Synthesis, Characterization and Application of Transition Metal Complexes in Bio-inorganic Chemistry

M.Sc. Thesis

By

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DISCIPLINE OF CHEMISTRY

INDIAN INSTITUTE OF TECHNOLOGY INDORE

June 2017

Synthesis, Characterization and Application of Transition Metal Complexes in Bio-inorganic Chemistry

A THESIS

Submitted in partial fulfillment of the requirements for the award of the degree
of
Master of Science

by
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DISCIPLINE OF CHEMISTRY
INDIAN INSTITUTE OF TECHNOLOGY INDORE
June, 2017

INDIAN INSTITUTE OF TECHNOLOGY INDORE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **Synthesis, Characterization and Application of Transition Metal Complexes in Bio-inorganic Chemistry** in the partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** and submitted in the **DISCIPLINE OF CHEMISTRY, Indian Institute of Technology Indore**, is an authentic record of my own work carried out during the time period from July 2016 to June 2017 under the supervision of Dr. Suman Mukhopadhyay, Associate Professor, IIT Indore.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other institute.

VAISHALI CHHABRA

This is to certify that the above statement made by the candidate is correct to the best of my/our knowledge.

Dr. SUMAN MUKHOPADHYAY

VAISHALI CHHABRA has successfully given his/her M.Sc. Oral Examination held on June 30, 2017

Signature of Supervisor
Date:

Convener, DPGC
Date:

Signature of PSPC
Date:

Signature of PSPC
Date:

ACKNOWLEDGEMENTS

With deep regards and profound respect, I avail this opportunity to express my deep sense of gratitude and indebtedness to Dr. Suman Mukhopadhyay for introducing the present project topic and for his inspiring guidance, constructive criticism and valuable suggestion throughout the project work. I gratefully acknowledge his constant encouragement and help in different ways to complete this project successfully. I would also like to extend my gratitude to my PSPC committee members Dr. Sampak Samanta and Dr. Sudeshna Chattopadhyay and DPGC convener Dr. Apurba K. Das for their valuable suggestion and guidance.

I also take this opportunity to express a deep sense of gratitude towards our honourable Director, Prof. Pradeep Mathur and head of Chemistry discipline Dr. Satya S. Bulusu for constantly motivating us for research and providing various facilities and a healthy environment, which helped me to focus on the project exclusively. I would also like to acknowledge all the faculty members of Chemistry discipline IIT Indore for their supportive and humble gesture. Furthermore, I would like to thank SIC IIT Indore for instrumentation facilities.

I extend my regards to my mentor Mr. Bidyut Kumar Kundu for his intense involvement in shaping up this project and transforming it to a conclusive form. All my group members hold an indispensable part in this work of mine. Their cooperative and friendly behaviour, down to earth attitude has made this one year experience a memory to cherish, with a special note of thanks to Dr. Komal Vyas whose support helped me to overcome every difficult situation I got stuck in.

I am thankful to Ms. Sarita Batra, Mr. Kinney Pandey, Mr. Ghanshyam Bhavsar and Mr. Manish Kushwaha for their technical help and support.

I also need to express my deepest love and gratitude to my lovable parents and friends near and far for their unconditional love and patience during

this tenure. A special thanks to my elder brother Mr. Ankit Chhabra for believing in my abilities and for being a constant source of motivation.

Vaishali Chhabra

Discipline of chemistry

IIT Indore

DEDICATED TO.....

MY ELDER BROTHER

For his unwavering belief in my potential!

Abstract

Metal complexes of Schiff base ligands possess suitable biometric properties that can mimic the active sites and hence have wide applications in treatment of various ailments, biochemical reactions and also as biological regulators. The present work of this thesis stems from our interest to explore the structural and functional properties of Schiff base complexes. Henceforth four new monomeric complexes from Schiff base ligand [(E)-1-(((2-hydroxypropyl)imino)methyl)naphthalen-2-ol] (H_2L) have been synthesized. Two of which are of Cu (complexes **1** and **2**) and the remaining two are of Ni and Mn (complexes **3** and **4**) respectively. All the complexes are characterized by ESI-MS, IR spectroscopy and elemental analyses. X-ray crystallography studies done for complexes **1** and **2** revealed that complex **1** is monoclinic in nature with square pyramidal geometry whereas the complex **2** is square planar triclinic crystal system. All the complexes were screened for catechol oxidation using 3,5-ditertiarybutyl catechol (3,5-DTBC) as model substrate. In addition to their potential use as a bio-mimic catalyst in catechol oxidation, the interaction of all the complexes with proteins (BSA) was also studied to establish their potent role as metal-drug system.

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NOMENCLATURE

θ	Angle
λ	Wavelength
α	Alpha
β	Beta
γ	Gamma
Å	Angstrom
δ	Chemical shift (NMR)
ν	Frequency
ºC	Degree Centigrade
$\bar{\upsilon}$	Wavenumber
cm	Centiimeter
%	Percentage
g	gram
mol	Mole
mmol	Milimole
M	Molar
mM	Milimolar
µM	Micromolar
L	Litre
mL	Mililitre
µL	Microlitre
K	Kelvin
nm	Nanometre

ACRONYMS

bpy	Bipyridine
ppy	Phenylpyridine
OLED	Organic Light Emitting Diode
N	Nitrogen
O	Oxygen
S	Sulfur
C	Carbon
H	Hydrogen
His	Histidine
Mn	Manganese
Cu	Copper
Zn	Zinc
Ni	Nickel
BSA	Bovine Serum Albumin
HSA	Human Serum Albumin
DTBC	di-tertbutyl catechol
DTBQ	di-tertbutyl quinone
OEC	Oxygen evolving complex
a.m.u	Atomic mass unit
KBr	Potassium Bromide
DMSO	dimethylsulphoxide
H_2O_2	Hydrogen peroxide
UV-vis	Ultraviolet-visible spectroscopy
KI	Potassium Iodide
HCl	Hydrochloric acid
H_2SO_4	Sulphuric acid

Cl	Chlorine
Na	Sodium
MeOH	Methanol
SCN	Thiocyanate
TOF	Turn Over Frequency
Tris-HCl	Tris(hydroxymethyl)aminomethane-hydrochloride
ORTEP	Oak Ridge Thermal Ellipsoid Plot Program
ESI-MS	Electron Spray Ionosation- Mass Spectrometry
IR	Infrared spectrometry
NMR	Nuclear Magnetic Resonance

Chapter 1

Introduction

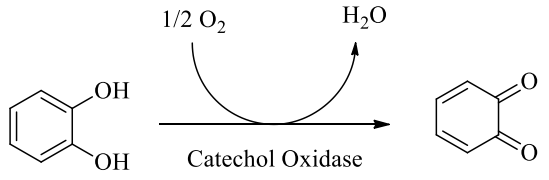
1.1 General Introduction:

Ever since the foundation of coordination chemistry has been laid by Alfred Werner *[1]*, the role of transition metal complexes in different biological processes has been understood in a more prominent way. The chemistry of transition metal complexes is greatly influenced by the central metal ion as well as the ligand surrounding the metal center. Thus, the design and synthesis of novel ligands has become one of the most important aspects of coordination chemistry owing to its great influence on the structures and functionality of metal complexes. In addition, the ligand environment may also affect the rate of reactions at a metal center by stabilizing the transition state and/or by destabilizing the ground state. *[2-3]* Like for instance, biological activities of transition metal complexes derived from Schiff base ligands are one of the most exhaustively studied area in coordination chemistry, due to their different metal coordination behaviours, simple and inexpensive synthesis with various substituents and enhanced activities compared to non-Schiff base complexes *[4-7]*. Schiff base complexes show important physiological and pharmacological activities due to their favourable cell membrane permeability, which has led to its use in the designing of many useful pharmacophores *[8-11]*. On the other hand the requirement of ligand for making OLEDs demand strong σ-donor property of ligand together with the π- accepting ability for which ligands such as 2,2-bipyridine (bpy) *[12-14]* and 2-phenylpyridine (ppy) *[15-16]* are ideally suitable. Thus the ample amount of variations possible with the ligand and the metal itself keeps the research challenging and provide motivation for the synthesis of new complexes with improved functions in their respective fields.

1.2 Applications of Transition metal complexes:

1.2.1 Transition metal complexes as Biomimics:

Metallo-enzymes are fascinating natural factories able to catalyze a great variety of reactions under very mild conditions and with high chemo- and stereo-selectivity. *[17]* Designing biomimetic complexes for the modelling of metallo-enzyme active sites is a fruitful strategy for obtaining fundamental information and a better understanding of the molecular mechanisms at work in natural chemistry. It also allows discovering involved reactive species and/or unknown reactivity patterns associated with a metal ion. All this information may also lead to the development of bio-inspired systems displaying interesting and exploitable properties. *[18]* The classical way to design biomimetic complexes consists in using a ligand that mimics the natural environment of the metal ion at work in the enzyme. *[19-20]* It could be a porphyrin for modelling heme enzymes or a tripodal ligand with N/O/S donors for mimicking amino-acid residues holding the metal ion in the active site. The design and synthesis of coordination compound models of metalloenzymes with oxidase activity has received a great deal of attention in recent years for the development of bio-inspired catalysts *[21]* that could be as efficient as the enzyme itself. *[22-24]* Catechol oxidase, a lesser explored member of type-III copper proteins has been the subject of a large number of model studies. It is a ubiquitous globular plant enzyme, the bio-function of which is to catalyze the oxidation of *o*-benzenediols (catechols) to the corresponding quinones through the four-electron reduction of molecular oxygen to water. *[25-26]*



o- Benzenediol *o*- Benzoquinone

Figure 1.1: Schematic representation of catalytic activity of metalloenzyme catechol oxidase.

When plant tissue is damaged, the chloroplast may rupture and release catechol oxidase into the plant cytoplasm, and vacuoles may also rupture, releasing stored catechol into the cytoplasm. The tissue damage also allows oxygen to penetrate into the cell. Thus, tissue damage facilitates the interaction of catechol oxidase with its substrate to produce *o*-quinone. Quinones being highly reactive intermediates, polymerize non-enzymatically to a brown pigment melanin that forms an insoluble barrier for wound protection *[27]* and thus acts as a natural antiseptic for the protection of damaged tissues against pathogens and insects. *[28]* Apart from this, the oxidation of phenols to quinones has been the subject of many studies because of industrial interest as well. *[29-33]* Various quinones are used as intermediates in the synthesis of fine organic materials such as drugs, vitamins, dyes and perfume aromas. *[34-36]* It is well documented that the active center of the catechol oxidase consists of a hydroxo-bridged dicopper (II) center in which each copper(II) center is coordinated to three imidazole nitrogens of histidine residue *[38]* with the nitrogens on the imidazole side chains of His88, His109, and His118 coordinating with the first catalytic copper while the nitrogens on the imidazole side chains on His240, His244 and His274 coordinating with the second catalytic copper ion. Although the active site of catecholase enzyme consists of a hydroxo-bridged dicopper(II) center, literature study shows that several monometallic complexes of copper(II) *[39]* and some other metal ions, such as Mn(II), *[40-41]* Fe(II), *[42]* and Ni(II), *[43-45]* are also found to show catecholase activity.

1.2.2 Transition metal complexes as protein binding agents:

The interaction between bio-macromolecules and drugs has been drawing attention among researchers with great interest during last two decades *[46-48]*. Interest in this area is a fundamental requirement, not only for gaining some insight into the mechanism involved in biochemical procedures governing protein sequencing, but also for understanding the reactive models for protein–nucleic acid interactions

as well as obtaining information about the rational design and synthesis of new types of pharmaceutical molecules. *[49-51]* Therefore, it is important to investigate the potential drug–protein interactions. Proteins are the most abundant macromolecules in cells and are crucial to maintain normal cell functions. Among bio macromolecules, the serum albumins are the major soluble protein constituent of the circulatory system; they have many physiological functions such as they contribute to osmotic blood pressure *[52]* and can play a main role in drug transportation and disposition. *[53]* Bovine serum albumin (BSA) has been one of the most extensively studied proteins, especially because of its structural homology with human serum albumin *[54]*. BSA consists of three homologous domains (I, II, III) and each domain in turn is the product of two sub-domains. BSA has two tryptophan residues, Trp–134 and Trp–212, which are embedded in the first sub-domain IB and sub-domain IIA, respectively.

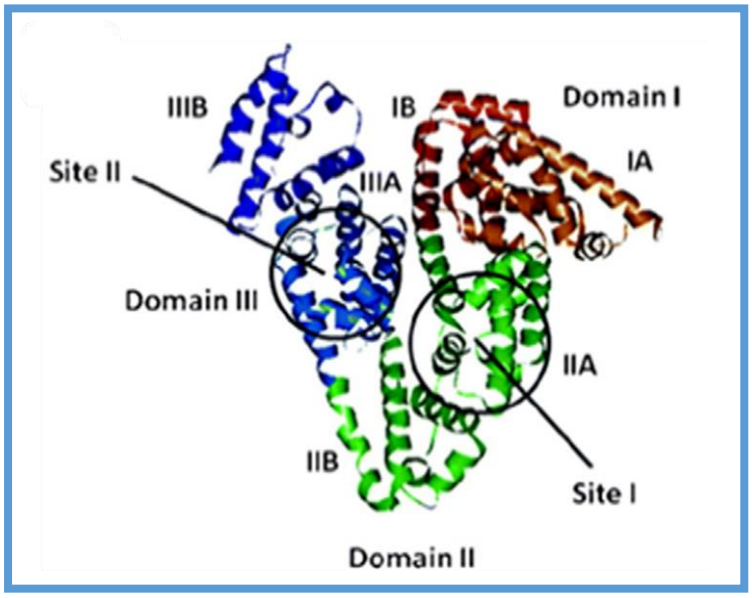


Figure 1.2: The ribbon model of the BSA derived from X-ray diffraction crystallography.

Since the binding ability of a drug to serum albumin may have an importance in pharmacokinetics as well as the determination of the dosage form of the drug, the changes in fluorescence intensities of BSA-

drug complex could give considerable information regarding the binding characteristics and the therapeutic effectiveness of drugs. Therefore, the binding of drugs to serum albumin *in vitro,* is considered as a model in protein chemistry to study the binding behavior of proteins, research field in chemistry, life sciences and clinical medicine. *[54]*

1.2.3 Transition metal complexes as catalysts in organic reactions:

Catalysts in general are the substances which alter the rate of the reaction without themselves getting changed. There are two types of the catalyst namely positive catalyst which increases the rate of the reaction and the negative catalyst which decreases the rate of the reaction. Transition metals complexes are known for their remarkable involvement in the catalytic processes. In recent days the development of environmental benign methodologies *[55-56]* has become one of the important tasks for the organic and inorganic chemists. *[57]* For fulfilling the needs of green chemistry, certain valuable challenges remain, such as

- Reduce the cost of reaction
- High efficiency of catalyst
- Avoiding the use of harsh reaction conditions
- Using green reagents
- The replacement of expensive noble metals with cheap and affordable catalysts.

Complexes of transition metals like Zn, Cu, Fe, Ni are cheap, abundant, and nontoxic. Therefore, the use of these metal cores as catalysts instead of catalysts based on noble metals (Pd, Ir, Ru, etc.) is much more economical and useful. Transition metal complexes assist in a variety of industrially important organic synthesis reactions such as hydrogenation and dehydrogenation, hydrosilation, hydroformylation, polymerization, isomerization, acylation oxidative hydrolysis of olefins, oxidation of alcohols to aldehydes and ketones.*[58-60]* Further improvement of current catalysts and the design of new metal based complexes for highly efficient reactions are based on understanding

related mechanistic insights and kinetics of the reaction. With the development of modern electronic structure theory and computer science, computational quantum chemistry has become a powerful tool for understanding the structures and properties of compounds and elucidating detailed mechanisms of chemical reactions, and therefore, holds great promise in the design of new catalysts. *[61]*

1.3 Growing importance of Cu metal complexes in bio-inorganic chemistry

Copper is one among the essential bio-relevant transition metal (in addition to iron and zinc) and is present in several metalloenzymes and metalloproteins *[62-64]* that are involved in electron transfer, oxygen transport and oxygenation reactions. Active copper centers dominate the field of biological oxygen chemistry and play a vital role in catalysis. *[65-67]* Given that copper is a metal present in the active site of a wide variety of enzymes that perform vital chemical transformations, there is great interest in understanding both the structural and mechanistic aspects of these complex metalloproteins. During the last decades, a large number of relatively simple copper coordination compounds have been described and studied as models for some of these biological catalysts. The aim in these model studies was either to mimic the structural and spectroscopic features of the metal sites and/or to obtain catalytic behavior from these complexes by using model substrates. Catechol oxidase has been the subject of a large number of model studies. The accessibility of catecholase activity has significantly broadened recently as the researchers are intensively studying mononuclear copper systems as potential applicants. *[68-69]* Furthermore, the biocompatibility and versatility in coordination of copper has led to its use in metal-based drugs. As copper is an essential element for most aerobic organisms, an assumption that this endogenous metal may be less toxic for normal cells than cancer cells is raised. It is

reported that the metabolism and cell response to copper between normal and tumor cells are generally different, which ground the basis of copper complexes endowed with antineoplastic characteristics. *[70]*

1.4 Extending the horizon with different transition metals like Mn, Ni

Nickel(II) has very fascinating coordination chemistry owing to its inherent ability to adopt various geometries. In recent years the development of nickel bio-chemistry have given a thrust in this area. Similarly manganese being one of the most important trace element in biological systems draws attention for the syntheses of its complexes and for the study of their physical and chemical properties owing to its potential abilities in biological modeling application, e.g. to mimic the active sites of oxygen evolving complexes (OEC) in photosystem II (PS II) of green plants, in which dioxygen is evolved by water oxidation in photosynthesis [71-72]. From a literature survey, it is possible to recognize that some complexes of Mn(III), Mn(IV), few ones of Mn(II) *[40-41]* and even fewer of Ni(II) *[43-45]* are found to mimic catecholase-like activity. Another potential application of Ni and Mn complexes is their ability to bind with plasma protein especially serum albumin which leads to the transport of metal ions and metal complexes of drugs through the blood stream.

1.5 Organization of thesis:

The aim of this project is to synthesize different transition metal complexes and to study their application in biochemistry. This was to be achieved by reproducing a Schiff base ligand and carrying out its complexation reaction with various metal ions.

Chapter 2: This chapter includes review of past work and project motivation:

Chapter 3: This chapter includes materials, instruments and the experimental procedure used to synthesize the metal complexes. It also includes experimental techniques employed to study their catecholase like and BSA binding properties.

Chapter 4: In this chapter results have been discussed which were obtained after the synthesis and application study of the metal complexes.

Chapter 5: This chapter concludes the described work and also looks for possible future scope and applications.

Chapter 2

Review of past work and project motivation

The ability of copper complexes to oxidize phenols and catechols has been known for at least 40 years. For example, in 1964 Grinstead *et al.* reported the oxidation of 3,5-di-tert-butylcatechol (3,5-DTBCH_2) to the respective 3,5-di-tert-butyl-o-benzoquinone (3,5-DTBQ) in aqueous methanol in the presence of 1% of copper(II) chloride.*[72]* One of the pioneering mechanistic studies on catechol oxidation catalyzed by copper(II) complexes was presented by Lintvedt and Thuruya *[73]* through their study of the kinetics of the reaction of 3,5-DTBCH_2 with dioxygen catalyzed by bis(1-phenyl-1,3,5-hexanetrionato)dicopper(II) complex. Although authors like Oishi *et al.*, Malachowski *[74]* and Casellato *et al. [75]* have reported the higher activities of dinuclear copper(II) complexes in the oxidation of 3,5-DTBCH_2 in comparison to their mononuclear analogues, there have been reasonable amount of studies focused on achieving better catecholase activity with mononuclear Cu (II) complexes.*[39,76]* The literature survey done unravelled reports by Merry Mitra *et al. [40]* and Abhijit Pal *et al. [45]* showing Mn and Ni complexes also being studied for catecholase like activity.

Also one of the fundamental goals in medicinal chemistry is the development of new anticancer and antimicrobial therapeutic agents as cancer remains the second most common cause of death worldwide, accounting for about one of every four deaths. Barnett Rosenberg, Van Camp *et al.* of Michigan State University in 1967 reported different ionic species of platinum (IV) complexes which successfully inhibited growth or cell division in Escherichia coli. *[77-78]* Since then, platinum complexes have been at the centre of research studies as chemotherapy agents. *[79-80]* However, the several side effects of platinum drugs such as nephrotoxicity, neurotoxicity, *[81-82]* inherited or acquired resistance phenomena limited its comprehensive application in the

therapy of cancers. Bioinorganic and medicinal chemists have since then focused on the design and synthesis of new metal-based anticancer agents with a better biological activity, better selectivity, lower toxicities and different mechanisms of action to overcome the unresolved clinical problems of cisplatin analogues drugs. *[83]* As proteins are one the main cellular targets for anticancer drugs so the study of drug–protein interactions is very important to understand the activity and toxicity of drugs. An anticancer drug may work by binding to carrier proteins in the blood which increases its solubility in the blood plasma and results in deliver of drugs to its target cells so Jimmy Flarakos *et. al.* (2005) developed method for determining the relative binding of drug candidates in small, focused medicinal libraries against human serum albumin (HSA), as it is the most abundant proteins in the systemic circulation, with HSA comprising 60% in plasma.*[84]* One of the most investigated non-Pt compounds as potentially attractive anticancer agents were copper complexes.*[70]* For many years, a lot of research studies have actively investigated copper compounds based on the assumption that endogenous metals may be less toxic. Nickel ion, known for its essential role in biological living system *[85]* and also being placed in palladium and platinum elements group has also been gaining attention for long time. The biological properties of nickel complexes have been extensively studied because some nickel complexes were found to exhibit antibacterial, antifungal, antimicrobial, and anticancer activities.

Being motivated from these reports, four new complexes; two of Cu and one of Mn and Ni each have been synthesised by complexing the suitable salts with the Schiff base ligand (((2-hydroxypropyl)imino)methyl)naphthalen-2-ol (H_2L) reported by Atena Naeimi *et al*. (2015) *[86]* and their catecholase like and BSA binding activity have been explored. Their corresponding Kcat values and turn over number were established using Michaelis-Menten equation and

binding constant (K_b) and bimolecular quenching rate constant (k_q) were found using Stern-Volmer equation.

Chapter 3

Experimental Section

3.1 Reagent and Chemicals:

All of the chemicals [except Copper perchlorate] were of analytical grade and used as received without further purification. These chemicals included 2-hydroxynaphthaldehyde (Sigma Aldrich), 1-amino-2-propanol (Sigma Aldrich, assay 93%), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$) (Merck assay 99%), trimethylamine (Avantor, assay 99.5%), sodium thiocyanate (Fluka, assay 98%), manganese chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$) (Loba Chemie assay 97%), nickel acetate tetrahydrate [$Ni(OAc)_2.4H_2O$] (Finar Chemicals assay 98%). Copper perchlorate hexahydrate $Cu(ClO_4)_2.6H_2O$ was prepared in laboratory.

3.2 Instrumentation:

Infrared spectra (4000 to 500 cm−1) were recorded with a BRUKER TENSOR 27 instrument in KBr pellets. NMR spectra were recorded on an AVANCE III 400 Ascend Bruker BioSpin machine at ambient temperature. Mass spectrometric analyses were done on Bruker-Daltonics, microTOF-Q II mass spectrometer. Spectrophotometric measurements were performed on a Varian UV-Vis spectrophotometer (Model: Cary 100) (for absorption) and a Fluoromax-4p spectrofluorometer from Horiba JobinYvon (Model: FM-100) (for emission) using a quartz cuvette with path length of 1 cm. Single-crystal X-ray structural studies were performed on an Agilent Technology Supernova CCD diffractometer equipped with a low-temperature attachment.

3.3 Synthesis of Schiff-base Ligand and its Metal complexes:

3.3.1 Synthesis of Schiff-base ligand (H_2L)

The tridentate Schiff base H_2L was prepared according to the previously reported procedure *[86]* by mixing 5 mmol of 1-amino-2-propanol (0.37g) with 5 mmol of 2-hydroxynaphthalene-1-carbaldehyde (0.86 g) in 20 mL of ethanol. The reaction mixture was stirred under reflux condition for 4 h at 80˚C. The clear bright yellow solution obtained was dried off completely and crude was collected, characterized and used further.

3.3.2 Synthesis of Complex 1

The mononuclear complex **1** was prepared by reacting 0.11g (0.5 mmol) of H_2L with 0.10g (1 mmol) of triethylammine followed by addition of 0.08g (0.5 mmol) of $CuCl_2.2H_2O$ in 25 mL of methanol. The reaction mixture was stirred under reflux condition for 4 hrs. The green precipitate formed upon complexation was removed *via* filtration and the resulting filtrate was allowed to stand for crystallization at 298K. Upon very slow evaporation of the solvent, green coloured needle like crystals were obtained after 7-8 days.

3.3.3 Synthesis of Complex 2

The mononuclear complex **2** was prepared by reacting 0.11g (0.5 mmol) of H_2L with 0.10g (1 mmol) of triethylammine followed by addition of 0.18g (0.5 mmol) of $Cu(ClO_4)_2.6H_2O$ in 25 mL of methanol. To this reaction mixture methanolic solution of 0.10g (1.2 mmol) of sodium thiocyanate was added and the entire mixture was stirred under reflux condition for 4 hrs. The precipitate formed was removed *via* filtration and the resulting filtrate was concentrated and allowed to stand for crystallization at room temperature. Upon slow evaporation of the solvent, dark green coloured block like crystals were obtained within 4-5 days.

3.3.4 Synthesis of Complex 3

The mononuclear complex **3** was prepared by reacting 0.11g (0.5 mmol) of H_2L with 0.10g (1 mmol) of triethylammine followed by addition of 0.06g (0.25 mmol) of $Ni(OAc)_2$ in 25 mL of methanol. The reaction mixture was refluxed for 4 hrs. Upon complexation, a green coloured precipitate was formed which was removed by filtration and the resulting filtrate was layered with diethyl ether and kept for crystallization. Green coloured needle like crystals were obtained after 2 days.

3.3.5 Synthesis of Complex 4

The mononuclear complex **4** was prepared by reacting 0.11g (0.5 mmol) of H_2L with 0.10g (1 mmol) of triethylammine followed by addition of 0.04g (0.25 mmol) of $MnCl_2$ in 25 mL of methanol. The reaction mixture was stirred under reflux condition for 4 hrs. The precipitate formed was removed by filtration and the filtrate was used for crystallization *via* slow evaporation.

3.4 BSA study

The protein binding studies of these complexes were investigated using bovine serum albumin (BSA) by means of fluorescence spectroscopy recording excitation at 295 nm and the corresponding emission at 340 nm. The excitation and emission slit widths and scan rates were kept constant throughout the experiment. A 10 μM stock solution of BSA was prepared using Tris-HCl buffer (pH ~ 7.4) solution and stored at 4°C for further use. Stock solutions of complexes **1**-**4** (1 mM in strength) were also prepared in Tris-HCl buffer and 5% DMSO. Fluorescence intensity of 2 mL stock solution of BSA was measured and recorded as blank. Thereafter it was titrated by successive additions of 10 μL of the respective stock solution of complexes (upto 200 μL). The fluorescence data was further analyzed by the Stern–Volmer equation.

3.5 Catecholase activity study

The catalytic experiments were conducted in aerobic condition at room temperature with 3,5-di-tert-butylcatechol (3,5-DTBC) in methanol as model substrate. For this purpose, 10^{-4} M methanolic solution of the complexes was added to 100 equivalents of 3,5-DTBC separately and the time course of the reaction was monitored spectrophotometrically. As the reaction progresses, a gradual increase in the absorbance band at 400 nm owing to the increase in concentration of 3,5-DTBQ was observed. The initial rate method was applied to determine the rate of reaction and Michaelis-Menten approach was used to calculate TOF (Turn Over Frequency).

3.6. Detection of hydrogen peroxide in the catalytic reaction of catechol oxidase activity

The formation of H_2O_2 during the catalytic reaction of catechol oxidation by the complexes **1** and **2** was detected iodometrically by assaying I_3^- that was formed from the reaction of KI with the reaction mixture. For this purpose the reaction mixtures were prepared as in the kinetics experiments and after 1 h of reaction, formed quinone was extracted using dichloromethane and water. Quinone gets separated with dichloromethane while the aqueous layer was acidified with H_2SO_4 to pH ≈ 2 to stop further oxidation, and 1 mL of 10% solution of KI was added to it followed by addition of catalytic amount of ammonium molybdate (3 drops of 3% solution) to accelerate the formation of I_3^- . In the presence of hydrogen peroxide, I^- is oxidised to I_2, and with an excess of iodide ions, the tri-iodide ion is formed. The formation of I_3^- was monitored by UV-Vis spectroscopy due to the development of the

characteristic I_3^- band at 350 nm for complex **1** and 353 nm for complex **2**.

3.7 X-ray crystallography:

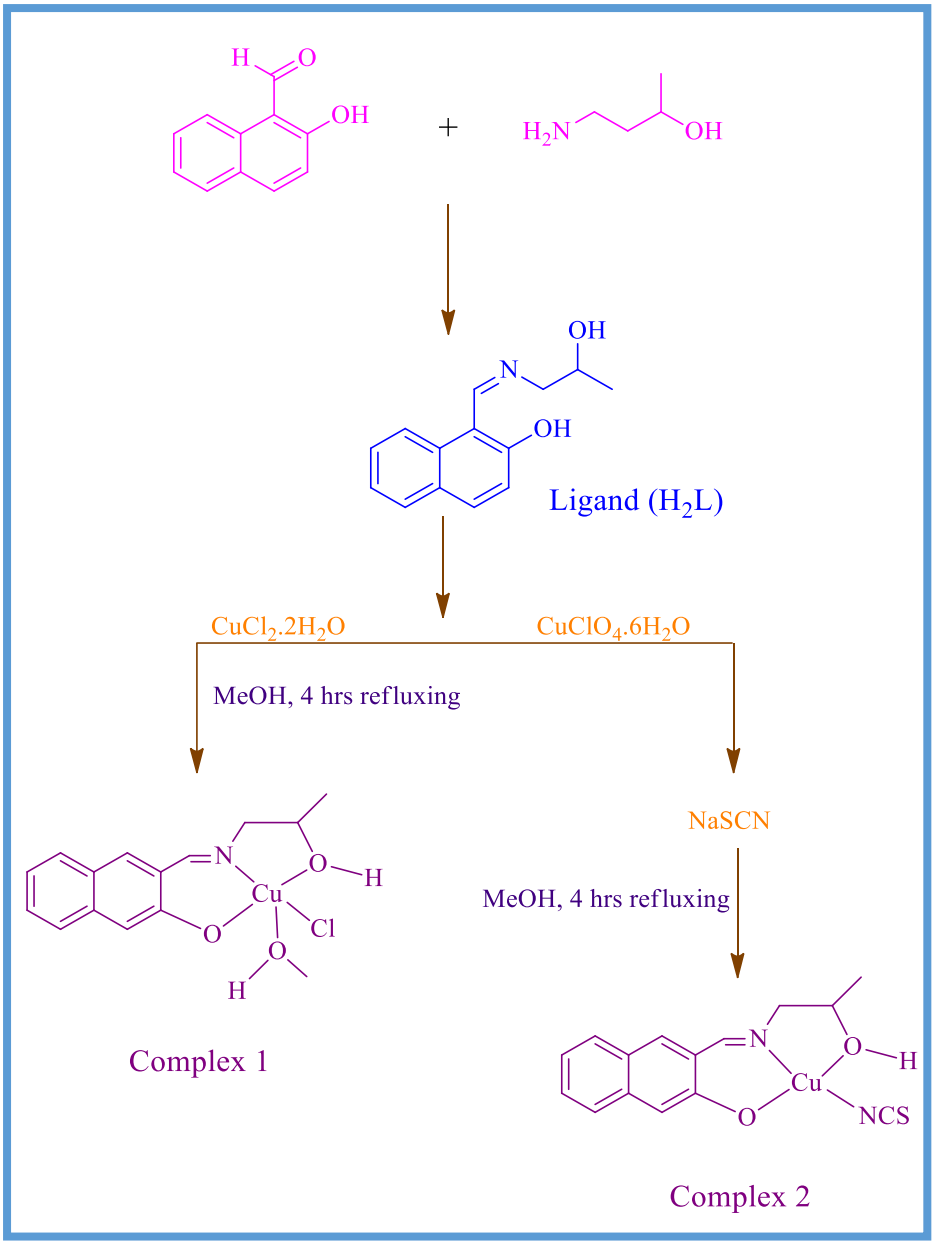
A dark green needle like specimen of complex **1** with approximate dimensions 0.330 mm x 0.260 mm x 0.210 mm and a black block like specimen of complex **2** having dimensions 0.230 mm x 0.190 mm x 0.150 mm were used for the X-ray crystallographic analysis. The crystals of complex **4** could not be obtained and that obtained for complex **3** were not diffracted. The X-ray structural studies were performed on a CCD Agilent Technologies (Oxford Diffraction) SUPER NOVA diffractometer. Data for all the complexes were collected at 293(2) K using graphite-monochromated CuKα radiation (λα = 1.54184 Å). The strategy for the data collection was evaluated by using the CrysAlisPro CCD software. The data were collected by the standard 'phi-omega' scan techniques and were scaled and reduced using CrysAlis- Pro RED software. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least squares with SHELXL-97, refining on F^2. The positions of all the atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The remaining hydrogen atoms were placed in geometrically constrained positions and refined with isotropic temperature factors, generally 1.2Ueq of their parent atoms.

Chapter 4

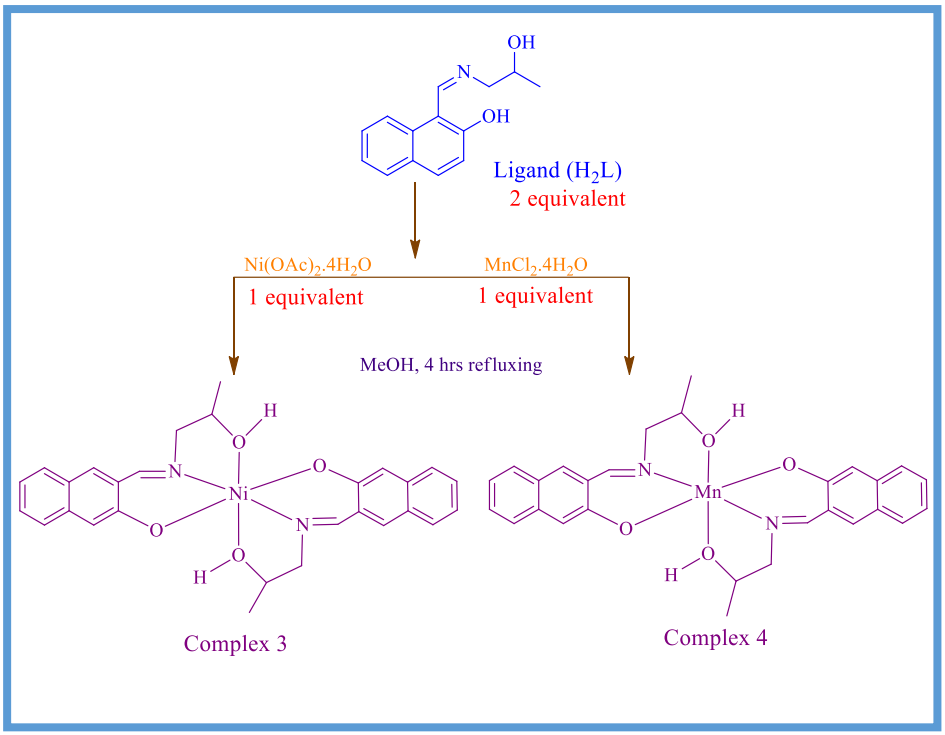
Results and Discussion

4.1 Syntheses and characterization:

Reaction of 2-hydroxynaphthaldehyde with 1-amino-2-propanol in 1:1 molar ratio in ethanol led to the formation of Schiff base ligand H_2L (Scheme 4.1). The ligand H_2L was reported previously. *[86]* Upon reaction of H_2L with copper chloride (1:1 molar ratio) in methanol in presence of triethylamine, a green coloured solution was obtained, which upon further concentration and slow evaporation furnished green needle shaped crystals of [Cu(HL)Cl(MeOH)] (Complex **1**) (Scheme 1). However, use of copper perchlorate salt with H_2L instead of copper chloride and adoption of a similar procedure like the synthesis of complex **1** with further addition of sodium thiocyanate in the reaction mixture resulted in the formation of crystals of [Cu(HL)(SCN)] (Complex **2**) (Scheme 4.1). For preparation of complex **3** $[Ni(HL)_2]$ and complex **4** $[Mn(HL)_2]$, 1: 2 molar ratio of ligand to salt (nickel acetate and manganese chloride respectively) was used in the same reaction conditions as those employed for complex **1** (Scheme 4.2). The ligand H_2L has been characterized by ^{1}H and ^{13}C NMR and ESI-MS spectroscopy. Both the complexes **1** and **2** have been characterized by IR and ESI-MS spectroscopy, elemental analyses and single crystal X-ray crystallography. Good quality crystals could not be obtained for complexes **3** and **4** but satisfactory results were obtained from IR and ESI-MS spectroscopy and elemental analyses.


H
O
OH
+
H2N
OH
OH
N
OH
Ligand (H2L)
CuCl2.2H2O
CuClO4.6H2O
MeOH, 4 hrs refluxing
N
O
H
Cu
Cl
O
O
H
Complex 1
NaSCN
MeOH, 4 hrs refluxing
N
O
H
Cu
O
NCS
Complex 2

Scheme 4.1



Scheme 4.2

4.1.1. FT-IR spectra:

The IR spectra of ligand and all the complexes (**1- 4**) have a prominent band around 1600 cm^{-1} assignable to $\bar{\upsilon}$(C=N) imine stretching mode *[87]* (Figure 4.1 – 4.5). The free ligand has the imine stretching band at $\bar{\upsilon}$ = 1636 cm^{-1} whereas for complexes **1-4**, the same band occurs at $\bar{\upsilon}$= 1621 cm^{-1}, 1623 cm^{-1}, 1618 cm^{-1}, and 1617 cm^{-1} respectively. The shift of this band towards lower frequency on complexation with the metal suggests coordination to the metal ion through imine nitrogen atom. Moreover, for complex **2** a strong band at $\bar{\upsilon}$= 2084 cm^{-1} has also been observed which can be attributed to stretching frequency of isothiocyanate group (N=C=S). *[87]*

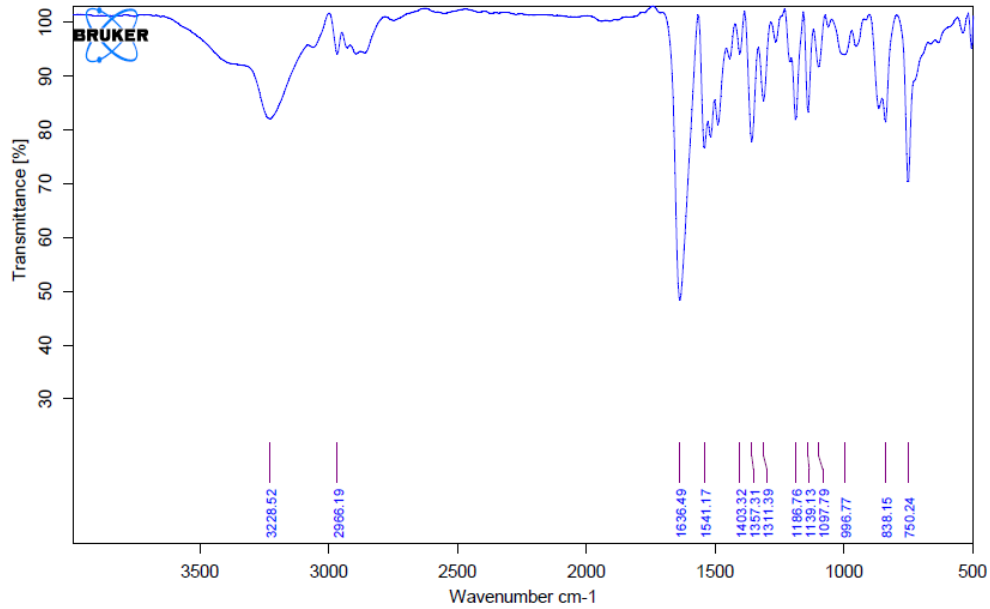


Figure 4.1: IR spectrum of ligand

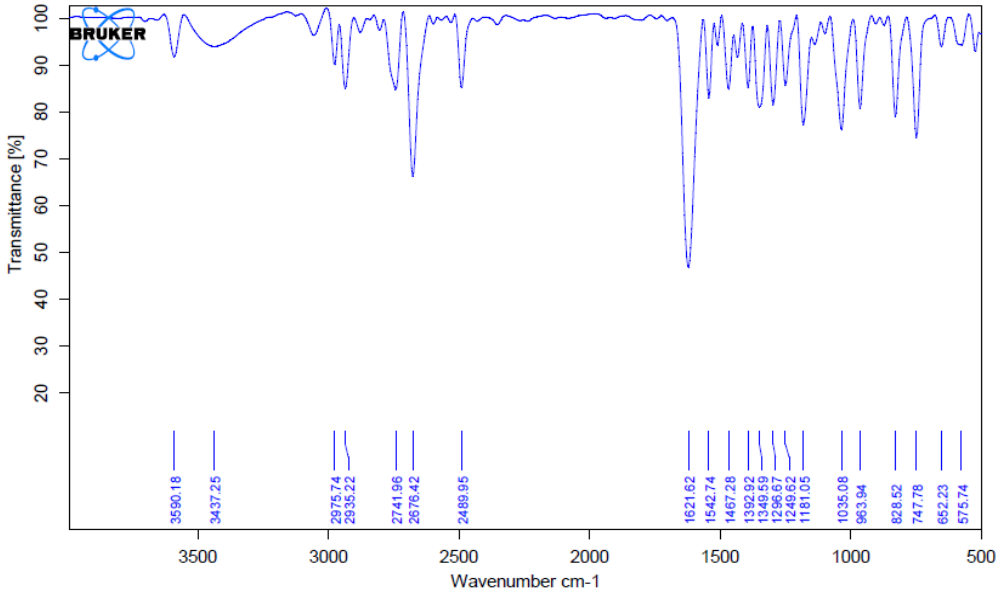


Figure 4.2: IR spectrum of Complex **1**

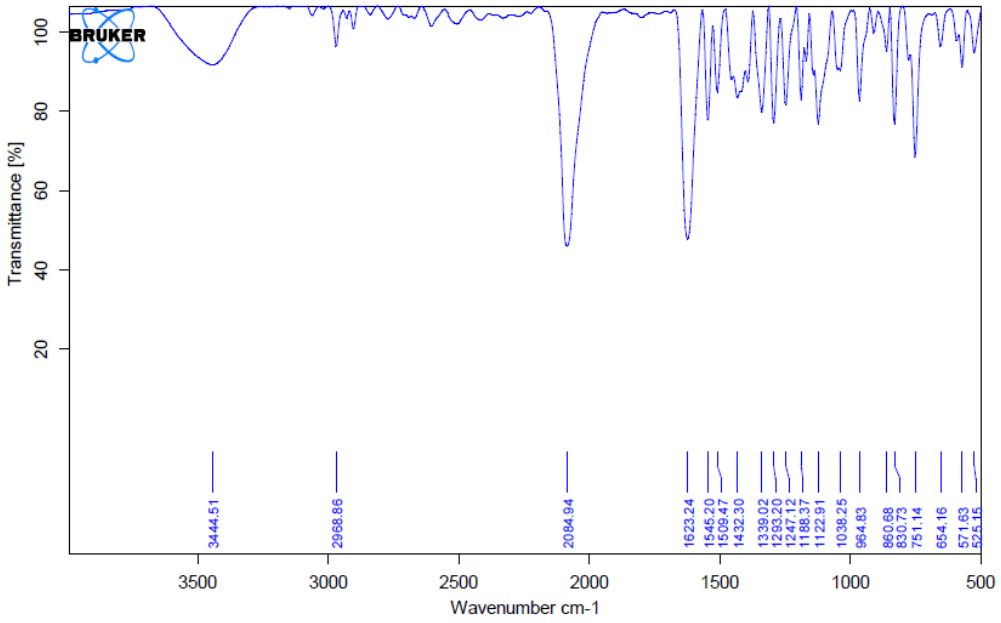


Figure 4.3: IR spectra of complex **2**

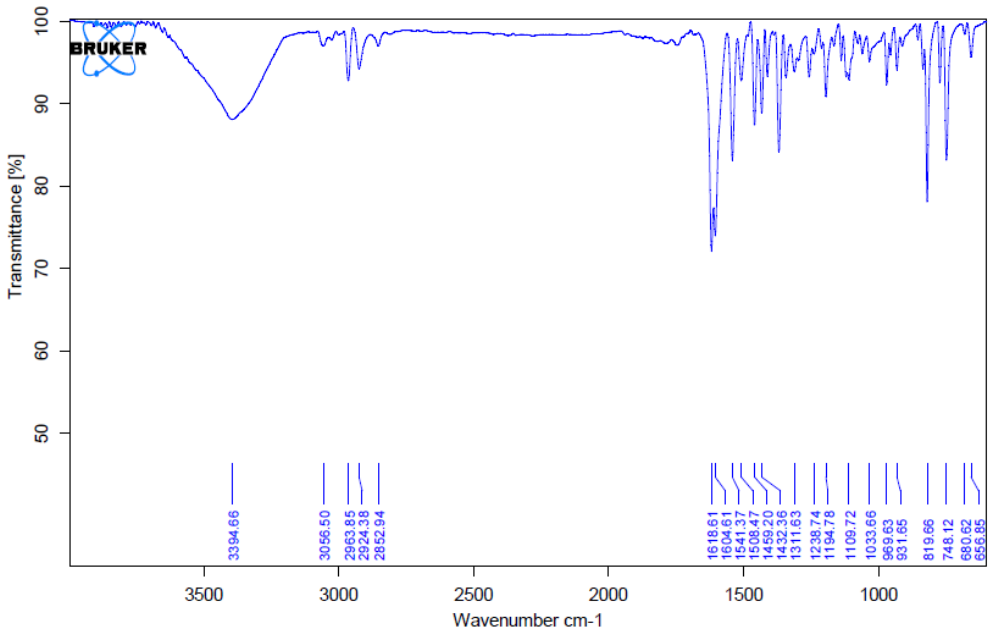


Figure 4.4: IR spectrum of Complex **3**

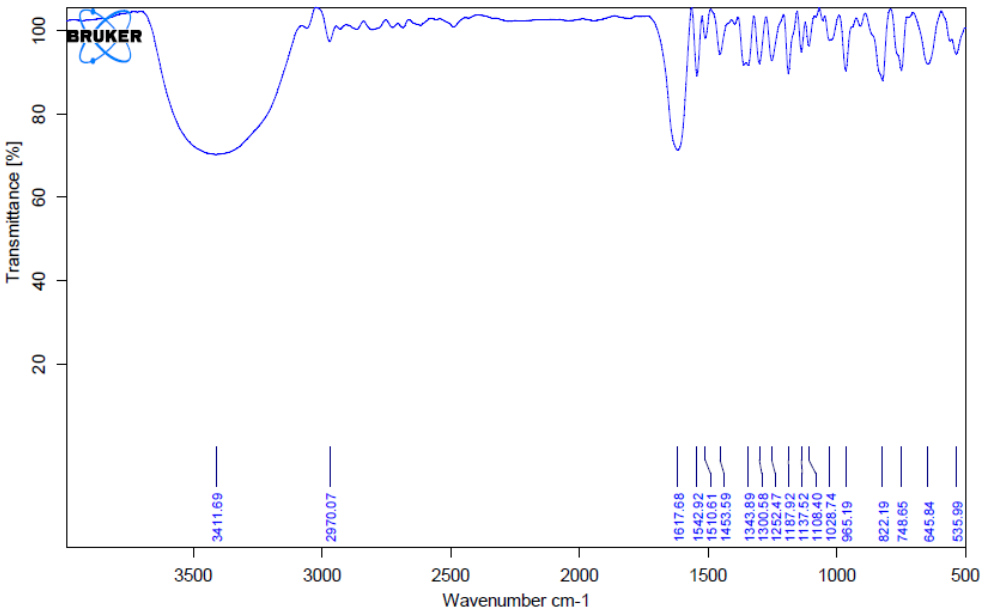


Figure 4.5: IR spectrum of Complex **4**

4.1.2. NMR spectra:

^{1}H NMR and ^{13}C NMR data were found to be in good agreement with the structure proposed for the ligand H_2L (Figure 4.6 – 4.7). For ^{1}H NMR, all the six aromatic protons lie in the range of δ = 6.5 to 8 ppm. The peak observed at δ = 8.63 ppm is the characteristic peak for benzylidenimine. Phenolic proton is observed as a broad peak at δ = 14.1 ppm. The chemical shift values for all the proton are given as:

*δ*ppm: 1.36 (d, *J* = 8 Hz, 3H,), 3.4 (dd, 1H), 3.7 (m, 2H), 4.14 (s, 1H), 6.75 (d, *J* = 8 Hz, 1H), 7.14 (t, *J* = 8 Hz, 1H), 7.37 (m, 2H), 7.44 (d, *J* = 8 Hz 1H), 7.73 (d, *J* = 8 Hz, 1H), 8.64 (s, 1H), 14.1 (s, 1H)

In ^{13}C NMR, a total of 14 peaks were obtained. All of the naphthalene carbons were observed in the range of δ = 118 to 135 ppm, except for the carbon directly attached to –OH group, which was observed at δ = 159 ppm.

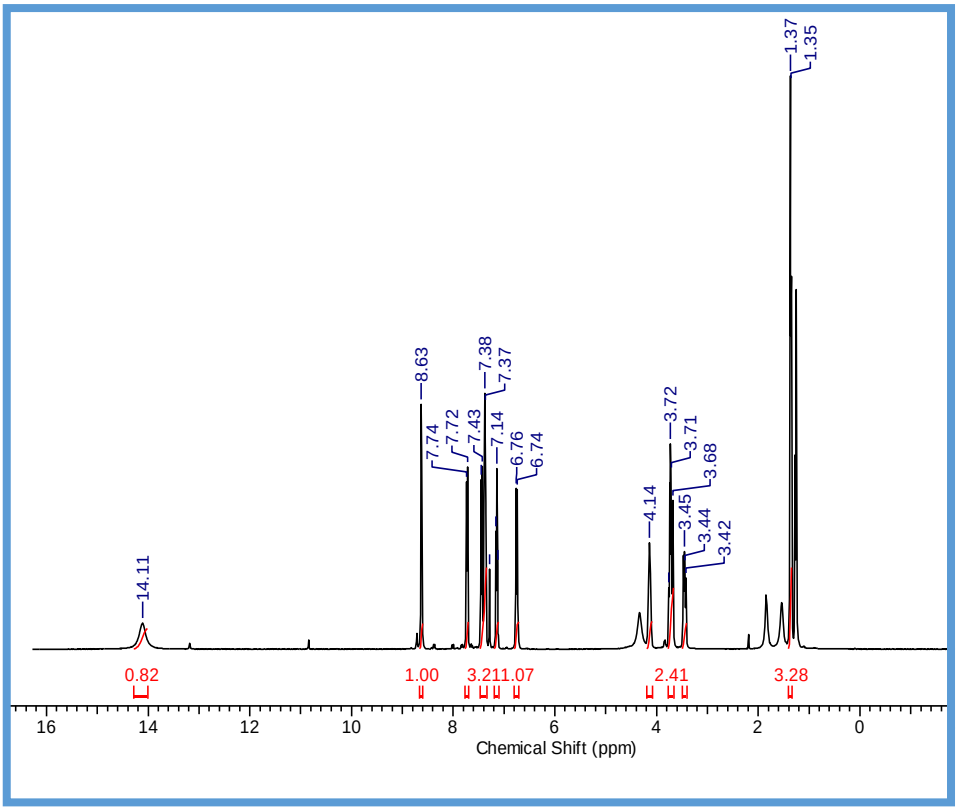


Figure 4.6: ^{1}H NMR data of ligand H_2L

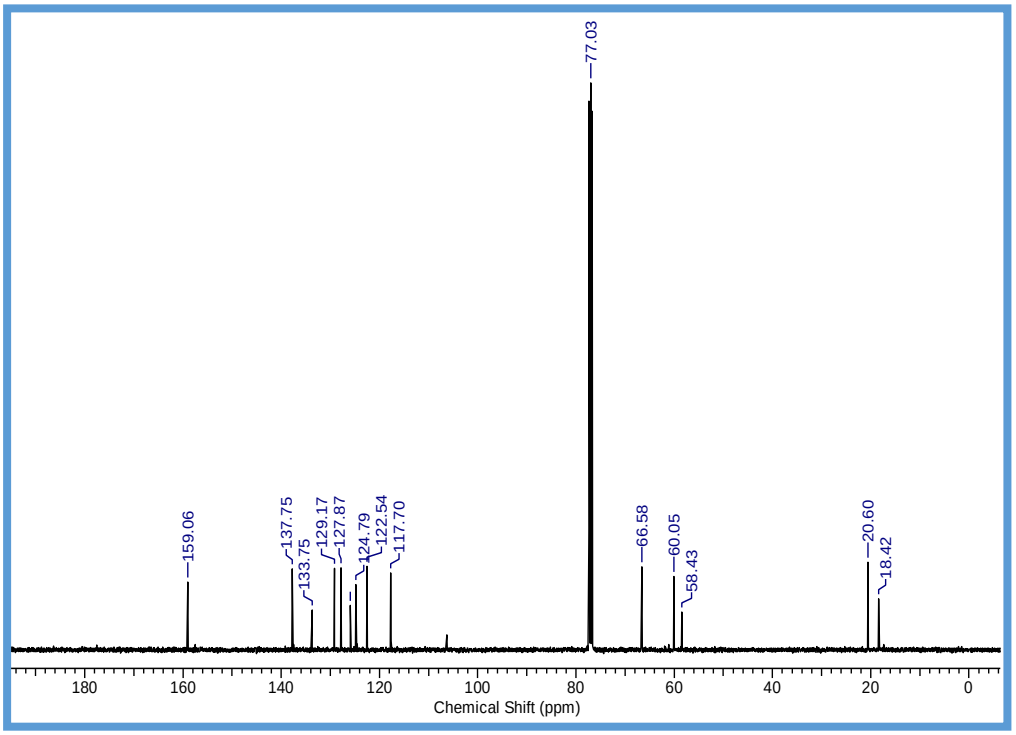


Figure 4.7: ^{13}C NMR data of ligand H_2L

4.1.3. Mass spectra:

The electrospray ionization mass (ESI-MS positive) spectra of Schiff base ligand and their metal complexes provide reliable evidence for the formation of the suggested structures (Figure 4.8 – 4.12). The spectra of ligand H_2L complex **3** and complex **4** showed $[M + H]^+$ molecular ion peak at m/z = 230.4, 515.1, 511.2 a.m.u. respectively and complex **2** showed $[M + Na]^+$ molecular ion peak at m/z = 372. However in the spectra of complex **1**, base peak at m/z = 291 a.m.u. was observed which can be assigned to monocationic specie with formula $[Cu(HL)]^+$.

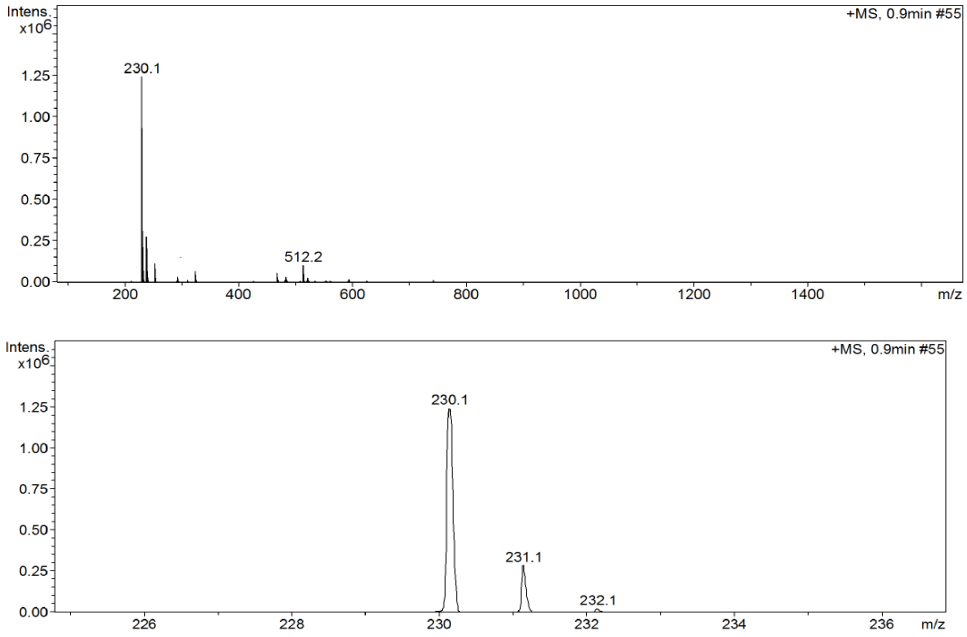


Figure 4.8: ESI-MS spectra of ligand H_2L

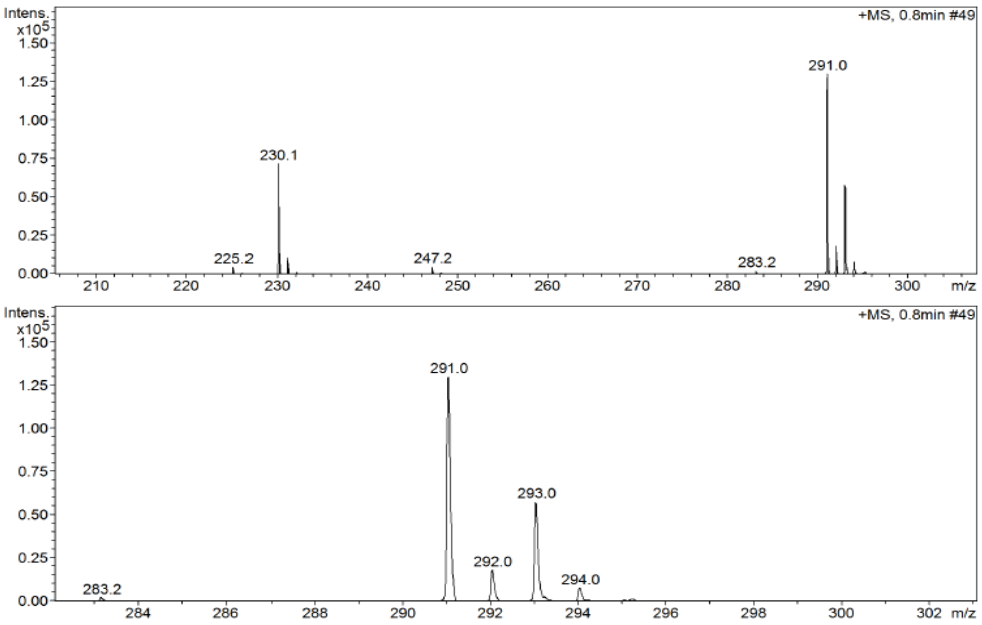


Figure 4.9: ESI-MS spectra of complex **1**

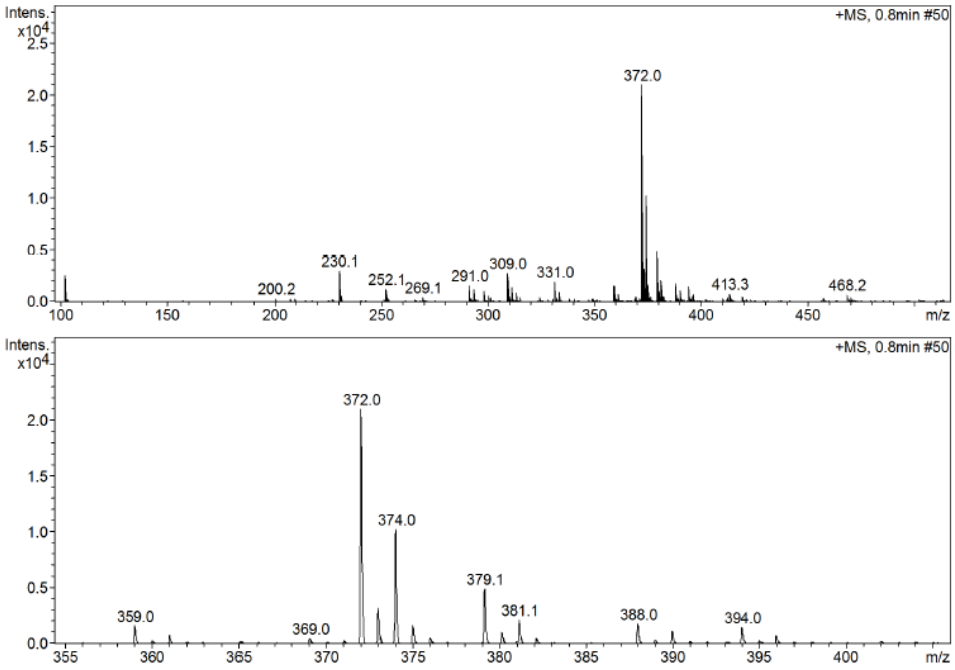


Figure 4.10: ESI-MS spectra of complex **2**

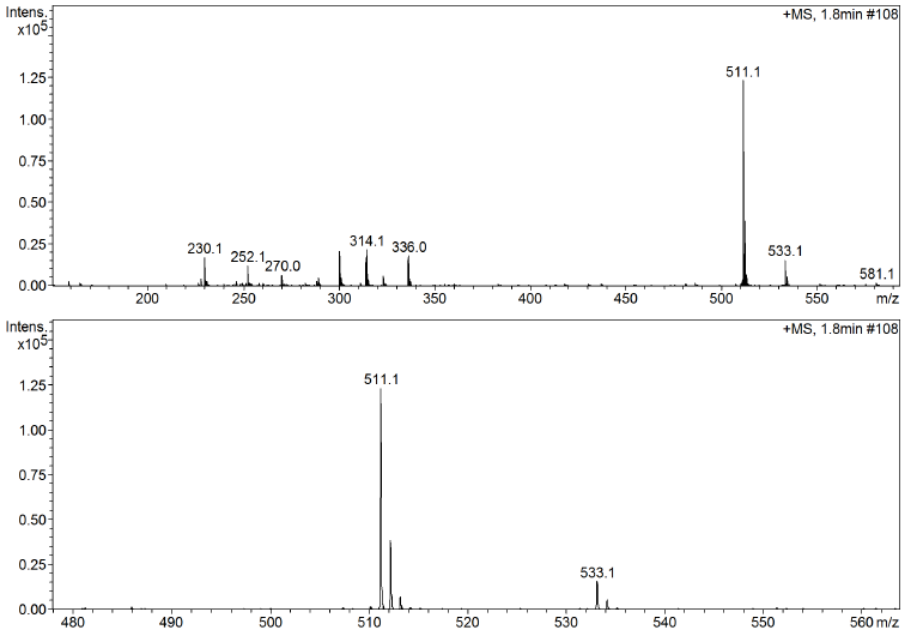


Figure 4.11: ESI-MS spectra of complex **3**

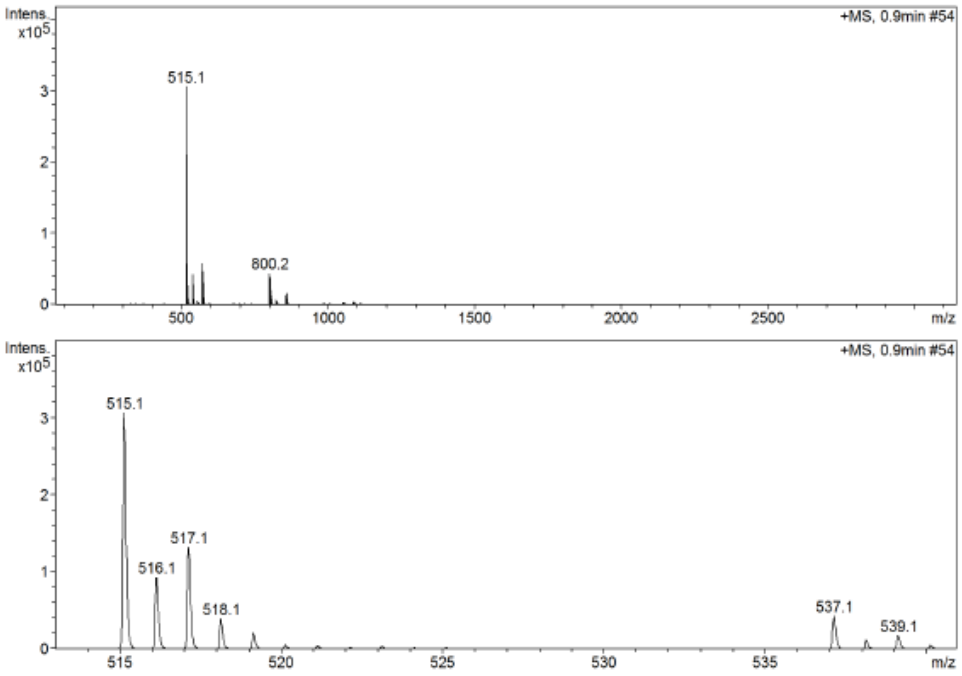


Figure 4.12: ESI-MS spectra of complex **4**

4.1.4. X-ray crystallography:

Complexes **1** and **2** have been structurally characterized by X-ray crystallography. The selected bond lengths and angles are given in Table 4.1 and the details of data collection conditions and parameters of refinement process are given in Table 4.2

Table 4.1: Selected bond lengths (Å) and bond angles (˚) for complex **1** and **2**.

Complex 1		**Complex 2**	
Cu(1)-O(1)	1.915(5)	Cu(1)-N(1)	1.9015(18)
Cu(1)-N(1)	1.928(5)	Cu(1)-O(1)	1.9129(15)
Cu(1)-O(2)	2.028(5)	Cu(1)-N(2)	1.919(2)
Cu(1)-Cl(1)	2.2392(19)	Cu(1)-O(2)	2.0265(17)
Cu(1)-O(4)	2.331(6)	N(1)-Cu(1)-O(1)	94.05(7)
O(1)-Cu(1)-N(1)	92.9(2)	N(1)-Cu(1)-N(2)	171.35(8)
O(1)-Cu(1)-O(2)	168.0(2)	O(1)-Cu(1)-N(2)	93.60(8)
N(1)-Cu(1)-O(2)	82.1(2)	N(1)-Cu(1)-O(2)	82.96(7)
O(1)-Cu(1)-Cl(1)	91.52(15)	O(1)-Cu(1)-O(2)	160.41(8)
N(1)-Cu(1)-Cl(1)	171.47(18)	N(2)-Cu(1)-O(2)	91.14(8)
O(2)-Cu(1)-Cl(1)	92.22(14)	C(1)-O(1)-Cu(1)	125.85(13)
O(1)-Cu(1)-O(4)	98.9(2)	C(13)-O(2)-Cu(1)	112.08(13)
N(1)-Cu(1)-O(4)	86.1(2)	Cu(1)-O(2)-H(101)	106(2)
O(2)-Cu(1)-O(4)	91.6(2)	C(11)-N(1)-Cu(1)	125.63(15)
Cl(1)-Cu(1)-O(4)	100.43(16)	C(12)-N(1)-Cu(1)	113.92(14)
C(1)-O(1)-Cu(1)	125.1(4)	C(15)-N(2)-Cu(1)	170.4(2)
C(13)-O(2)-Cu(1)	110.9(4)		
Cu(1)-O(2)-H(2)	124.5		
C(15)-O(4)-Cu(1)	119.5(6)		
Cu(1)-O(4)-H(101)	109(7)		
C(11)-N(1)-Cu(1)	125.6(4)		
C(12)-N(1)-Cu(1)	113.9(4)		

Table 4.2: Crystal refinement data for complex **1** and **2**

Complex	**1**	**2**
Empirical Formula	$C_{15}H_{18}ClCuNO_3$	$C_{15}H_{14}CuN_2O_2S$
Formula weight	359.29	349.88
Crystal system	Monoclinic	Triclinic
Space group	P 21/n	P -1
a (Å)	14.3109(7)	8.4523(4)
b (Å)	7.8159(3)	8.8494(5)
c (Å)	14.7975(7)	10.3691(7)
α (°)	90	102.354(5)
β (°)	111.500(6)	101.454(5)
γ(°)	90	101.476(5)
V (Å^3)	1539.97(13)	718.54(8)
λ (Å)	1.54184	1.54184
ρ_{calcd} (mg m^{-3})	1.550	1.617
Z	4	2
T (K)	293(2) K	293(2)
μ (mm^{-1})	3.677	3.549
F(0 0 0)	740	358
Crystal size (mm^3)	0.330 x 0.260 x 0.210	0.230 x 0.190 x 0.150
θ ranges (°)	5.402 - 71.298	4.511 - 71.355
h/k/l	-16,17/-9,8/-15,18	-10,8/-10,10/-12,12
Reflections collected	9608	4378

Independent reflections	2943	2714
T_{max} and T_{min}	1.00000 and 0.38750	1.00000 and 0.63802
Data/restraints/parameters	2943 / 7 / 196	2714 / 0 / 196
Goodness-of-fit (GOF) on F^2	1.139	1.037
Final R indices [I > 2σ(I)]	R1 = 0.0849, wR2 = 0.2602	R1 = 0.0359 , wR2 = 0.1066
R indices (all data)	R1 = 0.0865, wR2 = 0.2608	R1 = 0.0363, wR2 = 0.1070
Largest peak and hole(e $Å^{-3}$)	2.009 and -1.015	0.637 and -0.404
CCDC No.	1544784	1544785

i) Crystal structure of [Cu(HL)(MeOH)Cl] (**1**)

Complex **1** is mononuclear with the monoclinic crystal system and P 21/n space group. Copper atom is located in a five-coordinated environment completed by one N atom and two O atom from H_2L ligand and the remaining sites are occupied by chloride ion and MeOH group. The distance between metal and coordination atoms were: Cu(1)-O(1) = 1.915(5) Å, Cu(1)-N(1) = 1.928(5) Å, Cu(1)-O(2) = 2.028(5) Å, Cu(1)-Cl(1) = 2.2392(19) Å, Cu(1)-O(4) = 2.331(6) Å. The selected angles between metal and coordination atoms are found to be to the tune of previously reported structures. The space configuration of five-coordinated complexes could be well defined by the τ value [$\tau = (\beta - \alpha)/60$ where α and β being the two largest coordination angles], $\tau = 0$ for an ideal square pyramid as well as $\tau = 1$ for an ideal triangular bipyramid. For complex **1** the value of $\tau = 0.058$, thus it forms slightly distorted square pyramidal geometry. Figure 4.13 shows the ORTEP diagram of complex **1** upto 70% ellipsoidal probability level. Two discrete

molecules of complex **1** were closely packed *via* H-bonding through O(4)-H(101)….Cl(1) atoms to complete a 1D polymeric chain (Figure 4.14).

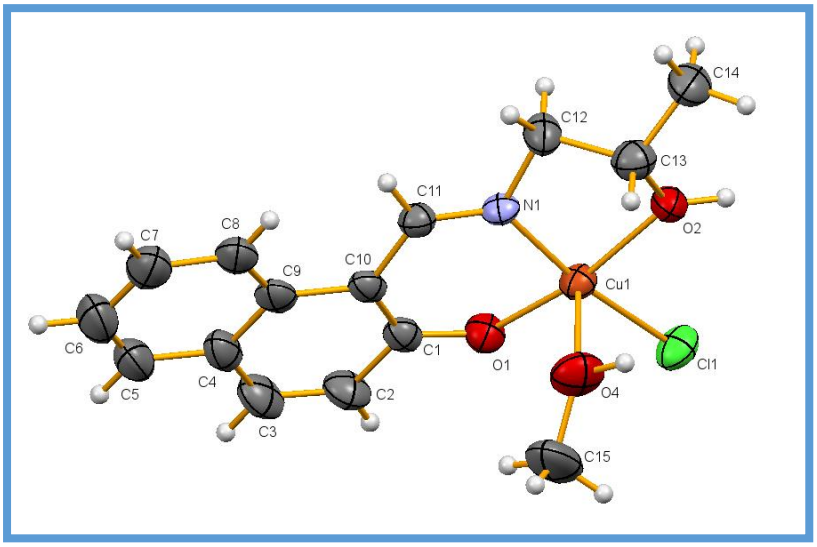


Figure 4.13: ORTEP diagram of complex **1**

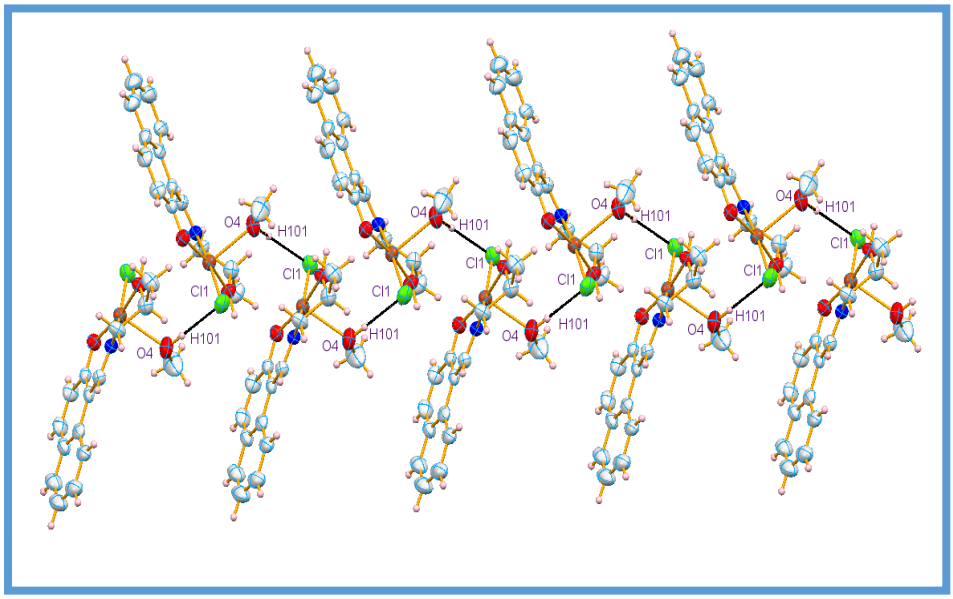


Figure 4.14: 1D polymeric chain of complex **1** closely packed via H-bonding through O(4)-H(101)….Cl(1) atoms.

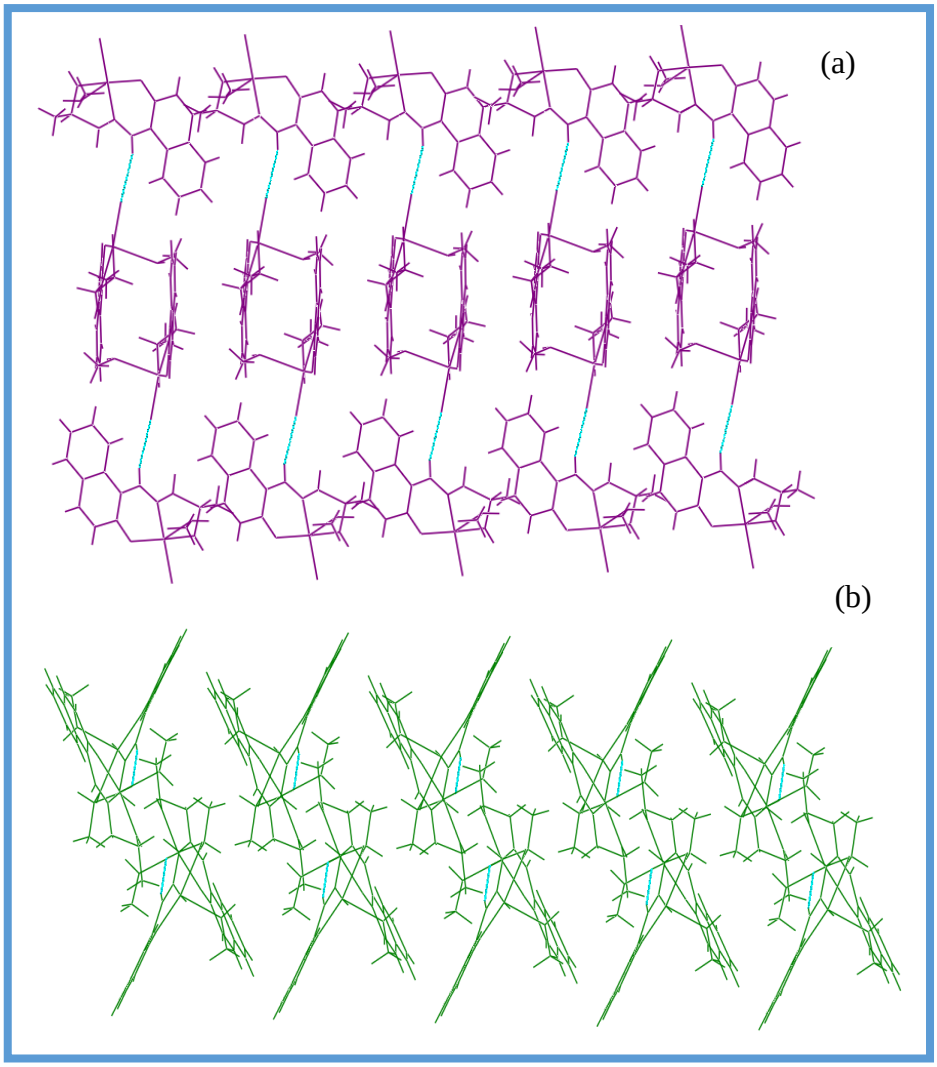


Figure 4.15: Different representations (a,b) of H- bonding between two discrete molecules of Complex **1**

ii) Crystal structure of [Cu(HL)(NCS)] (**2**)

Complex 2 is mononuclear with the triclinic crystal system and P -1 space group. The central Cu metal atom is tetra-coordinated with three of the coordination sites fulfilled by one N atom and two O atom of ligand H_2L. The remaining one position is occupied by N atom of isothiocyanate group. The bond distances and angles are similar to that earlier reported structures. The bond angles reveal that complex **2** occurs in distorted square planar geometry. Figure 4.16 shows the ORTEP diagram of complex **2** upto 70% ellipsoidal probability level.

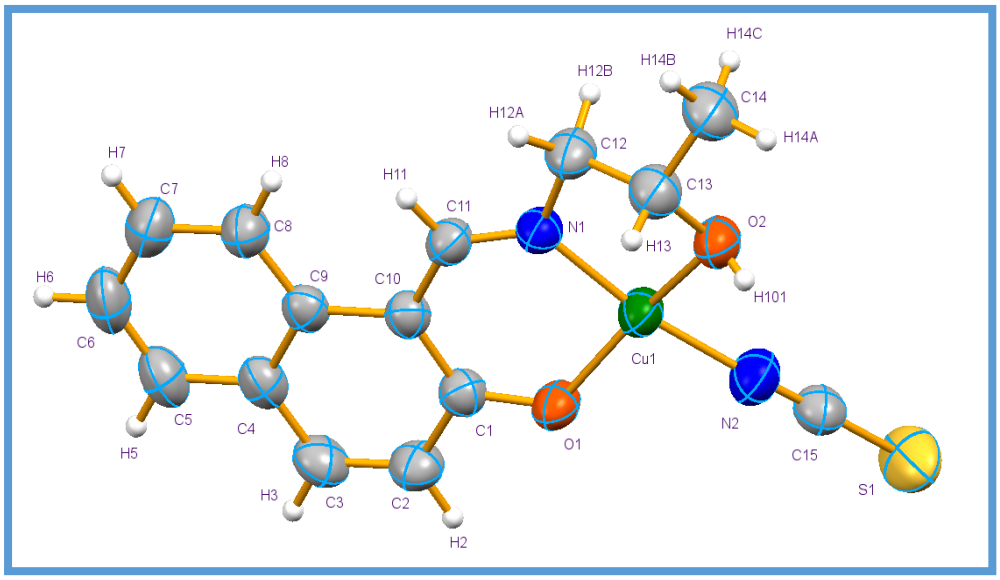


Figure 4.16: ORTEP diagram of complex **2**

4.2 BSA study:

To understand the interaction of synthesized complexes with proteins, their effect on the intrinsic fluorescence emission band of bovine serum albumin has been analyzed. For this purpose separate solution of BSA was titrated against addition of complexes **1**-**4**. It was observed that significant decrease of initial fluorescence intensity (figure 4.17 – 4.20) is caused by the addition of complexes to the respective protein solution.

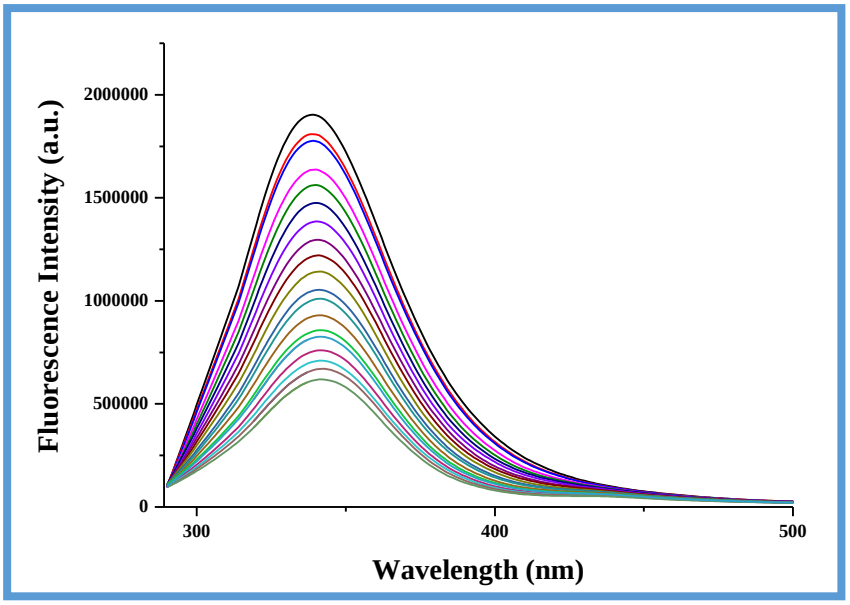


Figure 4.17: Fluorescence quenching of BSA by complex **1**

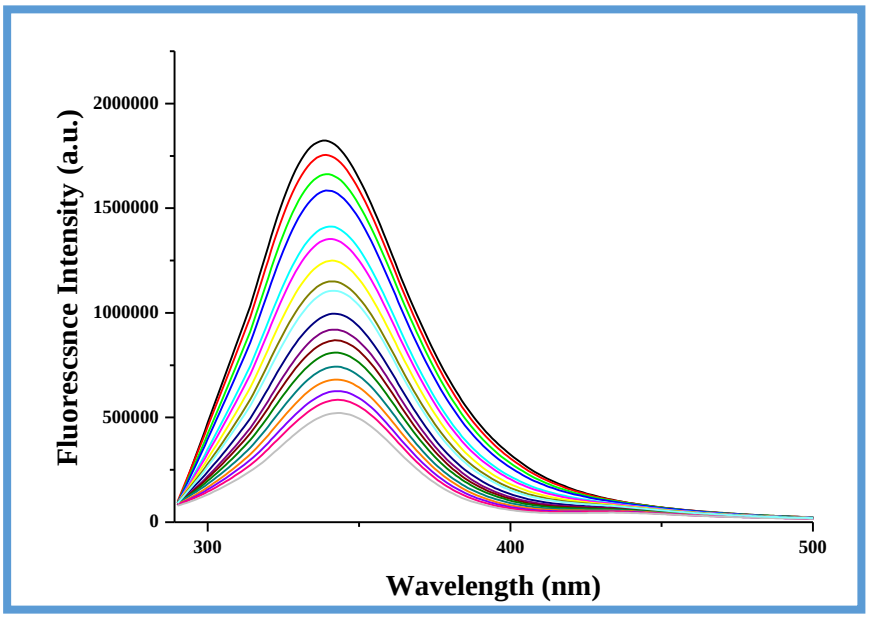


Figure 4.18: Fluorescence quenching of BSA by complex **2**

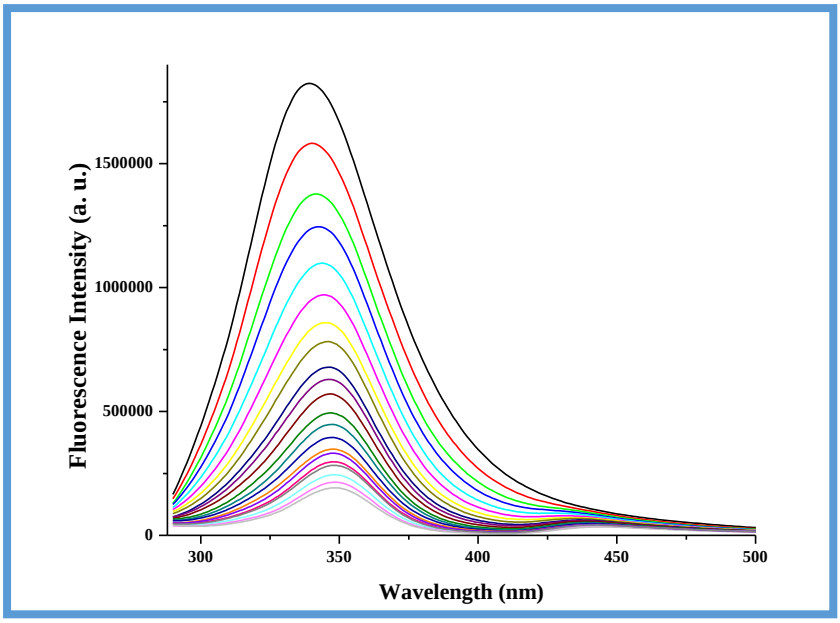


Figure 4.19: Fluorescence quenching of BSA by complex **3**

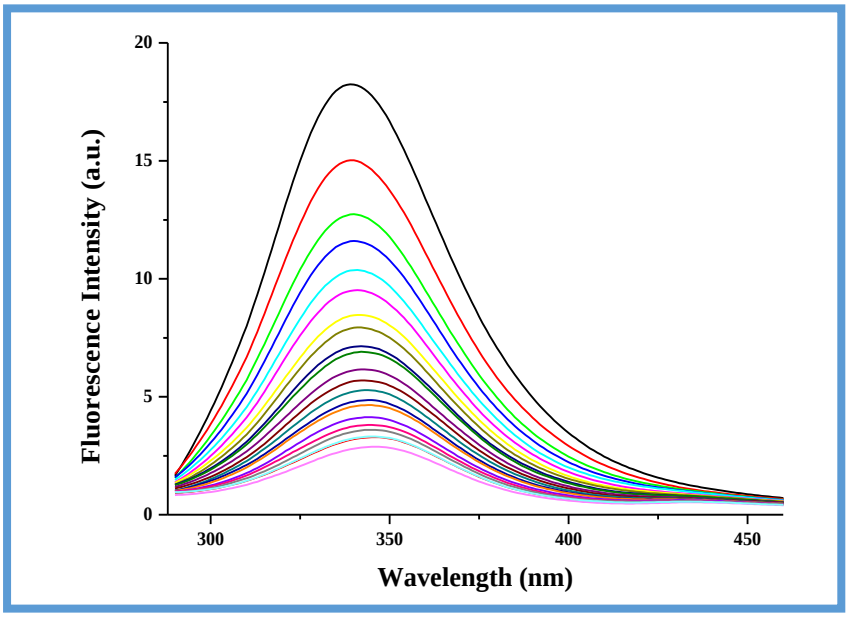


Figure 4.20: Fluorescence quenching of BSA by complex **4**

To get further insight in the quenching process, the fluorescence quenching data were analyzed with Stern-Volmer equation, (graphs shown in figure 4.21 – 4.24) according to which:

$$\frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} [Q]$$

where F_0 and F are the fluorescence intensities in the absence and the presence of a quencher, k_q is the bimolecular quenching rate constant, τ_0 is the average lifetime of fluorophore in the absence of a quencher and [Q] is the concentration of a quencher (metal complexes). K_{SV} is the Stern–Volmer quenching constant in M^{-1}.The K_b value obtained for complexes **1-4** are of order 10^4-10^6 (M^{-1}) with complex **1** showing maximum affinity for protein binding with $K_b = 2.798 \times 10^6$ (M^{-1})

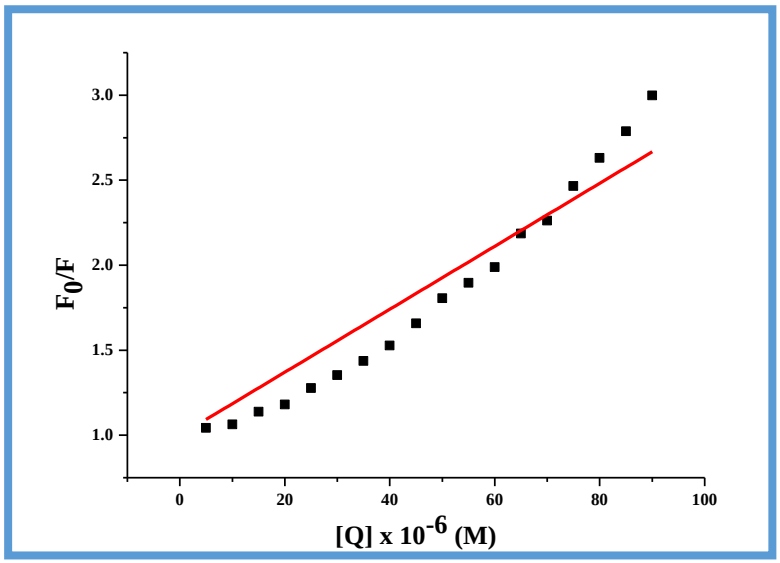


Figure 4.21: Stern volmer plot for complex **1**

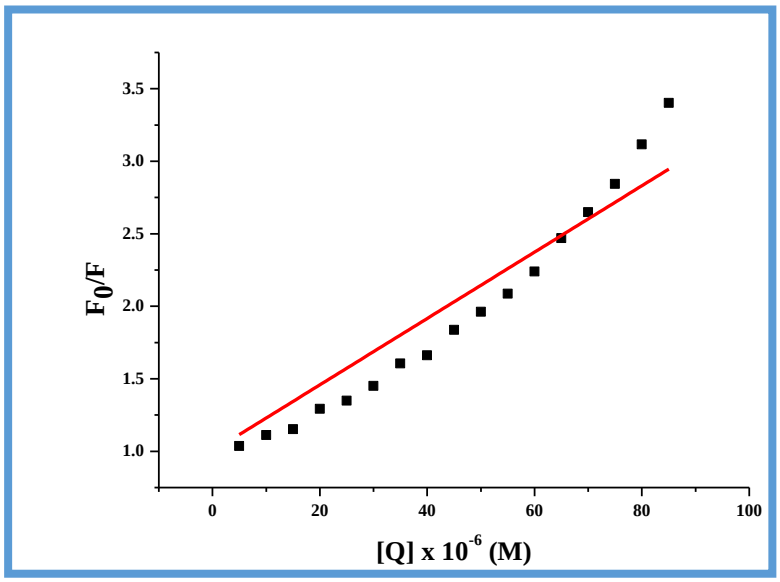


Figure 4.22: Stern volmer plot for complex **2**

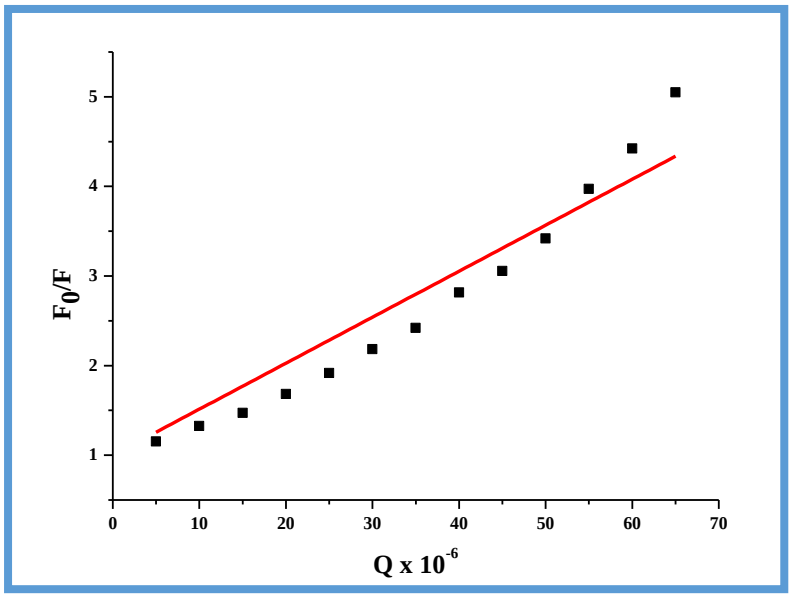


Figure 4.23: Stern volmer plot for complex **3**

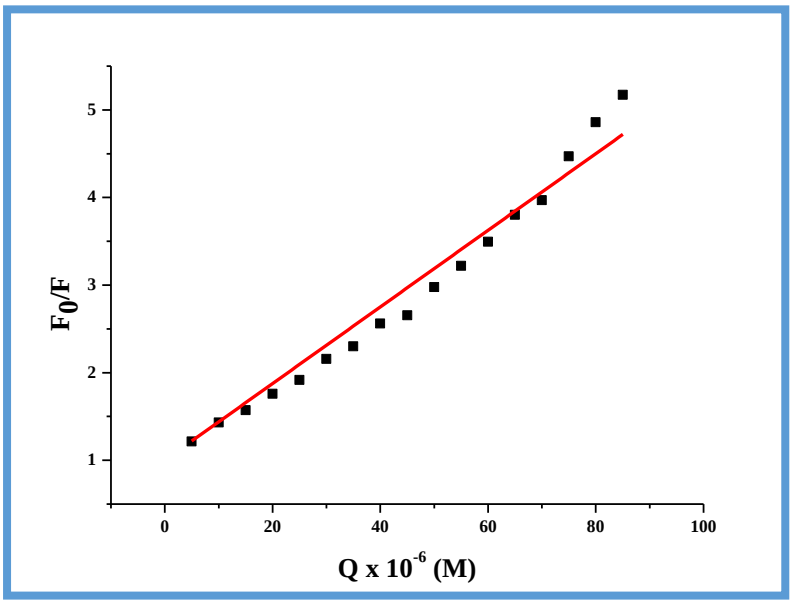


Figure 4.24: Stern volmer plot for complex **4**

The binding constant (K_b) and number of binding sites (n) have been determined by Scatchard plot (figure 4.25 – 4.28), equation of which is given by:

$$\log \left[\frac{F_0 - F}{F} \right] = \log K_b + n \log [Q]$$

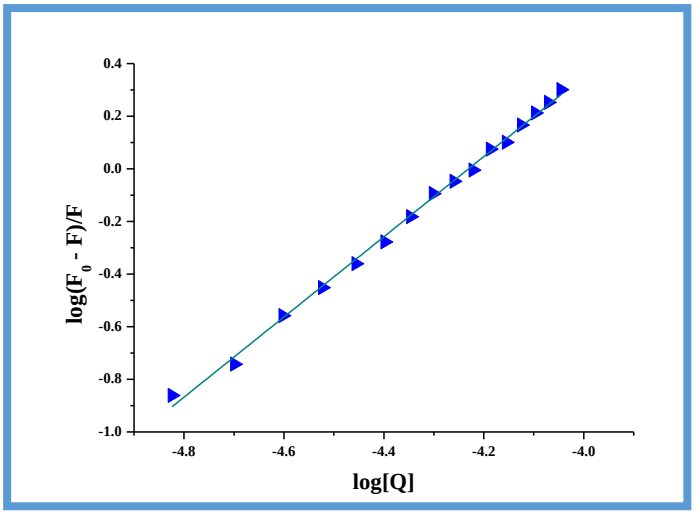


Figure 4.25: Scatchard plot for complex **1**

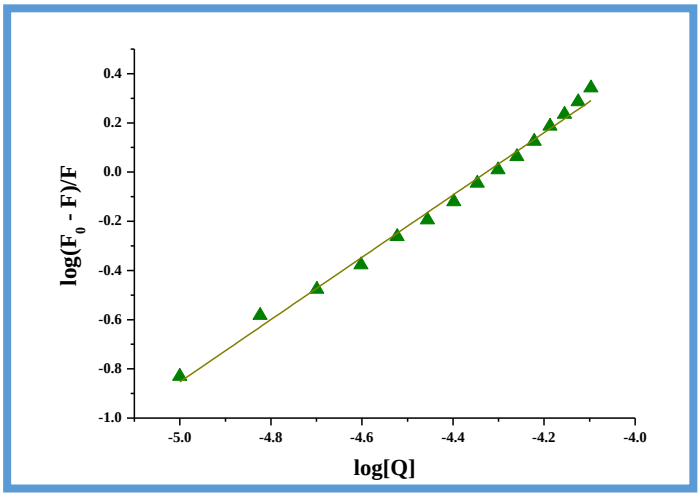


Figure 4.26: Scatchard plot for complex **2**

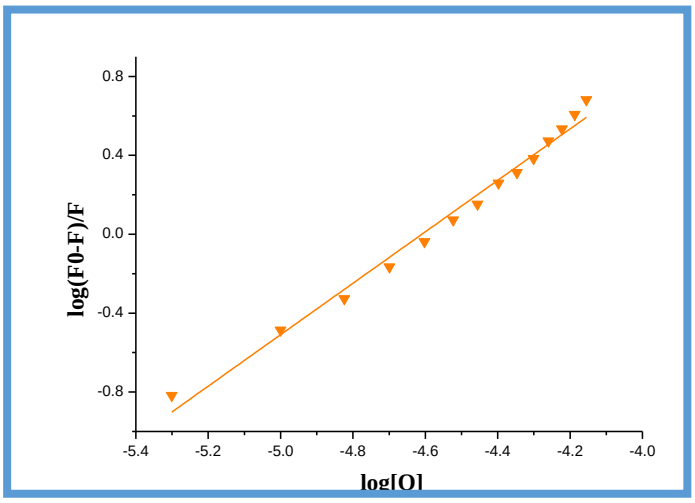


Figure 4.27: Scatchard plot for complex **3**

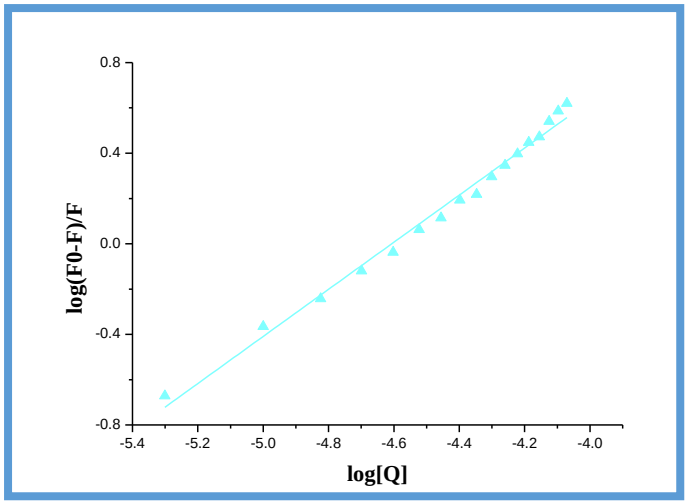


Figure 4.28: Scatchard plot for complex **4**

The calculated values of Ksv, n and kq for the interaction of the complexes with the BSA are given in Table 4.3 indicating substantial BSA binding affinity of the complexes.

Table 4.3: Table for Stern–Volmer quenching constant, binding constant and binding site

Catalyst	**K_{SV} (M^{-1})**	**k_q (M^{-1} s^{-1})**	**K_b (M^{-1})**	**n**
Complex 1	1.8521×10^4	3.0868×10^{12}	2.7918×10^6	1.52
Complex 2	2.2895×10^4	3.8158×10^{12}	0.3010×10^6	1.26
Complex 3	5.1334×10^4	8.5557×10^{12}	1.0295×10^6	1.30
Complex 4	4.3767×10^4	7.2945×10^{12}	6.1944×10^4	1.04

4.3 Catalytic activity and kinetic study:

For investigating the catecholase activities of synthesized metal complexes, solution of the complex in methanol was treated with 3,5-di-*tert*-butylcatechol (3,5-DTBC). The reactions were carried out at 25

°C in aerobic conditions. The oxidation product, 3,5-DTBQ, is considerably stable and has a strong absorption at λ_{max} = 400 nm. Therefore, activities and reaction rates were determined using electronic spectroscopy by following the appearance of the absorption maximum of the quinone. Upon addition of 100 equivalents of catecholic substrate to 10^{-4} M solution of complexes **1-4** in methanol, a new band starts to gradually appear at 398 nm for complex **1** and at 397 nm for complex **2** (figure 4.29 – 4.30) . With time, the intensity of the band increases indicating the increase in the concentration of the oxidised product 3,5-DTBQ. Complex **3** and complex **4** do not show appreciable increment of the DTBQ band. To understand the kinetic aspects of catalysis, the rate constant for a catalyst complex was determined by traditional initial rate methods. The observed rate versus substrate concentration data were then analyzed on the basis of the Michaelis–Menten approach of enzymatic kinetics. The Michaelis–Menten constant (K_M) and maximum initial rate (V_{max}) were determined by linearization using Lineweaver–Burk plots (Figure 4.33 – 4.34). The turnover number values (k_{cat}) were obtained by dividing the V*max* values by the concentration of the corresponding complexes. All the data explicitly establish that both the complexes **1** and **2** are very much active.

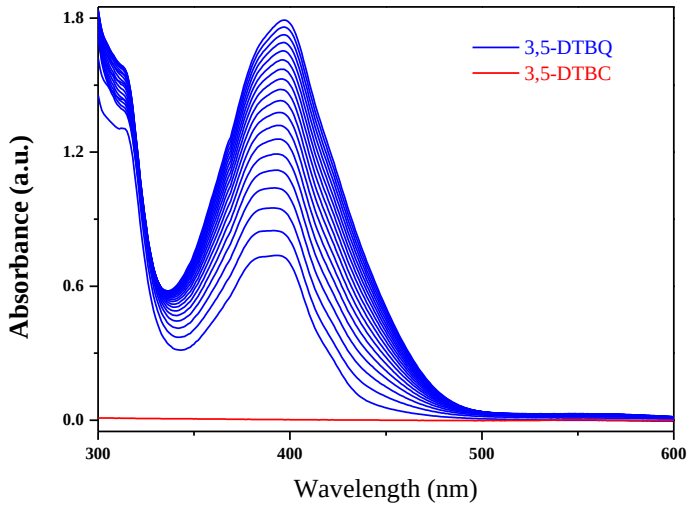


Figure 4.29: UV-Vis spectra of oxidation of 3,5-DTBC to 3,5-DTBQ with time using complex **1**

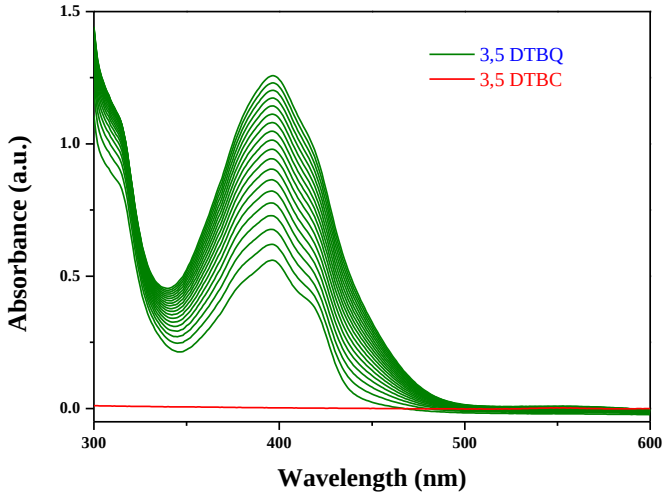


Figure 4.30: UV-Vis spectra of oxidation of 3,5-DTBC to 3,5-DTBQ with time using complex **2**

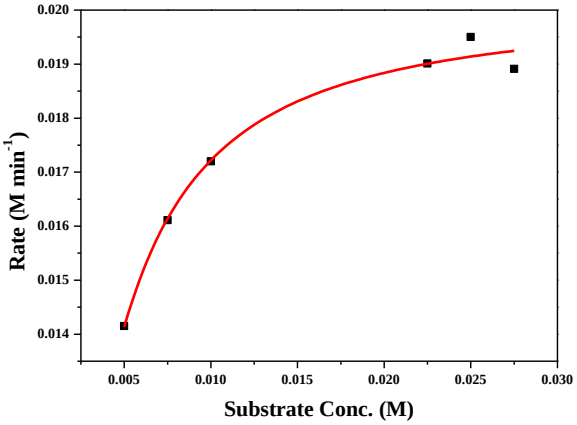


Figure 4.31: Plot of rate vs. substrate concentration for complex **1**

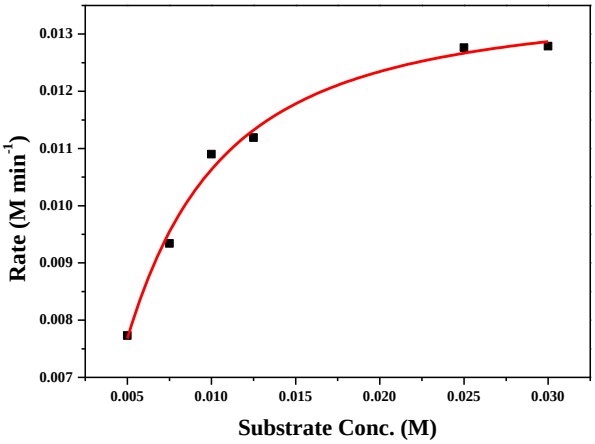


Figure 4.32: Plot of rate vs. substrate concentration for complex **2**

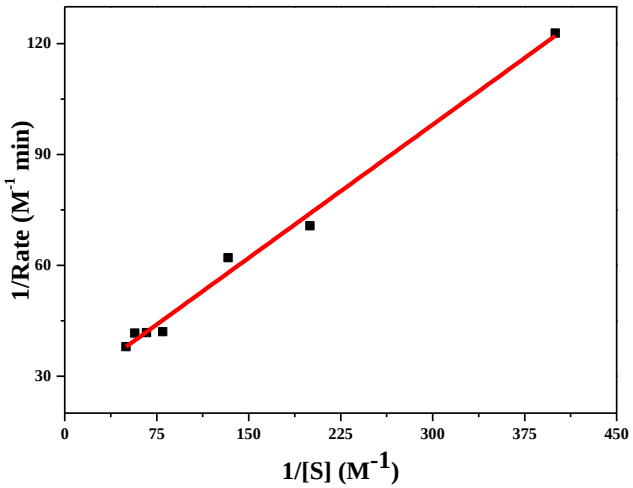


Figure 4.33: Line weaver Burk plot for complex **1**

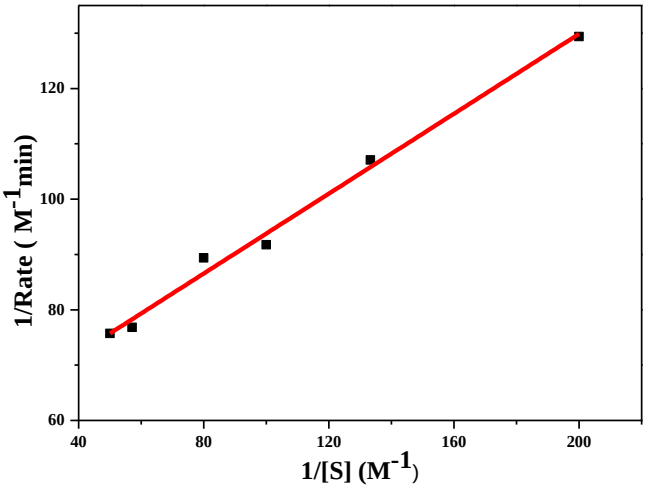


Figure 4.34: Line weaver Burk plot for complex **2**

The details of different enzyme kinetic parameters at complex concentration of 10^{-4} (M) in MeOH are tabulated in Table 4.4 which demonstrates the k_{cat} values as 1.2036 x 10^{4} h^{-1} for **1** and 0.8142 x 10^{4} h^{-1} for **2**.

Table 4.4: Different enzyme kinetic parameter for complex **1** and complex **2** in MeOH

Catalyst	**Vmax** **(M min^{-1})**	**Std. Error**	**K_m (M)**	**K_{cat}/T.O.F** **(h^{-1})**
Complex 1	0.02006	5.9415 x 10^{-4}	0.00262	1.2036 x 10^4
Complex 2	0.01357	4.1992 x 10^{-4}	0.00418	0.8142 x 10^4

To examine the probable reaction pathway and intermediate formed during the oxidation reaction, the probable complex-substrate intermediate was analyzed through ESI-MS (Figure 4.35). The ESI-MS spectrum of complex **1** recorded after 10 min of mixing exhibited two peaks of considerable intensity at m/z = 243 and 511 owing to the species $[(3,5\text{-DTBQ}) + Na]^+$ and complex-product [(HL)Cu-3,5-DTBQ] aggregate respectively and a small peak at m/z = 463 corresponding to $[(3,5\text{-DTBQ})_2 + Na]^+$.

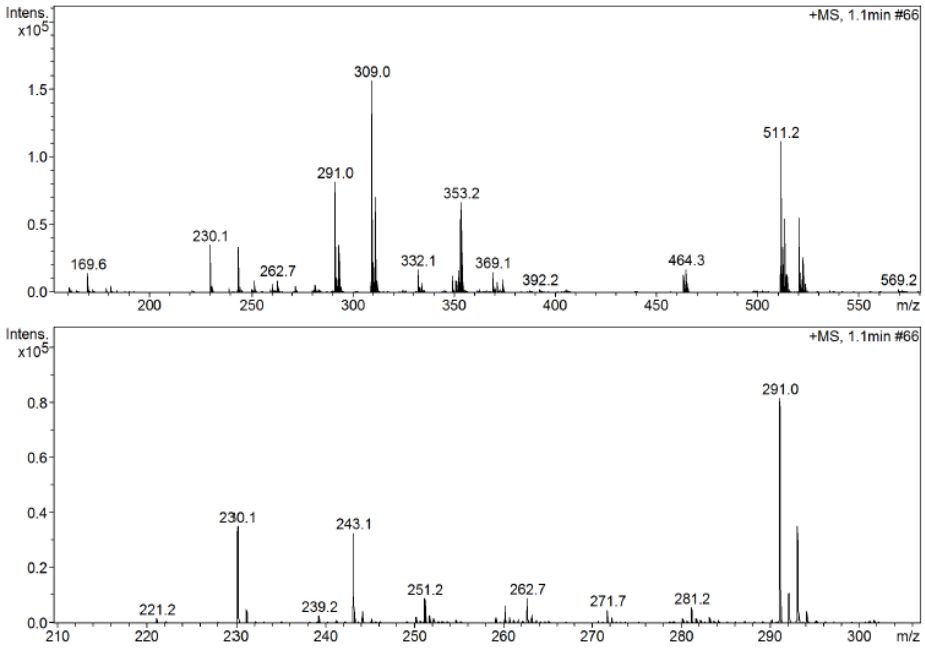
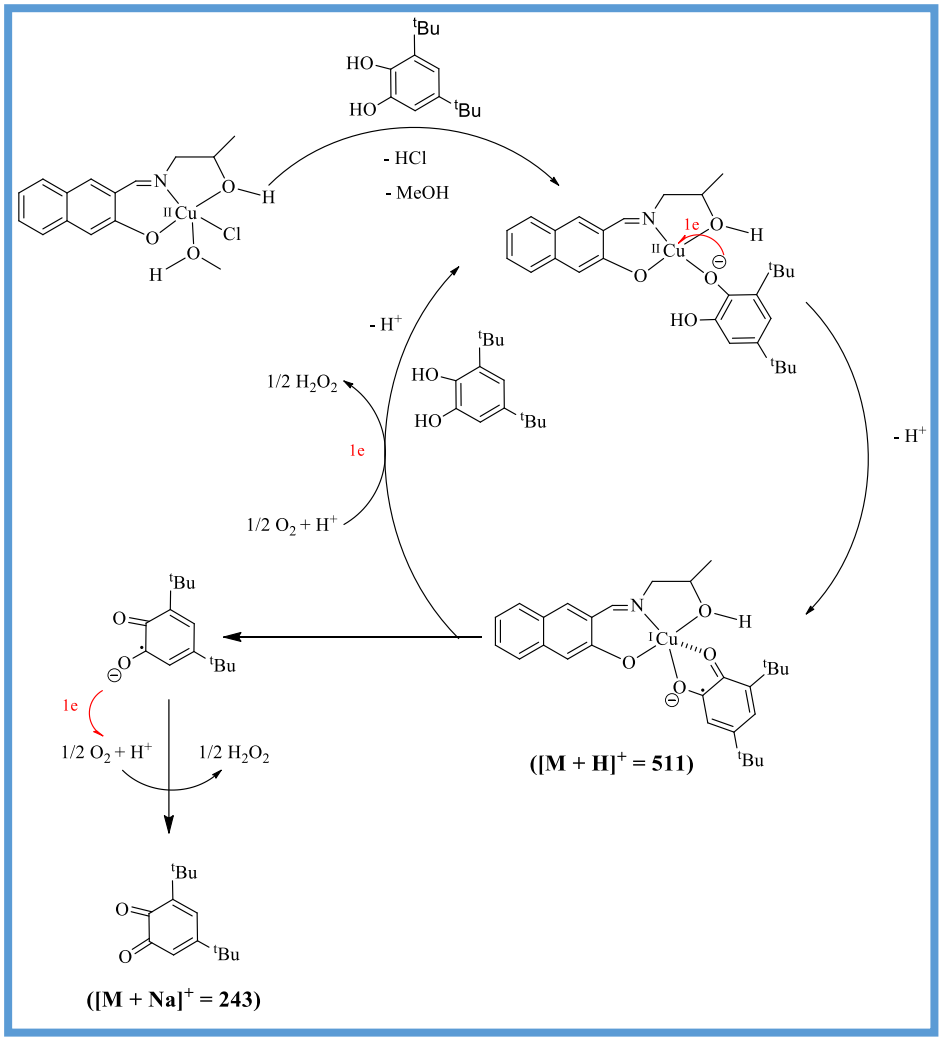


Figure 4.35: ESI-MS spectrum of the reaction mixture obtained 10 min after the addition of substrate 3,5-DTBC to the methanolic solution of complex **1**, showing the peak of product as well as intermediate.

Based on the information obtained from ESI-MS and previously reported database,*[88]* a reaction mechanism was fashioned out, in which it was proposed that oxidation of catechol using complex **1** was following a radical pathway and possibly through a pentacoordinated intermediate as represented in scheme 4.3.



Scheme 4.3

According to the reported generalized catecholase reaction mechanism, *[89]* electron transfer is mainly facilitated by metal center and then further delocalized via C=N bond of metal Schiff-base complex to the adjacent conjugate system.

4.4 H_2O_2 detection:

Another interesting feature is formation of H_2O_2 from the reduction of atmospheric oxygen during the catechol oxidation reaction. Modified iodometric method was used for the qualitative detection of H_2O_2 formed during the catalytic reaction. After the subsequent work-up of the reaction mixture containing catechol substrate and complex followed by addition of KI (detailed description provided in the experimental section) resulted in oxidation of I^- to I_2 due to the presence of H_2O_2. In the presence of excess of I^-, I_2 is converted to I_3^-. The reactions are given as:

$$H_2O_2 + 2I^- + 2H^+ \rightarrow 2H_2O + I_2 \quad \text{and}$$

$$I_2\,(aq.) + I^- \rightarrow I_3^-$$

The formation of I_3^- was monitored by UV-Vis spectroscopy due to the development of the characteristic I_3^- band at 350 nm for complex **1** and 353 nm for complex **2** (Figure 4.36 – 4.37).

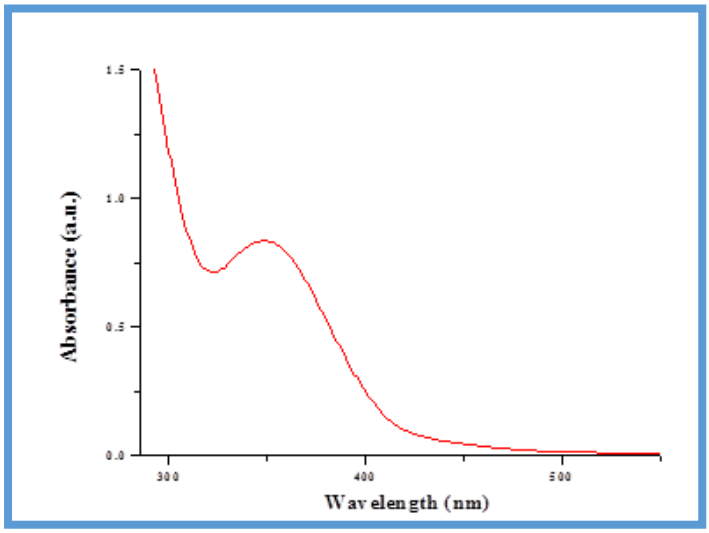


Figure 4.36: Characterized peak of I_3^- for qualitative detection of H_2O_2 in complex **1** during catalytic oxidation process.

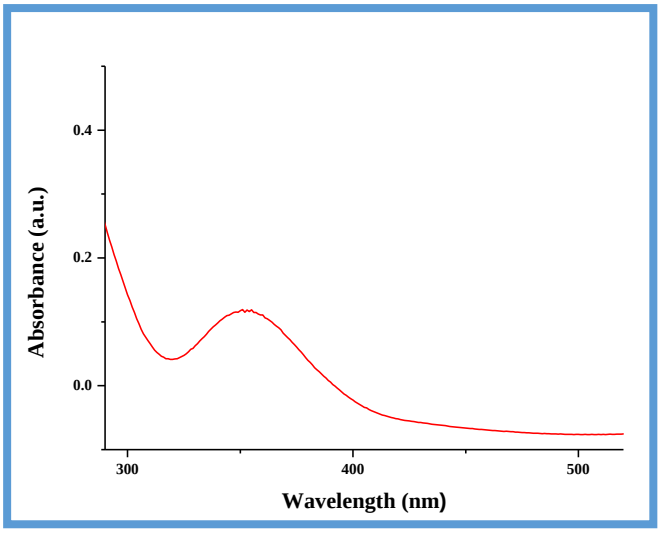


Figure 4.37: Characterized peak of I_3^- for qualitative detection of H_2O_2 in complex **2** during catalytic oxidation process.

Chapter 5

Conclusion and Future Scope

In a nutshell we can say, four new monomeric complexes based on schiff-base ligand have been synthesised successfully. All the four complexes showed appreciable protein binding activity when examined using BSA protein with Complex **3** showing maximum quenching of intrinsic fluroescence of BSA as compared to the remaining complexes.

The complexes were also studied for catechol oxidase mimic activity by observing the oxidation of 3,5-DTBC to 3,5-DTBQ spectrophotometrically and it was found that while complexes **1** and **2** showed excellent catalytic activity towards oxidation of 3,5-DTBC, complexes **3** and **4** showed negligible activity.

The results obtained indicate that Schiff Base ligand with "ONO" donor set can form interesting copper based complexes which can show high affinity towards protein and strong catecholase mimicking activity. In future more compounds with different co-ligands can be prepared to fine tune the properties of the complexes to maximize the benefit out of it.

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