SYNTHESIS AND CHARACTERIZATION OF MIXED LIGANDS OF ANTIDIABETIC ZINC(II) COMPLEXES

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Dissertation submitted in fulfilment of the requirements for the Degree Master of Science in Chemistry at the Mafikeng Campus of the North-West University

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December 2016

DECLARATION

I declare that this project which is submitted in fulfillment of the requirements for the Degree of Master of Science in Chemistry (MSc) at North West University, Mafikeng Campus has not been previously submitted for a degree at this university or any other University.

The following research project was compiled, collated and written by me, Thato Medupe. All the quotations are indicated by appropriate punctuation marks. Sources of my information are acknowledged in the reference pages.

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ACKNOWLEDGEMENTS

To my supervisor Professor D.A Isabirye, I would like to express my deepest appreciation, for being such a great mentor and for your tremendous assistance on making my project such a success. Your expertise, understanding, patience, and persistence added considerably to my graduate experience. Your vast knowledge and skill in many areas, your assistance in writing reports (i.e. grant proposals, bursary applications and this thesis), your words of encouragement, kindness and inspiration are highly appreciated.

To Professor Eno. E Ebenso, I would like to express my sincere gratitude and appreciation to you. I'll forever be thankful for your support, encouragement and vote of confidence in me.

To Professor Mukwevho and diabetes and obesity therapeutics research group at large, I would like to thank you for your contribution and assistance with regards to the application work of the project.

I would like to thank Dr. Aiyelabola and Dr Damian Onwudiwe, for their assistance at all levels of the research project. To the laboratory technicians of the department of chemistry, thank you for your assistance.

I recognize that this research project would not have been possible without the financial assistance of the Sasol Inzalo Foundation, the National Research Foundation (N.R.F) and the North-West University. For that, I thank you.

To my mother and sister, thank you for the encouragement and support that you have always provided me with through my entire life. Thanks a million!

To all my friends, I thank you for your support during my darkest hours. You are all special to me.

To the Almighty God, till to this day, I have no idea as to what I have done to deserve your forever merciful, greatest tender love and such undeserved grace. I thank you so much for being a great part of my life. With love and much thanks, your son Thato.

I, Thato Medupe dedicate this work to my very own special mother and sister, Maggy Keikantsemang and Mpho Abigail Medupe, as well as my brother and friend Teddy Mark Saunders.

ABSTRACT

Diabetes Mellitus (DM), one of the most pandemic, universal and life-style related disease across the globe. It is described as a metabolic disorder of multiple aetiology, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolisms which may result from defects in insulin secretion and insulin action. The disease may be classified as Type 1 and Type 2 DM according to the National Diabetes Data Group of the USA and the 2nd World Health Organization (WHO) Expert Committee on DM. The clinical method utilized to treat both types of the disease were reported to be defective: daily insulin injections several times a day are painful and elevate the levels of patient stress especially in young people and synthetic therapeutic agents often have some severe side effects. To this date, many research studies have been conducted to develop a new class of metallopharmaceutical compounds that may be able to minimize or eradicate the problematic situations reported on this clinical method.

Zinc(II) metal ion, which has many nutritional and pharmacological roles, along with its complexes has been identified to exhibit insulin mimetic activities. The mono ligand antidiabetic zinc(II) complexes consisting of amino acids such maltol and picolinic acid were reported to exhibit greater *in vitro* insulin mimetic activities and *in vivo* antidiabetic activities in diabetic rat animals. Mixed ligands zinc(II) complexes have been lagging behind. In this study, the complexes bis(maltolato)zinc(II), bis(picolinato)zinc(II) and the new complexes maltolato(picolinato)zinc(II), (N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II) and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] were synthesized and characterized using Infrared and Ultraviolet-visible spectroscopy, single crystal X-ray diffraction and microanalysis. In vitro evaluation tests were carried out by making use of C2C12 (skeletal muscle) cell lines. Cells were induced with Type 2 Diabetes Mellitus and treated with the synthesized zinc(II) coordination compounds. The results of the C2C12 cell line culture plates have shown the presence of insulin mimetic activities and are in line with the published work.

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LIST OF ABBREVIATIONS

DM	Diabetes mellitus
WHO	World Health Organization
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non-insulin dependent diabetes mellitus
MRDM	Malnutrition related diabetes mellitus
IGT	Impaired glucose tolerance
GDM	Gestational diabetes mellitus
IND	International nomenclature of diseases
ICD-10	The tenth revision of international classification of diseases
TZDs	Thiazolidinediones
GLP-1	Glucagon-like peptide-1
GLP-1 RA	Glucagon-like peptide-1 receptor agonist
PPAR γ	Peroxisome proliferator-activated receptor γ
HbA1c	Haemoglobin A1c
SGlT 2i	Sodium glucose co-transporter 2 inhibitor
DPP4i	Dipeptidyl peptides-4 inhibitor
STZ rats	Streptozotocin-induced diabetic rats
PI3K	Phosphatidylinositol 3-kinase
Akt/PKB	Akt/protein kinase B
$ZnCl_2$	Zinc chloride
Ob/Ob mice	Obese mice
His-Pro	Histidyl-Proline
IR	Insulin receptor
PI3K	Phosphatidylinositol 3-kinase
GLUT4	Glucose transport 4
$Zn(mal)_2$	Bis(maltolato)zinc(II)
$Zn(pic)_2$	Bis(picolinato)zinc(II)
$ZnSO_4$	Zinc sulphate
$VOSO_4$	Vanadyl sulpahte

His	Histidine
FFA	Free fatty acids
ASN	Asparagine
PRO	Proline
THR	Threonine
VAL	Valine
TGA	Thermogravimetric
FTIR	Fourier transform infrared spectroscopy
DMEM	Dulbecco’s Modified Eagle’s Medium
FBS	Fetal Bovine Serum
RNA	Ribonucleic Acid

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CHAPTER 1

1. INTRODUCTION

1.1 Diabetes mellitus

Diabetes Mellitus (DM), one of the most pandemic, universal and lifestyle related diseases across the globe, is described as a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolisms which may result from defects in insulin secretion and insulin action.[1] The illness is commonly known to be associated with absolute or relative insulin deficiency.[2] The effects of the illness include progressive development of specific complications such as nephropathy leading to renal dysfunction, cardiac abnormalities, diabetic retinopathy with potential loss of vision, neuropathy with the risk of foot ulcers and amputations, blood vessels and ocular disorders.[3]

1.2 Classification of diabetes mellitus

1.2.1 Former classification

The disease diabetes mellitus was classified as insulin dependent diabetes mellitus (IDDM) or Type 1, and non-insulin dependent diabetes mellitus (NIDDM) or Type 2. This type of classification was brought into order by the National Diabetes Data Group of the USA and the second World Health Organization (WHO) Expert Committee on Diabetes Mellitus.[1] However, this type of classification by both authorities was altered and modified and the new classification system was widely accepted all around the world and had been put to practice for a longer period of time.[4] In this classification, the degree of insulin deficiency and etiology was utilized in conjugation to classify the chronic metabolic disorder. The terms Type 1 and 2 were eliminated from the classification, but the terms IDDM and NIDDM were retained, and a new class of Malnutrition Related Diabetes Mellitus (MRDM) had emerged.[7] The terms IDDM and NIDDM imply stages of diabetes which represent different degrees of insulin deficiency. Both reports that were organized by these authorities consisted of various classes of diabetes mellitus which included Other Types, Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were revealed in the subsequent International Nomenclature of Diseases (IND) and the tenth revision of the International Classification of Diseases (ICD-10).

1.2.2 Present Classification

This classification consists of both clinical stages and etiological types of diabetes mellitus and other classes of hyperglycemia as recommended by Kuzuya and Matsuda.[7] The clinical stages previously mentioned in classification reports of diabetes,[1,8] signifies that diabetes mellitus advances through numerous clinical stages during its natural history irrespective of its etiology. Furthermore, individual subjects may shift from stage to stage in either direction. Individuals who may be developing or have already been diagnosed with the disease can be classified by stage according to the clinical characteristics, even in the absence of the relevant information concerning the underlying etiology.[1] The stage of glycaemia may change over a period of time depending on the extent of the underlying disease process. Figure 1 displays the relationship between etiological mechanisms along with the clinical stages of the illness.

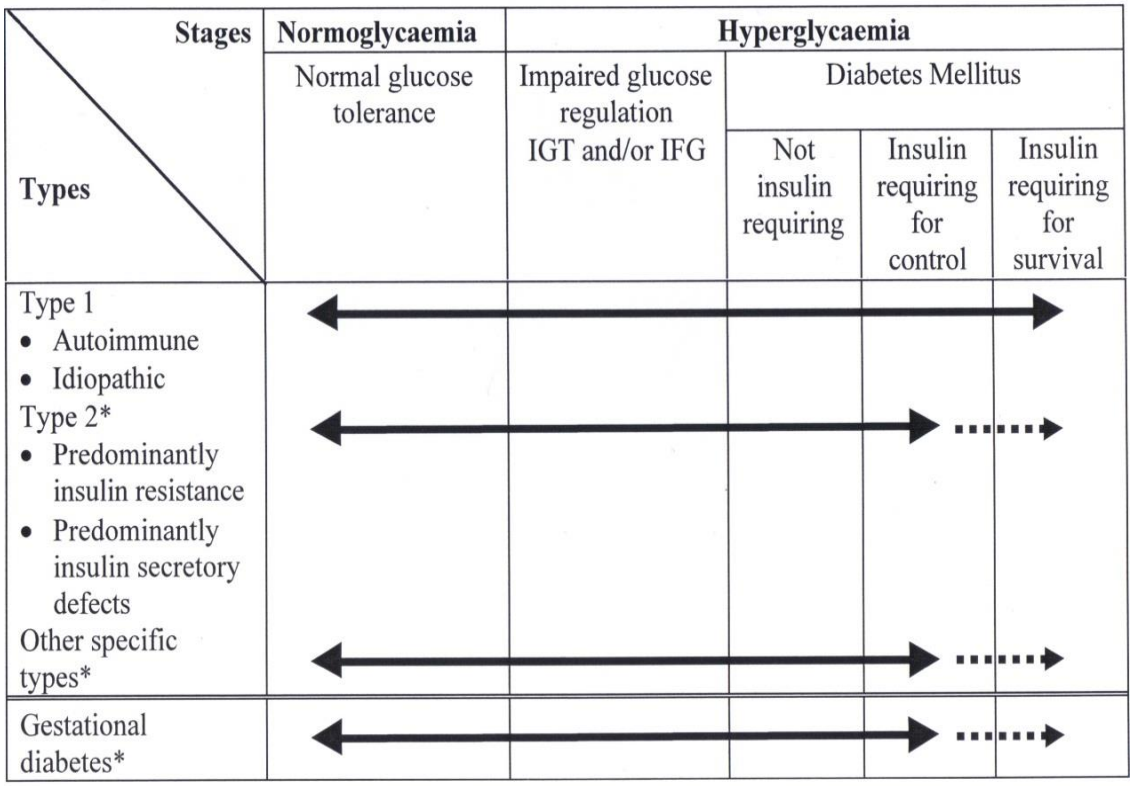


Figure 1 Disorders of glycaemia: etiological types and clinical stages.[7]

The arrows towards the right indicate deterioration of glucose metabolism, including the development of diabetes. The filled portion of solid and broken lines represents the stages regarded as diabetes. The broken lines indicate uncommon phenomena. For example, the life of a patient with Type 2 diabetes does not depend on several injections of insulin, but may develop ketoacidosis in association with severe infection. The arrows towards the left are filled for their full length, implying that a patient, who had previously been diagnosed with diabetes, should be regarded to have diabetes, even if he/she improves to resume normal glucose tolerance.[1,6,7]

This classification by etiology results from enhanced understanding of the causes of diabetes mellitus. The classification itself is profound. However, the methods utilized to determine the pathogenesis of diabetes are not yet reliable to an extent that it is impossible to always identify the etiology of diabetes in each patient.[8] The etiological mechanisms displayed in Figure 1 consists of Type 1, Type 2, other specific types and gestational diabetes mellitus. In this classification, the terms IDDM and NIDDM are omitted and are replaced by the terms Type 1 and Type 2 diabetes mellitus.

1.3 Types of diabetes mellitus

1.3.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus evolves as a result of absolute deficiency of insulin which is primarily due to the damage of the pancreatic β-cells.[6] In most reported cases of this type of diabetes, it has been established that an autoimmune process plays a vital role in β-cell destruction. Autoantibodies as well as islet cell antigens are discovered in many patients at earlier stages after onset. Individuals who are likely to suffer from ketoacidosis/idiopathic condition may also be classified under this type of diabetes. Ketoacidosis is a diabetic condition in which both the etiology and pathogenesis of the illness are known due to failure to detect both auto-antibodies and islet cell antigens. Type 1 diabetes mellitus may further be subdivided into autoimmune and idiopathic classes.[8,9] The idiopathic class of Type 1 diabetes mellitus is not popular as compared to the autoimmune class. The pathogenesis and etiology of the disease are not well understood, but patients normally lack insulin production and are susceptible to ketoacidosis in the absence of antibodies to β-cells.[10]

1.3.2 Type 2 diabetes mellitus

This type of diabetes is the most prevalent form of the illness across the globe and accounts for approximately 90-95% of those individuals diagnosed with diabetes mellitus. It is defined as an adult onset illness that is caused by defective insulin sensitivity, aging, obesity, spiritual stress, environmental factors, combination of resistance to insulin action and an inadequate compensatory insulin secretory response.[5,6] Type 2 diabetes mellitus is used for individuals who have relative (rather than absolute) insulin deficiency. Individuals are constantly resistant to the action of insulin. This implies that individuals do not need insulin treatment to survive at least at early stages of detection and often throughout their livelihood.[1] In this type, the pancreatic β-cells are maintained for a period of time and injections of insulin are rarely required to sustain life. Ketoacidosis may occur in the presence of severe infection or environmental stress. The disease has been reported[4] to have a strong genetic predisposition with a very complex genetic makeup that is unclearly defined. Most patients suffering from this type of diabetes are currently obese or were previously diagnosed with obesity.

1.3.3 Other specific types[8]

Other specific types consist of those complications that are not identified to be common causes of diabetes mellitus, but are those in which the underlying defect can be identified in a relatively specific manner. An example of this class of diabetes is the fibrocalculous pancreatopathy, a form of diabetes that was initially classified as one type of malnutrition-related diabetes mellitus. This type of category consists of:

- Diabetes in which specific mutations have been identified as a cause of genetic susceptibility.
- Diabetes associated with other pathologic conditions or diseases.

1.3.4 Gestational Diabetes Mellitus (GDM)[8]

Gestational diabetes mellitus (GDM) is defined as a state of carbohydrate intolerance that results in hyperglycemia of variable severity during pregnancy. Etiologically, most patients suffering from GDM may possibly have common genetic constitutions with Type 1 or Type 2 diabetes mellitus and the metabolic effect of pregnancy initiates their regularity of glucose tolerance. The

clinical importance of GDM itself provides a solid based reason as to why this type of diabetes is being categorized independently. During pregnancy, the mother together with her unborn baby may be affected adversely by this irregularity in the glucose tolerance. In most cases, glucose is usually normalized after the birth of the child. However, patients become at a higher risk of developing the disease shortly afterwards.

1.4 Diagnostic criteria

Diabetic patients are identified clinically by evaluating their blood glucose concentrations for a period of time. In most cases, these patients have glucose concentrations that exceed the normal limit. In order to classify and assign a type of diabetes to a potential patient, the clinician must often observe the circumstances present at the time of diagnosis.[11] Most diabetic individuals do not fit into a single category of the illness for example, individuals treated with thiazides may develop type 2 diabetes mellitus within a couple of years later. It is known that thiazides themselves rarely cause severe hyperglycemia and since the illness is made worse by the drug, these individuals may be diagnosed with Type 2 diabetes.[4] Therefore, for both the clinician and the patient, it is less important to identify a particular type of diabetes than it is to understand the pathogenesis of the disease and to find effective ways of treating and managing the illness.

The most favoured method utilized to diagnose a diabetic patient is based on measuring the glucose levels in the blood at different situations described:

- Random plasma glucose $\geq$ 200 mg/dL (11.1 mmol/L)[11]
- Fasting plasma glucose $\geq$ 126 mg/dL (7 mmol/L)[11,12]
- Oral glucose tolerance test (measure of plasma glucose levels 2 hours after glucose is given orally) $\geq$ 200 mg/dL (11.1 mmol/L)[11]

For pregnant woman suffering from diabetes mellitus, the World Health Organization (WHO) composed a criterion based on a 2-hour 75-g oral glucose tolerance test which highlights and specifies that the fasting plasma glucose concentrations is > 126 mg/dL or the 2-hour fasting plasma glucose is > 140 mg/dL.[13]

Type 2 diabetes mellitus has been the most challenging to diagnosis for many years. This is due to the fact that the hyperglycemia is often not severe enough to provoke noticeable symptoms of the illness.[14]

1.5 Management and treatment

In critical cases, the life of an individual who has recently been diagnosed with the disease depends on regular intraperitoneal injections of insulin, a regular pattern of meals, and a suitably adjusted lifestyle. At greater extremes, a weight reducing diet may suffice to correct the metabolic disturbance completely. Adequate physical activity or exercising and reduction or avoidance of obesity are primary ways of managing the disease. Educating the patient and motivating them to become part of an antidiabetic program and maintenance of general physical and emotional health is of essence if the therapeutic measures are to be effective.

Medical professionals often prescribe insulin to patients who are diagnosed with Type 1 diabetes mellitus. Insulin can be administered orally, in injectable, or with novel delivery systems based on nanotechnology. Methods of managing the illness include the use of self-monitoring devices for blood glucose levels in order to adjust insulin dosage and regular monitoring of risk factors to prevent complications associated with diabetes mellitus.

Individuals suffering from type 2 diabetes mellitus may be prescribed Metformin[15] (a biguanide) which is the most widely used first line type 2 diabetes drug, sulfonylurea insulin secretagogues which stimulate insulin release,[16] thiazolidinediones[17] (TZDS) drugs responsible for enhancing insulin sensitivity in skeletal muscle and reducing hepatic glucose production as well as drugs focused on the incretion system.[18] The injectable GLP-1 stimulates pancreatic insulin secretion in a glucose dependent fashion, thereby suppressing pancreatic glucagon production which in turn slows down the gastric emptying and lastly decreasing appetite. Due to the progressive β-cell dysfunction that characterizes Type 2 diabetes mellitus, patients are strongly advised to undergo insulin replacement therapy as it is required regularly.[19]

For women suffering from gestational diabetes mellitus, maintaining a good healthy diet is the primary source of treatment. The maternal blood glucose profile which is vital during gestation is

maintained and regulated by decreasing fat intake and the replacement of complex carbohydrates for refined carbohydrates. In order to achieve this, there are two approaches recommended:

- Lowering the proportion of carbohydrates to 40% in a 3 course meal daily with only three or four snacks, or
- Decreasing the glycemic index in order for carbohydrates to make up approximately 60% of the daily intake.

Individuals are also advised to consult a registered dietician to develop and discuss the nutrition plan suitable for the patient based on weight and height.[20-21]

1.6 Mixed ligand complexes

The development of zinc(II) coordination compounds exhibiting antidiabetic effects or blood glucose lowering effects by making use of experimental diabetic animals has been of prior investigation to research scientists.[22] The zinc(II) metal ion is of great interest within this field of research due to the fact that the metal ion is an essential trace elements in all biological systems and is less toxic compared to transition elements. The metal ion was found to trigger lipogenesis in rat adipocytes in a manner comparable to mimicking insulin activity. A research study[3] conducted on the administration of $ZnCl_2$ to diabetic rat animals has shown that $ZnCl_2$ lowers the high blood glucose levels present in the blood stream of the model mice. This study led to the uprising investigations based on zinc(II) complexes which are less toxic, contain no side effects, possess the ability to increase lipogenesis and contain blood glucose lowering effects. Compounds coordinated to amino acids such as the bis(maltolato)zinc(II) and bis(allixinato)zinc(II), with the $Zn(O_4)$ coordination modes were reported to exhibit higher insulin mimetic activities compared to the free zinc(II) metal cation.[23] Moreover, the compounds bis(thioallixin-N-methyl)zinc(II) and bis(l-oxy-2-pyridine-thiolato)zinc(II) with $Zn(S_2O_2)$ coordination mode were reported to possess not only the highest *in vitro* insulin mimetic activities and but also a potent hypoglycemic effect *in vivo*.[24] In order to determine the possible antidiabetic therapeutic agents that may enhance the insulin mimetic activities of coordination compounds, mixed ligand complexes of this nature have been synthesized and evaluated for their antidiabetic effects.

Mixed ligand complex formation comprises of 2 or more different ligands coordinating to a central metal atom/ion. Complexes of this nature play an essential function in living organisms and have been studied for decades. The ideology of conducting projects focusing on the synthesis of such coordination compounds is based on the idea of enhancing the application work in relation to the biological activities of these complexes.[25] Research studies[25] conducted on these coordination compounds indicate that most complexes of this sort possess antimicrobial, antifungal and cytotoxic properties and are more biologically active than the mono-ligand complexes due to chelation.

Amino acids are the primary components and the building blocks of proteins found in tissues within the human body. Mixed ligand complexes of amino acids are extensively studied, applied and employed in biology and pharmaceutical industries as well as laboratory reagents. Complexes are involved in several biological processes in living organisms such as neurotransmitter function, pH regulation, transamination, cholesterol metabolism, decarboxylation, pain administer, detoxification and regulation of inflammation.[26] Many research projects focus on their structures, chemical composition and physiochemical properties in order to better understand their behavior and potential applications.[27] The metal oxidation state, the nature and number of donor atoms, as well as the their relative positions within the ligand are key factors to investigating the relation between the activity and structure of the amino acid.[28,29]

Amino acids easily form stable complexes with transition metal ions and have been [30] to exhibit anticarcinogenic and antidiabetic activities. For example, when coordinated to Zn(II) metal cation, the ligand's biological activities are enhanced as compared to when coordinated to a single free ligand.

In this work, mixed ligand complexes of the form $[Zn(L_1)(L_2)\cdot 2H_2O]$ as well as dithiocarbamate complexes of the form (2.2-bipyridine)$[Zn(L_1)(L_2)]$ have been synthesized and characterized successfully by using spectrophotometric methods. These compounds contain nitrogen, oxygen and sulphur binding sites such as L-proline, picolinic acid, maltol, [N-methyl-N-phenyl] and [N-butyl-N-phenyl]ammonium dithiocarbamate ligands, hence they are extensively studied for their biological properties. This was done in order to better understand their molecular structures and

the nature of binding and coordination with the zinc(II) metal ion. This work seeks to add more knowledge to the present coordination chemistry with the three new mixed ligand complexes being studied in order to screen investigate such complexes for antidiabetic biological activities.

1.7 Research problem statement

It has become clearer that diabetes mellitus is a serious life threatening disease and a social health problem all around the world. A research study conducted recently has shown that the statistical number of patients suffering from the disease increases daily indicating the widespread presence of diabetes in the human population from children to adults which has increased to over 14 million.[31] In adults only, the prevalence in the world is estimated to reach 5.4% while the number is expected to increase to approximately 300 million worldwide by 2025.[32] For a very long time, the disease has been controlled by daily insulin injections for patients suffering from type 1 diabetes along with several types of pharmaceutics for patients suffering from type 2 diabetes. Unfortunately, these methods of treatment have given rise to other complications. For example, injections of insulin several times a day can be painful both physically and mentally by elevating the levels of patient stress especially in young people. Further, pharmaceutics used for a longer duration often induce some severe side effects. In order to reduce if not to eradicate the complications experienced when treating diabetes mellitus, the current methods of treatment need to be improved in such a way that they will reduce complications and discomfort among patients.

1.8 Aim

In this century, research work is being conducted on synthetic therapeutic agents that are much more effective to treat and manage diabetes without having to cause serious complications as well as to mimic the action of the insulin hormone. This work seeks to increase knowledge and educate the population at large about the disease at hand. It also seeks to investigate and showcase the metallopharmaceutical agents that may contain insulin mimetic activities and serve as synthetic agents with probable or no severe side effects to manage type 2 diabetes mellitus. This should ultimately improve the quality of the life of patients diagnosed with type 2 diabetes mellitus.

1.9 Objectives

The research project was therefore carried out with the following objectives:

- To prepare and obtain stable antidiabetic agents consisting of the zinc(II) metal ion coordinating to different amino acids as well as dithiocarbamate ligands;
- To characterize these compounds using Infrared spectroscopy, Ultraviolet-Visible spectroscopy, microanalysis and single crystal XRD in order to validate their purity, their identity as well as their structures;
- To test these compounds for their antidiabetic properties via *in vitro* evaluation.

CHAPTER 2

2. LITERATURE REVIEW

2.1 Background

There are numerous pathogenic processes that contribute to the development of Type 2 diabetes mellitus. To mention a few, Type 2 diabetes mellitus is caused by a combination of inadequate insulin secretion along with a decrease in insulin-stimulated glucose uptake in peripheral (muscle) tissues which results from a defect in β-cell function.[33] It is also caused by metabolic deformations which include impaired insulin secretion in response to glucose and increased hepatic glucose production. Diabetes research studies indicate that within a small group of patients diagnosed with the illness, the condition is caused by the preparation of an abnormal, biologically less-active, insulin molecule. As a result of the cause, a sudden, random change of the insulin gene (mutant insulin) occurs. Genetic makeups and environmental factors also contribute to the evolution and severity of diabetic complications.[34] Therefore, the physiology of Type 2 diabetes mellitus is not only focused on β-cell-, muscle-, and liver-related deficiencies as previously contemplated.[33] Figure 2 below indicates multiple defects leading to the occurrence of hyperglycemic Type 2 diabetes mellitus.

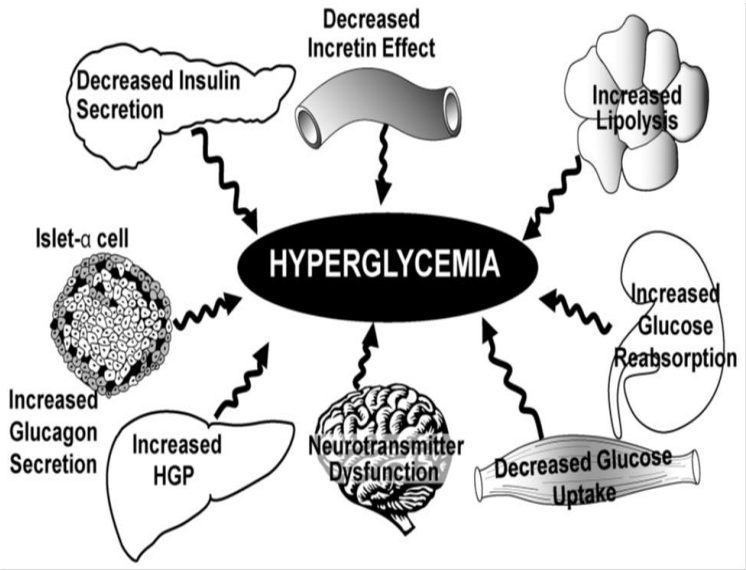


Figure 2 Multiple defects contributing to the development of glucose in Type 2 diabetes mellitus.[33]

Symptoms of the illness are often not severe, or may be absent, and therefore hyperglycemia of sufficient degree to cause pathological and functional changes may be present to some extent before the disease is detected, irrespective of the type of diabetes mellitus a particular person may suffer from.[1] However, characteristic symptoms may include blurring of vision, polydipsia, weight loss, sometimes with polyphagia and polyuria.[6] When the disease graduates to its severe forms, ketoacidosis or non-ketotic hyperosmolar state may advance and lead to stupor, coma and, in the absence of effective treatment, death may occur. Children often experience severe symptoms of diabetes mellitus. These include increased blood glucose levels, marked glycosuria and ketonuria.[1]

The treatment and management of hyperglycemia in Type 2 diabetes mellitus has become of greater priority, recognition and concern. The statistical data of the prevalence of the disease reveals increasing numbers worldwide, particularly in developing countries. This has resulted in the search for new classes of drugs suppressing blood glucose levels to supplement the other therapies. This has increased the knowledge based on the pharmacological therapeutic agents that are now available and are extensively used worldwide to manage Type 2 diabetes mellitus.[35] Figure 3 displays the antidiabetic therapeutic medications utilized in most developed and developing countries which target the dysfunctional physiological actions in Type 2 diabetes mellitus.

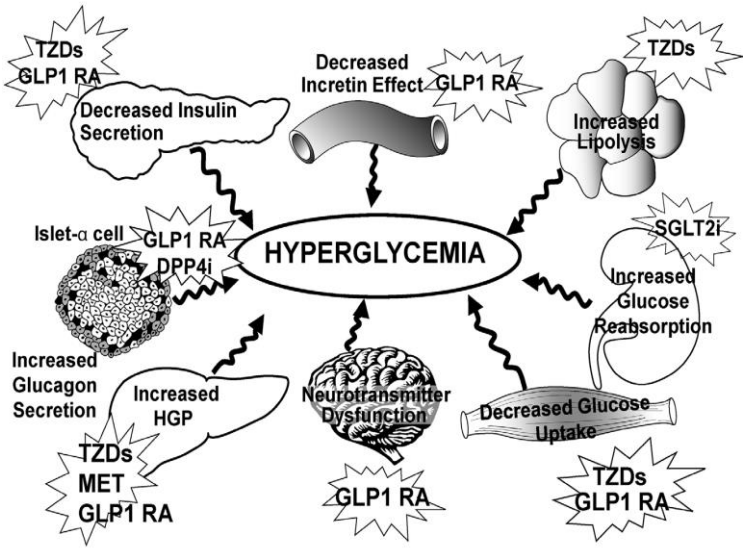


Figure 3 Antidiabetic agents targeting different pathophysiological disorders.[33]

The antidiabetic agents displayed in Figure 3 are mentioned and described below:

- Thiazolidinedione (TZDs or glitazones)[36]

Thiazolidinediones are peroxisome proliferator-activated receptor γ (PPAR γ) modulators. These agents are known to decrease blood glucose and haemoglobin A1c (HbA1c) levels, insulin levels, and may preserve or even enhance β-cell function. They also increase muscle, fat and liver sensitivity to endogenous and exogenous insulin.

- Glucagon-like peptide-1 receptor agonist (GLP1 RA)[37]

The glucagon-like peptide 1 agonists (exenatide) are gut derived incretin peptides, which occur naturally and are produced by the L cells found in small intestines. These peptides potentiate insulin secretion, inhibit gastric emptying, and reduce appetite and food intake. The therapeutic agents carry the same function of the GLP-1 hormone and contribute to an increase in the incretin effect in patients diagnosed with Type 2 diabetes mellitus, and hence stimulating insulin secretion. The infusions of the hormone have been reported to decrease blood glucose levels in Type 2 hyperglycemic patients through a transient glucose dependent stimulation of insulin and suppression of glucagon secretion and gastric emptying. Some beneficial side effects include glucagon reduction, slowing gastric emptying and inducing satiety.

- Sodium glucose co-transporter 2 inhibitor (SGLT 2i)[38]

Sodium glucose co-transport 2 is a low affinity, high capacity SGLT positioned exclusively at the kidney cortex, specifically at the apical domain of the epithelial cells in the premature proximal convoluted tubule. SGLTs take part in the regulation of steady state glycaemia through the mediation of the absorption of glucose from the proximal tubules of the kidney and due to such; their inhibition has therapeutic potential in Type 2 diabetes.

- Metformin (MET)[39]

Metformin is a biguanide that has been used worldwide for decades. It serves as an antihyperglycemic therapeutic agent for treating patients diagnosed with Type 2 diabetes mellitus. The therapeutic agent enhances cardiovascular risk factors and is considered as an ‘insulin sensitizer’ because it decreases the rate of glucose without having to increase insulin secretion. It also decreases the production of endogenous glucose at the level of the liver. In Type 2 diabetic adults, treating patients with metformin is beneficial because the agent also

contributes to weight reduction/loss, decreased hyperinsulinaemia, enhanced lipid profiles, augmented fibrinolysis and improved endothelial function. The use of metformin along with its advantageous effects has led to the ideology of prescribing metformin in insulin resistant states.

- Dipeptidyl peptides 4-inhibitor (DPP4-i)[33,40]

DPP4-inhibitors are also considered to be a novel class of oral anti-hyperglycemic agents (OHAs). These inhibitors are tiny molecules that intensify the GLP-1 effect, thus increasing glucose-mediated insulin production and subdue the glucagon secretion. DPP4 is an ubiquitous cell membrane protein that is widely expressed in various tissues, such as liver, lung, kidney, intestinal brush-border membranes, lymphocytes, and endothelial cells. In clinical practice studies, DPP4-inhibitors mime the mechanism ascribed to GLP-1R agonists, which includes insulin stimulation and inhibition of glucagon secretion and preservation of β-cell mass through the stimulation of cell proliferation and inhibition of apoptosis.

The evolution of these therapeutic agents in addition to the existing treatment, such as lifestyle-directed interventions, insulin and metformin, has increased the number of treatment options available for managing type 2 diabetes. These pharmaceutical interventions have been reported to increase the life span of diabetic patients and have given hope to the human population that the illness is indeed controllable. Although researchers have reported vast knowledge and information based on the treatment and management of Type 2 diabetes, achieving novel agents with probable or no side effects to reduce the mortality rate related to long term complications of Type 2 diabetic cases has been of greater priority and concern.

2.2 Metallopharmaceutical agents

The invention and development of metallopharmaceutics has become an important and exceptionally desirable investigation in the 21st century.[3] It has become necessary because the measures of treatment that are being utilized to manage a handful of diseases that are lethal need to be enhanced. There are various therapeutic agents that have been identified and developed for clinical measures in order to treat a number of abnormal physiological disorders. For example, synthetic metallopharmaceutics such as gold-containing antirheumatoid arthritis drugs, auranofin, platinum containing anticancer drugs, cisplatin as well as the aluminium- and zinc-

containing scralfates and polaprezincs as antiulcer actives, respectively.[41] These coordination compounds are all metal-ligand complexes where the metal ion is expected to be incorporated in human organs or cell-tissues by complex formation greater than that of each metal ion itself. The existence of such compounds as well as the research studies that have been conducted on these compounds serve as motivation to develop alternatives to treat diseases that pose a threat to the human population at large.

In relation to this study, a number of research studies have been conducted to seek metallo-pharmaceutical agents that may not cause any side effects when administered to patients, suppress high glucose levels and display insulin mimetic activity. Before the discovery that insulin along with its clinical trial was able to treat and manage the disease, a fascinating outcome was announced in which orally administered sodium vanadate ($NaVO_3$) was reported to be successful in enhancing the conditions of patients infected with the disease.[42] This report gave assurance to research scientists that vanadium could be utilized in the treatment of diabetes mellitus.

2.3 Metal ions with insulin mimetic activity

There is a large number of metal ions that are identified to play a vital role in biological processes especially within the human body itself.[43] The majority of these metal ions display insulin mimetic activities or effects. For example, vanadium ions were confirmed to contain the *in vitro* insulin-mimetic effect in 1979.[44] Vanadium has been discovered to have normoglycemic activity in streptozotocin-induced Type 1 DM rats (STZ rats).[35] Besides the vanadium ion, it was proposed that chromium (Cr),[46,47] manganese (Mn),[48] cobalt (Co),[49] zinc (Zn),[50,51] selenium (Se),[52] molybdenum (Mo)[53] and tungsten (W),[54] along with their complexes display insulin-mimetic activity in *in vitro* animal experiments. Table 1 summarizes the effective chemical forms of the metal ions and complexes causing antidiabetic activities in experimental animals and subjects with diabetes mellitus.

Table 1 Metal ions and complexes causing anti-diabetic activities in experimental animals and subjects with diabetes mellitus.

Metal	Ionic Form	Complex Form	Reference
V	Vanadyl sulfate ($VOSO_4$)	Bis(methyl cysteinato)oxovanadium(IV)	42, 44,45
	Sodium Vanadate ($NaVO_3$)	Bis(maltolato)oxovanadium(IV)	
		Bis(picolinato)oxovanadium(IV)	
Cr	-	Bis(picolinato)chromium(III)	46, 47
		Chromium polynicotinate	
Mn	Manganese chloride ($MnCl_2$)	-	48
Co	Cobalt chloride ($CoCl_2$)	-	49
Zn	Zinc chloride ($ZnCl_2$)	Bis(picolinato)zinc(II)	50, 51
		Bis(maltolato)zinc(II)	
Se	Sodium selenite (Na_2SeO_3)	-	52
Mo	Sodium molybdate (Na_2MoO_4)	-	53
W	Sodium tungstate (Na_2WO_4)	-	54

Chromium ion was reported by Schwarz and Mertz to better the glucose tolerance and arouse the function of insulin,[55] Tuvemo *et al.*[56] and Paolisso *et al.*[57] recommended that the deficiency of magnesium in the blood (hypomagnesaemia) had some form of relationship with diabetes mellitus and that oral consumption of magnesium improved insulin sensitivity.

2.4 Biological properties of zinc(II) metal ion

Zinc with atomic number 30, atomic weight of 65.39 with an oxidation state of positive II, is a fundamental element in biological systems and is present in numerous proteins and enzymes which exist in living organisms.[3] Some of these proteins and enzymes carry regulatory functions such as insulin synthesis, insulin secretion and signaling.[58]

2.5 Relationship between zinc(II) and insulin

A close relationship between zinc(II) and insulin exists due to the fact that zinc(II) is essential for the normalization of the insulin precursor (pro-insulin) and is transported into the pancreas and secreted to the blood with insulin.[59] Furthermore, it was observed that the concentration of zinc(II) ion in the fingernails of patients suffering from this disease decreased,[60] resulting in an increase of zinc(II) excretion in urine.[61] The metal ion functions as a lewis acid and contains physicochemical properties entirely different from those of vanadium(IV) ion which exhibit a redox property and insulin mimetic activity. The zinc(II) ion is known to be less toxic than those of vanadium(IV) ion. It is generally known that the complexation of free metal ions decreases the toxicity of the metal ions and encourages their absorption into the blood.[62]

2.6 Zinc(II) ion as an insulin mime

Zinc(II) ion has many nutritional and pharmacological roles.[63] A pharmacological role of interest linked to this project is that the ion acts as an insulin mime. In 1980, Coulston and Dandona[64] reported that an increase in lipogenesis in rat adipocytes is stimulated by zinc(II) ion which is comparable of mimicking the action of insulin. Ezaki[65] also stated that the insulin mimetic activity of zinc on rat adipocytes stimulates glucose transport by post receptor/kinase mechanism. In 1989, Tang *et al*.[66] reported the insulin mimetic effect of zinc on glucose transport which was shown by phosphatidylinositol 3-kinase (PI3K) and Akt/protein kinase B (Akt/PKB) in rat adipocytes. This implies that zinc does not only mimic the action of insulin but may be used as a substitute for insulin.[67]

Shisheva*et al*.[68] and Chen *et al*.[69] reported that the oral administration of zinc chloride ($ZnCl_2$) to streptozotocin-induced diabetic rats (STZ rats) or obese (ob/ob) mice for a longer period (8 weeks) of time, and at a high dosage of the agent resulted in greater stabilization of their high blood glucose levels by as much as 50%. Song *et al*.[70] reported that the blood glucose levels of STZ rats, which were given drinking water containing zinc(II) with cyclo histidyl-proline (His-Pro), were lower than those of the rats given zinc(II) ion alone. Moreover, past research projects indicated that zinc acts on adipocytes and boosts the induction of leptine and also acted on the pancreas, thus helping insulin to attach with the insulin receptor. This resulted in improvement of the conditions of Type 2 diabetes mellitus.[71,72]

It has been proposed[3,73] that zinc complexes with several coordination modes do exhibit greater *in vitro* insulin mimetic activity and *in vivo* antidiabetic activity in diabetic animals. In rat adipocytes, such zinc complexes were determined to act on the insulin receptor (IR) and phosphatidylinositol 3-kinase (PI3K), which in turn affected glucose transport 4 (GLUT4) and phosphodiesterase, thereby resulting in stabilization of the blood glucose levels in experimental diabetic rat animals.[5] Hider *et al.*[74] reported that the absorption rate in red blood cells was increased by the complex bis(maltolato)zinc(II) or rather $Zn(mal)_2$ more than the free zinc(II) ions. Therefore, it was concluded that $Zn(mal)_2$ contained greater insulin mimetic activity compared to the free zinc(II) ions as approximated in *in vitro* animal experiments.[73] Besides the $Zn(mal)_2$ complex, other zinc(II) complexes with overall stability constants (log β) lower than 10.5 were found to exhibit higher insulin mimetic activities as well than those of zinc sulphate ($ZnSO_4$) and vanadyl sulphate ($VOSO_4$) or were comparable to them except for Zn(GtG) (IC_{50} = 3.18) (Table 2). On the other hand, zinc complexes with His, GeG and mGeGm (log β = 12.05, 11.22 and 11.83) with log β values higher than 11.0 displayed no insulin mimetic action. Table 2 shows the overall stability constants (log β) of zinc complexes and the estimated IC_{50} values for the free fatty acids (FFA) release from isolated rat adipocytes in the presence of glucose.

In addition, zinc complexes with L- and D- amino acids, Asn, Pro, Thr and Val exhibited similar insulin mimetic activities to each other (Table 2). Accordingly, the difference in the insulin mimetic activities of zinc complexes was not seen on the basis of the absolute configurations of the alpha-amino acids. From all these findings, it can be concluded that zinc(II) complexes have promise as new insulin mimes.

Table 2 Over-all stability constants (log β) of zinc complexes and the estimated IC_{50} values for the free fatty acids (FFA) release from isolated rat adipocytes in the presence of glucose.[3]

Complex	IC_{50}(nM) (± S.D.[a])	log β	Complex	IC_{50}(nM) (± S.D.[a])	log β
$Zn(_L\text{-Asn})_2$	0.65 (0.03) *	8.55	$Zn(_D\text{-Asn})_2$	0.65 (0.09)*	8.55
$Zn(_L\text{-Pro})_2$	0.89 (0.07)	9.75	$Zn(_D\text{-Pro})_2$	0.89 (0.07)	9.75
$Zn(_L\text{-Thr})_2$	0.54 (0.03)**	8.46	$Zn(_D\text{-Thr})_2$	0.48 (0.03)**	8.46
$Zn(_L\text{-Val})_2$	0.77 (0.08)	8.24	$Zn(_D\text{-Val})_2$	0.87 (0.04)	8.24
$Zn(_L\text{-His})_2$	None	12.05			
$Zn(pic)_2$	0.64 (0.13)*	9.52	$Zn(6mpa)_2$	0.31 (0.05)**	_[b]
$Zn(ma)_2$	0.59 (0.10)**	10.4			
Zn(GeG)	None	11.22	Zn(MGeGm)	None	11.83
Zn(βAeAβ)	0.82 (0.05)	7.6			
Zn(GtG)	3.18 (0.04)	10.27	Zn(VtV)	0.92 (0.04)	8.63
$VOSO_4$	1.00		$ZnSO_4$	0.81 (0.10)	

[a]Each value is expressed as the mean ± S.D. for three experiments.

[b]The value could not be obtained because of the precipitations occurred during the course of titration.

* Significance at $P < 0.05$ vs. $ZnSO_4$.

** Significance at $P < 0.01$ vs. $ZnSO_4$.

The research work previously reported[75] on the zinc(II) metal ion has served as evidence to indicate that the metal ion has the ability and high flexibility to form coordinate bonds with amino acids to a variety of amino acids side-chains and substrates in a different geometry in order to enhance their biological activities.

CHAPTER 3

3. EXPERIMENTAL

3.1 Reagents

The reagents used were supplied by Sigma-Alderich, MERCK chemical (Pty) Ltd, Promark Chemicals, Saarchem Suppliers, CJ Chem Suppliers, ThermoFisher. They were used as commercially supplied.

3.2 Preparation of compounds

3.2.1 Bis(maltolato)zinc(II) complex[76]

Maltol (2.5 g, 20.0 mmol) was dissolved in a mixture of water and methanol (1:1) 50 cm^3. Zinc chloride (1.4 g, 10.0 mmol) was dissolved in the same solvent mixture and added to the maltol solution with vigorous stirring. The resulting mixture was refluxed at 60°C for approximately 4 hours. The solution was concentrated to minimum quantity using a rotary evaporator and allowed to stand for 48 hours under room temperature. The crystalline solid that separated out was filtered and washed with minimum quantity of diethyl ether. Yield (2.8 g). Recrystallization from 1:1 methanol:water gave colourless needle-shaped crystals.

3.2.2 Bis(picolinato)zinc(II) complex[76]

Picolinic acid (0.5 g, 4.3 mmol) was dissolved in water (20 cm^3). Zinc sulphate (0.6 g, 2.1 mmol) was dissolved in 20.0 cm^3 of water. The picolinic acid solution was then added to the zinc sulphate solution and stirred for approximately 5 minutes. The pH of the solution was then adjusted to 4.4 with drop wise addition of sodium hydroxide (1 $mol.dm^{-3}$) solution. The white precipitate that formed was isolated by filtration, washed with methanol and ether. Yield (0.8 g). Recrystallization from 1:1 methanol:water gave colourless needle-shaped crystals.

3.2.3 Maltolato(picolinato)zinc(II) complex

Picolinic acid (0.3 g, 2.0 mmol) was dissolved in a mixture of water and ethanol (1:1, 10.0 cm^3). Maltol (0.3 g, 2.0 mmol) was dissolved in a mixture of water and ethanol (1:1, 10.0 cm^3). An aqueous solution of zinc chloride (0.1 g, 1 mmol) was prepared. The ligand mixture solutions were added simultaneously to the zinc chloride solution. The pH of the solution was then adjusted to basic conditions (pH=8.4) by adding sodium hydroxide (0.1 M) dropwise. The

resulting mixture was refluxed at 60°C for approximately 20 minutes. The product formed was isolated by filtration, washed with aqueous ethanol (1:1) and allowed to stand in a vacuum desiccator. Yield (1.0 g). Crystallization of the compound for single crystal X-ray analysis was unsuccessful.

3.2.4 Dithiocarbamate ligands[77,78,79]

- Ammonium N-methyl-N-phenyldithiocarbamate

N-methyl-aniline (0.1 mol) was added to concentrated aqueous ammonia (30.0 cm^3) and the mixture was stirred for 10 minutes. The reaction was performed in ice cold temperatures. To this mixture, carbon disulfide (0.1 mol) was added gradually to the solution. The solution was stirred for 6-7 hours. The yellowish solid product formed was then filtered off and washed 3 times with cold ethanol (5.0 cm^3). The product was found to be air and thermally unstable. Attempts to obtain crystals were hindered by rapid decomposition. Yield (1.7 g).

- Ammonium N-butyl-N-phenyldithiocarbamate

N-butyl-aniline (0.1 mol) was added to concentrated aqueous ammonia (30.0 cm^3) and the mixture was stirred for 10 minutes. The reaction was performed in ice cold temperatures. To this mixture, carbon disulfide (0.1 mol) was added gradually to the solution. The solution was stirred for 6-7 hours. The orange solid product formed was then filtered off and washed 3 times with cold ethanol (5.0 cm^3). The product was found to be air and thermally unstable. Attempts to obtain elemental analysis were hindered by rapid decomposition. Yield (1.9 g).

3.2.5 (N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II) complex

Ammonium N-methyl-N-phenyldithiocarbamate (0.25g, 1.25 mmol) and ammonium N-butyl-N-phenyldithiocarbamate (0.3 g, 1.6 mmol) were separately dissolved in water (20 cm^3). The ligands were mixed together and stirred for 5 minutes. Zinc chloride (0.2 g, 1.3 mmol) was dissolved in water and added to the mixture of the two dithiocarbamate ligands. The white precipitate that immediately formed was vigorously stirred for 1 hour at room temperature. The product was filtered, rinsed with small quantities of water and dried in a vacuum desiccator. Yield (0.2 g).

3.2.6 (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex

2,2-bipyridine (0.1 g, 0.4 mmol) was dissolved in hot chloroform (20.0 cm^3). The complex (N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II) (0.2 g, 0.4 mmol) was dissolved in hot chloroform (20.0 cm^3). The two solutions were mixed together and the resulting yellow solution was refluxed for 20 minutes, concentrated to about 10.0 cm^3 and filtered. The pale yellow solid which separated out from the solution, after 48 hours, was filtered and dried in a vacuum desiccator. Single crystals suitable for X-ray analysis were obtained from slow evaporation of dichloromethane-ethanol solvent mixture of the complex. Yield (0.2 g).

3.3 Characterization of compounds

Compounds were characterized using Infrared spectroscopy, Ultraviolet-visible spectroscopy, single crystal X-ray diffractometer and microanalysis.

3.3.1 Infrared spectroscopy

The infrared spectra of the complexes bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato (picolinato)zinc(II), (N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II) and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] were measured with Bruker Alpha and the Cary 670 series FTIR spectrometers in the region, 3500-400 cm^{-1}. The spectra are collected in Figures 4, 5, 6, 7 and 8.

3.3.2. Ultraviolet-visible spectroscopy

The ultraviolet-visible electronic spectra of freshly prepared solutions of the complexes bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato (picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] dissolved in suitable solvents such as methanol, ethanol and chloroform was measured in 1 cm quartz cell using a Varian Cary 50 ultraviolet-visible spectrometer in the range of 300-700 (λ_{max}). The relevant spectra of the complexes are shown in Figures 9, 10, 11, 12 and 13.

3.3.3 X-ray crystallography

The single X-ray crystallography of bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato (picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] complexes were performed by instrumental analysis laboratory of the Nelson Mandela Metropolitan University. The single crystal X-ray results are compiled in Figure 14, 15, 16, 17 and 18.

3.3.3.1 Mono and di-aqua molecules of bis(maltolato)zinc(II)

X-ray diffraction studies were performed at 200K using a Bruker Kappa Apex II diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). APEX II was used for data collection and SAINT for cell refinement and data reduction.[80] The structures were solved using SHELXT-2014,[81] and refined by least-squares procedures using SHELXL-2014[81] with SHELXLE[82] as a graphical interface. All non-hydrogen atoms were refined anisotropically. Carbon-bound hydrogen atoms were placed in calculated positions and were included in the refinement in the riding model approximation, with U_{iso}(H) set to 1.2 U_{eq}(C). The hydrogen atoms of the methyl groups were allowed to rotate with a fixed angle around the C-C bonds to best fit the experimental electron density (HFIX 137 in the SHELX program suite[82]), with U_{iso}(H) set to 1.5 U_{eq}(C). The hydrogen atoms of the water molecules were located on a different Fourier map and refined with the O-H bond length and the H-O-H bond angle restrained to 0.84 Å and 109° respectively. The data were corrected for absorption effects by the numerical method using SADABS[80]. The relevant X-ray diffraction ortep diagrams of the mono and di-aqua molecules of bis(maltolato)zinc(II) complexes are shown in Figures 14 and 15.

3.3.3.2 Mono and di-aqua molecules of bis(picolinato)zinc(II)

X-ray diffraction studies were performed at 200K using a Bruker Kappa Apex II diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). APEX II was used for data collection and SAINT for cell refinement and data reduction.[80] The structures were solved using SHELXT-2014[81], and refined by least-squares procedures using SHELXL-2014[81] with SHELXLE[82] as a graphical interface. All non-hydrogen atoms were refined anisotropically. Carbon-bound hydrogen atoms were placed in calculated positions and were included in the refinement in the riding model approximation, with U_{iso}(H) set to 1.2 U_{eq}(C). The hydrogen

atoms of the water molecules were located on a difference Fourier map and refined with the O-H bond length and the H-O-H bond angle restrained to 0.84 Å and 109 ° respectively. The data were corrected for absorption effects by the numerical method using SADABS.[80] The relevant X-ray diffraction ortep diagrams of the mono and di-aqua molecules of bis(picolinato)zinc(II) complexes are shown in Figures 16 and 17.

3.3.3.3 (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]

X-ray diffraction studies were performed at 200K using a Bruker Kappa Apex II diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). APEX II was used for data collection and SAINT for cell refinement and data reduction.[80] The structures were solved using SHELXT-2014[81], and refined by least-squares procedures using SHELXL-2014[81] with SHELXLE[82] as a graphical interface. All non-hydrogen atoms were refined anisotropically. Carbon-bound hydrogen atoms were placed in calculated positions and were included in the refinement in the riding model approximation, with U_{iso}(H) set to 1.2 U_{eq}(C). The hydrogen atoms of the butyl molecule were located on a difference Fourier map and refined with the C-H bond length and the H-C-H bond angle restrained to 0.84 Å and 109 ° respectively. The data were corrected for absorption effects by the numerical method using SADABS.[80] The relevant X-ray diffraction ortep diagram of the complex (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] is shown in Figure 18.

3.3.4 Microanalysis

The microanalysis of bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato (picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyldithiocarbamato)zinc(II)] complexes were performed by the instrumental analysis laboratory of the University of Cape Town. The microanalytical results are displayed in Tables 4, 5, 6, 7 and 8.

3.4 Anti-diabetic biological studies

The coordination compounds bis(maltolato)zinc(II) (10^{-4} M), bis(picolinato)zinc(II) (10^{-4} M), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] (10^{-4} M), maltolato (picolinato)zinc(II) (10^{-4} M) and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N

phenyl)dithiocarbamatozinc(II)] (10^{-4}M) were dissolved in water (100 ml) and used as test samples for treating the C2C12 (muscle) cell lines.

3.4.1 Cell culture

The experiment was carried out with reference to published methods with a few changes and modifications[83]. In vitro testing for the biological antidiabetic activity of the synthesized zinc(II) complexes was performed. C2C12 (skeletal muscle) cell culture lines were used in this study to investigate the potential of the synthesized zinc(II) coordination compounds on glucose uptake. Growth medium was prepared and primarily constituted of Dulbecco's Modified Eagle's Medium (DMEM (90 %)) supplemented with Fetal Bovine Serum (FBS (9 %)) and an antibiotic (1 %) incubated at 37˚C.

3.4.2 Differentiation of C2C12 (skeletal muscle) cell lines[84]

For differentiation, cells were grown to confluence and the differential medium was changed to DMEM (97 %) supplemented with FBS (2 %) and antibiotic (1 %). Cells were incubated with this medium for 1 week and every 2 days, the culture medium was substituted with fresh medium until the cells fully differentiated. After 1 week of incubation, sodium palmitate (0.75 mM, 5 µL) dissolved in ethanol (95 %) was then introduced to the culture plates in order to induce type 2 diabetes. The culture plates were then incubated at 37°C for 10 hours.

3.4.2 Treatment of C1C12 (skeletal muscle) cell lines[84]

For treatment, the growth medium was changed and coordination compounds in liquid form (10^{-4} M, 5 µL) were added to 4 differentiated cell cultured plates. The control plate was not treated and metformin (5 µL) was added to one of the test culture plate. Cell lines were then incubated for 48 hours after treatment. After treatment, the cells reached 80-100 % confluence. The cells are shown in Figures 19, 20, 21, 22, 23 and 24.

CHAPTER 4

4. RESULTS

4.1 Preparation of compounds

The percentage yields of the solid complexes i.e. bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato(picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complexes that were obtained during synthesis are given in Table 3.

Table 3 Percentage yields of the synthesized compounds

Coordination compound	Percentage yields %
Bis(maltolato)zinc(II)	72
Bis(picolinato)zinc(II)	74
Maltolato(picolinato)zinc(II)	52
[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]	66
(2.2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]	48

4.2 Characterization of compounds

4.2.1 Infrared spectroscopy

The absorption peaks of bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato(picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] are recorded in Table 4 while the corresponding spectra are compiled in Figures 4, 5, 6, 7 and 8.

Table 4 Infrared spectra of the compounds and their corresponding assignments

Coordination compound	Ligand Vibration (cm^{-1})	Assignments[76-79,83]
Bis(maltolato)zinc(II)	3254	υ_s(O-H)
	1611	δ_{as}(O-H)
	1364	υ_s(C-O)
	1305	υ_s(C-O)
	1455	υ_{as}(C=C)
	712	υ(C-C)
	478	υ_s(Zn-O)
Bis(picolinato)zinc(II)	3097	υ_s(O-H)
	1621	υ(C=N)
	1565	υ_{as}(C=C)
	1589	υ_s(C-O)
	1477	υ_s(C-N)
Maltolato(picolinato)zinc(II)	1606	δ_{as}(O-H)
	1480	υ_s(C-N)
	1455	υ_{as}(C=C)
	1362	υ_s(C-O)
	710	υ(C-C)
[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]	1485	υ(C-N)
	1449	υ(C=N)
	1253	$\upsilon(C_2\text{-}N)$
	1071	υ(C-S)
	942	υ(C=S)
(2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]	2927	υ(-CH)
	1593	υ_s(C-H)
	1489	δs(C-H)
	1438	υ(C=N)
	1284	$\upsilon(C_2\text{-}N)$
	958	υ(C=S)

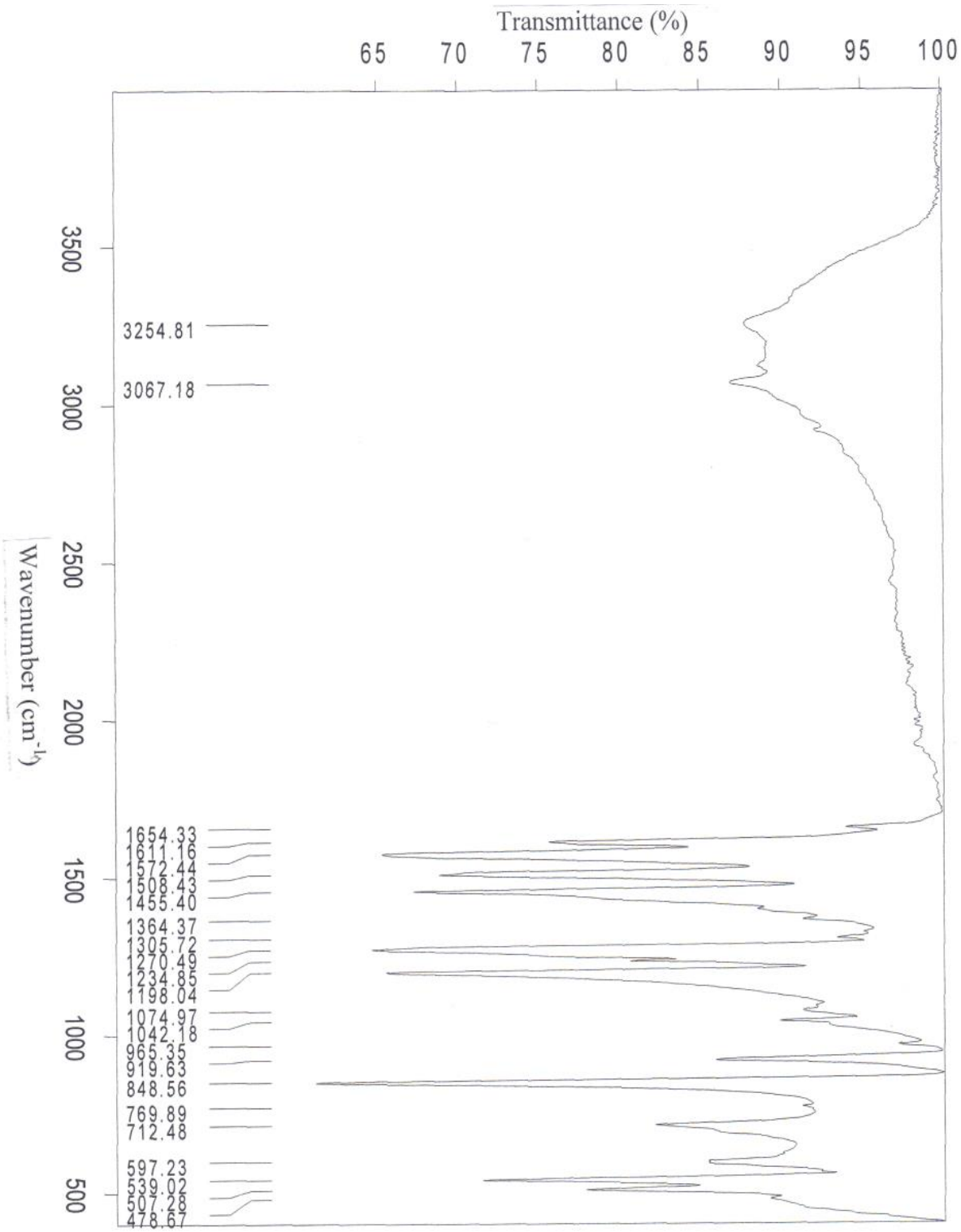


Figure 4 Infrared spectrum of bis(maltolato)zinc(II) complex.

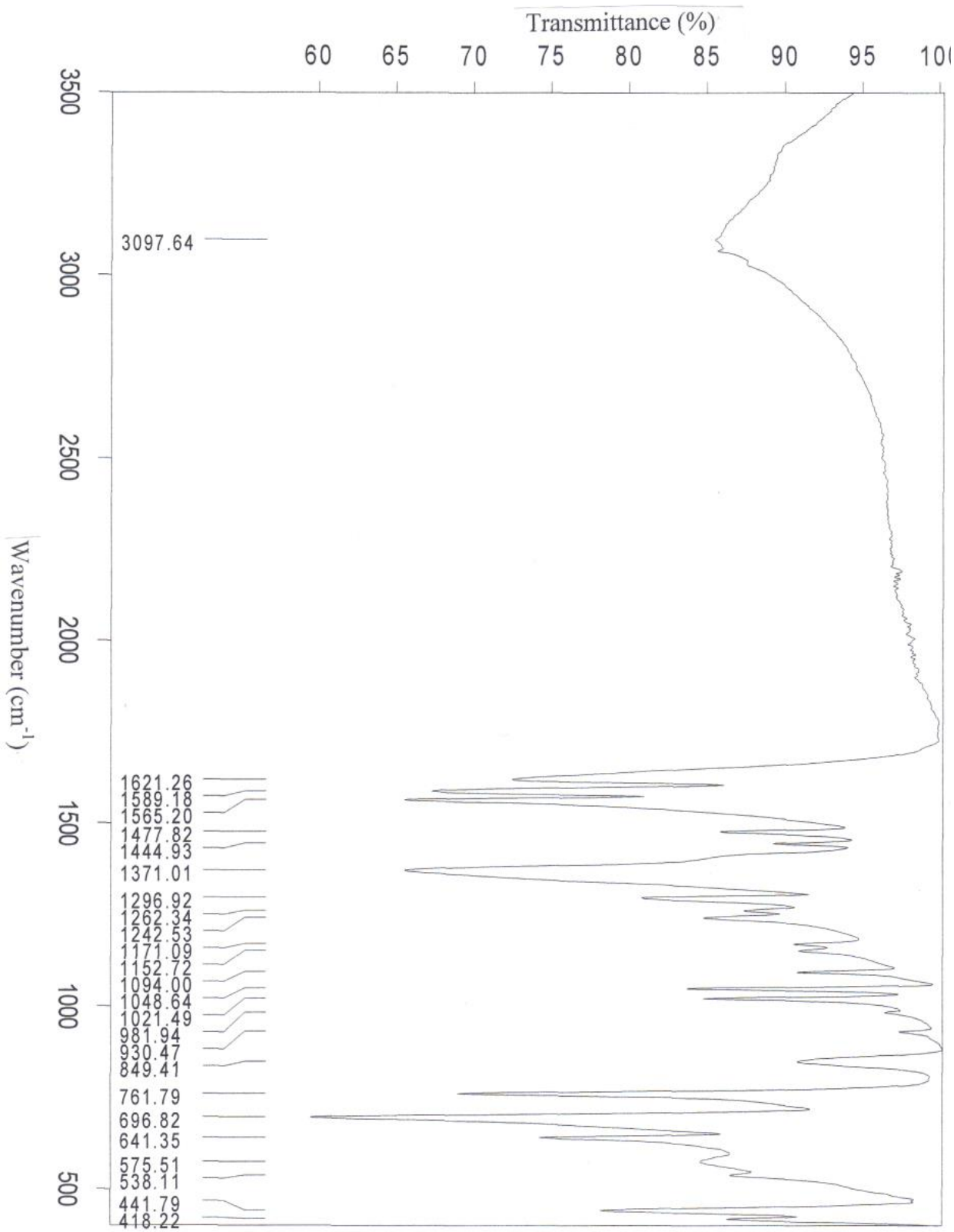


Figure 5 Infrared spectrum of bis(picolinato)zinc(II) complex.

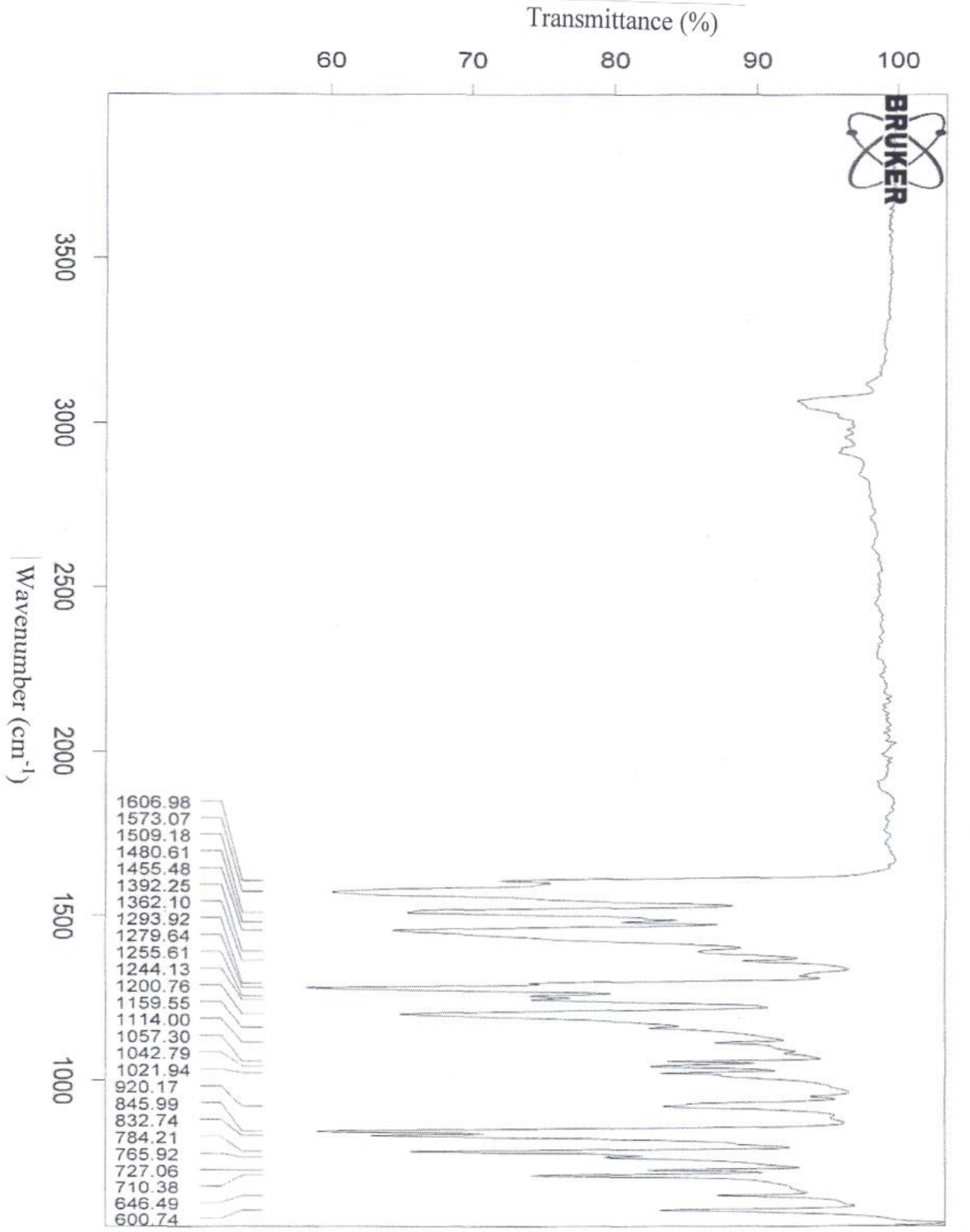


Figure 6 Infrared spectrum of maltolato(picolinato)zinc(II) complex.

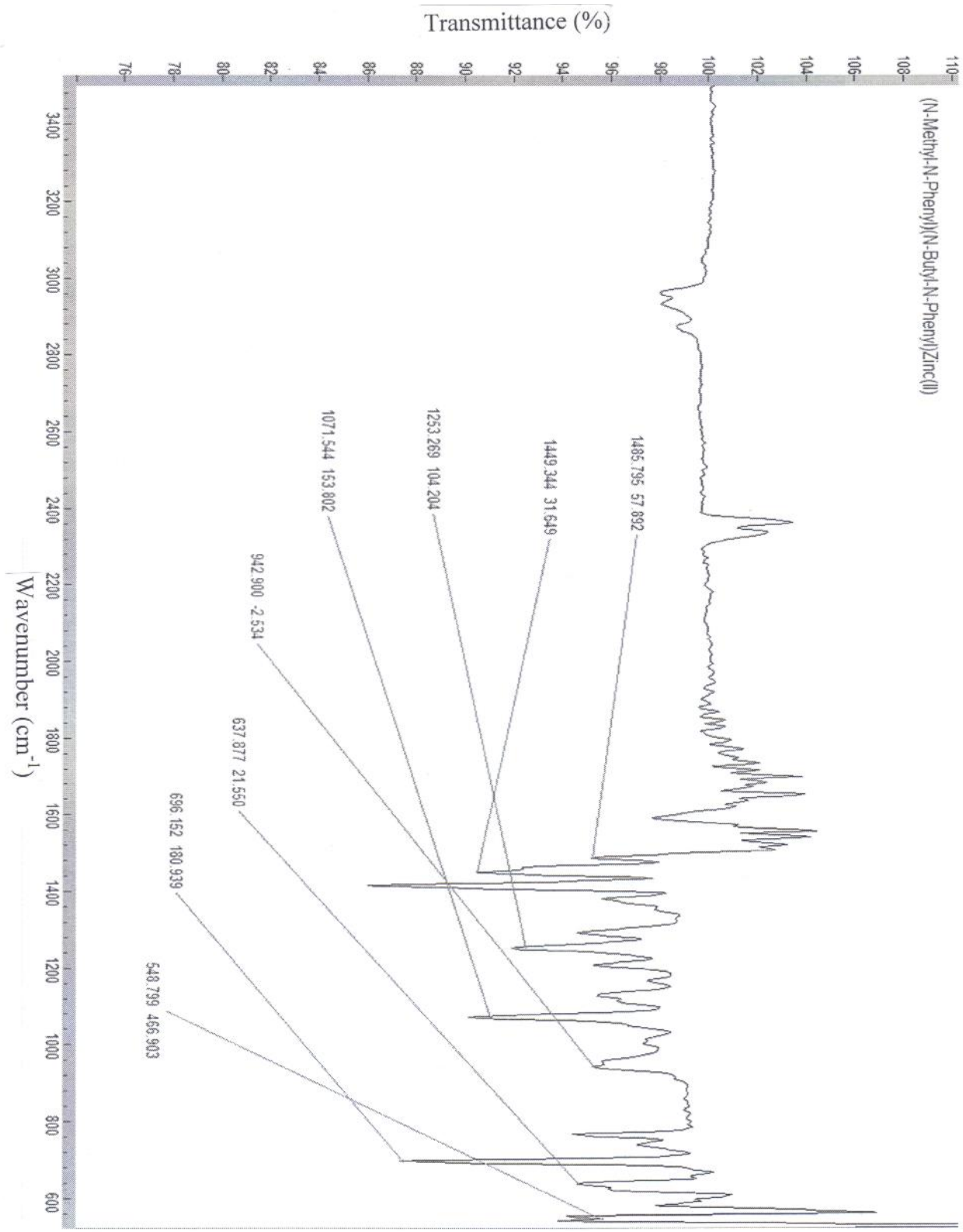


Figure 7 Infrared spectrum of [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

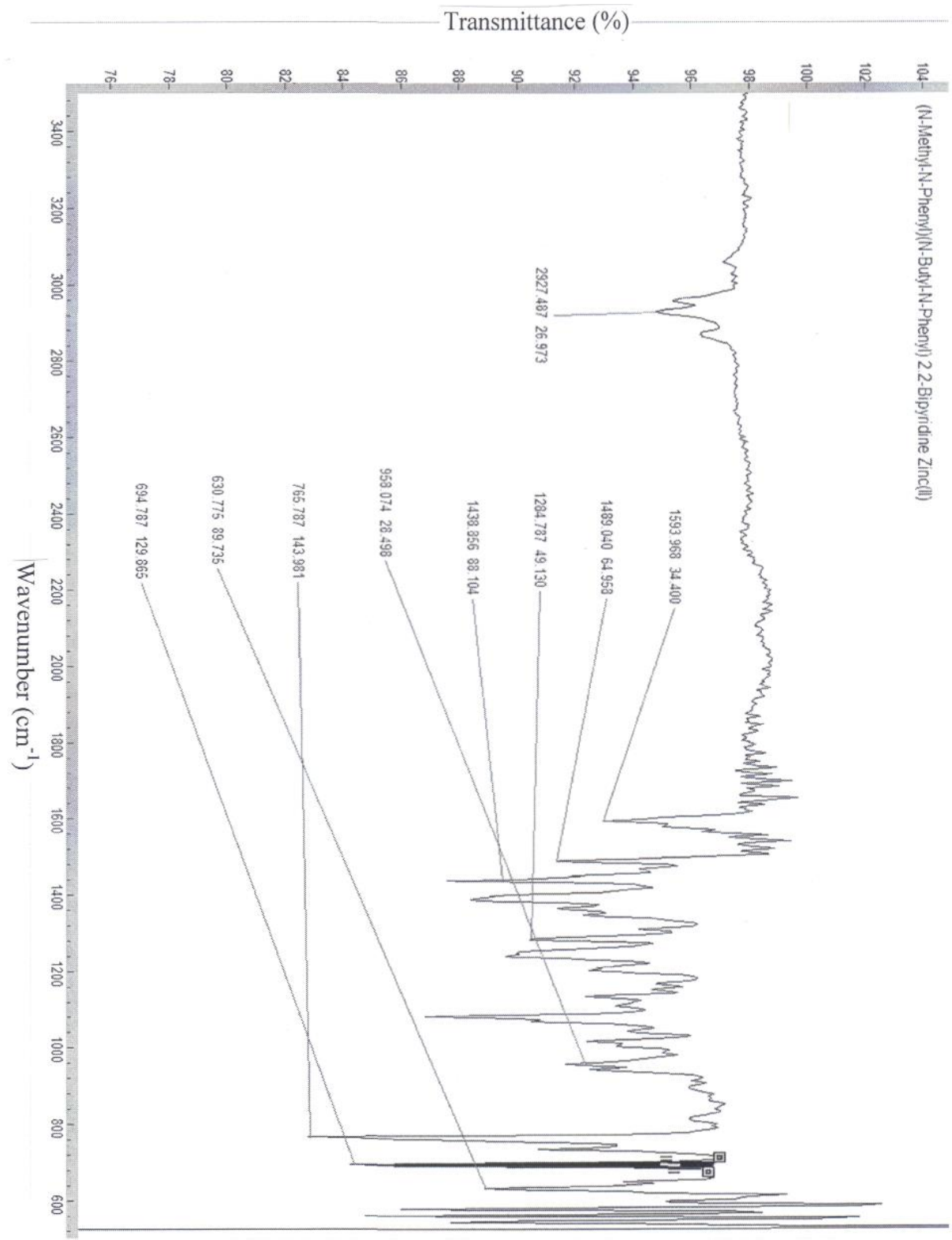


Figure 8 Infrared spectrum of (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex**.**

4.2.2 Ultraviolet-visible spectroscopy

The ultraviolet-visible spectra of the complexes bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato(picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] are displayed in Figures 9, 10, 11, 12 and 13.

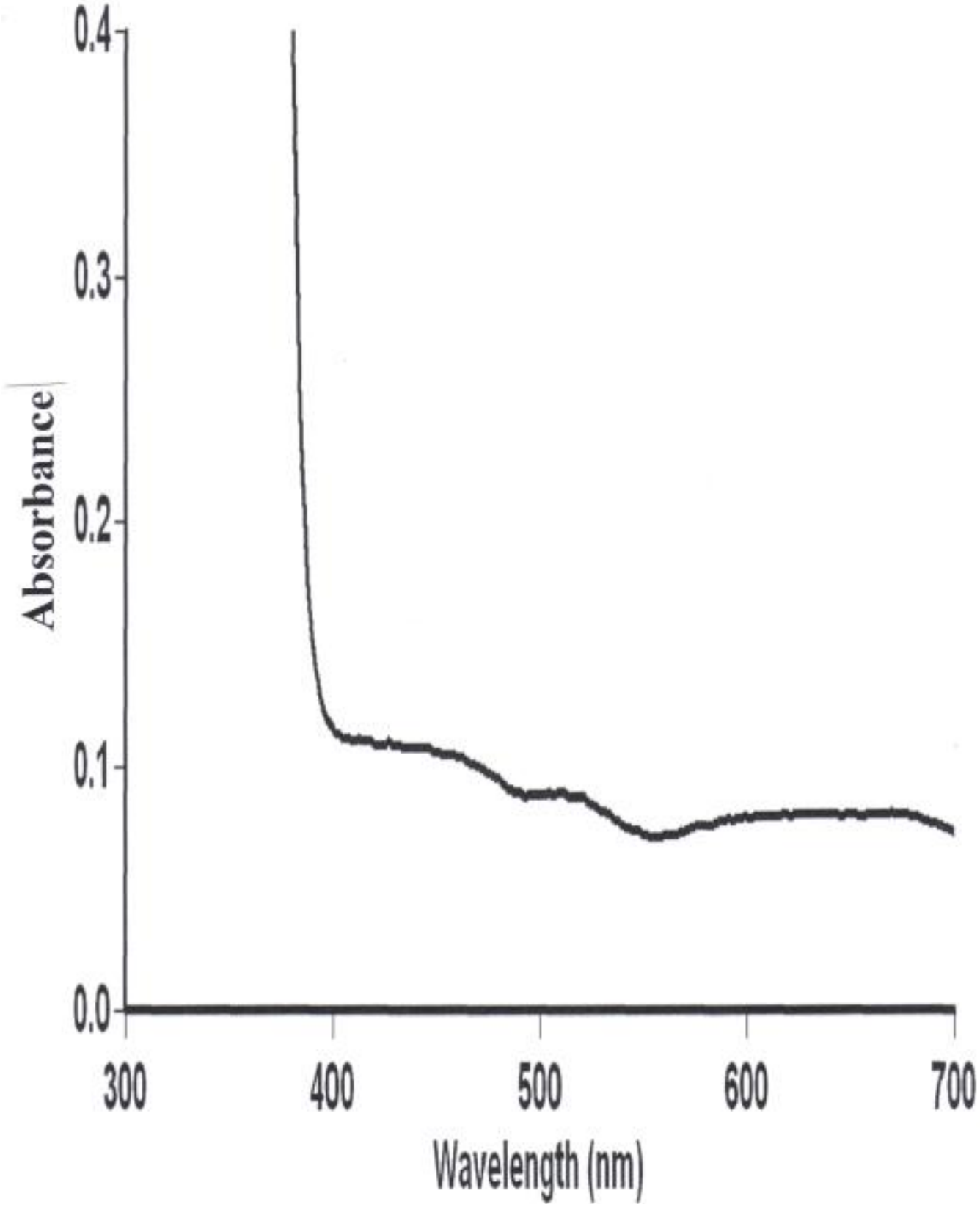


Figure 9 Ultraviolet-visible spectrum of bis(maltolato)zinc(II) complex.

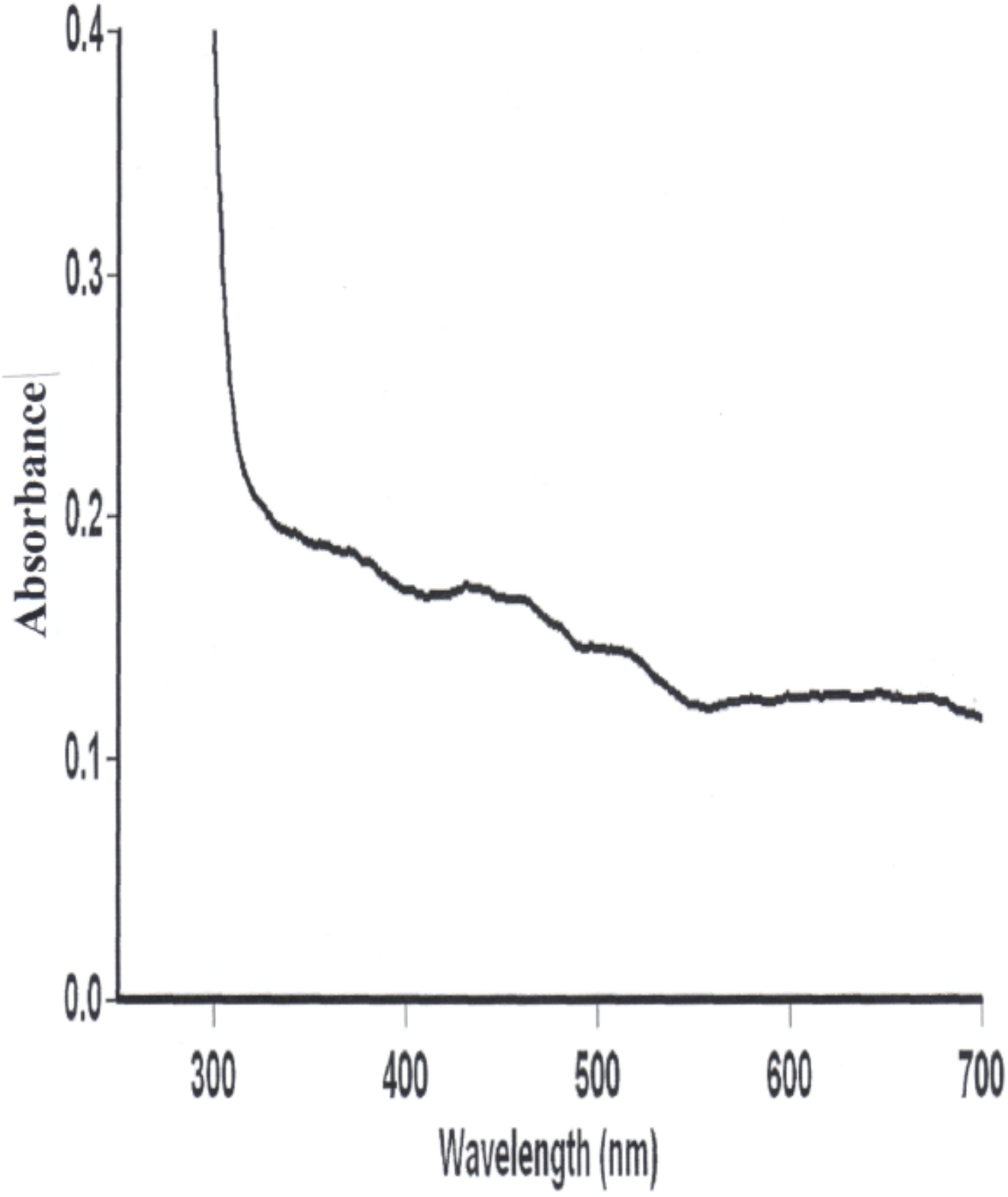


Figure 10 Ultraviolet-visible spectrum of bis(picolinato)zinc(II) complex

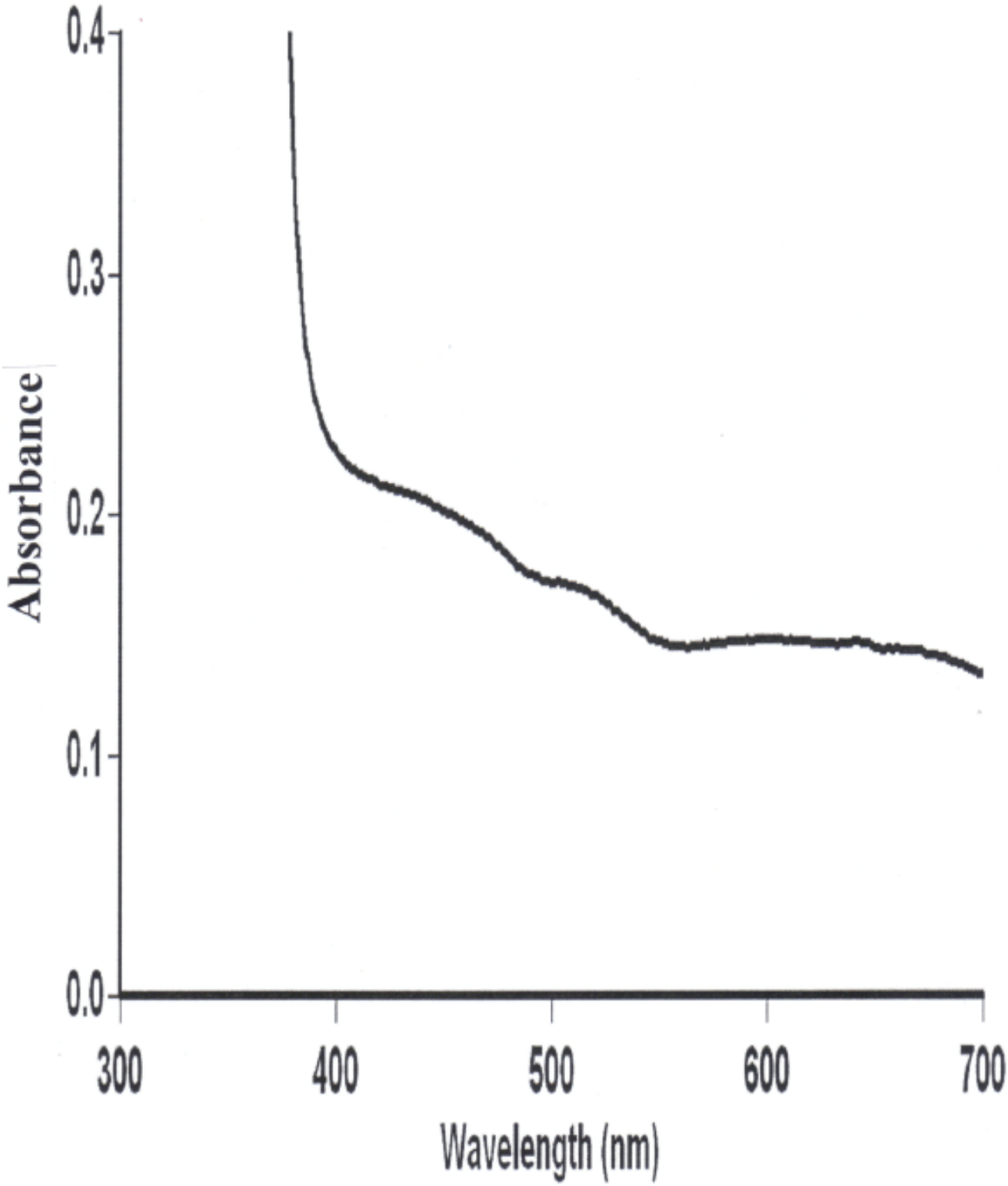


Figure 11 Ultraviolet-visible spectrum of maltolato(picolinato)zinc(II) complex

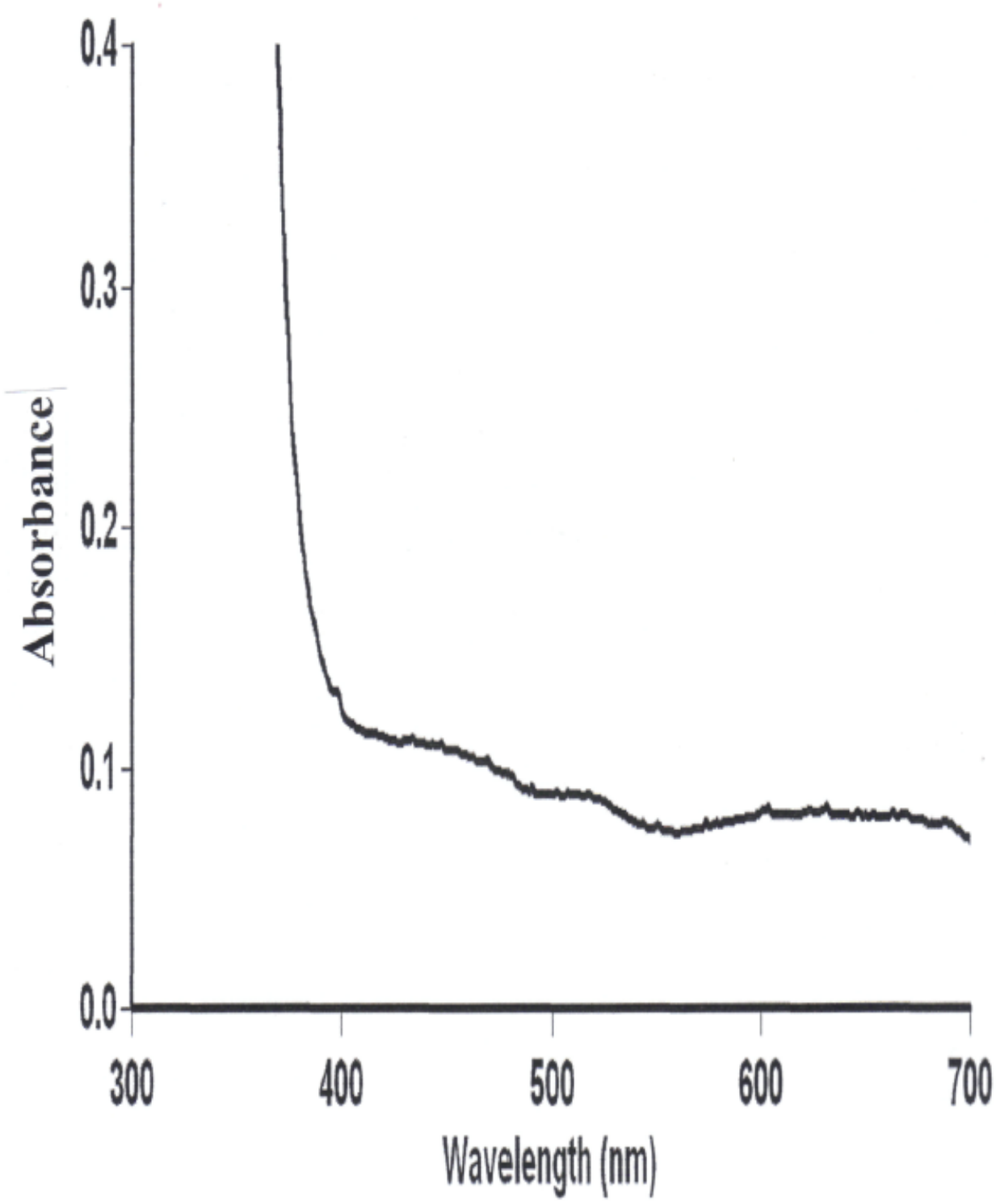


Figure 12 Ultraviolet-visible spectrum of [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

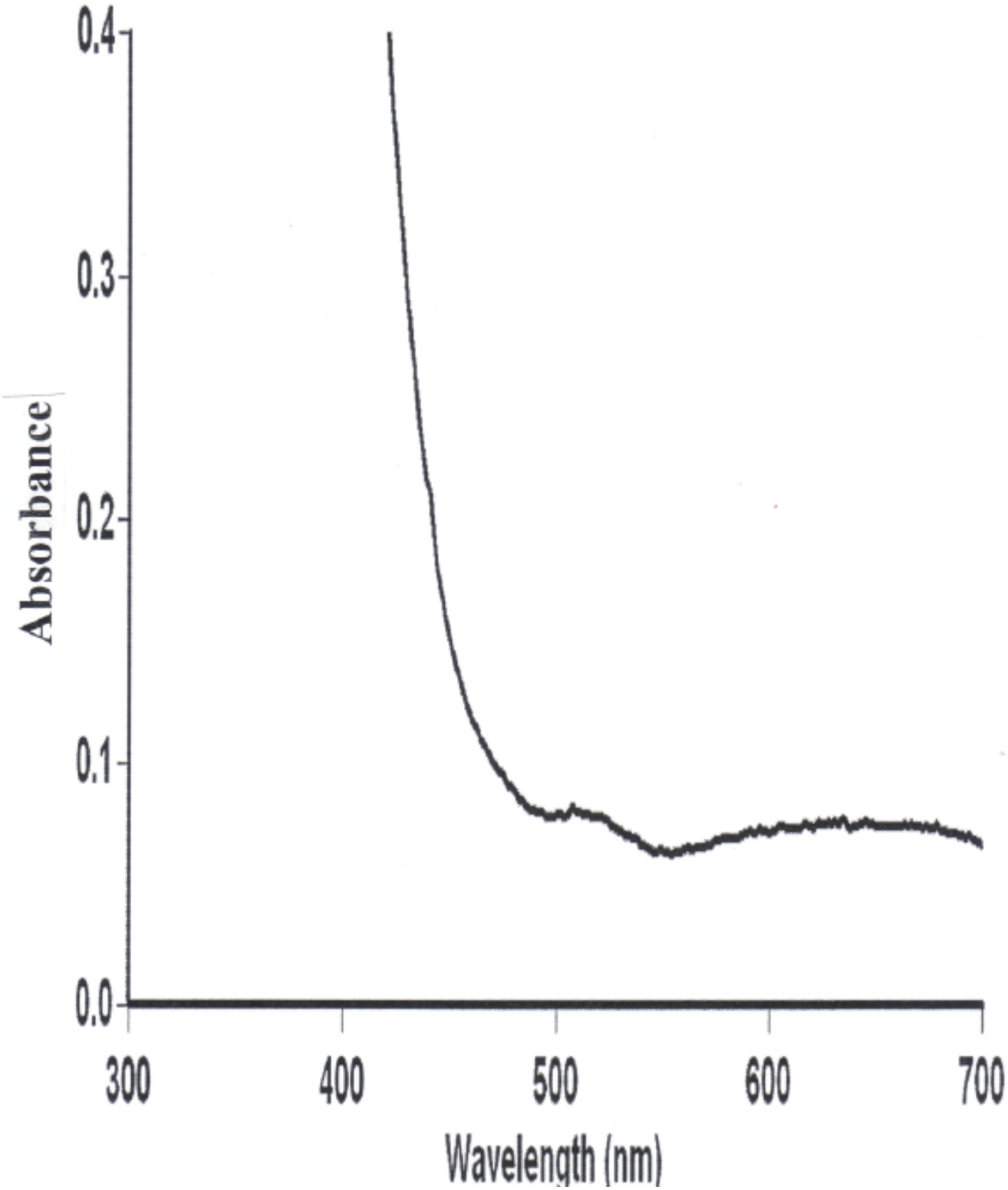


Figure 13 Ultraviolet-visible spectrum of (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

4.2.3 X-ray crystallography

The single-crystal XRD ortep diagrams of the mono(aqua)bis(maltolato)zinc(II), di(aqua)bis(maltolato)zinc(II), mono(aqua)bis(picolinato)zinc(II), di(aqua)bis(picolinato)zinc(II) and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complexes are compiled in Figures 14, 15, 16, 17 and 18.

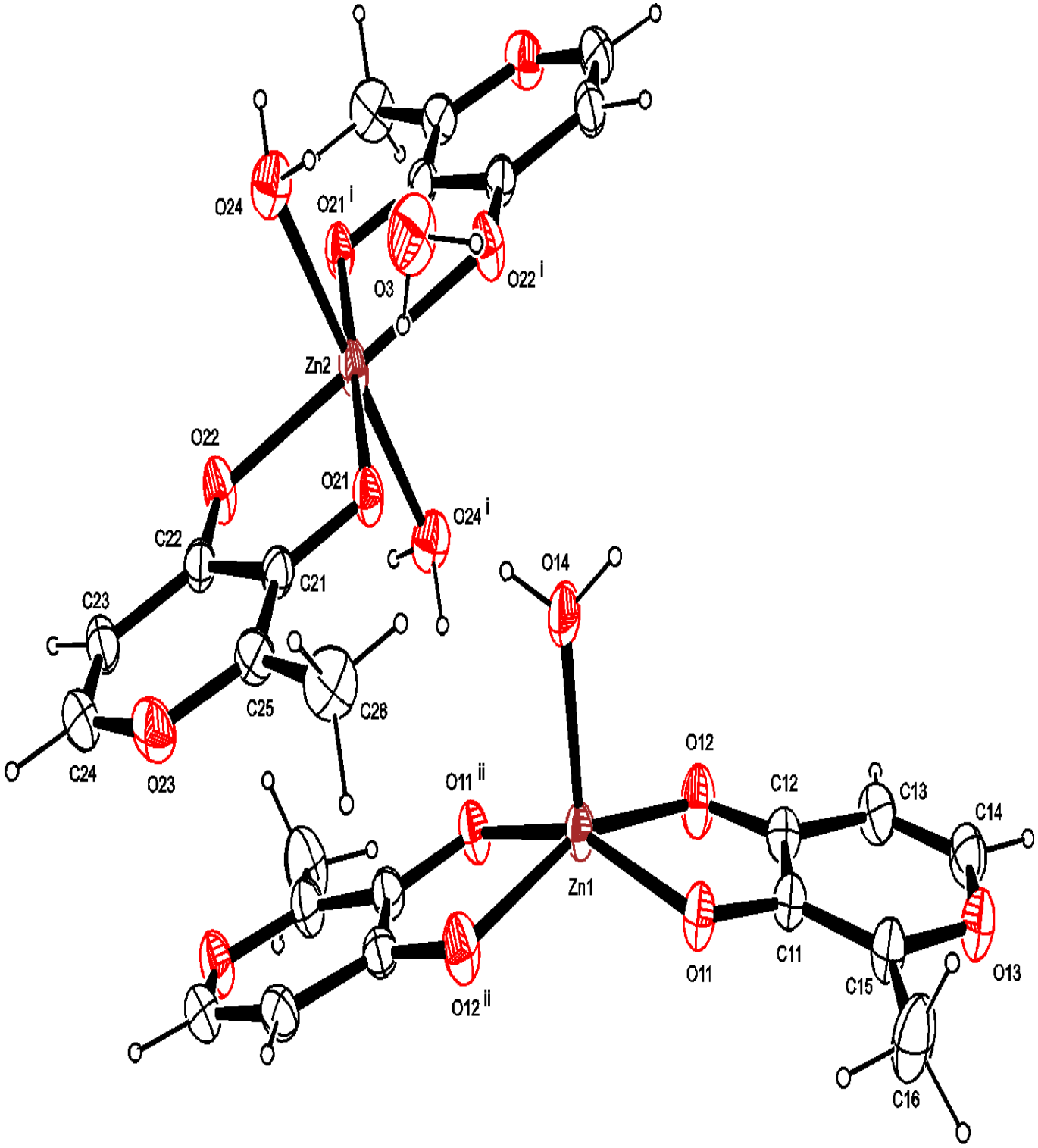


Figure 14 Crystal structure of mono(aqua)bis(maltolato)zinc(II) complex.

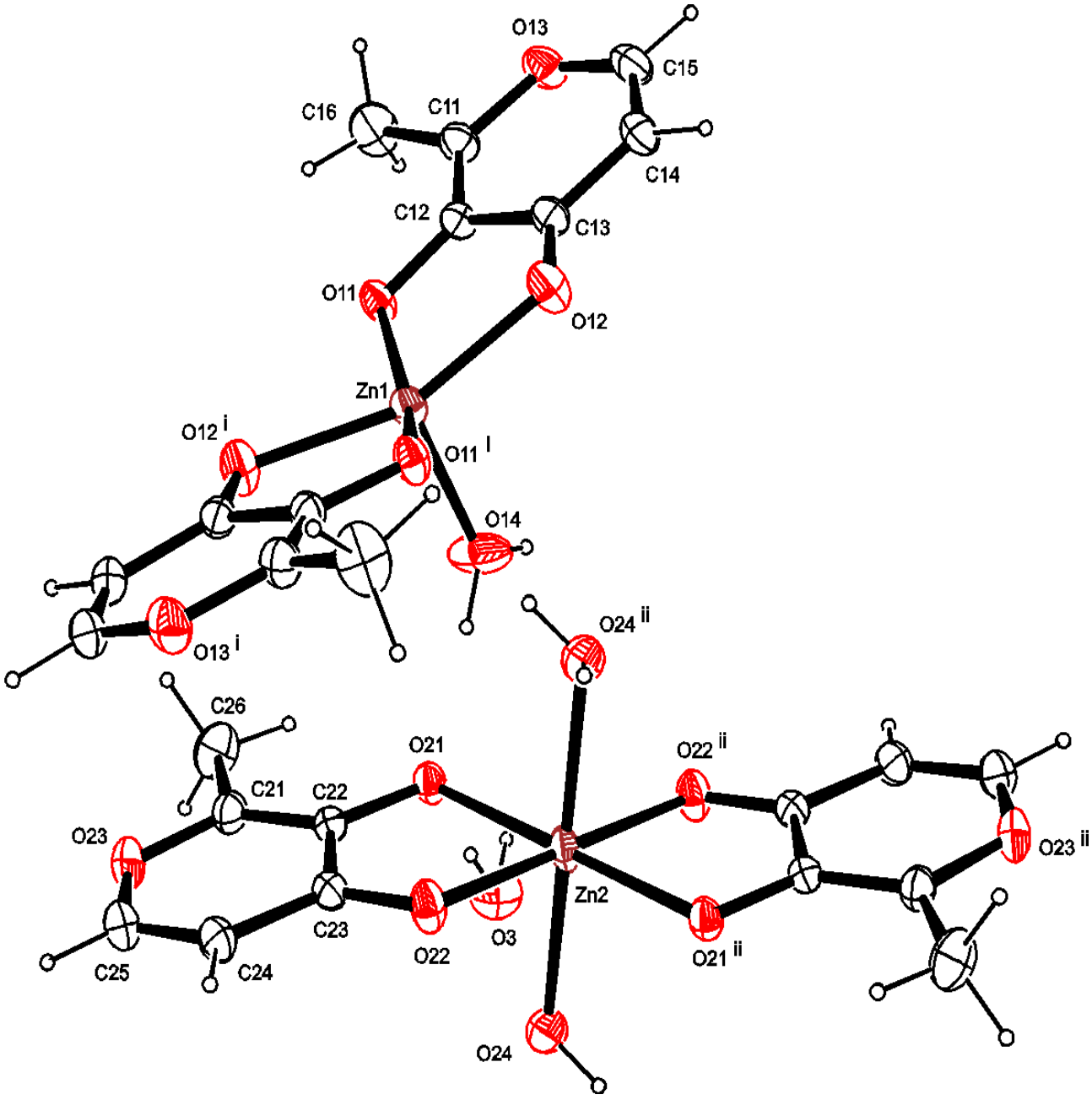


Figure 25 Crystal structure of di(aqua)bis(maltolato)zinc(II) comlplex**.**

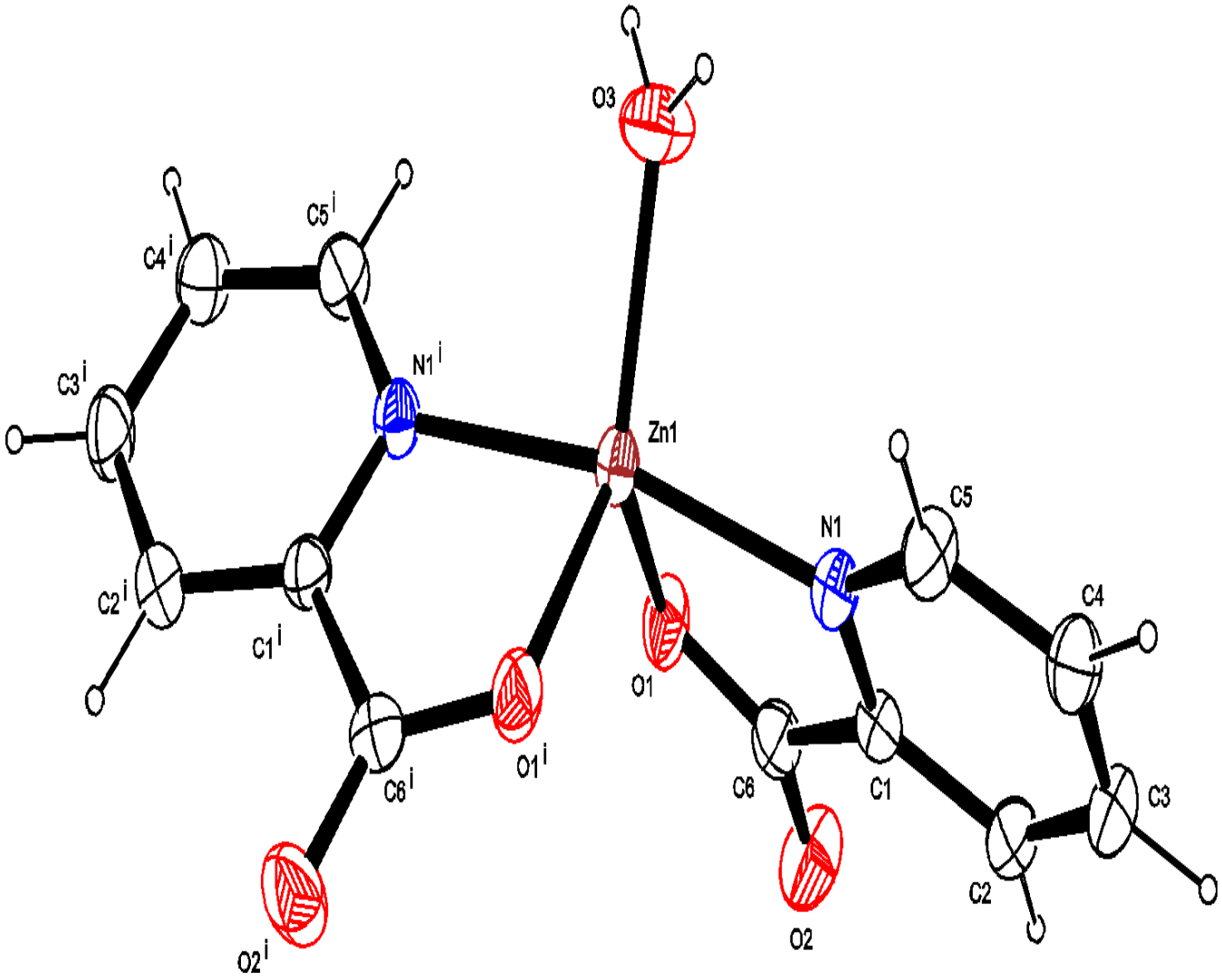


Figure 16 Crystal structure of mono(aqua)bis(picolinato)zinc(II) complex.

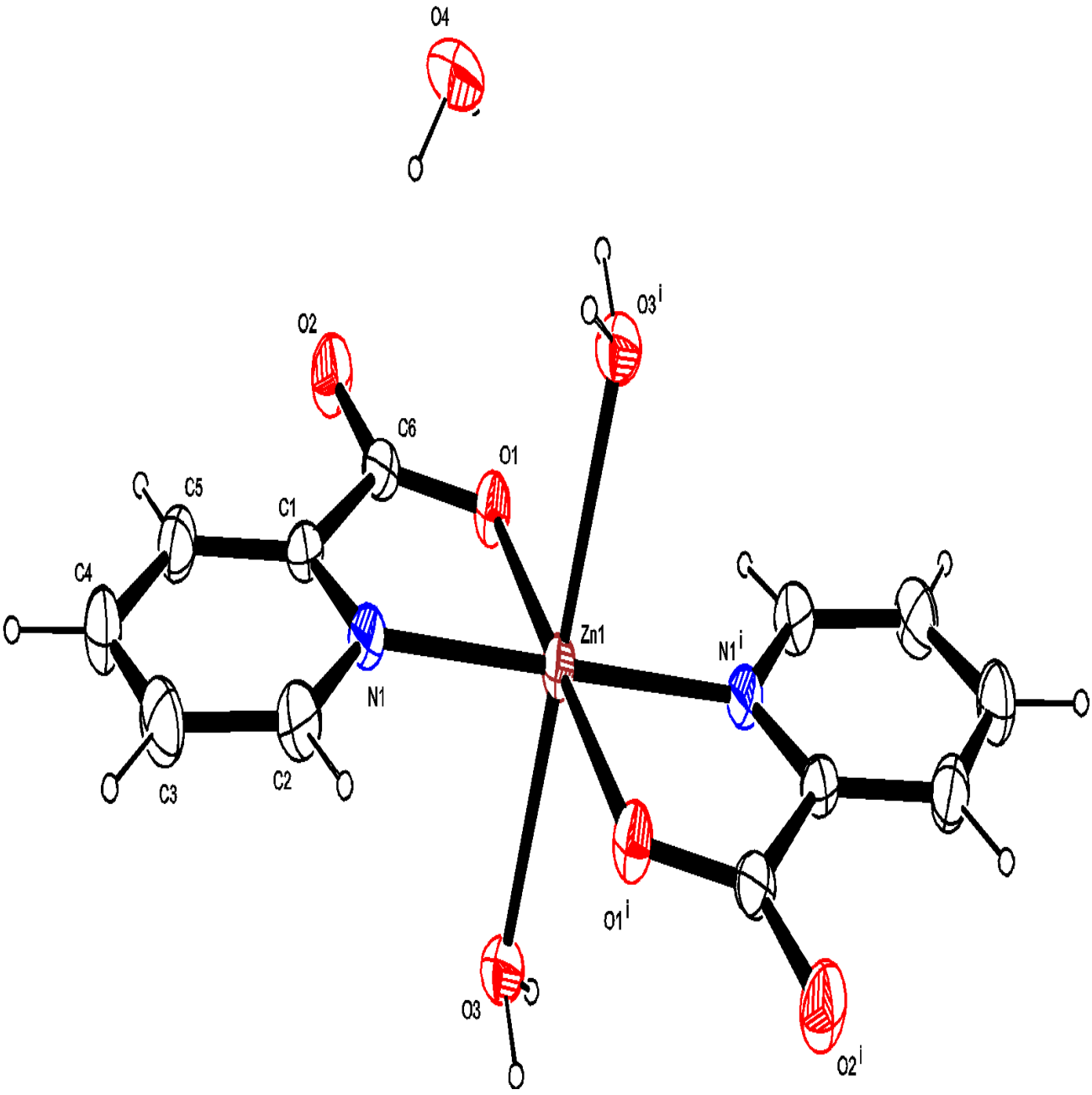


Figure 17 Crystal structure of di(aqua)bis(picolinato)zinc(II) complex.

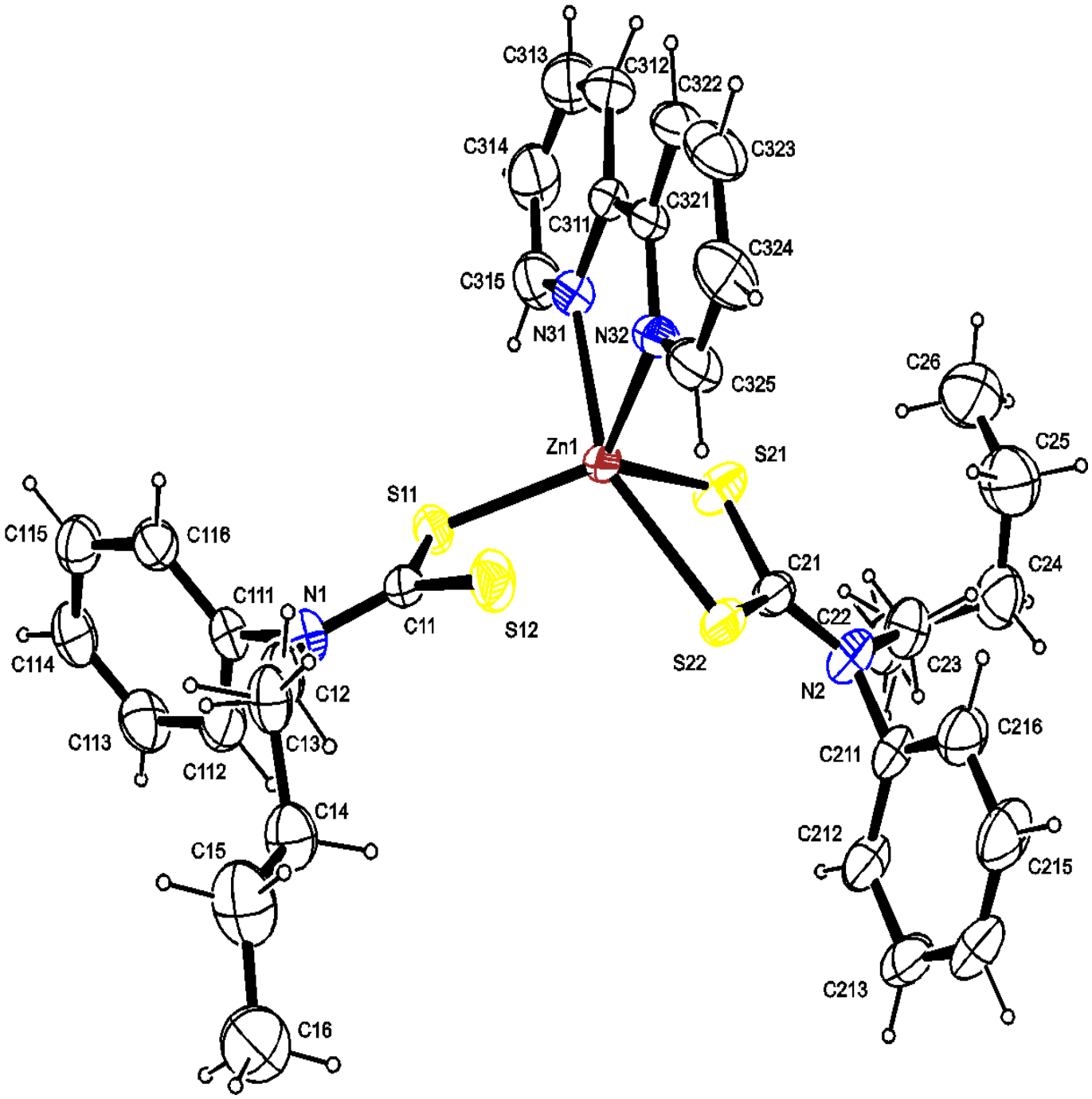


Figure 18 Crystal structure of (2,2-bipyridine)[(N-methyl-N-phenyl)(N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

4.2.4 Microanalysis

Microanalysis of the synthesized zinc(II) coordination compounds produced results that are in close agreement with the calculated percentages of carbons, hydrogens, nitrogens and oxygens and the results for the compounds are summarized in Tables 5, 6, 7, 8, 9, 10 and 11.

Table 5 Microanalysis of mono(aqua)bis(maltolato)zinc(II)

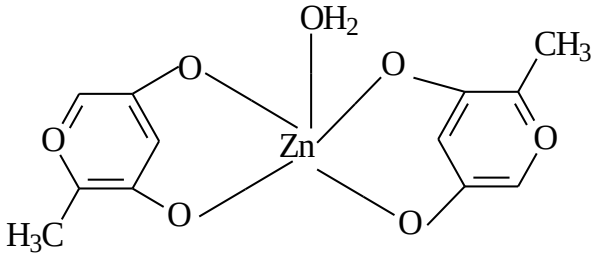
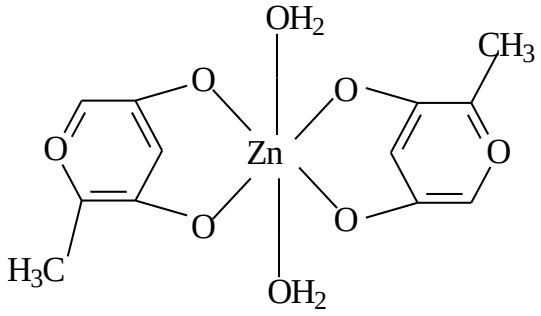
Formula	**$C_{12}H_{12}O_7Zn$(II)**		
Molecular Weight (g/mol)	334.52		
Structure			
	Microanalysis		
	C	**H**	**O**
Found	43.06	3.59	33.46
Calculated	43.09	3.62	33.48

Table 6 Microanalysis of di(aqua)bis(maltolato)zinc(II)

Formula	**$C_{12}H_{14}O_8Zn$(II)**		
Molecular Weight (g/mol)	351.62		
Structure			
	Microanalysis		
	C	**H**	**O**
Found	40.97	3.97	36.38

Calculated	40.99	3.99	36.40

Table 7 Microanalysis of mono(aqua)bis(picolinato)zinc(II)

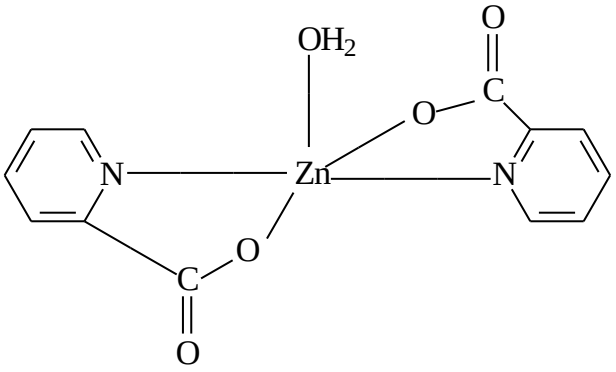
Formula	**$C_{12}H_{10}O_5N_2Zn(II)$**			
Molecular Weight (g/mol)	327.61			
Structure				
	Microanalysis			
	C	**H**	**N**	**O**
Found	43.97	3.06	24.40	8.53
Calculated	43.99	3.08	24.42	8.55

Table 8 Microanalysis of di(aqua)bis(picolinato)zinc(II)

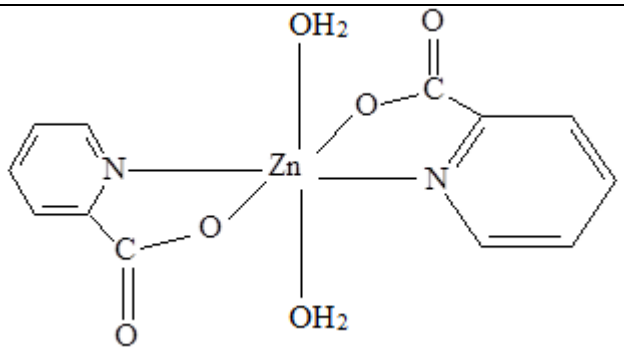
Formula	**$C_{12}H_{12}O_6N_2Zn(II)$**			
Molecular Weight (g/mol)	345.58			
Structure				
	Microanalysis			
	C	**H**	**N**	**O**
Found	41.68	3.48	8.13	14.85
Calculated	41.71	3.50	8.15	14.87

Table 9 Microanalysis of di(aqua)maltolato(picolinato)zinc(II)

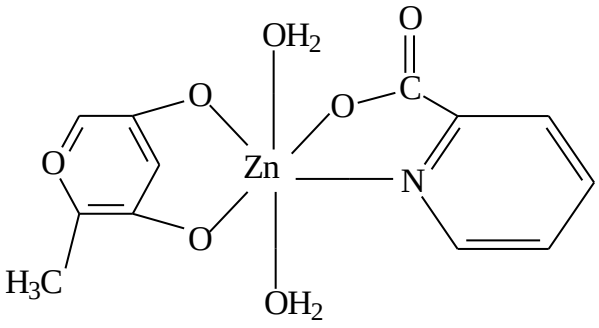
Formula	**$C_{12}H_{13}O_7NZn(II)$**			
Molecular Weight (g/mol)	348.62			
Structure				
	Microanalysis			
	C	**H**	**N**	**O**
Found	41.31	3.74	4.00	32.10
Calculated	41.34	3.76	4.02	32.12

Table 10 Microanalysis of [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]

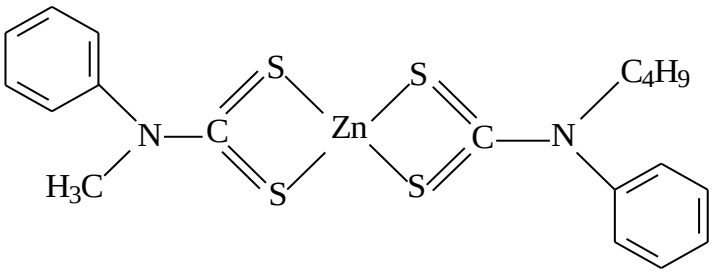
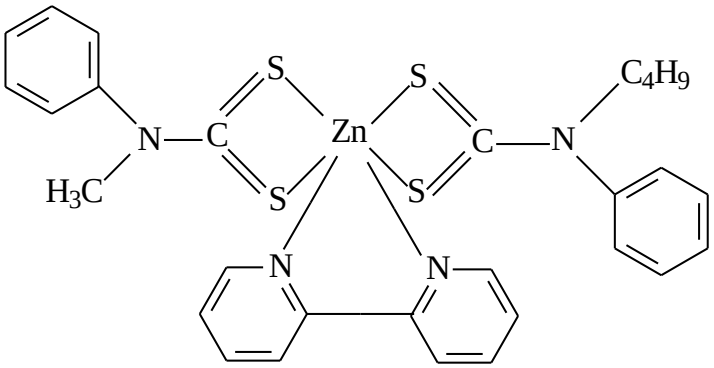
Formula	**$C_{19}H_{22}N_2S_4Zn(II)$**			
Molecular Weight (g/mol)	472.05			
Structure				
	Microanalysis			
	C	**H**	**N**	**S**
Found	48.30	4.67	5.89	27.13
Calculated	48.34	4.70	5.93	27.17

Table 11 Microanalysis of (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)]

Formula	**$C_{29}H_{30}N_4S_4Zn(II)$**			
Molecular Weight (g/mol)	628.238			
Structure				
	Microanalysis			
	C	**H**	**N**	**S**
Found	55.40	4.79	8.90	20.40
Calculated	55.44	4.81	8.92	20.42

4.3 Anti-diabetic biological studies

A total of 6 cell culture plates were prepared for 4 coordination complexes, the control plate and the positive test plate (metformin) to treat the cells after inducing Type 2 Diabetes Mellitus.

4.3.1 Cell Culture

The C2C12 (muscle) cell lines were cultured and maintained successfully. The cells reached 80-100% confluency.

4.3.2 Differentiation and treatment of C2C12 (skeletal muscle) cell lines

Sodium palmitate (0.75mM, 1mL) was then introduced to the cell lines in order to induce Type 2 Diabetes Mellitus and incubated at 37°C for 10 hours. After the incubation period, the cells were then treated with liquefied coordination compounds (10^{-4}MC, 5µL). Cell lines treated with complexes bis(maltolato)zinc(II), bis(picolinato)zinc(II), maltolato(picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] were observed using a microscope and are compiled in Figures 19, 20, 21, 22, 23 and 24.

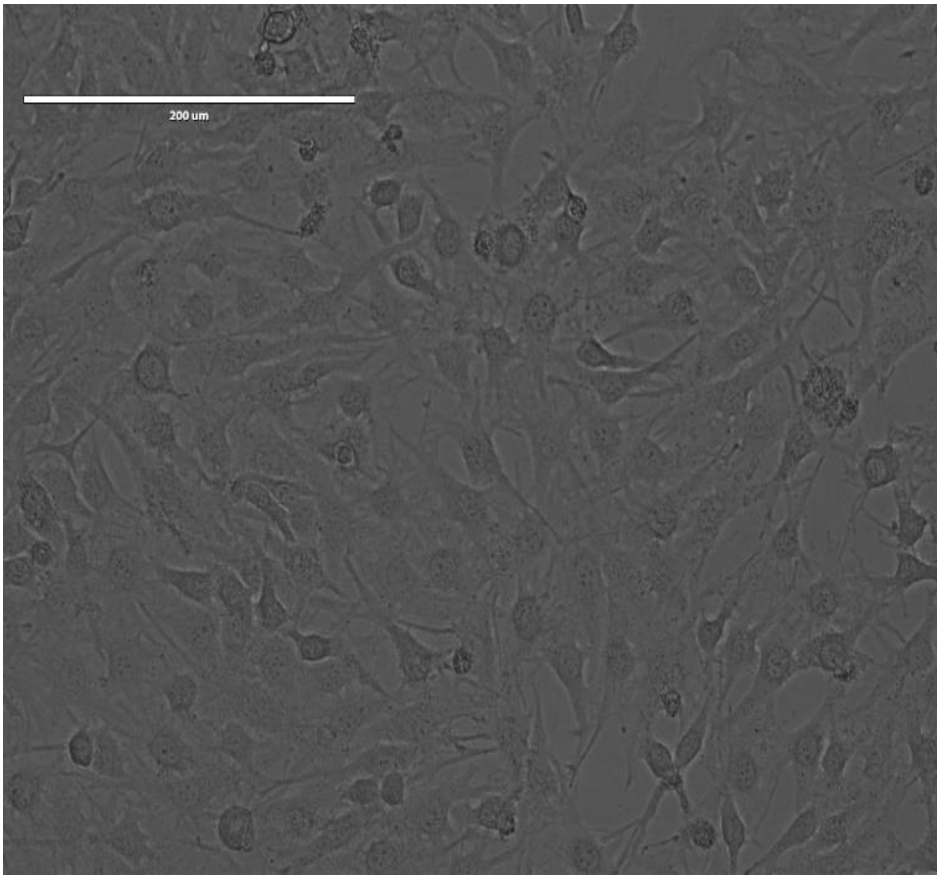


Figure 19 Culture plate prepared for bis(maltolato)zinc(II) complex

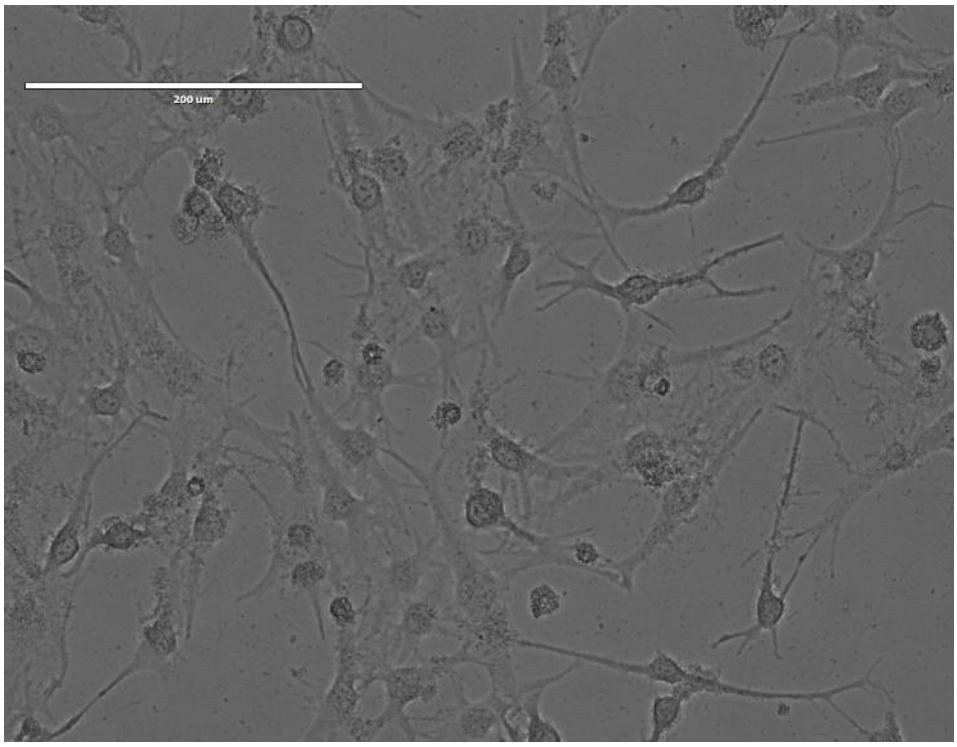


Figure 20 Culture plate prepared for bis(picolinato)zinc(II) complex

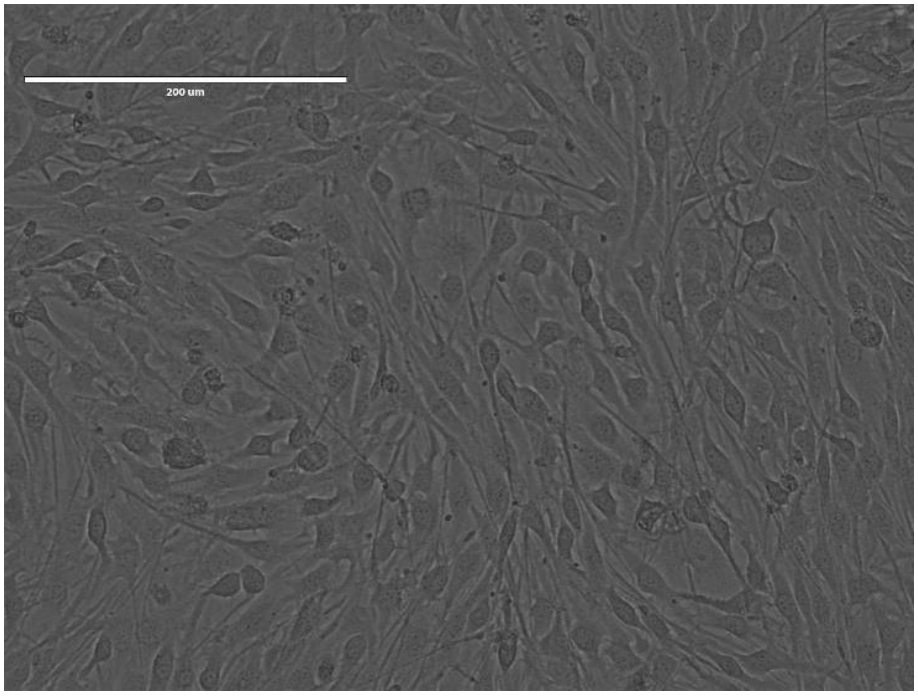


Figure 21 Culture plate prepared for [(N-methyl-N-phenyl)(N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

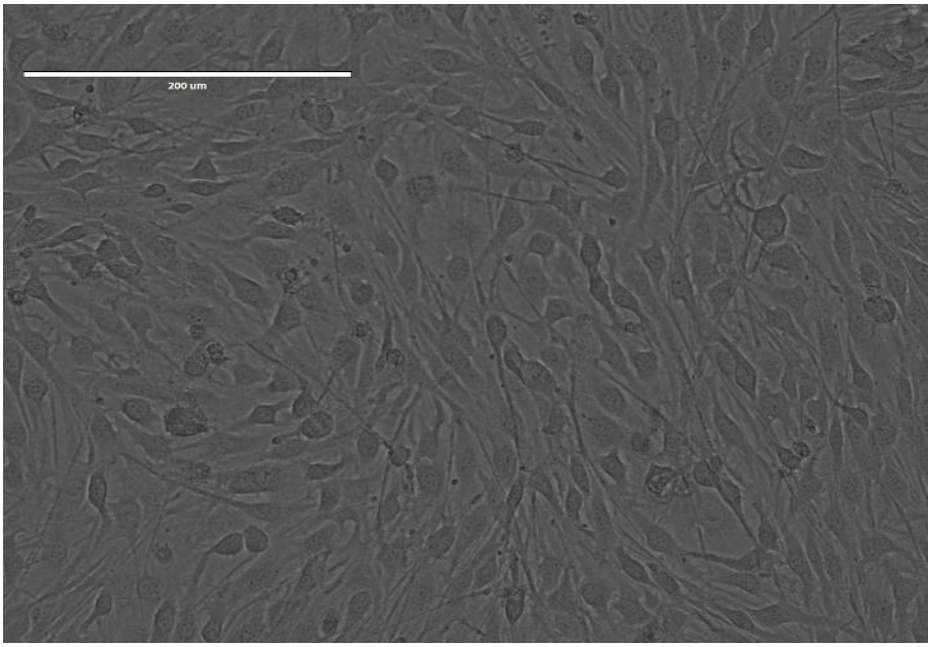


Figure 22 Culture plate prepared for (2,2-bipyridine)[(N-methyl-N-phenyl)(N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

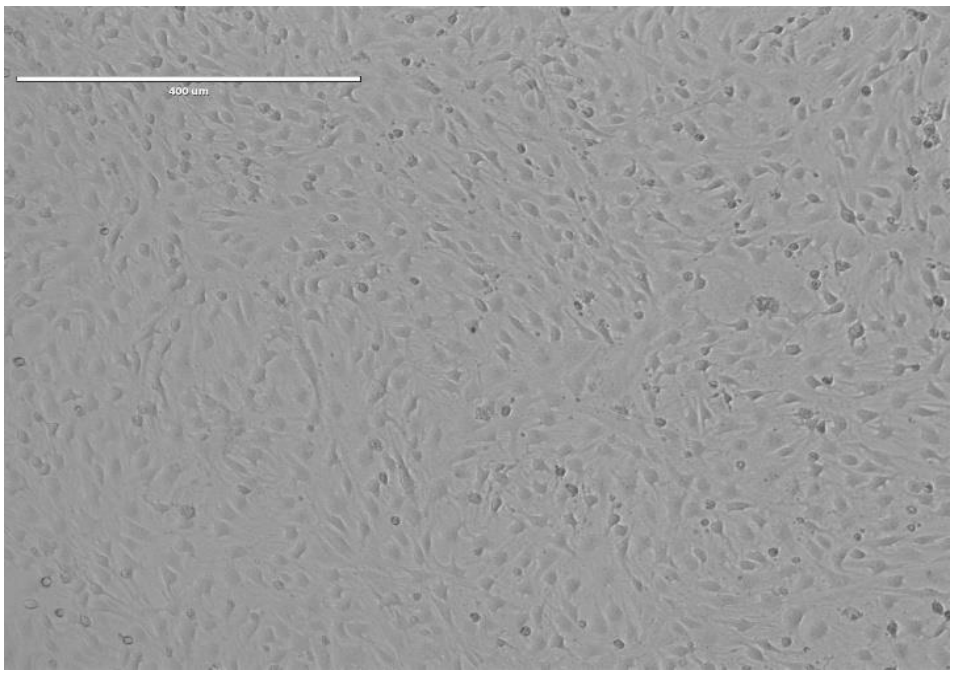


Figure 33 Culture plate prepared for metformin

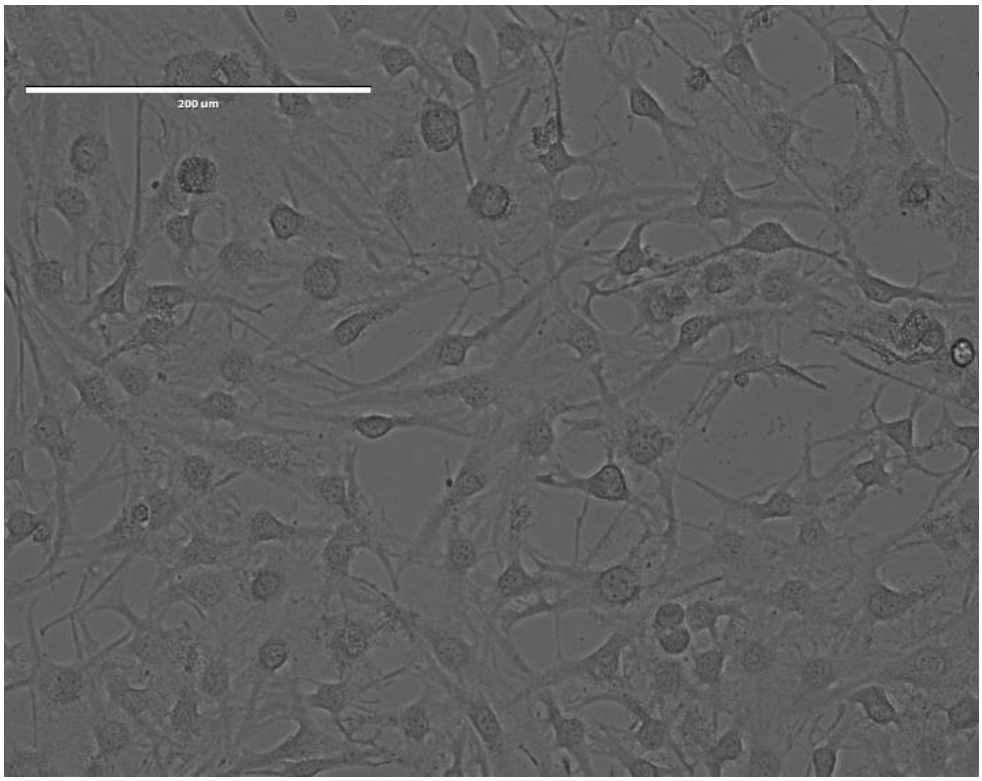


Figure 24 Culture plate prepared for control

CHAPTER 5

5. DISCUSSION

5.1 Preparation of new compounds

The preparation of new compounds maltolato (picolinato)zinc(II), [(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] was a mile stone in the work of antidiabetic zinc(II) complexes. These compounds were characterized by Infrared spectroscopy, Ultraviolet-visible spectroscopy, single crystal X-ray diffraction and microanalysis.

5.2 Characterization of compounds

5.2.1 Infrared spectra of the compounds

The use of infrared spectroscopy in the identification of functional groups of the coordination compounds of the metal-amino acid and dithiocarbamate complexes is well documented.[68-71,75] The peaks identified and listed below indicate the frequencies of typical zinc(II) metal-amino acid and dithiocarbamate coordination compounds.

Table 12 Infrared frequency (cm^{-1}) and band assignments of amino acid complexes.

Coordination compound	Stretching vibration (O-H) (cm^{-1})	Antisymmetric vibration (C=C) (cm^{-1})	Stretching vibration (C-O) (cm^{-1})	Stretching vibration (C-N) (cm^{-1})	Stretching vibration (Zn-X) (cm^{-1})	Reference
Bis(maltolato) oxovanadium(IV)	3290	1485	1335	-	545	68,75
Bis(maltolato)zinc(II)	3254	1455	1364, 1305	-	478	This work
Bis(picolinato) oxovandium(IV)	3500	1640	1600	1548	440	68,75

Bis(picolinato)zinc(II)	3097	1565	1589	1477	418	This work
Maltolato (picolinato)zinc(II)	1606	1565	1362	1480	-	This work

X=O, N

- The symmetric vibrations of the O-H molecules generate broad and weak IR-bands which appear in the region 3097-3500 cm^{-1}. In the maltolato(picolinato)zinc(II) complex, the O-H molecule generates a small weak peak recorded at 1606 cm^{-1}.
- Antisymmetric vibrations of the carbon (C=C) groups, existing within the pyrone ring of maltol ligand and the pyridine ring of picoline ligand are well represented at 1455 cm^{-1} and 1565 cm^{-1}. In the mixed ligand complex, it is represented at 1565 cm^{-1}.
- The occurrence of strong sharp bands at 1364 cm^{-1}, 1305 cm^{-1}, 1589 cm^{-1} corresponds to C-O symmetric vibrations. In the maltolato (picolinato)zinc(II) complex, a small shoulder band is also indicated at 1362 cm^{-1}.
- In the spectrum of bis(picolinato)zinc(II) complex, the occurrence of the C-N stretching vibration peak at 1477 cm^{-1} is a characteristic assignment of the carbon-nitrogen (C-N) bond formation. In the mixed ligand complex, the carbon-nitrogen bond is well represented at 1480 cm^{-1}.
- In the spectrum of the bis(maltolato)zinc(II) complex, the appearance of the Zn-O stretching vibration peak at 478 cm^{-1} is a characteristic assignment for the coordination bond existing between the metal ion and oxygen atoms of the carbonyl groups from the 2 maltol ligands. This assignment is not shown in the mixed ligand complex as it is not included in the visible region.
- In the spectrum of the bis(picolinato)zinc(II) complex, a small, short and sharp band at 418 cm^{-1} corresponds to symmetric stretching mode for the Zn-N bond. In the spectrum of the mixed ligand complex, the Zn-N is not catered for in the visible region.

The assignments of the zinc(II) amino acid compounds were found to be in good agreement with bis(maltolato)oxovanadium(IV)[68,75] and bis(picolinato)oxovandium(IV) [6,75] complexes.

Table 13 Infrared frequency (cm^{-1}) and band assignments of dithiocarabamate complexes.

Coordination compound	Stretching vibration (C=N) (cm^{-1})	Stretching vibration (C=S) (cm^{-1})	Stretching vibration (C_2-N) (cm^{-1})	Reference
[(N-methyl-N-phenyl, N-ethyl-N-phenyl) dithiocarbamatozinc(II)]	-	995	1279	69-71
[(N-methyl-N-phenyl, N-butyl-N-phenyl) dithiocarbamatozinc(II)]	-	942	1253	This work
(2,2-bipyridine)[(N-methyl-N-phenyl,N-ethyl-N-phenyl) dithiocarbamatozinc(II)]	1465	969	1263	70,71
(2,2-bipyridine)[(N-methyl-N-phenyl,N-butyl-N-phenyl) dithiocarbamatozinc(II)]	1438	958	1284	This work

- In the spectra of the 2,2-bipyridine adducts of dithiocarbamate complexes, the single and strong peaks at 1465 cm^{-1} and 1438 cm^{-1} correspond to the C=N stretching vibration.
- The single and strong symmetric vibration peaks of the C=S dithiocarbamate molecules are outlined at 995 cm^{-1}, 942 cm^{-1}, 969 cm^{-1} and 958 cm^{-1}.
- The symmetric vibrations of the single C−N bonds are outlined at 1263, 1253.27 and 1284 cm^{-1} for the above mentioned complexes in Table 11.
- Bands associated with υ(M−S) in dithiocarbamate complexes are usually observed at 420-250 cm^{-1}. These peaks are not catered for within the visible region of the infrared spectra.

The assignments of these dithiocarbamate complexes were found to be in good agreement with [(N-methyl-N-phenyl,N-ethyl-N-phenyl)dithiocarbamatozinc(II)][69-71] and (2,2-bipyridine)[(N-methyl-N-phenyl,N-ethyl-N-phenyl)dithiocarbamatozinc(II)][70,71] coordination compounds.

5.2.2 Ultraviolet-visible spectra of the compounds

The ultraviolet-visible spectra of the zinc metal-complexes discussed are well documented and shown in Figures 9-13. It is evident enough to notice that there are no ultraviolet-visible absorption bands observed in Figures 9-13 due to the fact that zinc is a diamagnetic metal with $[Ar]d^{10}4s^2$ electron configuration. There are no d-d electron transitions in d^{10} metal complexes because the d orbitals are completely filled. Therefore, a Tanabe-Sugano diagram for d^{10} metal complexes is non-existent.

The UV-visisible spectra of the amino acid and dithiocarbamate ligands used to complex with the zinc(II) metal ion in this work, would be expected to absorb and show bands indicating their presence.

5.2.2.1 Maltol ligand

Maltol ligand absorption bands may be observed in the UV-vis spectra which displays intense absorption bands around 200-276 nm due to intraligand charge transfer. This also indicates the bathochromic shift of $\pi \rightarrow \pi^*$ electron transition which is ascribed to the interaction of the metal ions with the chromophores O-C=O when bonded with the zinc(II) metal ion through both hydroxyl and carbonyl groups.[88] The broad bands may also be assigned to Zn-dipic charge-transfer transitions, which mainly involve the ligand rings. Upon complexation with the metal ion, the absorbance maximum is expected to shift to around 300 nm. Zinc(II) coordination compounds are colourless, making it impossible to record Visible spectra.

5.2.2.2 Picolinic acid ligand

Picolinic acid ligand absorption bands may be observed in the UV-vis spectra which indicates the bathochromic shift of $\pi \rightarrow \pi^*$ electron transition, ascribed to the interaction of the metal ions with the chromophores C=N when bonded with the zinc(II) metal ion through both hydroxyl and carbonyl groups.[88] The broad bands may also be assigned to Zn-dipic charge-transfer transitions,

which mainly involve the ligand rings. When the ligand is complexed with the zinc(II) ion, the wavelength (λ_{nm}) is shifted to approximately 275 nm.

5.2.2.3 [(N-methyl-N-phenyl, N-butyl-N-phenyl) dithiocarbamatozinc(II)]

The UV-vis spectra of dithiocarbamate complexes normally display high-intense absorption bands because of the NCS_2 groups.[89] The only absorption bands expected are those due to π-π* of the phenyl ring and n-π* of an electron of the lone pair on the sulphur atom to an antibonding p-orbital transitions. Two absorption bands resulting from the π-π* transition are observed at 234 nm and 374 nm in the UV spectrum of the ammonium N-methyl-N-phenyldithiocarbamate and ammonium N-methyl-N-phenyldithiocarbamate ligands. They are assigned to the N-C-S and S-C-S groups. When complexed with the zinc(II) metal, these bands undergo bathochromic shift and appear at 302 nm. This may result due to the ligand → d-orbital transition.[90]

5.2.2.4 (2,2-bipyridine)[(N-methyl-N-phenyl,N-butyl-N-phenyl) dithiocarbamatozinc(II)]

As stated about the UV-vis spectra of the [(N-methyl-N-phenyl, N-butyl-N-phenyl) dithiocarbamatozinc(II)] complex, the only absorption bands expected are those due to π-π* and n-π* transitions. The n-π* transition involves the transition of an electron of the lone pair on the sulphur atom to an antibonding p-orbital. The (2,2-bipyridine)[(N-methyl-N-phenyl,N-butyl-N-phenyl) dithiocarbamatozinc(II)] complex undergoes a peak splitting which indicates the non-equivalence of the C–S bond.[91] When complexed with the zinc(II) metal, these bands undergo bathochromic shift and appear at 302 nm.

Useful information on ligand spectra is expected below the ultraviolet-visible region < 300 nm. Spectra was re-run below 1.0 absorbance in the ultraviolet-visible region (< 300 nm) and found that there was no substantial absorption attributed to the ligands.

5.2.3 X-ray crystallography

5.2.3.1 Monoaquabis(maltolato)zinc(II) complex

In this complex (Figure 12), zinc (Zn1) is coordinated to two bidentate maltolato anions and one water molecule. The two maltolato ligands are symmetry related through a 2-fold rotational axis that passes through the zinc atom and the oxygen of the coordinated water.

The complex has a square pyramidal geometry with the four coordinating maltolato oxygen atoms (O11, O12, $O11^{i}$, and $O12^{i}$) in the equatorial positions and the water oxygen (O14) in the axial position (i:1-x, +y, 3/2-z). Tau-Descriptor for 5-Coordination is 0.01 indicating that the square pyramidal geometry has little distortion.[84] The zinc atom Zn1 is 0.3851(3) Å above the equatorial plane. The two least square planes through the two maltolato ligands make an obtuse angle of 158.430(13)˚ with each other and 10.785(8)˚ with the equatorial plane. The Zn1−O14 bond with the coordinated water molecule is the shortest bond at 1.9929(16) Å while the Zn1−O12 bond with the maltolato carbonyl oxygen is the longest at 2.0717(10) Å.

5.2.3.2 Diaquabis(maltolato)zinc(II) complex

In this complex (Figure 13), zinc (Zn2) is coordinated to two bidentate maltolato anions and two water molecules. The structure is centrosymmetric with the inversion point placed at the zinc atom position.

The structure has an octahedral geometry with the four coordinating maltolato oxygen atoms (O21, O22, $O21^{ii}$, $O22^{ii}$) atoms on the equatorial plane, and the oxygen atoms (O24, $O24^{ii}$) of the coordinating water molecules in the axial positions (ii:1-x, 1-y, 2-z). The two least square planes through the two maltolato ligands make angles of 12.41(4)˚ with the equatorial plane. The Zn2−O21 bond with the maltolatohydroxyl atom is the shortest at 2.0331(8) Å while the Zn2-O24 bond with the coordinated is the longest at 2.2438(10) Å.

There are a number of intermolecular hydrogen interactions with the water molecules and complexes in the structure. The shortest interaction is O14− H14A...O21 with a length of 1.851(15) Å. There is an infinite change of π...π ring interactions between adjacent maltolato rings. The centroid to centroid distance alternates between 3.4869(7) and 3.8416(7) Å along the b-axis.

5.2.3.3 Monoaquabis(picolinato)zinc(II) complex

In this complex (Figure 14), zinc is coordinated to two bidentatepicolinate anions and one water molecule. The two picolinate ligands are symmetry related through a 2-fold rotational axis that passes through the znc atom and the oxygen of the coordinated water.

The complex has a trigonalbipyramidal geometry with the three oxygen atoms (O1, O3, $O1^{i}$) in the equatorial positions and the nitrogens (N1, $N1^{i}$) in the axial positions (i: 1-x, y, 3/2-z). The Tau-Descriptor for 5-coordination is 0.75 indicating a distorted trigonalbipyramidal geometry.[84] The zinc atom lies on the equatorial plane and the least square plane through the pyridine group of the ligand makes an angle of 88.45(5)˚ with the equatorial plane. The Zn-O bond lengths with the picolinate ligand is 2.0238(10) Å while the bond with the water molecule is shorter at 1.9591(15) Å. The Zn-O bond lengths with the picolinate ligand is 2.0238(10) Å while the bond with the water molecule is shorter at 1.9591(15) Å. The Zn−N bond lengths are 2.1290(10) Å[84].

The non-coordinating oxygen O2 of the picolinate ligand is the acceptor for two intermolecular hydrogen interactions: O3−H3a...$O2^{ii}$ and C5−H5...$O2^{ii}$ with lengths of 1.773(16) and 2.44 Å respectively (ii: 1/2+x, 1/2+y, z). The shortest interaction between adjacent rings is between the ligand pyridine ring and the ring formed by Z1, N1, C1, C6 and O1 with a centroid distance of 3.7070(7) Å.

5.2.3.4 Diaquabis(picolinato)zinc(II) complex

This structure (Figure 15) is a coordinated two bidentate picolinate anions and two water molecules. The structure is centrosymmetric with the inversion point placed at the zinc metal atom position. There is also one solvent water molecule. This structure has been reported.[85]

The structure has an octahedral geometry with the coordinating picolinate nitrogen (N1, $N1^{i}$) and oxygen (O1, $O1^{i}$) atoms on the equatorial plane, and the oxygen atoms (O3, $O3^{i}$) of the coordinating water molecules in the axial positions (i: 1-x, 1-y, 1-z). The least square plane through the pyridine rings makes an angle of 9.55(7)˚ with the equatorial plane. The Zn-O and Zn− N bond lengths for the picolinate coordinating atoms are 2.0772(8) and 2.1175(9) Å respectively which are similar to the bond lengths obtained for the monoaqua complex. The Zn-O bond length for the coordinated water molecules are however longer at 2.1612(8) Å compared to the monoaqua complex.

There are a number of intermolecular hydrogen interactions with the water molecules in the structure. The shortest interaction is O4−H4A...O2 with a length of 1.860(11) Å. There is one long intermolecular interaction directly between adjacent picolinate ligands: C5−H5...$O2^{ii}$ with a length of 2.49 Å (ii: -x, -y, 1-z).

5.2.3.5 (2,2-bipyridine)[(N-methyl-N-phenyl,N-butyl-N-phenyl)dithiocarbamatozinc(II)] complex.

The crystal structure consists of discrete molecular species in which the zinc atom is coordinated by a methyl dithiocarbamate ligand acting as a monodentate ligand, a butyl dithiocarbamate ligand acting as a bidentate (S, S) and a 2,2-bipyridine acting as a bidentate ligand through the nitrogen atoms. The zinc atom is in a distorted [ZnS_2SN_2] trigonal bipyramidal environment. The distortion of the regular trigonal bipyramidal geometry is due to the small bite angle of the two chelating ligands: dithiocarbamate and 2,2-bipyridine whose S(22)-Zn(1)-S(21) and N(32)-Zn(1)-S-N(31) chelate angles (71.381(16)° and 75.72(5)°, respectively) deviate significantly from the expected 90°. The bond angles for N(32)-Zn(1)-S(21) [113.58(4)°], N(32)–Zn(1)–S(11) [114.48(4)°], N(32)-Zn(1)-S(12) [114.48(4)°] and
S(11)-Zn(1)-S(12) [105.87(2)°] are significantly different from the expected 90° while N(31)-Zn(1)-S(11) [90.05(4)°] and N(31)-Zn-S(22) [87.71(4)°] are very close to the ideal value. The angle described by the equatorial positions should be close to 120°; while S(11)-Zn-S(22) [129.62(2)°] is greater than 120°, the other angles involving S(11) are less than 120°. This may be due to the steric requirement of the dithiocarbamate and 2,2-bipyridine chelate but it also significantly affect the N(31)-Zn(1)-S(12) [160.44(4)°] bond angle which is far from ideal value of 180°. The rest of the angles around the Zn atom range from 73–44(2)–129.62(2)° significantly from the ideal right angle geometry. The Zn–S bond length in (2.2-bipyridine)[(N-methyl-N-phenyl,N-butyl-N-phenyl)dithiocarbamatozinc(II)]; S(11)-Zn(1) [2.3444(6)° and S(12)-Zn(1) [2.4228(6)°] are greater than the average value of tetracoordinated complexes and much more shorter than those reported for hexacoordinated complexes but the values agree well with those found in other pentacoordinated complex containing similar ligands.[92-94]

A crystal structure of new coordination compounds maltolato(picolinato)zinc(II) and [(N-methyl-N-phenyl)(N-butyl-N-phenyl)dithiocarbamatozinc(II)] could not be obtained because the powdered compounds could not crystallize due to the fact that the compounds were highly insoluble in the chosen solvents utilized for growing the crystal. The crystallization process was also disturbed by sound vibrations from the fume hoods and hence the environment was not conducive enough for crystal formation. Such disturbances contribute greatly to the formation of crystals.

5.2.4 Microanalysis

Microanalysis of all the prepared compounds corresponded well with the calculated results as given by in Tables 5, 6, 7, 8, 9, 10 and 11. This confirmed the purity and identity of the complexes.

5.3 Anti-diabetic biological studies

The application of coordination compounds in biological systems has been the center of research for many decades[88]. Several metal ions have been reported [95] to play vital roles in biological processes in humans. For example, the metal ions selenium, chromium, manganese, molybdenum, tungsten and vanadium have been reported to possess blood glucose lowering effects when coordinated to organic compounds. However, these metal ions in their different oxidation states can be toxic and harmful. Zinc(II) metal ion is one of the most abundant transition metal ion and is an essential trace element in the human body. The metal ion is less toxic, has many nutritional and pharmacological roles and exists in the form of complexes with proteins and nucleic acids. The metal ion takes part in all aspects of intermediary metabolism, transmission and regulation of the expression of genetic information, storage, synthesis and action of peptide hormones and structural maintenance of chromatin, biomembranes and extracellular matrices.[96,97].

In relation to this work, one of the essential biological processes carried by the zinc(II) metal ion is its ability to function as an insulin mime with regard to its biosynthesis, stability, and secretion.[64] Ever since the discovery of this unique biological property of zinc(II) metal ion, the development of zinc(II) coordination compounds has been of key focus to research scientists in order to investigate their antidiabetic properties.

The evaluation tests carried out via *in vitro* study methods play a vital role in investigating the insulin mimetic activities or antidiabetic properties as well as the biological properties of compounds at large. This study method provide knowledge based on the mode of action of the synthetic drugs proposed. The necessary biological material normally consists of perfused whole organs, isolated tissues, cell culture systems, or tissue slice preparations. *In vivo* biological study

methods on the other hand, seek to determine the behavior of the mode of action under clinical or pathophysiological environments. Information obtained from experimental methods can predict the cytotoxic properties within model animals and establish the mode of action of the synthetic therapeutic drugs proposed.[98]

For this project, *in vitro* evaluation study method was considered and highly favourable compared to *in vivo* evaluation method because of the efficiency of the method with minimum costs spent on purchasing reagents and biological cell tissues and muscles. The method also assess product performance and offers benefits in terms of ethical clearance considerations.[99] When utilizing model animals, acquiring ethical clearance can be troublesome and can take longer durations to possess and get approved for a specific research study. Hence, in vitro evaluation study method was highly considered.

C2C12 (skeletal muscle) cell lines were utilized to investigate the relationship of the synthesized zinc(II) coordination compounds with metformin treated as the existing pharmaceutical medication prescribed to patients suffering from Type 2 Diabetes Mellitus. Skeletal muscle cell lines play a vital role in body energy balance and are the pre-eminent tissues affected by insulin for glucose uptake. Hence, C2C12 cell lines are suitable target tissues for the synthesized zinc(II) coordination compounds.[95] The culture plates were prepared while the cell lines were maintained and grown successfully. This was observed under a microscope and the cell lines reached 80-100 % confluence. The C2C12 (skeletal muscle) cell lines were differentiated successfully. Sodium palmitate (0.75 mM, 1mL) was then introduced to the cell lines successfully in order to induce Type 2 Diabetes Mellitus. The cell lines were incubated at 37°C for 10 hours. After the incubation period, cells lines were treated successfully with liquefied coordination compounds (10^{-4} M, 5 µL). Type 2 diabetes mellitus was successfully induced with sodium palmitate (0.75 mM, 1 mL). As depicted in Figures 19-22, the cell lines were able to incorporate the treatment into their organelles and survive. Since the results obtained of the treated culture plates were in line with the published work,[83,100] it can be argued that the coordination compounds enhanced glucose uptake at the suggested concentration, equalling or surpassing the effects of metformin, the standard drug used to serve as treatment for patients diagnosed with Type 2 Diabetes Mellitus.

CHAPTER 6

6. CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Conclusions

The coordination compounds bis(maltolato)zinc(II), bis(picolinato)zinc(II) and the new coordination compounds maltolato(picolinato)zinc(II), [(N-methyl-N-phenyl)(N-butyl-N-phenyl)dithiocarbamatozinc(II)] and (2,2-bipyridine)[(N-methyl-N-phenyl, N-butyl-N-phenyl)dithiocarbamatozinc(II)] complexes were synthesized and characterized by Infrared and Ultraviolet-visible spectroscopy, single crystal X-ray diffraction and microanalysis methods successfully. This served as evidence that the complexes were identified and were found to be pure. These zinc(II) coordination compounds were then utilized to treat C2C12 (skeletal muscle) cell lines. The cells were viewed under a microscope and reached 80-100 % confluency after treatment. This serves as evidence that the cells were able to survive the treatment at such concentrations of the coordination compounds. The results obtained were in line with the published results obtained after treatment and this implies that the coordination compounds may serve as treatment for Type 2 Diabetes Mellitus.

6.2 Suggestions for further work

The amino acid ligand zinc(II) metal complexes that are reported on and other coordination compounds such as bis(oxalato)zinc(II), bis(L-prolinato)zinc(II) and bis(D-prolinato)zinc(II) may be screened and investigated for their cytotoxic properties and cell viability tests (MTT assay) in order to determine non-toxic concentrations of the complexes. Zinc(II) complexes such as bis(1-oxy-2-pyridonato)zinc(II) and bis(1-oxy-2-pyridinethiolato)zinc(II), mixed ligand amino acid complexes such as oxalato(prolinato)zinc(II), oxalato(maltolato)zinc(II), oxalate(picolinato)zinc(II) and dithiocarbamate complexes of the nature (N-alkyl-N-phenyl)zinc(II) may also be tested for their insulin mimetic anti-diabetic effect or blood glucose-lowering activity by making using use of model mice (in vivo evaluation method). This method involves mode mice being injected with i.p. injections of the mixed ligand zinc(II) complexes whilst monitoring their blood glucose levels, body weight, food intake, and water consumption.After treatment of zinc(II) coordination compounds, blood samples may be collected from the tail vein to measure the blood glucose levels of the model mice

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