizes for all the complexes were estimated using the Debye-Scherrer relationship (Warren, 1990; Kavitha *et al.*, 2013).

The obtained structural data thus allows for the proposition of the structural formula for the studied metal(II) complexes as in Figures 5.1 -5.3. Subsequently, the molecular geometry and crystal classof the complexes are proposed in Table 5.1.

Table 5.1: Proposed molecular geometry and crystal class of metal(II) complexes

Complex	Formula weight ($gmol^{-1}$)	Proposed Molecular geometry	Proposed crystal class
$[Cu(\mathbf{L})_2].H_2O$**(1)**	377.97	Square planar	Monoclinic
$[Cu(\mathbf{L^1})_2].H_2O$**(2)**	341.91	Square planar	Monoclinic
$[Co(\mathbf{L})_2(H_2O)_2].H_2O$**(3)**	409.35	Octahedral	Monoclinic
$[Co(\mathbf{L^1})_2(H_2O)_2].H_2O$**(4)**	373.29	Octahedral	Monoclinic
$[Zn(\mathbf{L})_2].H_2O$**(5)**	379.80	Tetrahedral	Monoclinic
$[Zn(\mathbf{L^1})_2].H_2O$**(6)**	343.74	Tetrahedral	Cubic
$[Ni(\mathbf{L})_2].2H_2O$**(7)**	391.11	Tetrahedral	−
$[Ni(\mathbf{L^1})_2].2H_2O$**(8)**	355.05	Tetrahedral	−

− = not investigated

Fig.5.1: Proposed structure of M(II) complex

whereM=Cu(II), M=Zn(II), R=CH_3−S−CH_2− for L-methionine and R=CH_3−CH_2−CH(CH_3)− for L-leucine.

Fig.5.2: Proposed structure of M(II) complex

whereM=Co(II), R=CH_3−S−CH_2− for L-methionine and R=CH_3−CH_2−CH (CH_3)− L-leucine.

Fig. 5.3: Proposed structure of M(II) complex

where M=Ni(II), R=CH_3−S−CH_2− for L-methionine and R=CH_3−CH_2−CH (CH_3)− for L-leucine.

5.10 Antimicrobial Screening

A comparative evaluation of the antimicrobial activity of the ligands, **L** and $\mathbf{L^1}$, and the synthesized compounds, **1-8** was carried out against three Gram-positive bacteria, three Gram-negative bacteria and four fungi (Tables 4.9-4.17). The standard drug showed slightly better activity than all the synthesized complexes except for the fact that *B. cereus* showed resistance to the standard drug but was susceptible to complexes **2, 3, 4, 5** and **6**. Moreover, *S.pyogenes* proved susceptible to complexes **2, 3, 4, 7** and **8** but also resistant to the standard drug (Table 4.9). The ligand, **L** proved intermediately active against *S. aureus* but $\mathbf{L^1}$ do not. However, except for complexes **2** and **4**, all the other complexes exhibited enhanced activity against the organism.

The result of zone of inhibition also indicated that both **L** and $\mathbf{L^1}$ were inactive against *S. typhi* and *S. dysentriae* (Table 4.10). The synthesized complexes also showed no activity against these organisms with the exception of complexes **3** and **8**, which proved potent against *S. dysentriae* (Table 4.10). The ligands exhibited intermediate activity against *E. coli*; however, two of the complexes, **3** and **8** lacked potencies against the organism. All the other complexes proved potent against the microbe and are more active than the parent ligand, this suggest the enhanced lipophilicity of the complexes on coordination (Sadlers *et al.*, 2007; Chang *et al.*, 2010).

The ligands showed no activity against *A. niger* and *F. oxyparum*. The complexes of cobalt, **2** and **4** however, proved potent against *A. niger*, and complexes **4, 5, 6** and **7** were also active against *F. oxyparum.* This supports the hypothesis that metal centers induce or enhance the activity of biologically important moieties(Sadler and Guo, 1998; Sadlers *et*

al., 2007). The standard drug, fulcin showed better activity against all the fungi strains studied than the complexes and the ligands respectively, except for the complexes **1, 2, 5**and **8** which showed similar zones of inhibition with the standard drug against *C. albicans*(Table 4.11). Thismay be a strong indication that the activity of these complex compounds against *C. albicans* is at par with that of the standard drug indicating their potential as alternative antifungal agents (Asemave *et al.,* 2015). In addition, the ligands and complexes **1, 2, 5** and **6**proved active against *A. flavus* with the activity of the complexes increased on complexation. This outcome is in strong agreement with earlier findings of other researchers (Sadlers *et al.,* 2007). However, the fungi (*A. flavus*) proved resistant to the complexes of cobalt and nickel (**3, 4, 7** and **8**). The reason for the resistance of the microbes to some of the complexes may be vested on the fact that activity does not solely depend on the ease of movement of an antimicrobial agent through the cell walls of the microbes but rather a synergistic effect of many factors (Walsh, 2003; Floss and Yu, 2005; Drlica *et al.,* 2008).

Generally, the Gram-positive bacteria proved more susceptible to the amino acid complexes than the Gram-negative bacteria. The weak antibacterial activity against Gram-negative bacteria may be ascribed to the presence of an outer membrane in these microorganisms, which poses hydrophilic polysaccharide chains as a barrier to the amino acid complexes (Walsh *et al.,* 1996; Kabbani, *et al.,* 2007).The comparative studies of the amino acids and their metal complexes thus indicate that the complexes showed significantly enhanced antimicrobial activity against the studied microbial strains in comparison to the free ligands.

Among the complexes studied, complex **8** was found to demonstrate the best antimicrobial potency exhibiting the lowest MIC, 25µgmL^{-1} which is akin to that of the standard drug against *S. aureus,*and *S. dysentriae* (Tables 4.12 and 4.13). The result of this study is in accordance with the report of earlier workers, which showed that the antimicrobial activity of the ligands is greatly enhanced when coordinated to metal ions (Patil *et al.* 2011; Kasuga *et al.*, 2012).The overtone's concept (Raman *et al.,* 2002) and Tweedy's chelation theory (Dharmaraj *et al.,* 2001) can be used to explain the enhanced antimicrobial activity of the metal complexes. According to the Overtone's concept of cell permeability, the lipid membrane surrounding the cell favors the passage of only lipid-soluble materials; therefore, liposolubility is an important factor that controls the antimicrobial activity. On chelation, polarity of the metal ion is largely reduced due the overlapping of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Moreover, delocalization of the π-electrons over the whole chelate ring is increased, and lipophilicity of the complexes is enhanced. Although the standard drug (Ciprofloxacin) has less MIC, 25 µgmL^{-1}compared to the metal complexes in most cases, the result of this study suggest the importance of amino acid complexes as potential alternative antibacterial agents for the control of resistant bacteria which are becoming a threat to human health.

On the other hand, complex **6** appeared to demonstrate the best antifungal candidate exhibiting MICs of 25µgmL^{-1} against *C. albicans,* and 50 µgmL^{-1} against *A. flavus* and *F. oxyparum.* The standard antifungal drug proved inactive against the fungi, *A. niger, A. flavus* and *F. oxyparum* but some of the complexes proved sensitive against these microbes. Such finding suggests the amino acid complexes under study to be promising potential antifungal agents. MBC results (Tables 4.15-4.17) also revealed that the metal complexes

are more effective against the microbial strains compared to the free ligands.The order of the activity of the ligands, complexes and standard drug against the tested pathogens can be represented as follows;

S. aureus; **L<1<3 = 5 = 7<6<8<C**

*S. pyogene;***L^1<4 = 8<2 = 3 = 7**

*B. cereus;***L^1<2 = 3 = 6<4<5**

*E. coli;***L^1<6<L1 = 4 = 7<2<1<5<C**

*S. typhi;***C**

*S. dysentriae;***3<8<C**

*C. albicans;***L<L^1<3<6<1 = 2 = 8<5<Q**

*A. niger;***4<3**

*A. flavus;***L^1<L = 6<2 = 5<1**

*F. oxyparum;***4 = 5<6 = 7**

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary and Conclusion

Eight metal(II) amino acids complexes in aqueous basic solution, $[CuL_2].H_2O$, $[CuL^1{}_2].H_2O$, $[CoL_2(H_2O)_2].H_2O$, $[CoL^1{}_2(H_2O)_2].H_2O$, $[ZnL_2].H_2O$, $[ZnL^1{}_2].H_2O$, $[NiL_2].2H_2O$ and $[NiL^1{}_2].2H_2O$ with L=L-methionine and L^1=L-leucine were synthesized and analyzed by metal content analysis, molar conductivity measurements, magnetic susceptibility measurements, powder X-ray diffraction analysis, UV-Vis and IR spectroscopies.

Molar conductivity measurements showed that the complexes are non-electrolytic and their composition correspond to a metal-ligand ratio of 1:2. The IR spectra showed that the amino acids act as bidentate ligands with coordination involving the carboxyl oxygen and the nitrogen of the amino group. The result also suggests the presence water of crystallization in all the complexes.

Electronic spectra and magnetic susceptibility measurements suggested a four coordinate local symmetry around the Cu (square planar), Zn (tetrahedral) and Ni (tetrahedral) ions, and octahedral local symmetry for Co ion. Magnetic susceptibility studies also revealed that

the complexes of Cu, Co and Ni are paramagnetic while those of Zn are diamagnetic. The result also suggested the ligands, L-methionine and L-leucine to be weak field ligands as they formed high spin complexes with Co and Ni ions.

Metal content and hydration water analyses showed that the complexes of Cu, Co and Zn contain one mole of hydration water while those of Ni contain two moles.Powder XRD studies confirmed that the amino acid complexes are crystalline in nature and that they largely crystallized in monoclinic fashion. It also suggested that the bonding mode in the complexes is similar. The obtained structural data thus allows for the proposition of the structural formula for the studied metal(II) complexes as shown in Figures 5.1 - 5.3.

The antimicrobial studies suggested that the amino acid ligands are biologically active and their metal complexes showed significantly enhanced antimicrobial sensitivity against the studied microbial strains in comparison to the free ligands. The amino acid complexes are therefore promising potent broad-spectrum antimicrobial agents, and that L-methionine and L-leucine can be used medically to arrest metal overloads or poisoning.

6.2 Recommendation

It is recommended that single crystal of these complexes be grown and X-ray crystallographic analysis be carried out on them to emphatically establish their geometry. Other techniques such as elemental analysis, thermogravimetric analysis (TGA) and electronic spin resonance (ESR) can as well be used to characterize the complexes for detailed structural elucidation.Also, toxicity test should be done to determine the lethal dose (LD_{50}) of the complexes followed by clinical trials (in vivo studies) to enable the exploration of their antimicrobial potential.

REFERENCES

Abou-Hussein, A. A. and Linert, W. (2015). Synthesis, spectroscopic studies and inhibitory activity against bactria and fungi of acyclic and macrocyclic transition metal complexes containing a triamine coumarine Schiff base ligand. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 141, 223-232.

Agbaje, O. B., Wakil, S. M. and Osowole, A. A. (2014). Synthesis, Spectroscopic Characte rization and Antimicrobial Activities of Some Mixed Drug Metal(II) Complexes of Sulfamethoxazole and Paracetamol. *Journal of Research & Developments in Chemistry,* 2014(2014): 1-12

Aiyelabola T. O., Ojo I. A., Adebajo A. C., Ogunlusi G. O., Oyetunji O., Akinkunmi E. O., Adeoye A. O. (2012a). Synthesis, characterization and antimicrobial activities of some metal(II) amino acids' complexes. *Advances in Biological Chemistry.*2, 268-273.

Aiyelabola T., Akinkunmi E., Obuotor E., Olawuni I., Isabirye D. and Jordaan J. (2016). Synthesis Characterization and Biological Activities of Coordination Compounds of 4-Hydroxy-3-nitro-2*H*-chromen-2-one and its Aminoethanoic Acid and Pyrrolidine-2-carboxylic Acid Mixed Ligand Complexes. *Bioinorganic Chemistry and Applications.*2016: 1-9.

Aiyelabola, T. O., Isaac, O. and Olugbenga, A. (2012b). Structural and antimicrobial studies of coordination compounds of phenylalanine and glycine. *International Journal of Chemistry*, *4*(2): 49-53.

Aliyu, H. N. and Abdullahi, H. J. (2009). Synthesis and Characterization of manganese(II), cobalt(II), nickel(II) and copper(II) n, n' – bis(benzoin)ethylenediiminato complexes. *Bayero Journal of Pure and Applied Sciences,* 2(2): 110 – 112.

Asemave K., Anhwange B. A., Anom T. J. (2015). Antibacterial studies of leucine complexes of Fe(III) and Cu(II). *International Journal of Science and Research,* 4(1): 1527-1529.

Banerjee, S., Ray, A., Sen, S., Mitra, S., Hughes, D. L., Butcher, R. J., Batten, S. R. and Turner, D. R. (2008). Pseudohalide-induced structural variations in hydrazone-based metal complexes: Syntheses, electrochemical studies and structural aspects. *Inorganica Chimica Acta*, 361(9): 2692-2700.

Batiu, C., Jelic, C., Leopold, N., Cozar, O. and David, L. (2005). Spectroscopic investigations of new Cu(II), Co(II), Ni(II) complexes with γ-L-glutamyl amide as ligand. *Journal of Molecular Structure*, 744: 325-330.

Bhushanavathi P. and Rao G. N. (2013). Speciation studies of Co(II), Ni(II) and Cu(II) complexes of L-Valine in acetonitrile-water mixtures. *Chemical Speciation and Bioavailability*.25(4): 258-264.

Bukharov, M. S., Shtyrlin, V. G., Mukhtarov, A. S., Mamin, G. V., Stapf, S., Mattea, C. and Serov, N. Y. (2014). Study of structural and dynamic characteristics of copper(II) amino acid complexes in solutions by combined EPR and NMR relaxation methods. *Physical Chemistry Chemical Physics*, 16(20), 9411-9421.

Bunaciu, A. A., UdriŞTioiu, E. G. and Aboul-Enein, H. Y. (2015). X-ray diffraction: instrumentation and applications. *Critical Reviews in Analytical Chemistry*, 45(4), 289-299.

Canpolat, E., and Kaya, M. (2002). Synthesis and characterization of a vic-dioxime derivative and investigation of its complexes with Ni(II), Co(II), Cu(II) and UO_2(VI) metals. *Journal of Coordination Chemistry*, 55(8), 961-968.

Cao, W. X., Cheng, Q. M., Fei, X. F., Li, S. F., Yin, H. R. and Lin, Y. Z. (2000). A study of preoperative methionine-depleting parenteral nutrition plus chemotherapy in gastric cancer patients. *World Journal of Gastroenterology*, 6(2), 255.

Chandraleka, S., Ramya, K., Chandramohan, G., Dhanasekaran, D., Priyadharshini, A. and Panneerselvam, A. (2014). Antimicrobial mechanism of copper(II) 1, 10-phenanthroline and 2, 2′-bipyridyl complex on bacterial and fungal pathogens. *Journal of Saudi Chemical Society*, 18(6), 953-962.

Chang, E. L., Simmers, C. and Knight, D. A. (2010). Cobalt complexes as antiviral and antibacterial agents. *Pharmaceuticals*, 3(6): 1711-1728.

Chen, Z. Lin, J. Uchiyama, K. and Hobo, T. (2000a) Reversal behaviours of the enantiomer migration order and stereo-selectivity of Cu(II) complex with amino acid enantiomers in ligand exchange. *AnalyticalScience*.16, 131-137.

Chen, Z. Uchiyama, K. and Hobo, T. (2000b). Estimation of formation constants of ternary Cu(II) complexes with mixed amino acid enantiomers based on ligand exchange. *Analytical Science.*16, 837-841.

Chohan Z. H., Arif M., Akhtar M. A. and Supuran, C. T. (2006). Metal-based antibacterial and antifungal agents; Synthesis, Characterization and in vitro biological evaluation of Co(II), Cu(II), Ni(II) and Zn(II) complexes with amino acid-derived compounds. *Bioinorganic Chemistry and Applications.*2006, 1-11.

Clinical and Laboratory Standard Institute (CLSI) (2016). *Performance Standards for Antimicrobial Susceptibility Testing* (26^{th} Edition). CLSI Supplement M100S, Wayne P. A. USA.

Corbi, P. P., Melnikov, P., and Massabni, A. C. (2000). A solid nickel(II) complex with methionine sulfoxide. *Journal of Alloys and Compounds*, 308(1): 153-157.

Creaven, B. S., Egan, D. A., Kavanagh, K., McCann, M., Mahon, M., Noble, A. and Walsh, M. (2005). Synthesis and antimicrobial activity of copper(II) and silver(I) complexes of hydroxynitrocoumarins: X-ray crystal structures of $[Cu(hnc)_2(H_2O)_2]\cdot 2H_2O$ and [Ag(hnc)](hncH= 4-hydroxy-3-nitro-2H-chromen-2-one). *Polyhedron*, 24(8): 949-957.

Da Silva, J. F. and Williams, R. J. P. (1991). *The Biological Chemistry of the Elements: the Inorganic Chemistry of Life*. Oxford University Press.

Damaj, Z. Naveau, A. Dupont, L. Henon, E. Rogez, G. and Guillon, E. (2009) Co(II) (L-proline)$_2(H_2O)_2$ solid complex; characterization, magnetic properties and DFT computations. *Inorganic ChemistryCommunications*.12(1): 17-20.

Dharmaraj, N., Viswanathamurthi, P. andNatarajan, K. (2001). Ruthenium(II) complexes containing bidentate Schiff bases and their antifungal activity. *Transition Metal Chemistry*, 26(1-2):105-109.

Diekert, G., Gilles, H. H., Jaenchen, R. and Thauer, R. K. (1980). Incorporation of 8 succinates per mol nickel into factors F 430 by Methanobacterium thermoautotrophicum. *Archives of Microbiology*, 128(2): 256-262.

Dolinska, B. (2001). The properties of Zn(II)-amino acid complexes in the form of suspensions. *US National Library of Medicine, National Institutes of Health.* 56(10):737-40.

Dougherty, D.A. (2000) Unnatural amino acids as probes of protein structure and function. *Current Opinion in Chemical Biology*. 4: 645–652.

Drlica, K., Malik, M., Kerns, R. J. and Zhao, X. (2008). Quinolone-mediated bacterial death. *Antimicrobial Agents and Chemotherapy, 52*(2): 385-392.

El-Tabl, A. S., El-Saied, F. A. and Al-Hakimi, A. N. (2008). Spectroscopic characterization and biological activity of metal complexes with an ONO trifunctionalized hydrazone ligand. *Journal of Coordination Chemistry*, 61(15): 2380-2401.

Farrell, N., McCleverty, J. A. and Meyer, T. J. (2003). Metal complexes as drugs and chemotherapeutic agents, *Comprehensive Coordination Chemistry II*. Pergamon, Oxford, United Kingdom. Pp. 809-840.

Faúndez, G., Troncoso, M., Navarrete, P. and Figueroa, G. (2004). Antimicrobial activity of copper surfaces against suspensions of Salmonella enterica and Campylobacter jejuni. *BMC microbiology*, 4(1): 9-19.

Floss, H. G. and Yu, T. W. (2005). Rifamycin mode of action, resistance, and biosynthesis. *Chemical Reviews*, 105(2): 621-632.

Frassinetti, S., Bronzetti, G. L., Caltavuturo, L., Cini, M. and Della Croce, C. (2006). The role of zinc in life: a review. *Journal of Environmental Pathology, Toxicology and Oncology*, 25(3): 597-610.

Fuller, M. (2012). Determination of protein and amino acid digestibility in foods including implications of gut microbial amino acid synthesis. *British Journal of Nutrition*, 108(S2): S238-S246.

Fürst, P. and Stehle, P. (2004). What are the essential elements needed for the determination of amino acid requirements in humans? *The Journal of Nutrition*, 134(6): 1558S-1565S.

Garcia, J. S., de Magalhaes, C. S. and Arruda, M. A. Z. (2006). Trends in metal-binding and metalloprotein analysis. *Talanta*, 69(1): 1-15.

Gill, S. S. and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, 48(12): 909-930.

Gómez-Ruiz, S., Gallego, B., Žižak, Ž., Hey-Hawkins, E., Juranić, Z. D. and Kaluđerović, G. N. (2010). Titanium (IV) carboxylate complexes: Synthesis, structural characterization and cytotoxic activity. *Polyhedron*, 29(1), 354-360.

Grigoreva, L. N. and Dusheiko, A. G. (1970) Investigation of copper complexes of valine by optical spectroscopy and radio spectroscopy. *Journal of Structural Chemistry*.10: 702-705.

Hakimi, M.and Aliabadi, T. S. (2012). Coordination chemistry of copper α-amino acid complexes. *World Applied Programming,* 2(10): 431-443.

Harris, E. D. (1983). Copper in human and animal health. In *Trace elements in health: a review of current issues*. Butterworth, London, pp. 44-73.

Housecroft, C. E. and Sharpe, A. G. (2005) *Inorganic Chemistry* (Second Edition). Ashford Colour Press Ltd., Gosport, Pp. 579-587.

Jamil, W., Sorath, S., Muhammad, A., Khalid M.K., and Muhammad, T. (2015). Synthesis, Characterization, in vitro Antiglycation and DPPH Radical Scavenging Activities of isatin. *Journal of Applied Chemistry*. 10:206-208.

Kabbani, A. T., Hammud, H. H. and Ghannoum, A. M. (2007). Preparation and antibacterial activity of copper and cobalt complexes of 4-chloro-3-nitrobenzoate with a nitrogen donor ligand. *Chemical and Pharmaceutical Bulletin, 55*(3): 446-450.

Kasuga, N. C., Yoshikawa, R., Sakai, Y. and Nomiya, K. (2012). Syntheses, structures, and antimicrobial activities of remarkably light-stable and water-soluble silver complexes with amino acid derivatives, silver(I) N-acetylmethioninates. *Inorganic Chemistry*, 51(3), 1640-1647.

Kavitha, P., Saritha, M. and Reddy, K. L. (2013). Synthesis, structural characterization, fluorescence, antimicrobial, antioxidant and DNA cleavage studies of Cu(II) complexes of formyl chromone Schiff bases. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 102: 159-168.

Keypour, H., Shooshtari, A., Rezaeivala, M., Mohsenzadeh, F. and Rudbari, H. A. (2015). Synthesis and characterization of transition metal complexes of a hexadentate N_4O_2 donor Schiff base ligand: X-ray crystal structures of the copper (II)and zinc(II) complexes and their antibacterial properties. *Transition Metal Chemistry*, 40(7): 715-722.

Kulkarni, R.B., Rushi, A., Anis, M., Hussaini, S.S., Shirsat, M.D. (2014). Comparative study on Structural, Optical, Dielectric and Thermal Properties of Pure and L-valine Doped Bis-Thiourea Cadmium Acetate Crystal. *International Journal of Science and Research Publications.* 4(7): 1-5.

Kumar, R., Obrai, S., Sharma, A., Jassal, A. K., Hundal, M. S. and Mitra, J. (2014). Synthesis, structural, spectral characterization, DFT analysis and antimicrobial studies of aquabis (L-ornithine) copper (II) picrate. *Journal of Molecular Structure,* 1075: 43-48.

Kumar, S. (2011). Spectroscopic studies of valine and leucine molecules a comparative study. *Elixir Vibrational Spectroscopy,* 39: 4996-4999.

Küpper, H. and Kroneck, P. M. (2007). Nickel in the environment and its role in the metabolism of plants and cyanobacteria. *Metal Ions in Life Sciences*, 2*:* 31-62.

Latham M. C. (1997). "Chapter 8. Body composition, the functions of food, metabolism and energy". Human nutrition in the developing world. Food and Nutrition Series – No. 29. Rome: Food and Agriculture Organization of the United Nations. Pp.83-90.

Lee, J. D (2013) *Concise Inorganic Chemistry*. (Fifth Edition). Learning solutions specialty publications, Pp. 208-226.

Legler, A. V., Kazachenko, A. S., Kazbanov, V. I., Per'yanova, O. V. and Veselova, O. F. (2001). Synthesis and antimicrobial activity of silver complexes with arginine and glutamic acid. *Pharmaceutical Chemistry Journal*, 35(9): 501-503.

Liu, H., Wang, H., Gao, F., Niu, D. and Lu, Z. (2007). Self-assembly of copper(II) complexes with substituted aroylhydrazones and monodentate N-heterocycles: synthesis, structure and properties. *Journal of Coordination Chemistry*, *60*(24): 2671-2678.

Lotz, A. (2008). Variable Temperature Equipment for a Commercial Magnetic Susceptibility Balance. *Journal of Chemical Education*, 85(1): 107-108.

Maroney, M. J. (1999). Structure/function relationships in nickel metallobiochemistry. *Current Opinion in Chemical Biology*, 3(2): 188-199.

Metcoff, J. (1986). Intracellular amino acid levels as predictors of protein synthesis. *Journal of the American College of Nutrition*, 5(2): 107-120.

Miessler, G. L. and Tarr, D. A. (1999). "Coordination compounds," in *Inorganic Chemistry*, Pearson Prentice Hall, New York, NY, USA, vol. 642, pp. 315–316.

Mohamed, G. G., Omar, M. M. and Ibrahim, A. A. (2009). Biological activity studies on metal complexes of novel tridentate Schiff base ligand. Spectroscopic and thermal characterization. *European Journal of Medicinal Chemistry*, 44(12): 4801-4812.

Müller, A., Kögerler, P. and Kuhlmann, C. (1999). A variety of combinatorially linkable units as disposition: from a giant icosahedral Keplerate to multi-functional metal–oxide based network structures. *Chemical Communications*, (15): 1347-1358.

Muñoz-Espí, R., Dolcet, P., Rossow, T., Wagner, M., Landfester, K. and Crespy, D. (2011). Tin(IV) oxide coatings from hybrid organotin/polymer nanoparticles. *ACS Applied Materials &Interfaces*, 3(11): 4292-4298.

Nelson, D. L., Cox, M. M. and Lehninger, A. L. (2005). *Principles of biochemistry.*Fourth editionWH Freeman and Company, New York, pp. 75-111.

Nomiya, K., Takahashi, S., Noguchi, R., Nemoto, S., Takayama, T. and Oda, M. (2000). Synthesis and characterization of water-soluble silver(I) complexes with l-histidine (H_2his) and (S)-(−)-2-pyrrolidone-5-carboxylic acid (H_2pyrrld) showing a wide spectrum of effective antibacterial and antifungal activities. Crystal structures of chiral helical polymers [Ag(Hhis)]n and $[Ag(Hpyrrld)]_2$ n in the solid state. *Inorganic Chemistry*, 39(15): 3301-3311.

Noro, S. I., Kitagawa, S., Akutagawa, T., and Nakamura, T. (2009). Coordination polymers constructed from transition metal ions and organic N-containing heterocyclic ligands: Crystal structures and microporous properties. *Progress in Polymer Science*, 34(3), 240-279.

O'Shea, D. (2004). Synthesis, characterisation and biological activity of novel carboxylate complexes incorporating phenanthroline and benzimidazole ligands. *Doctoral*, 2.

Osowole, A. (2017). Synthesis, Physicochemical and Antioxidant Properties of Some Metal(II) Complexes of Mixed Drugs, Aspirin and Nicotinamide. *Ommega Internationals*, 2(1): 1-8.

Ozkan, E. G., Canpolat, E. and Kaya, M. (2005). Synthesis of new glyoxime derivatives, characterization and investigation of their complexes with Co(II), Ni(II), and Cu(II) metals and thermal studies. *Russian Journal of Coordination Chemistry*, 31(7): 506-510.

Pal, D. T., Gowda, N. K. S., Prasad, C. S., Amarnath, R., Bharadwaj, U., Babu, G. S. and Sampath, K. T. (2010). Effect of copper-and zinc-methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. *Journal of Trace Elements in Medicine and Biology*, 24(2), 89-94.

Patil, S. A., Unki, S. N., Kulkarni, A. D., Naik, V. H. and Badami, P. S. (2011). Co(II), Ni(II) and Cu(II) complexes with coumarin-8-yl Schiff-bases: spectroscopic, in vitro antimicrobial, DNA cleavage and fluorescence studies. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 79(5): 1128-1136.

Patil, S. S. Ganesh, Thakur, A. and Patil, R. R. (2009) Synthesis, spectral and biological studies on some mixed ligand Ni(II) complexes. *ActaPoloniae. Pharmaceutica*., 66: 271-277.

Petrucci, R.H.; Harwood, W.S. and Herring, F.G. (2002) *General Chemistry*. 8th edition, Prentice-Hall, pp. 341-2.

Pierpont, C. G. and Lange, C. W. (2007). The chemistry of transition metal complexes containing catechol and semiquinone ligands. *Progress in Inorganic Chemistry, Volume* 41: 331-442.

Pllana-Zeqiri M., Nura–Lama A., Plakolli M., Zeqiri L., Pllana F., Nikshiq–Kadriu S. (2012) Complexion of Fe^{3+} with Some Heterocyclic Ligands and Their Antibacterial Activities. I*nternational Journal of Modern Organic Chemistry*.1 (1):10–8.

Raman, N. (2002). Synthesis, structural characterization, redox and antimicrobial studies of Schiff base copper(II), nickel(II), cobalt(II), manganese(II), zinc(II) and oxovanadium(II) complexes derived from benzil and 2-aminobenzyl alcohol. *Polish Journal of Chemistry*, 76(8): 1085-1094.

Rayner-Canham, G. and Overtone, T. (2006). Descriptive Inorganic Chemistry (Fifth Edition). W. H. Freeman and Company, New York, pp 499-501.

Reddy P. R., Radhika M., Manjula P. (2005). Synthesis and characterization of mixed ligand complexes of Zn(II) and Co(II) with amino acids: Relevance to zinc binding sites in zinc fingers. *Journal of Chemical Science.*117 (3):239–46.

Refat, M. S. (2007). Synthesis and characterization of norfloxacin-transition metal complexes (group 11, IB): spectroscopic, thermal, kinetic measurements and biological activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *68*(5): 1393-1405.

Refat, M. S. (2010). Synthesis, characterization, thermal and antimicrobial studies of diabetic drug models: complexes of vanadyl(II) sulfate with ascorbic acid (vitamin C), riboflavin (vitamin B2) and nicotinamide (vitamin B3). *Journal of Molecular Structure*, 969(1): 163-171.

Refat, M. S., El-Deen, I. M., Zein, M. A., Adam, A. M. A. and Kobeasy, M. I. (2013b). Spectroscopic, structural and electrical conductivity studies of Co(II), Ni(II) and Cu(II) complexes derived from 4-acetylpyridine with thiosemicarbazide. *International Journal of Electrochemical Science*, 8(7), 9894-9917.

Refat, M. S., El-Sayed, M. Y. and Adam, A. M. A. (2013a). Cu(II), Co(II) and Ni(II) complexes of new Schiff base ligand: Synthesis, thermal and spectroscopic characterizations. *Journal of Molecular Structure*, 1038: 62-72.

Reynold, J. E. and Martindale, K. B. (1996). The extra pharmacopoeia 31st edition. *Published by the council of royal pharmaceutical society of Great Britain*, pp. 885-94.

Rodnina, M. V., Beringer, M. and Wintermeyer, W. (2007). How ribosomes make peptide bonds. *Trends in Biochemical Sciences*, 32(1): 20-26.

Rombach, M., Gelinsky, M. and Vahrenkamp, H. (2002). Coordination modes of amino acids to zinc. *Inorganica Chimica Acta*, 334: 25-33.

Roohani, N., Hurrell, R., Kelishadi, R. and Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences: the Official Journal of Isfahan University of Medical Sciences*, 18(2): 144-157.

Rosenthal, J., Angel, A. and Farkas, J. (1974). Metabolic fate of leucine: a significant sterol precursor in adipose tissue and muscle. *American Journal of Physiology--Legacy Content*, 226(2): 411-418.

Sadler, P. J and Guo, Z. (1998). Metal complexes in medicine: design and mechanism of action. *Pure and Applied Chemistry*, 70(4): 863-871.

Sadlers, P., Muncia, C. and Shipman,M. A. (2007) "Metals inmedicine,"in *Biological Inorganic Chemistry: Structure and Reactivity*, I. Bertini, H. Gray, E. Stiefel, and J. Valentine, Eds. University Science Books, London, UK, pp. 115–136.

Saha, S., Dhanasekaran, D., Chandraleka, S. and Panneerselvam, A. (2009). Synthesis, characterization and antimicrobial activity of cobalt metal complex against multi drug resistant bacterial and fungal pathogens. *Facta universitatis-series: Physics, Chemistry and Technology*, 7(1): 73-80.

Saha, S., Dhanasekaran, D., Chandraleka, S., Thajuddin, N. and Panneerselvam, A. (2010). Synthesis, characterization and antimicrobial activity of cobalt metal complex against drug resistant bacterial and fungal pathogens. *Advances in Biological Research*, 4(4): 224-229.

Sarkar, B. (1999). Treatment of Wilson and Menkes diseases. *Chemical reviews*, 99(9): 2535-2544.

Saxena, V. K., Gupta, M. and Srivastava, M. N. (1996). Synthesis and characterization of complexes of copper(II), nickel(II), cobalt(II) and zinc(II) with histidine and glycine or alanine. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, 26(10): 1661-1676.

Schwarz F. J., Kirchgessner M., Stangl G. I. (2000). Cobalt requirement of beef cattle-feed intake and growth at different levels of cobalt supply. *Journal of Animal Physiology and Animal Nutrition.* 83(3): 121-131.

Sharma, P., Bhale, J., Mishra, A. and Malviya, P. (2014). Synthesis and X-ray diffraction study of new copper(II) complexes of α-aminonitrile derived from P-methoxybenzaldehyde with aromatic amine. *Journal of Physics: Conference Series,* 534:1-4.

Sharwood-Scientific.com (2017). "Magnetic Susceptibility Balances"http://www.sherwood-scientific.com/msb/msbindex.html Retrieved 2017-11-30.

Sigel, A., Sigel, H. and Sigel, R. K. (Eds.). (2010). *Metal Ions in Life Sciences: Organometallics in Environment and Toxicology*. Royal Society of Chemistry. 605pp ISBN: 978-1-84755-177-1.

Singh, K., Kumar, Y., Puri, P. and Singh, G. (2012). Spectroscopic, thermal, and antimicrobial studies of Co(II), Ni(II), Cu(II), and Zn(II) complexes derived from bidentate ligands containing N and S donor atoms. *Bioinorganic Chemistry and Applications,* 2012: 1-10.

Sovago I., Osz K. and Varnagy K. (2003). Copper(II) Complexes of amino acids and peptides
containing chelating bis (imidazolyl) residues. *Bioinorganic Chemistry and Applicat ions.*1(2):123-139.

Sovago I., Kallay, C. and Varnagy, K. (2012). Peptides as complexing agents: Factors influencing the structure and thermodynamic stability of peptide complexes. *Coordination Chemistry Reviews,* 256(19): 2225-2233.

Stanila A., Marcu A., Rusu D., Rusu M., David L. (2007) Spectroscopic studies of some copper(II) complexes with amino acids. *Journal of Molecular Structure*. 834-836 (2007): 364-368.

Stanila, A., Braicu, C., Stanila, S. and Raluca, M. P. (2011). Antibacterial activity of copper and cobalt amino acids complexes. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 39(2): 124-129.

Stanila, A., Nagy, C., Marcu, A., Cozma, D., Rusu, D. and David, L. (2009). Spectroscopic investigations of new metallic complexes with leucine as ligand. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms,* 267(2): 419-421.

Sundberg, R. J. and Martin, R. B. (1974). Interactions of histidine and other imidazole derivatives with transition metal ions in chemical and biological systems. *Chemical Reviews*, 74(4), 471-517.

Thauer, R. K., Brandis-Heep, A., Diekert, G., Gilles, H. H., Graf, E. G., Jaenchen, R. and Schönheit, P. (1983). Dreineue Nickelenzyme ausanaeroben Bakterien. *Naturwissen schaften*, 70(2): 60-64.

Vallee, B. L. and Auld, D. S. (1993). Zinc: biological functions and coordination motifs. *Accounts of Chemical Research*, 26(10): 543-551.

Vemrlinov T., Arpadjan S., Karadjova I., Beattie J. (2006). Synthesis, structural, spectral characterization, DFT analysis and antimicrobial studies of aquabis(L-ornithine) copper(II) picrate. *Journal of Molecular Structure*. 56: 105-112.

Vilhena, F. S., Felcman, J., Szpoganicz, B. and Miranda, F. S. (2017). Potentiometric and DFT studies of Cu(II) complexes with glycylglycine and methionine of interest for the brain chemistry. *Journal of Molecular Structure*, 1127: 226-230.

Vogel, A. I. (1966). *Practical organic chemistry*, 3rd edition. Longman Companylimited. London, pp. 708-714.

Wagner I. andMusso H. (1983). "New Naturally Occurring Amino Acids". *Angewandte Chemie International Edition in English* 22(11): 816–28.

Walravens, P. A. (1980). Nutritional importance of copper and zinc in neonates and infants. *Clinical Chemistry*, 26(2): 185-189.

Walsh, C. (2003). Where will new antibiotics come from? *Nature Reviews Microbiology*, 1(1): 65-70.

Walsh, C. T., Fisher, S. L., Park, I. S., Prahalad, M. and Wu, Z. (1996). Bacterial resistance to vancomycin: five genes and one missing hydrogen bond tell the story. *Chemistry &Biology*, 3(1): 21-28.

Warren, B. E. (1990). *X-ray Diffraction*. Dover. New York, pp. *253*.

Whitney, E. and Rolfes, S. (2005). Supplements as Ergogenic Aids. *Understanding Nutrition* (Tenth edition). Thomson Learning Belmont. Pp. 447-50.

Wilson, G. J. and Wilson, J. (2006). Contemporary issues in protein requirements and consumption for resistance trained athletes. *Journal of the International Society of Sports Nutrition*, 3(1): 7-27.

Wokaun, A. (1996). B. Schrader: Infrared and Raman Spectroscopy- Methods and Applications. VCH, Weinheim, 1995, DM 298,- , ISBN 3- 527- 26446- 9. *Berichteder Bunsengesellschaft für physikalische Chemie*, 100(7): 1268-1268.

Xiao-Quan, C., Huan-Bin, L. and Guo-Bang, G. (2005). Preparation of nanometer crystalline TiO_2 with high photo-catalytic activity by pyrolysis of titanyl organic compounds and photo-catalytic mechanism. *Materials Chemistry and Physics*, 91(2): 317-324.

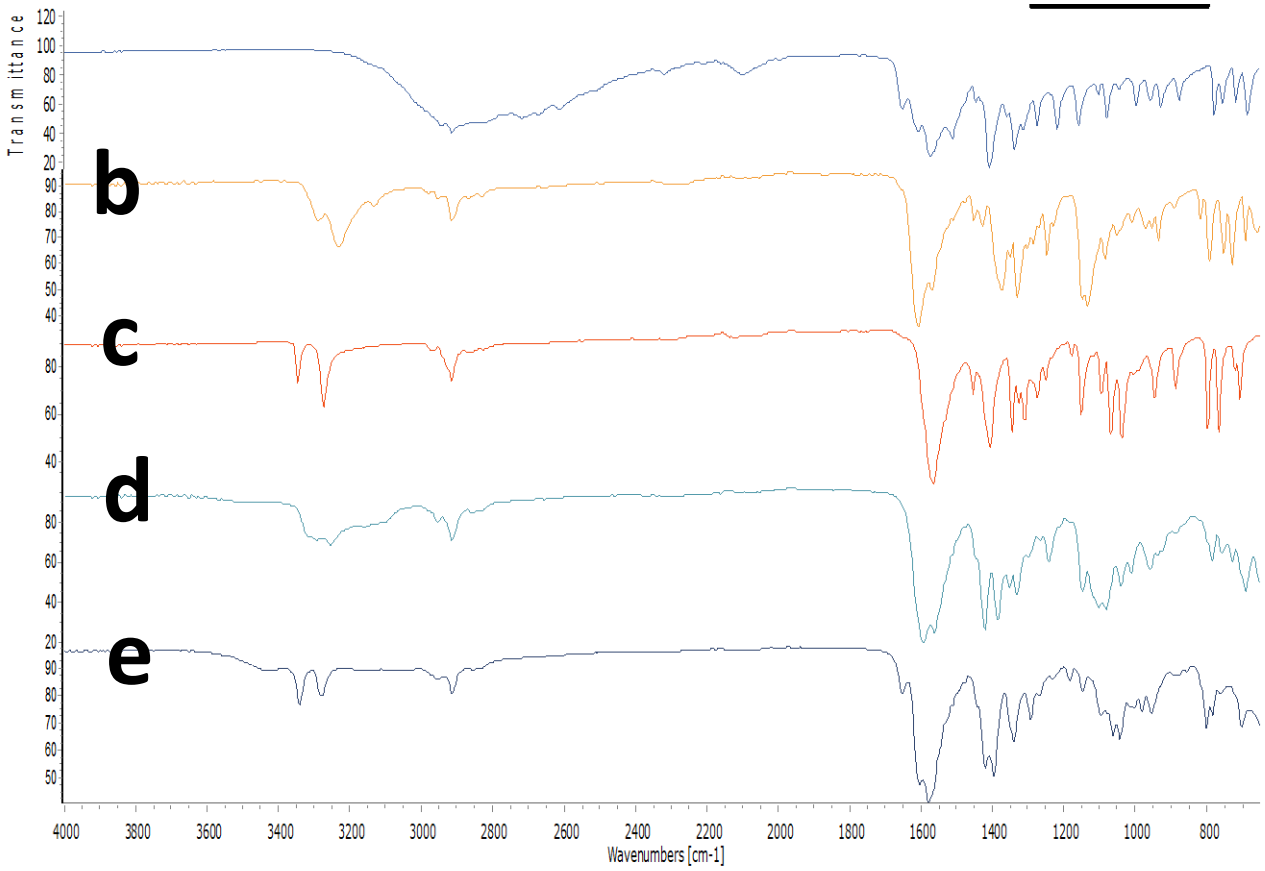
Yokota M., Kikuchi S., Sen J., Kamei T., Doki N. (2016). Cu(II) Complexes of L-Leucine favor a different type of crystal structure from Cu(II)-L-Val and Cu(II)-L-Ile. *Advances in Chemical Engineering and Science*, 6: 62-66.

Yudkoff, M., Daikhin, Y., Nissim, I., Horyn, O., Luhovyy, B., Lazarow, A. and Nissim, I. (2005). Brain amino acid requirements and toxicity: the example of leucine. *The Journal of Nutrition*, 135(6): 1531S-1538S.

APPENDICES

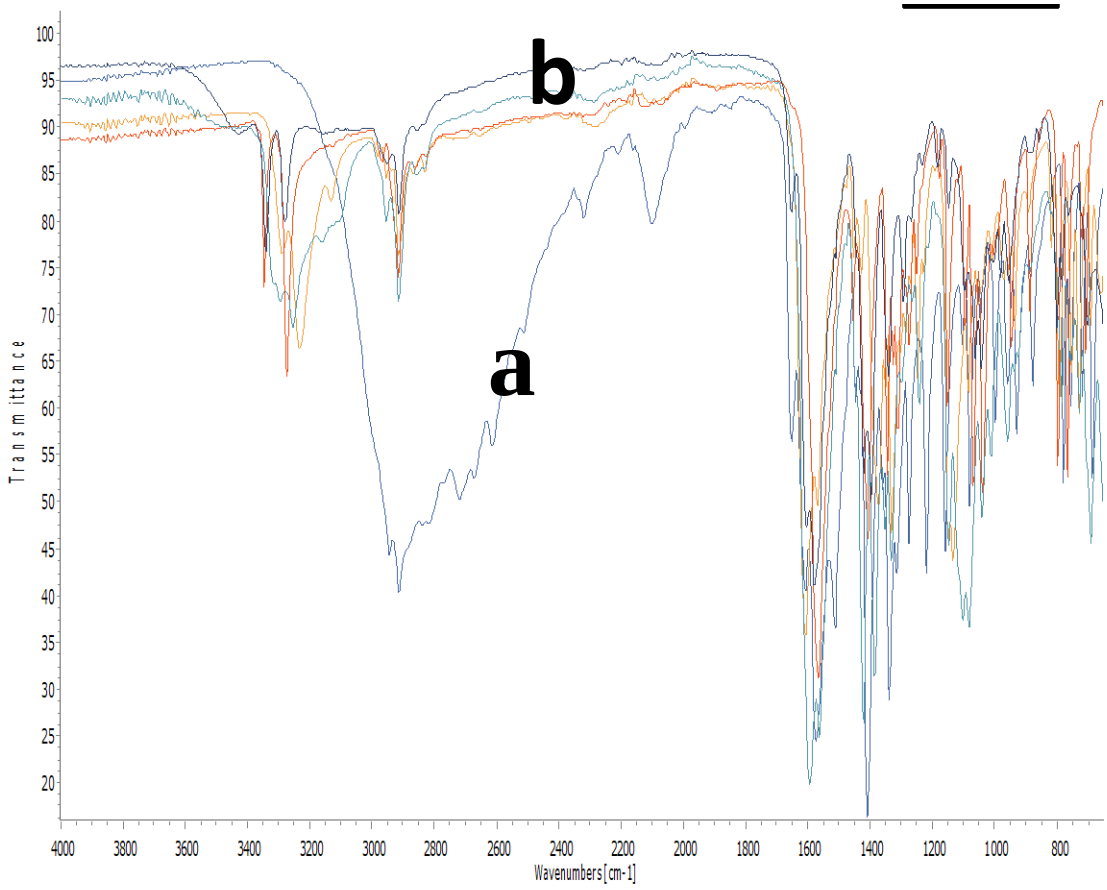
Appendix 1: Stacked IR spectra of L and ML complexes respectively

a



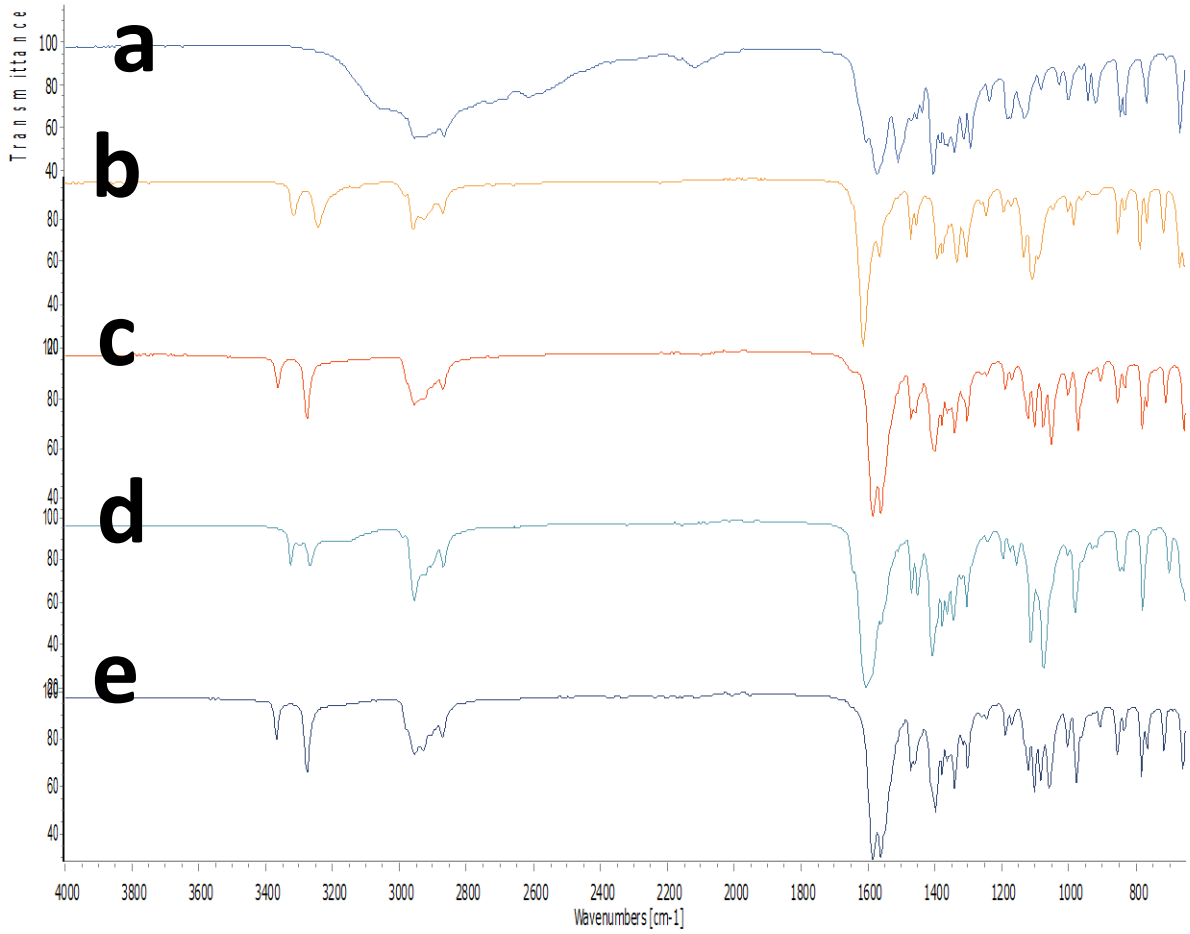
a = Met, b = Cu-Met, c = Co-Met, d = Zn-Met, e = Ni-Met

Appendix 2: Superimposed IR spectra of L with ML complexes respectively



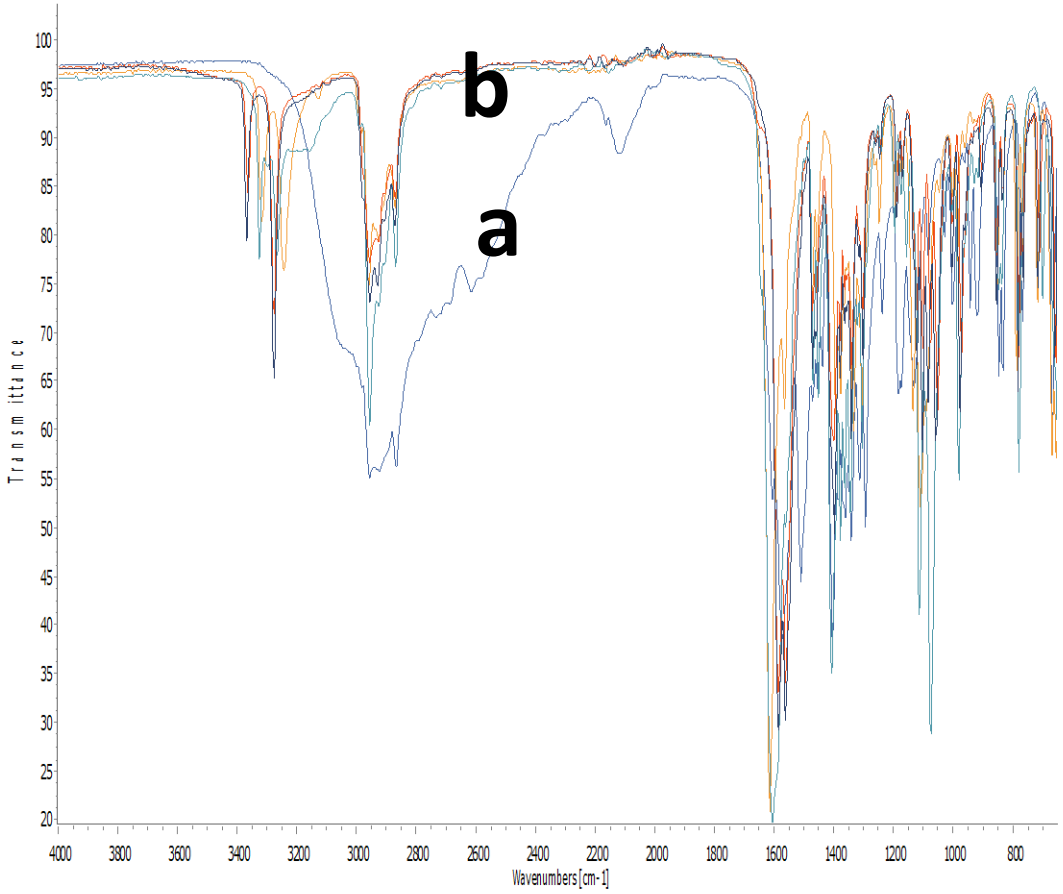
a = Met, b = Cu-Met, Co-Met, Zn-Met, Ni-Met

Appendix 3: Stacked IR spectra of L^1 and ML^1 complexes respectively

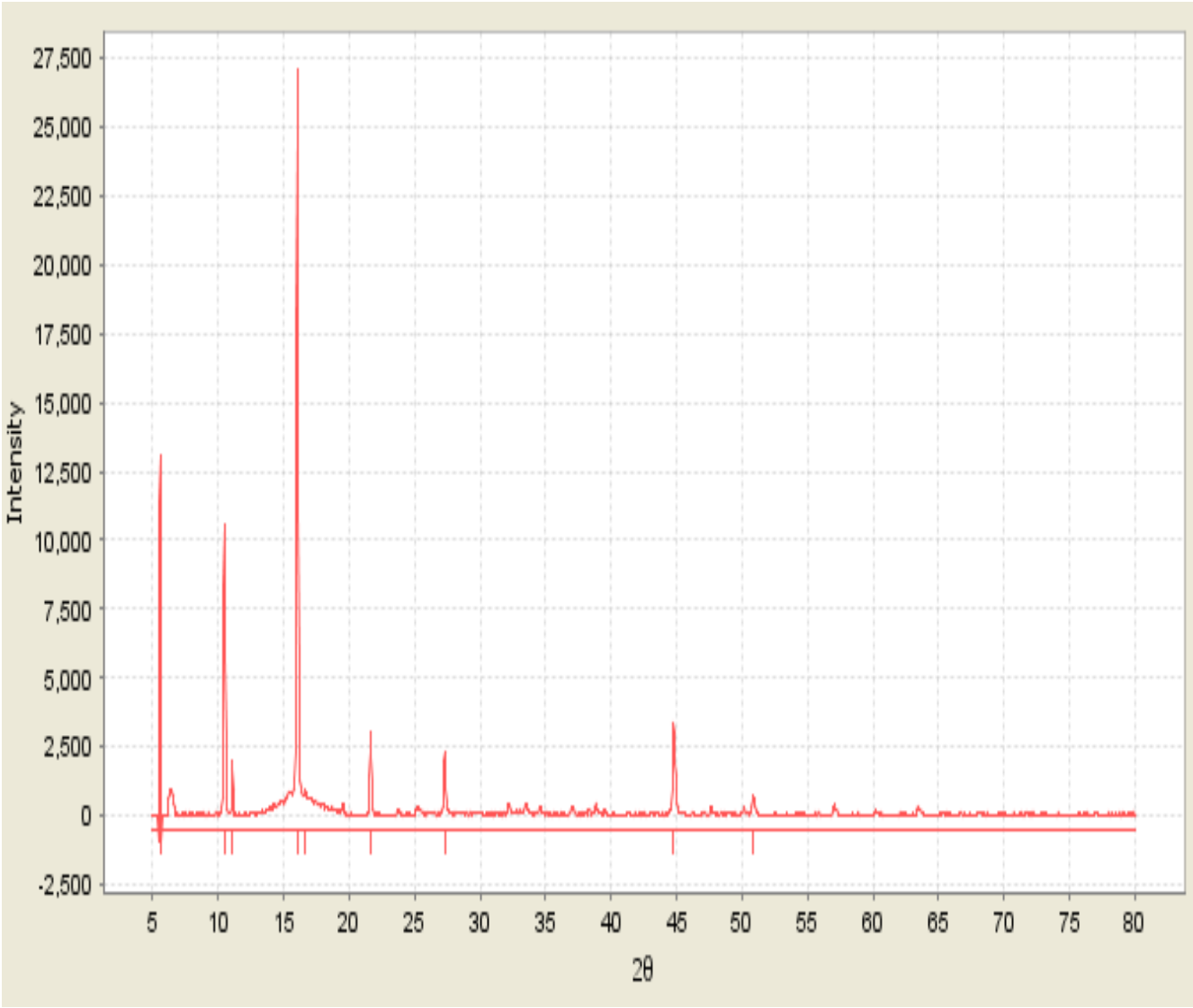


a = Leu, b = Cu-Leu, c = Co-Leu, d = Zn-Leu, e = Ni-Leu

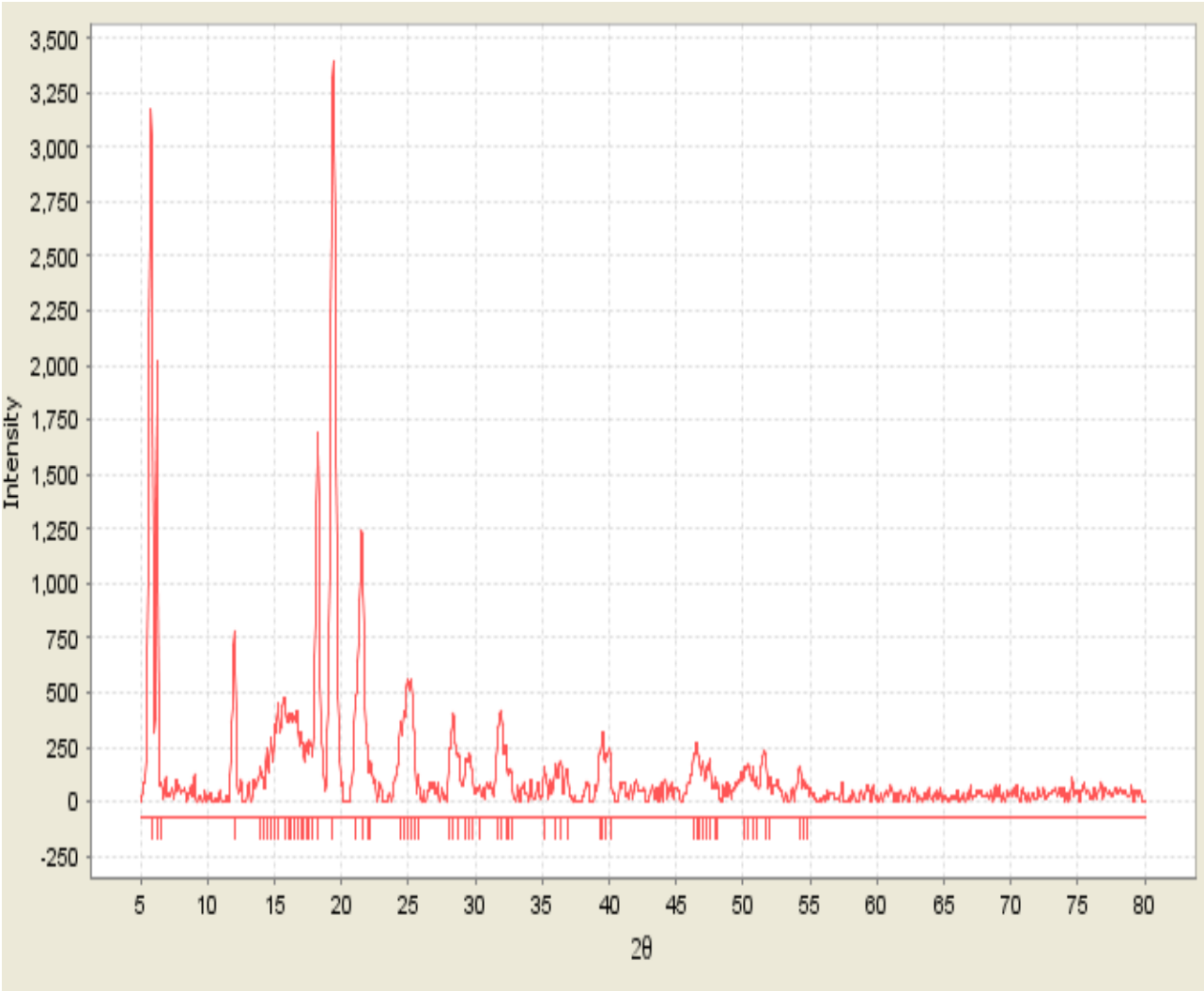
Appendix 4: Superimposed IR spectra of $L^1$with ML^1 complexes respectively



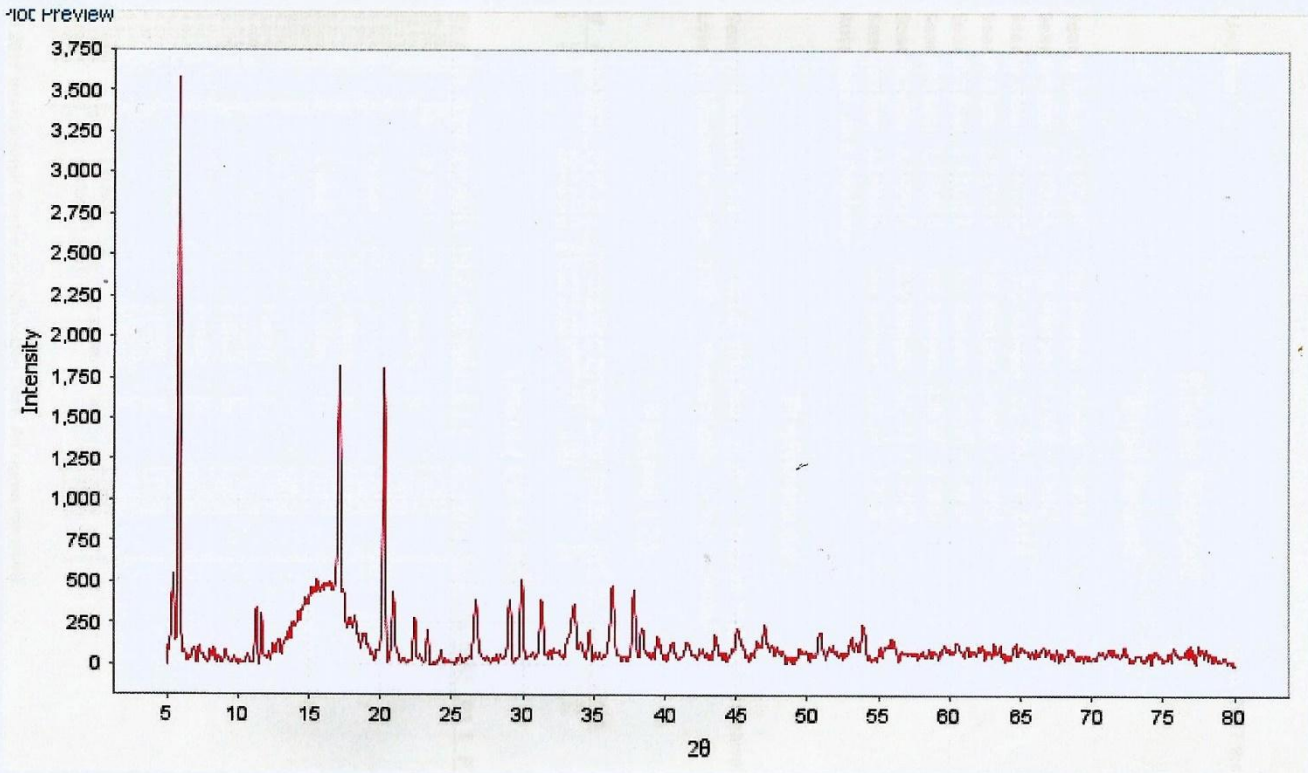
Appendix 5: XRD pattern of Cu-Met Complex (λ= Cu Kα 1 1.54056Å)



Appendix 6: XRD pattern of Cu-Leu Complex (λ= Cu Kα 1 1.54056Å)



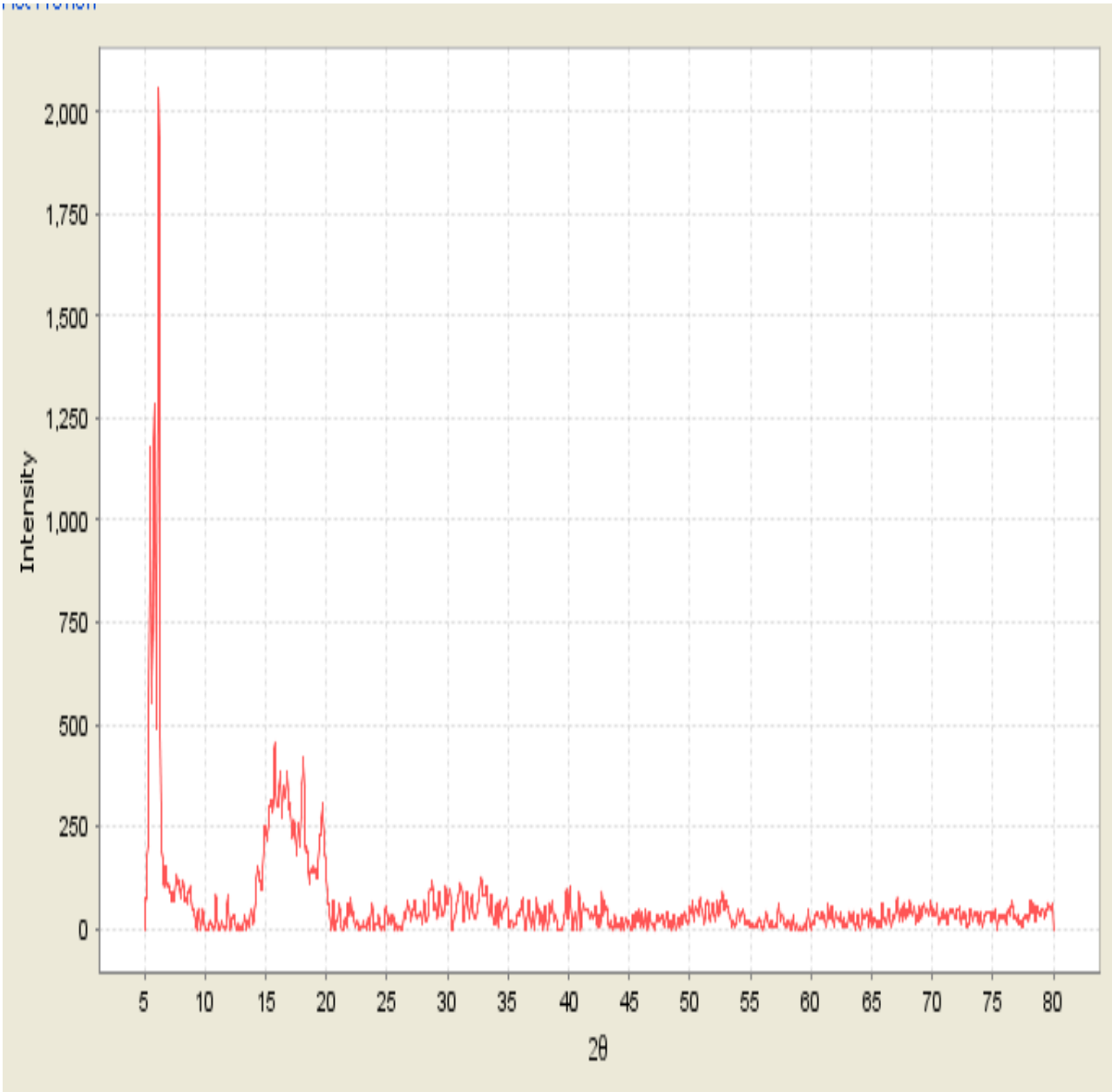
Appendix 7:XRD pattern of Co-Met Complex (λ= Cu Kα 1 1.54056Å)



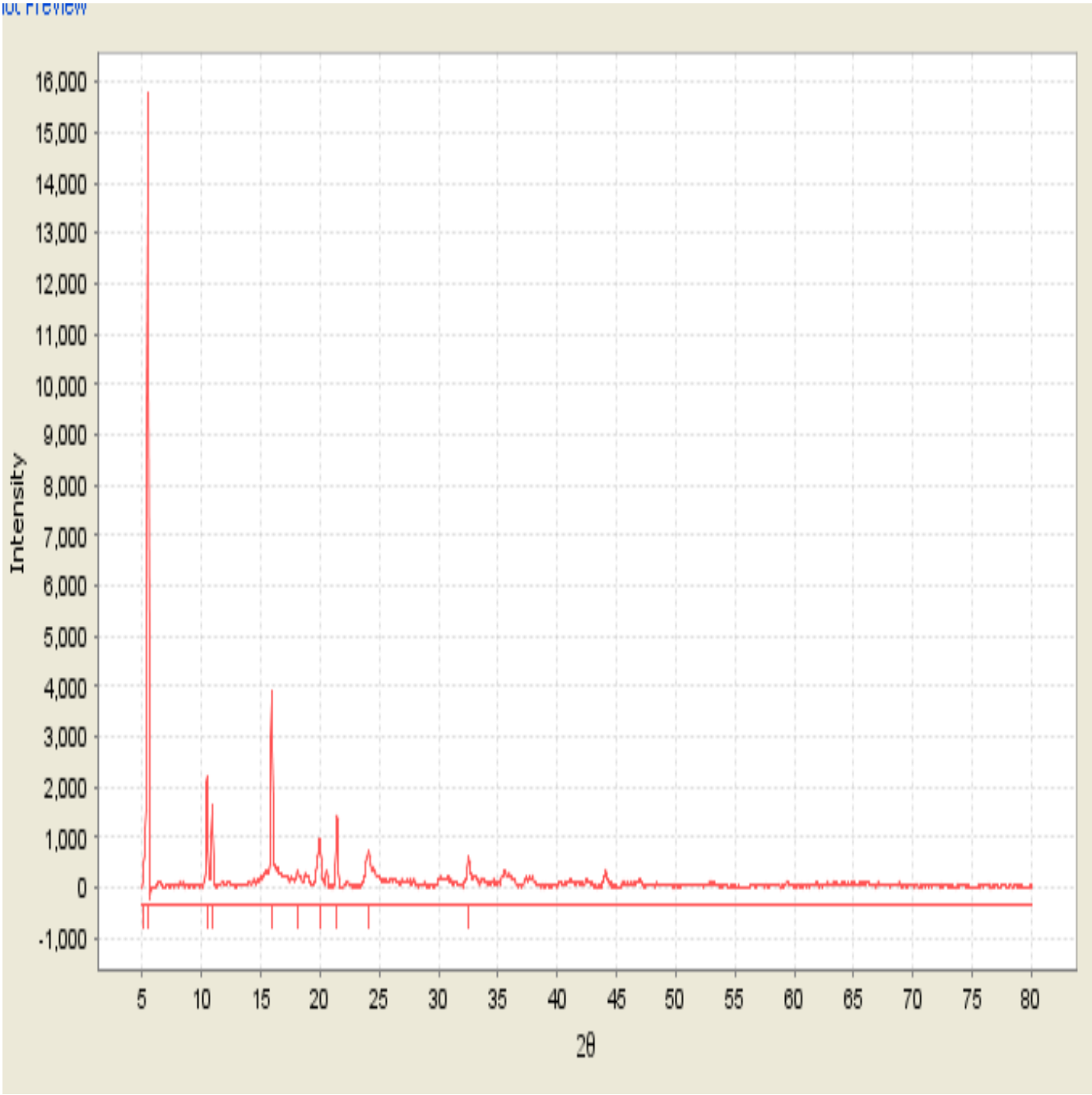
Sample Name:M2L1

Wavelength:Cuka1 1.54056Å

Appendix 8:XRD pattern of Co-Leu Complex (λ= Cu Kα 1 1.54056Å)



Appendix 9:XRD pattern of Zn-Met Complex (λ= Cu Kα 1 1.54056Å)



Appendix 10:XRD pattern of Zn-Leu Complex (λ= Cu Kα 1 1.54056Å)

