# Studies on Zinc Complexes in Nitrogen Rich Ligand Environment

## M.Sc. Thesis

By

**MAMTA KUNWAR**

**DISCIPLINE OF CHEMISTRY**

**INDIAN INSTITUTE OF TECHNOLOGY INDORE**

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# Studies on Zinc Complexes in Nitrogen Rich Ligand Environment

**A THESIS**

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

*by*
**MAMTA KUNWAR**

**DISCIPLINE OF CHEMISTRY**
**INDIAN INSTITUTE OF TECHNOLOGY INDORE**
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# INDIAN INSTITUTE OF TECHNOLOGY INDORE

## CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"Studies on Zinc Complexes in Nitrogen Rich Ligand Environment"** 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 2019 to June 2020 under the supervision of Prof. Suman Mukhopadhyay, 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.

Mamta

**MAMTA KUNWAR**

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

**Prof. SUMAN MUKHOPADHYAY**

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**MAMTA KUNWAR** has successfully given her M.Sc. Oral Examination held on 24.06.2020

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Date: 27.10.2020

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**MAMTA KUNWAR**

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DEDICATED TO………

***MY PARENTS***

# ABSTRACT

In the present work, the focus has been given to the synthesis and study of zinc complexes in nitrogen rich ligand environment. A Schiff base ligand HL was synthesized which was used to synthesize three new zinc complexes $[Zn(HL)(N_3)_2]•H_2O$, $[Zn(HL)(5\text{-phenyltetrazolato})_2]$ and $[Zn(HL)\{5\text{-}(4\text{-pyridyl})\text{-tetrazolato}\}_2]$. All the complexes are characterized through ESI-MS and IR spectroscopy. Single crystal X-ray diffraction analysis has been done for zinc-Schiff base complex $[Zn(HL)(N_3)_2]•H_2O$. The Schiff base ligand and complex $[Zn(HL)(N_3)_2]•H_2O$ have also been characterized through NMR spectroscopy. Sensing properties of complex $[Zn(HL)(N_3)_2]•H_2O$ have been studied using fluorescence and it is found that the complex shows good anion sensing ability for different phosphates and primarily dihydrogen phosphate ($H_2PO_4^-$) anion. The detection limit was found to be 243 nM. Recovery analysis was done for real-life water samples for determination of concentration of dihydrogen phosphate anion and the observed value was found to be in good agreement with the actual value.

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# NOMENCLATURE

| | |
|---|---|
| **α** | Alpha |
| **β** | Beta |
| **γ** | Gamma |
| **δ** | Chemical shift (N.M.R.) |
| **θ** | Angle |
| **Å** | Angstrom |
| **Λ** | Wavelength |
| **g** | Gram |
| **mg** | Milligram |
| **mL** | Millilitre |
| **μL** | Microlitre |
| **υ** | Frequency |
| **cm** | Centimeter |
| **nm** | Nanometer |
| **M** | Molar |
| **nM** | Nanomolar |
| **mol** | Moles |
| **mmol** | Millimole |
| **%** | Percentage |
| **ºC** | Degree Centigrade |
| **s** | Seconds |

# ACRONYMS

| | |
|---|---|
| **C** | Carbon |
| **N** | Nitrogen |
| **O** | Oxygen |
| **H** | Hydrogen |
| **$H_2PO_4^-$** | Dihydrogen phosphate ion |
| **$CN^-$** | Cyanide |
| **Al** | Aluminium |
| **Zn** | Zinc |
| **Cu** | Copper |
| **Ar** | Argon |
| **PPi** | Pyrophosphate |
| **$HSO_4^-$** | Hydrogen sulphate |
| **$PO_4^{3-}$** | Phosphate |
| **MeOH** | Methanol |
| **DMSO** | Dimethylsulphoxide |
| **DMF** | Dimethylformamide |
| **$H_2O$** | Water |
| **BSA** | Bovine Serum Albumin |
| **NMR** | Nuclear Magnetic Resonance |
| **FT-IR** | Fourier Transform-Infrared |
| **ESI-MS** | Electrospray Ionization-Mass Spectrometry |

# *CHAPTER 1*

# Introduction

## 1.1 General Introduction

The beginning of coordination chemistry was marked by the publication titled “*Contribution to the Constitution of Inorganic Compounds*” in 1893 by Alfred Werner [1]. He played a chief and dominant role for the development in the field of coordination chemistry and was honored with The Nobel Prize in 1913 for his remarkable work in this field. The exploration of this field not only provided a valid explanation of existing compounds but also led to the designing of complicated molecular systems and discovery of a number of new complexes which proved to be useful in diverse areas [2]. Transition elements are known to exhibit variable oxidation states and interact with organic and inorganic ligands which give rise to unique properties which can be utilized in several applications. The oxidation state of metals, geometry and electronic configuration substantially affects the chemistry of the complexes. The functionality of the complexes is also influenced by the position of ligands in coordination sphere and their electron donor properties. The metal complexes show a range of impressive applications in field of analytical chemistry [3], pharmaceuticals [4], dyes [5], nuclear fuels [6], magnetic materials [7], biology [8], sensing [9] and catalysts [10]. They are also attaining a considerable attention in development of target specific radiopharmaceuticals for imaging and therapy due to their impressive selectivity and specificity [11]. Ligands containing nitrogen atom as a donor in metal complexes are known to exhibit fascinating advantages with intermediate binding capacity which leads to its successful utilization in detection of analytes, catalysis and in biological applications.

Schiff bases have been examined over the years due to their flexibility, ability to stabilize metals and attractive physio-chemical properties [12].

The designing of Schiff bases with desirable binding properties can be relevantly done to have perfect control over denticity, donor properties and number of chelating moieties [13]. Thus, the ease in designing and preparation of these molecules attracts a huge attention. They are utilized as versatile metal complexing agents with large number of applications in different areas of research. They are used as multifaceted units in cycloaddition reactions [14], as anti-fungal [15], anti-malarial [16], anti-viral [17] and anti-cancer agents [18]. They are also widely studied for their sensing properties [19].

Utilization of heterocyclic ligands containing nitrogen for formation of metal complexes is widely done. It has shown remarkable application in different fields such as medicinal chemistry [20], catalysis [21,22], enzyme mimicking properties [23], material chemistry [24] and in formulation of anti-corrosion species [25]. Among the nitrogen containing heterocycles, tetrazoles attracted a huge attention due to their binding ability in nine different possible modes which can significantly contribute to synthesize mono- and polynuclear complexes [26-30]. Tetrazoles are five membered aromatic and planar nitrogen-containing heterocycle in which one carbon atom is present at 5-position of the ring and four nitrogen atoms are organized in regular manner. It exists in two tautomeric forms (Figure 1.1) out of which 1H-form is more stable in solution and 2H-form is more stable and predominant in gaseous phase.

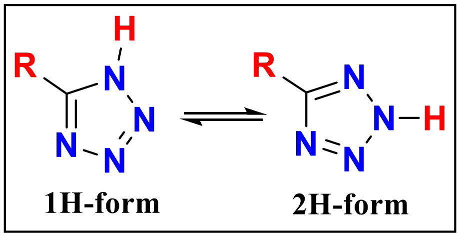


***Figure 1.1:*** *Tautomeric forms of tetrazole*

Tetrazoles are acidic in nature with a $pK_a$ of 4.89 which is comparable to aliphatic carboxylic acids which enables them to act as bioisosteres for carboxylate group [31]. There are several synthetic tetrazoles which are utilized as antihypertensive [32], anti-fungal [33], anti-inflammatory [34] and growth hormone stimulator drugs [35]. Some tetrazoles are used in the creation of solid-rocket propellent [36]. They are also employed as excellent high-energy performance explosives [37]. Tetrazoles can exhibit distinct coordination modes (Figure 1.2) and due to this unique ability, they are widely explored. Complexes of tetrazole are prepared by reaction of tetrazole ligands with metal salts or by [2+3] cycloaddition of metal coordinated azides and nitriles. It has been observed that in-situ [2+3] cycloaddition of metal coordinated azides with nitrile is more efficient method and can be done by microwave irradiation method, refluxing or by solvothermal methods [38-41]. Complexes of tetrazole are widely used in number of applications such as adsorption [42], magnetic materials [43,44], photoluminescence [45,46], catalysis [47-49], gas adsorption and storage [50,51] etc.

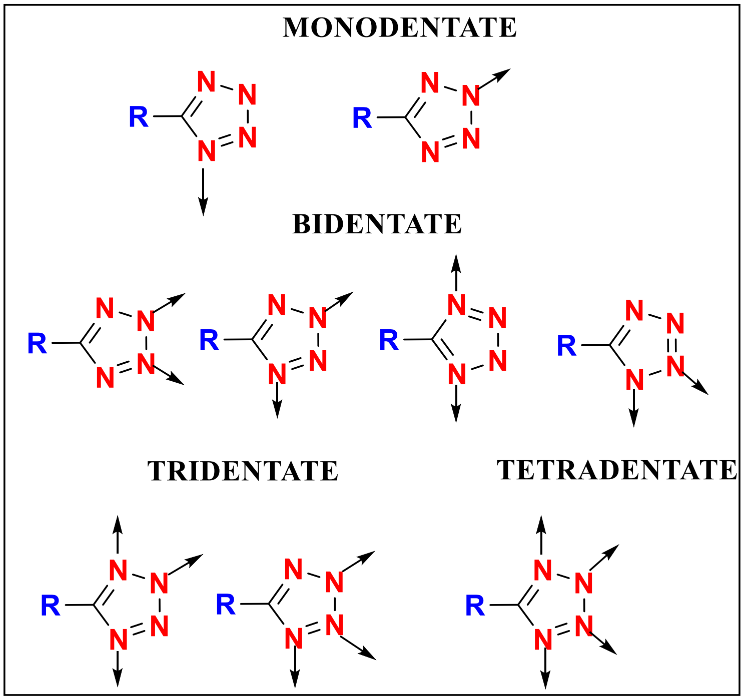


***Figure 1.2:*** *Nine different binding modes of substituted tetrazole ligand*

## 1.2 Schiff Bases: History and Preparation

Schiff bases were first invented by Hugo Schiff, a German chemist in 1864. Schiff bases have general formula $R_3NC=R_1R_2$ where R represents an alkyl group. They are nitrogen analogues of aldehyde or ketone and during synthesis carbonyl group is replaced by an imine group.

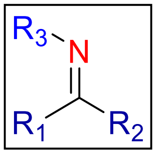
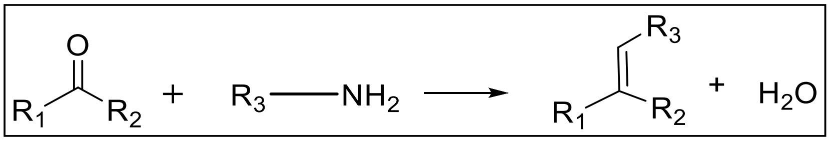


***Figure 1.3:*** *Representation of structure of a Schiff-base*

They are synthesized by the condensation reaction between primary amine and an aldehyde or ketone in the presence of basic or acidic condition or in the presence of heat [52].

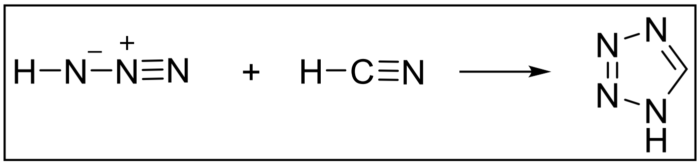


***Scheme 1.1:*** *Reaction scheme representing preparation of Schiff base*

Schiff base synthesis is a reversible process in which imine hydrolyzes under certain conditions and to overcome this problem, reaction is done in dry solvents and additional procedure is applied to remove the water formed as side product of the condensation reaction [53].

## 1.3 Tetrazoles: History and Preparation

Tetrazoles are magnificent class of five membered planar and aromatic heterocyclic compounds consisting of one carbon atom and four nitrogen atoms [54]. The nitrogen rich environment enables it to show impressive coordination ability with metal ions. Tetrazole was first synthesized by Bladin, a Swedish Chemist in 1885 by cycloaddition reaction of azide and nitrile group [55]. Since past two decades, synthesis of tetrazoles from nitriles and azides is efficiently carried out using new catalysts, solvents [56,57] etc.



***Scheme 1.2:*** *Reaction scheme representing synthesis of tetrazole*

## 1.4 Zinc(II) Metal Complexes

Zinc is a *d*-block bioavailable metal with an electronic configuration of $[Ar]3d^{10}4s^2$. It mainly exists as divalent metal cation Zn(II) after losing outer shell 4s electrons and achieves a stable closed shell electronic configuration $[Ar]3d^{10}$. The closed shell $d^{10}$ configuration of zinc makes it diamagnetic and is expected to create fluorescence characteristics controlled by the attached ligand. Zinc can form complexes with tetra-, penta- and hexa-coordinated geometries. Zinc is also incriminated as a prime cytotoxic suppressor agent in various cancers and thus its complexes could be employed as promising anticancer agents [58]. The fluorescent complexes of Zn(II)-Schiff base are also used as powerful tool to detect molecular events and thus are employed for bio-imaging [59].

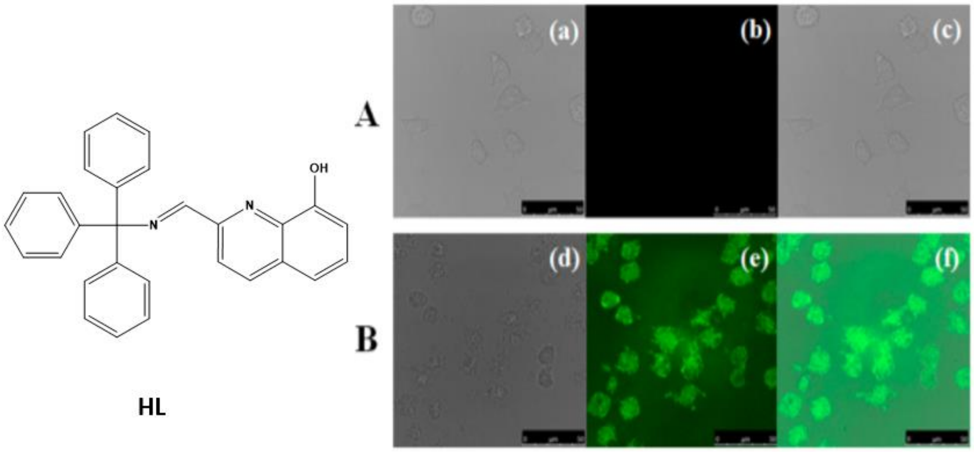


***Figure 1.4:*** *SH-SY5Y cells treated with HL (A) before incubation with zinc perchlorate and (B) after incubation with zinc perchlorate. Images (a) and (d) illustrate the bright-field cell images, (b) and (e) illustrate the fluorescence cell images, and (c) and (f) illustrate the overlay cell images (modified and adapted from ref. 59)*

Zinc forms very labile complexes with Schiff bases and thus it finds application in the development of anionic sensors as it can exchange ligands rapidly [60-62]. The sensing ability of Zn(II)-Schiff base based sensors also depends on weak forces such as O-H….O, N-H….O and C-

H….O, C-H….π, π- π interactions between the H-donor and acceptor groups situated on the ligands and the anions, respectively. Trinuclear complexes of Schiff bases coordinated to zinc(II) have also been synthesized as $F^-$ and $HSO_4^-$ sensors and practical application of the sensor was also checked and was found to respond very well [63].

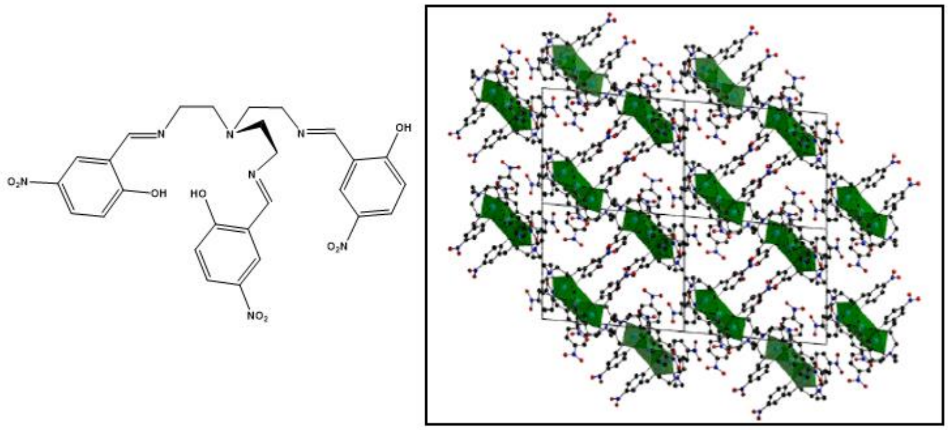


***Figure 1.5:*** *Structure of $H_3L$ and 3-Dimensional packing diagram of tri-nuclear Zn(II)-Schiff base complex, $Zn_3L$ (modified and adapted from ref. 63)*

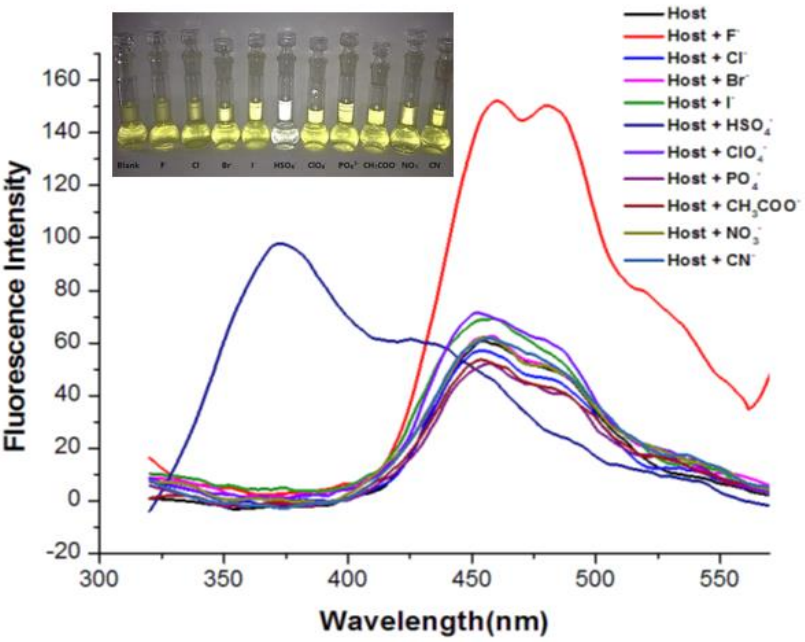


***Figure 1.6:*** *Visual detection of $HSO_4^-$ and fluorometric detection of $F^-$ and $HSO_4^-$ by tri-nuclear Zn(II)-Schiff base complex (modified and adapted from ref. 63)*

## 1.5 Organization of the Thesis

The aim of this project is to synthesize Zinc (II) complexes using nitrogen rich ligands such as Schiff bases and tetrazoles and to find their probable applications.

**Chapter 2:** This chapter comprises of past work done in the same field and motivation behind the work done during the project.

**Chapter 3:** This chapter comprises of materials, instrumentation and experimental procedure for the synthesis of ligands and complexes. This chapter also includes the techniques used to study sensing of anions and their recovery from aqueous solutions.

**Chapter 4:** This chapter comprises of the results obtained after the synthesis of ligands and complexes.

**Chapter 5:** This chapter comprises of the conclusion of the work done and possible future scope and applications.

# CHAPTER 2

## Review of Past Work and Project Motivation

### 2.1 Sensing

Analyte sensing is one of the widely explored area of supramolecular chemistry. The detection of the analyte can only be done when the sensor molecule can induce a response whether visible, optical or electrochemical. The most widely used method of sensing is optical sensing where the interaction of analyte with sensor molecule produces a change in the absorbance or fluorescence properties. This is a highly sensitive method as it allows the detection of a very small concentration of the analyte. A small change in the concentration will produce large difference in the optical response which can lead to the effective study of the sensing and its mechanism. The area of analyte sensing finds application in large number of areas including biological systems. Bidyut Kumar Kundu *et al*. have synthesized a Schiff base which was employed for the sensing of $Al^{3+}$ and $Zn^{2+}$ cations using fluorescence spectroscopy [64].

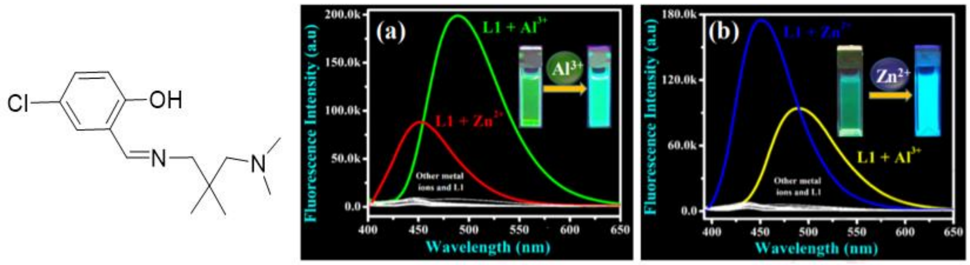


***Figure 2.1:*** *Schiff base ligand sensing $Al^{3+}$ and $Zn^{2+}$ (modified and adapted from ref. 64)*

Anionic recognition is an interesting field of interest due to role of anions in biological and environmental systems. This field is useful to sense biologically toxic anions like $CN^-$ and environment degrading anions like phosphates, sulphates, nitrates etc. Most of the sensors are soluble and work in organic solvents thus, major challenge is the detection and quantification of these anions in aqueous system. Phosphates are biologically important anions and at physiological pH dihydrogen phosphate, $H_2PO_4^-$ is the dominant anion. They play a major role in biological activities like construction of DNA and RNA, phospholipids, gene regulation, energy transfer and compose the backbone of nucleic acids [65]. They are also major component of fertilizers and medicinal drugs [66]. There is a negative consequence of excess of these anions in aquatic ecosystems. Their excess promotes algal growth and leads to eutrophication. This leads to the depletion of oxygen level in water body and leads to the death of aquatic plants and animals. Barnali Naskar *et. al.* synthesized a Schiff base $H_2L$ which was employed for sensing of $Zn^{2+}$ and to prepare a zinc(II) complex $[Zn_2(HL)_2(NO_3)_2]•CH_3OH$ which was found to sense pyrophosphate (PPi) anion [67].

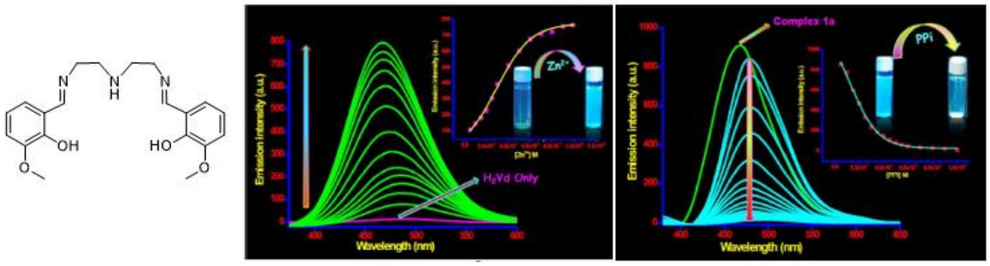


***Figure 2.2:*** *Figure showing sensing of* $Zn^{2+}$ *by Schiff base* $H_2L$ *and sensing of PPi by* $[Zn_2(HL)_2(NO_3)_2]•CH_3OH$ *(modified and adapted from ref. 67)*

## 2.2 Catalytic Activity

Metal tetrazole complexes are known to exhibit good catalytic properties. Manideepa Saha *et. al.* reported the preparation of a Cu(II)-tetrazole complex $[Cu_2(phen)_2(ptz)_4]$ (ptz= 5-phenyltetrazolate) which was found to catalyze oxidation of cyclohexane to cyclohexanol. It also exhibited catecholase-like activity and interaction with bovine serum albumin (BSA) [68].

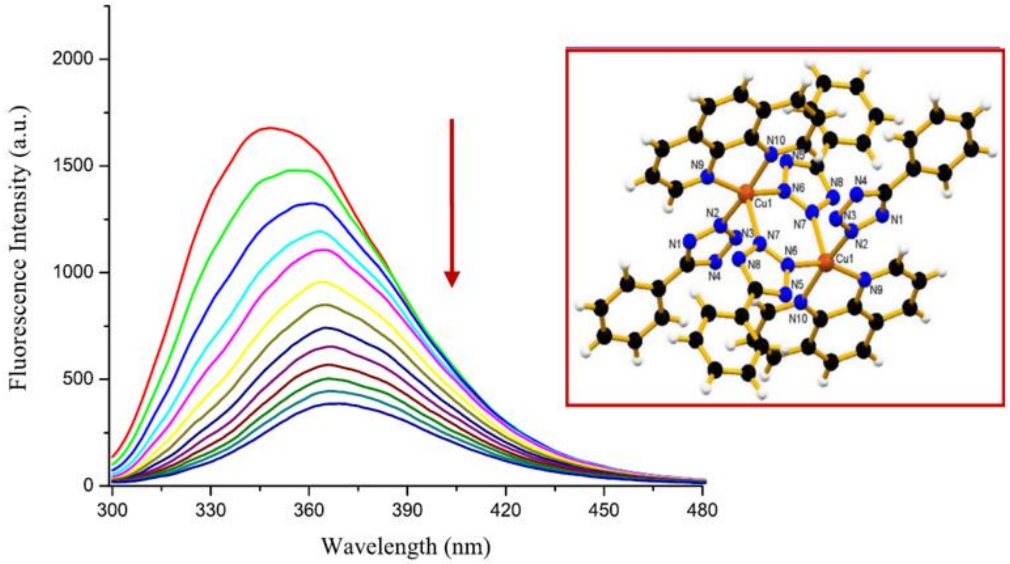


***Figure 2.3:*** *Fluorescence quenching of BSA by [$Cu_2(phen)_2(ptz)_4$] (modified and adapted from ref. 68)*

Novina Malviya *et. al.* synthesized two nickel complexes [NiL(5-phenyltetrazolato)] and [NiL-{5-(4-pyridyl)-tetrazolato] (L= 3-(2-diethylamino-ethylimino)-1-phenyl-butan-1-one)) by [3+2] cycloaddition reaction of nickel ligated azide and organonitriles which were found to exhibit moderate catecholase-like activity [69].

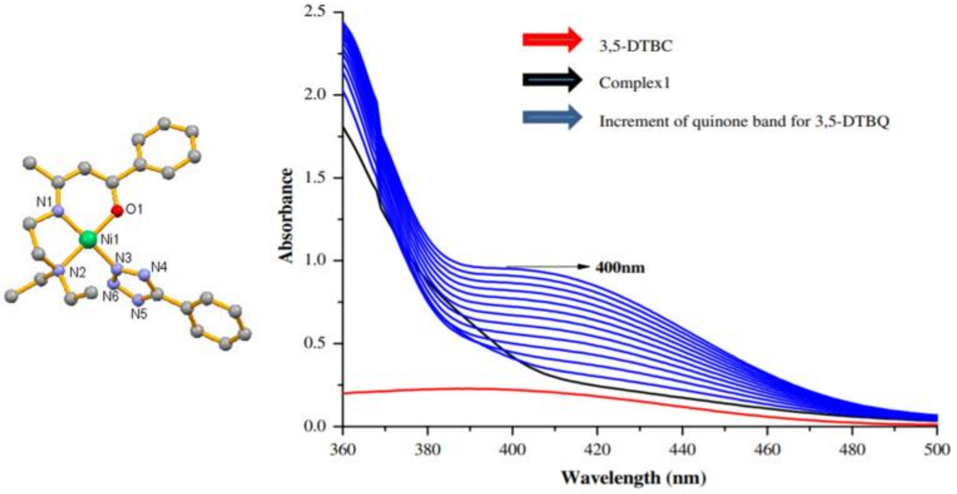
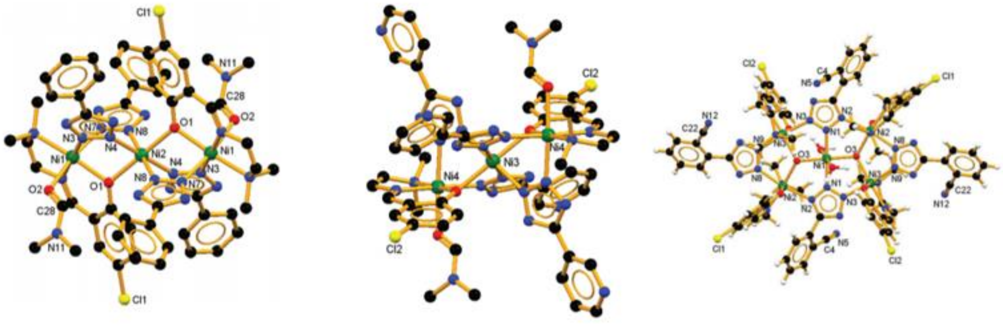


***Figure 2.4:*** *Crystal structure and spectral pattern of catecholase-activity study of tetrazole complex [NiL(5-phenyltetrazolato)] (modified and adapted from ref. 69)*

## 2.3 Magnetic Properties

Tetrazole complexes are widely investigated for their exciting magnetic properties. Manideepa Saha *et. al.* reported two nickel-trinuclear complexes [$Ni_3L_2$(5-phenyltetrazolato)$_4$(DMF)$_2$] and [$Ni_3L_2${5-(3-pyridyl)tetrazolato}$_4$(DMF)$_2$] and one pentanuclear complex [$Ni_5L_4${5-(2-cyanophenyl)-tetrazolato}$_4$(OH)$_2$($H_2O$)$_2$]·3$H_2O$·DMF whose magnetic susceptibility showed prevalent antiferromagnetic interactions among nickel centers in the synthesized complexes [70].



(a) (b) (c)

***Figure 2.5:*** *(a) Crystal structure of [$Ni_3L_2$(5-phenyltetrazolato)$_4$(DMF)$_2$] (b) Crystal structure of [$Ni_3L_2${5-(3-pyridyl)-tetrazolato}$_4$(DMF)$_2$] (c)Crystal structure of [$Ni_5L_4${5-(2-cyanophenyl)-tetrazolato}$_4$(OH)$_2$($H_2O$)$_2$]·3$H_2O$·DMF (modified and adapted from ref. 70)*

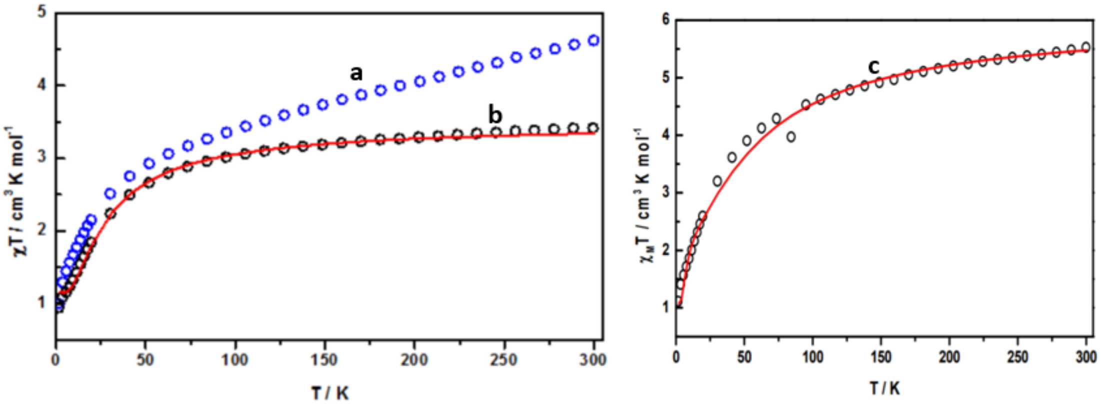


***Figure 2.6:*** *Molar susceptibility data for complex a, b and c (modified and adapted from ref. 70)*

## 2.4 Gas Adsorption Properties

Coordination polymers of tetrazoles are known to show good gas adsorption properties. Di-Chang Zhong *et. al.* proposed three coordination polymers based on 1*H*-tetrazole, $[Zn_3(Tz)_6(H_2O)_2]_n$, $[Cu_2(Tz)_3(OH)]_n$ and $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ (Tz = tetrazolate) prepared under hydrothermal conditions via *in situ* decarboxylation. $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ possessed 3D-porous framework and exhibit selective adsorption of $CO_2$ and $N_2$ over $H_2$ [71].

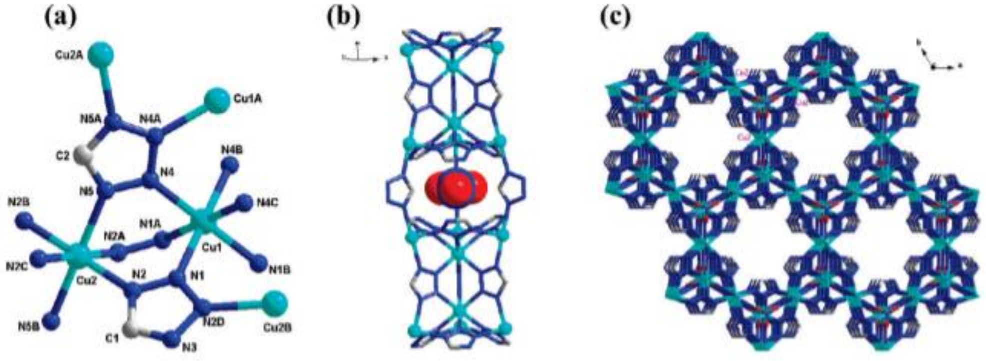


***Figure 2.7:*** *(a) Coordination environment of* $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ *(b)* $NO_3^-$ *occupied cage of* $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ *(c) 3D-framework with 1D-channels of* $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ *(modified and adapted from ref. 71)*

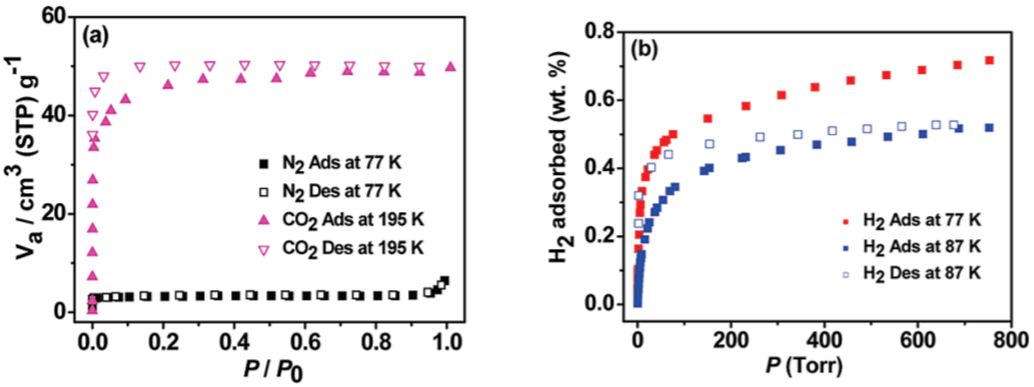


***Figure 2.8:*** *(a) Gas sorption isotherms of* $N_2$ *and* $CO_2$ *(b)* $H_2$ *adsorption isotherm for desolvated* $\{[Cu_5(Tz)_9](NO_3)_3•8H_2O\}_n$ *(modified and adapted from ref. 71)*

# CHAPTER 3

## Experimental Section

### 3.1 Reagents and Chemicals

All the reagents used were of analytical grade and were used further without any purification. All the experiments were carried out in open atmosphere. Salicylaldehyde, 1-(2-aminoethyl)piperazine, basic zinc carbonate, perchloric acid, sodium azide, 4-cyanopyridine, benzonitrile were bought from Merck-India Chemical Company. Zinc perchlorate hexahydrate was prepared in the laboratory.

***Caution:*** *Tetrazoles, azides and perchlorates are known to be potentially explosive. Small amount of these compounds should be used, prepared and handled with care.*

### 3.2 Methods and Instrumentation

Electrospray ionization mass spectrometry (ESI-MS) was done using Bruker-Daltonics, microTOF-Q II mass spectrometer. It was equipped with an electrospray ion source and was run with an ion-trap instrument. Infrared Spectra (IR) was done using FT-IR TENSOR 27 BRUKER instrument using KBr pellet method within range of 4000-500 $cm^{-1}$ over 64 scans at an interval of 1 s and resolution of 4 $cm^{-1}$. Nuclear Magnetic Resonance (NMR) spectra were recorded using ADVANCE III 400 Ascend Bruker BioSpin instrument at ambient temperatures using tetramethylsilane as an internal standard. Absorption spectra were recorded using a Varian Carry Carry 100 Bio UV-vis spectrophotometer and samples were taken in a quartz cuvette ($10 \times 10$ $mm^2$). Fluorescence spectra were recorded by taking sample in quartz cuvette ($10 \times 10$ $mm^2$) using a Fluoromax-4 spectrofluorometer (HORIBA Jobin Yvon, model FM-100) with data pitch of 1 nm and excitation and emission slit width of 2 nm. To calculate the detection limit, $3\sigma/k$ method was used and recovery analysis was done by spiking the water sample with ion to be detected and taking fluorescence of increasing concentrations of probe and spiked sample.

## 3.3 X-ray Crystallography

Orange colored cubic-shaped single crystal of complex $[Zn(HL)(N_3)_2]\cdot H_2O$ was used for the X-ray crystallographic analysis. The diffraction was performed using a CCD Agilent Technologies SUPER NOVA diffractometer. The data was collected using graphite-monochromatic MoKα radiation ($\lambda\alpha = 1.54184$ Å) at 293 K. The evaluation of the data collected was done using the CrysAlisPro CCD software. The data collected was scaled and reduced using CrysAlis-ProRED software. For solving the structure of the complex $[Zn(HL)(N_3)_2]\cdot H_2O$, direct methods from SHELXS-97 were used and the refinement was done using the full matrix-least square with SHELXS-97 refining on $F^2$.

The positions for all the atoms were obtained by direct methods and all non-hydrogen atoms were refined anisotropically. The residing hydrogen atoms were placed in geometrically constrained positions and were refined with isotropic temperature factors.

## 3.4 Synthesis

### 3.4.1 Synthesis of Schiff base ligand (HL)

The synthesis of Schiff base was done according to a previously reported procedure [72]. A solution of 1-(2-aminoethyl)piperazine (645.5 mg, 5 mmol) in 5 mL ethanol was added dropwise to a previously stirred solution of salicylaldehyde (612 mg, 5 mmol) in 5 mL ethanol. The reaction mixture was refluxed for 1 hour with stirring at 80 °C and an orange-colored solution was obtained. After the evaporation of the solvent a red-oil like liquid was obtained which on placing for some days in a vacuum desiccator gave a yellow colored hygroscopic solid and was used for further analysis and reactions. (Yield: 95%)

### 3.4.2 Synthesis of [Zn(HL)$(N_3)_2$]•$H_2O$

A solution of zinc perchlorate hexahydrate (186.2 mg, 0.5 mmol) in 5 mL methanol was added dropwise to previously stirred 2.5 mL methanolic solution of Schiff base ligand HL (116.7 mg, 0.5 mmol). The reaction mixture was refluxed with stirring for 20 min. The reaction mixture was filtered and cooled to room temperature. Then, aqueous solution of sodium azide (65 mg, 1 mmol) in 5 mL Milli-Q water was added to the reaction mixture and was left for 24 hours. Orange-colored cubic-shaped crystals were obtained which were washed with diethyl ether and used for further reactions and analysis. (Yield: 51%)

### 3.4.3 Synthesis of [Zn(HL)(5-phenyltetrazolato)$_2$]

A mixture of [Zn(HL)$(N_3)_2$]•$H_2O$ (100 mg, 0.262 mmol) and 10 mL of benzonitrile was refluxed with stirring at 195 °C for 24 hours. The reaction mixture became darker from colorless to brown to black as the reaction proceeded. After 24 hours, a dark brown precipitate was observed which was isolated, washed with diethyl ether and dried in a vacuum desiccator. The solution of the obtained precipitate in DMF was placed for 21 days to obtain orangish yellow colored crystals and were washed with diethyl ether and dried for further analysis. (Yield: 27%)

### 3.4.4 Synthesis of [Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$]

A solution of [Zn(HL)($N_3$)$_2$]•$H_2O$ (100 mg, 0.262 mmol) and 4-cyanopyridine (68.2 mg, 0.655 mmol) in 10 mL DMF was refluxed with stirring at 155 °C for 24 hours to obtain a reddish-brown solution. Excess solvent was evaporated using rotavapor and obtained oily residue was treated with ~10 mL of diethyl ether and then with ~10 mL of methanol. A light brown colored solid was obtained which was filtered, washed with diethyl ether and water and dried in a vacuum desiccator. The solution of the obtained precipitate in DMF was placed for 21 days to obtain brownish yellow colored crystals and were washed with diethyl ether and dried for further analysis. (Yield: 41%)

## 3.5 Sensing of Anions

The anion sensing studies of [Zn(HL)($N_3$)$_2$]•$H_2O$ were done using fluorescence spectroscopy by exciting [Zn(HL)($N_3$)$_2$]•$H_2O$ at 385 nm (emission at 400nm). Stock solution of the complex [Zn(HL)($N_3$)$_2$]•$H_2O$ (1mM) was prepared in DMSO and 1mM solution of sodium salts of different anions was prepared using Milli-Q water. The excitation and emission slit width were kept constant during whole experiment. For sensing experiment, first the fluorescence was recorded for 1.5 mL of stock solution of complex [Zn(HL)($N_3$)$_2$]•$H_2O$ and 1.5 mL of Milli-Q water. Then, fluorescence was recorded for 1.5 mL of stock solution of complex [Zn(HL)($N_3$)$_2$]•$H_2O$ and 1.5 mL of 1 mM stock solution of anion to make the total volume 3 mL. For calculation of detection limit of sensing of $H_2PO_4$-, fluorescence titrations were done by repeatedly adding 20 µL of 1 µM stock solution of $H_2PO_4$- to 2.5 µM solution of [Zn(HL)($N_3$)$_2$]•$H_2O$.

## 3.6 Recovery Analysis

Fluorescence titrations of the stock solution of $[Zn(HL)(N_3)_2]\bullet H_2O$ were done by adding stock solution of $H_2PO_4$- in increasing concentration. The real water samples were spiked with known concentrations and fluorescence spectra were recorded. Recovery analysis was done by comparing the values of decrease in fluorescence intensity with standard curve.

# CHAPTER 4

## Results and Discussion

### 4.1 Synthesis and Characterization

**(A)** The Schiff base ligand **HL** was synthesized by previously reported procedure [72]. It was prepared by the condensation reaction of salicylaldehyde and 1-(2-aminoethyl)piperazine employing ethanol as a solvent. The reaction was done at 80 °C for 1 hour. After the completion of reaction, excess solvent was evaporated and a red-oil like liquid was obtained which on standing for few days in vacuum desiccator produced a yellow solid.

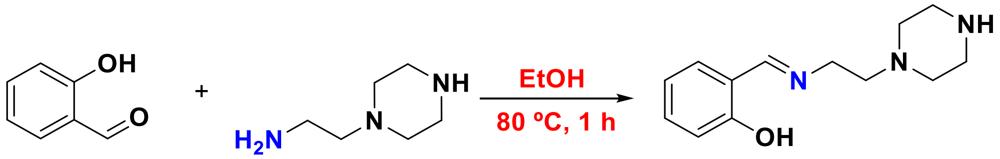
**(B)** A solution of $Zn(ClO_4)_2•6H_2O$ and **HL** in methanol was refluxed with stirring for 20 minutes at 70 ºC. The reaction mixture was filtered and cooled. Then, aqueous solution of $NaN_3$ was added to it and was left for 24 hours to obtain orange colored crystals. The characterization of $[Zn(HL)(N_3)_2]•H_2O$ was done using various analytical techniques and the structure was further confirmed by X-ray crystallography.

**(C)** A mixture of $[Zn(HL)(N_3)_2]•H_2O$ and benzonitrile was refluxed with stirring at 195 ºC for 24 hours to obtain a dark-brown colored precipitate. Orangish yellow colored crystals were obtained from DMF in 21 days and characterization of $[Zn(HL)(5\text{-phenyltetrazolato})_2]$ was done using various analytical techniques.

**(D)** A solution of $[Zn(HL)(N_3)_2]•H_2O$ and 4-cyanopyridine in DMF was refluxed with stirring at 155 ºC for 24 hours. Excess solvent was evaporated to obtain an oily residue which was treated with diethyl ether and methanol to obtain a light brown colored precipitate. Brownish yellow colored crystals were obtained from DMF in 21 days and the characterization of $[Zn(HL)\{5\text{-(4-pyridyl)-tetrazolato}\}_2]$ was done using various analytical techniques.

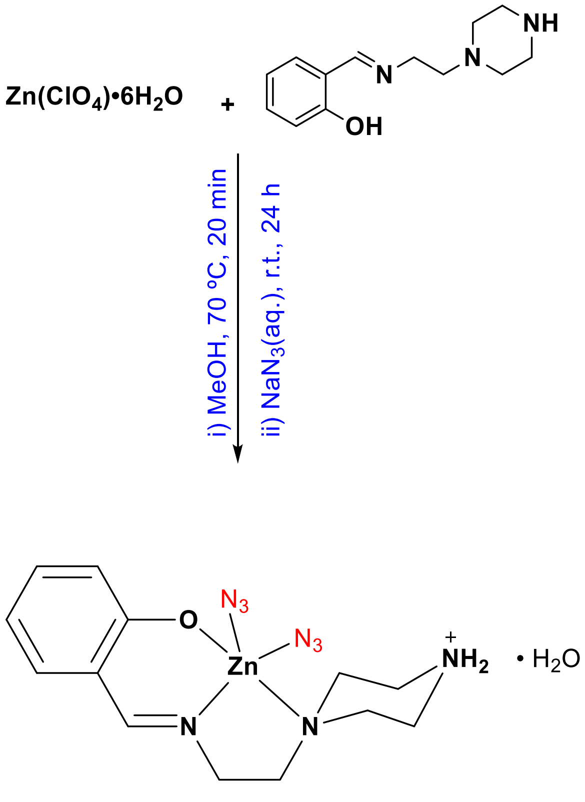
## 4.2 Reaction Schemes

### 4.2.1 Synthesis of Schiff base ligand HL



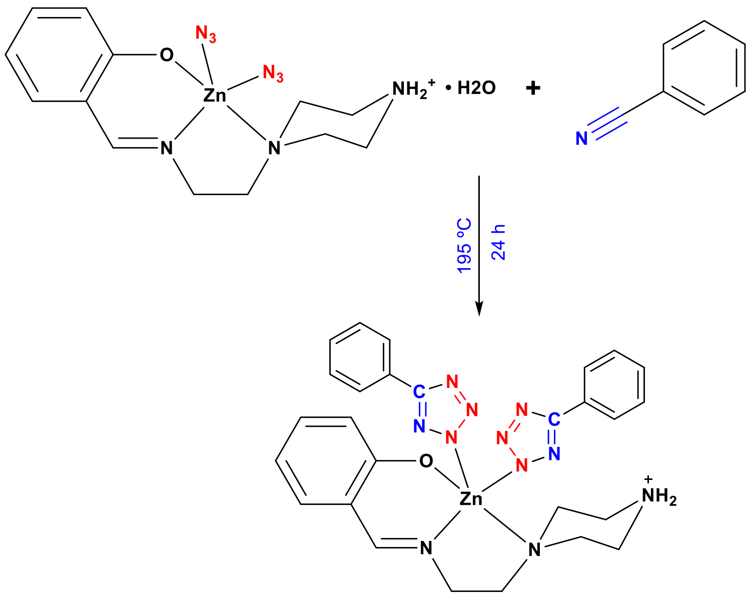
***Scheme 4.1:*** *Reaction scheme for synthesis of Schiff base ligand HL*

### 4.2.2 Synthesis of $[Zn(HL)(N_3)_2]\cdot H_2O$



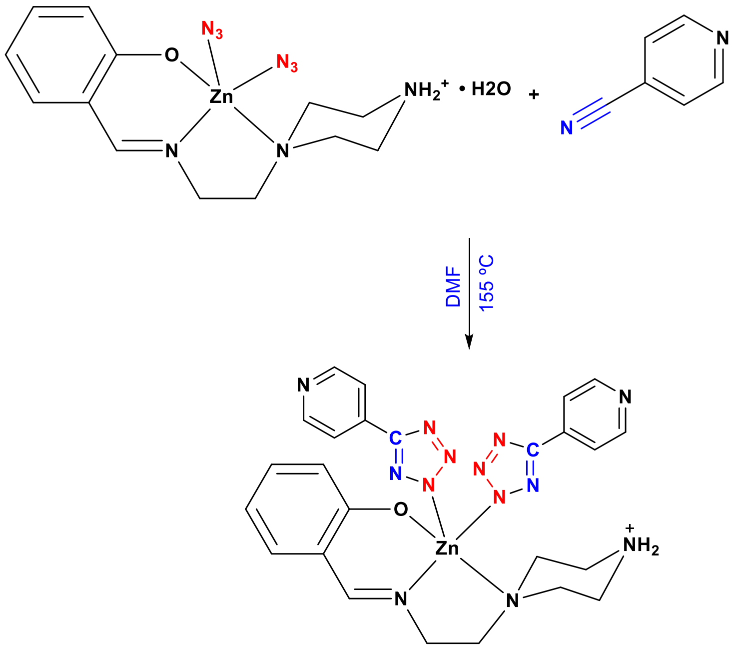
***Scheme 4.2:*** *Reaction scheme for synthesis of $[Zn(HL)(N_3)_2]\cdot H_2O$*

### 4.2.3 Synthesis of [Zn(HL)(5-phenyltetrazolato)$_2$]



*Scheme 4.3:* *Reaction scheme for synthesis of [Zn(HL)(5-phenyltetrazolato)$_2$]*

### 4.2.4 Synthesis of [Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$]



*Scheme 4.4:* *Reaction scheme for the synthesis of [Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$]*

## 4.3 Mass Spectra

The electrospray ionization mass spectrometry (ESI-MS) spectra of HL, [Zn(HL)($N_3$)$_2$]•$H_2O$, [Zn(HL)(5-phenyltetrazolato)$_2$], **[**Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$] provides reliable confirmation for the formation of these compounds. ESI-MS spectrum of Schiff base ligand HL shows $[M+H]^+$ peak at *m/z* 234.1665. However the spectrum of complex [Zn(HL)($N_3$)$_2$]•$H_2O$ shows $[Zn(HL)N_3]^+$ peak at *m/z* 340.1729, [Zn(HL)(5-phenyltetrazolato)$_2$] shows molecular ion $[M+H]^+$ peak at *m/z* 587.1063 and **[**Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$] shows $[M-(N_4CPy)]^+$ peak at 444.0.

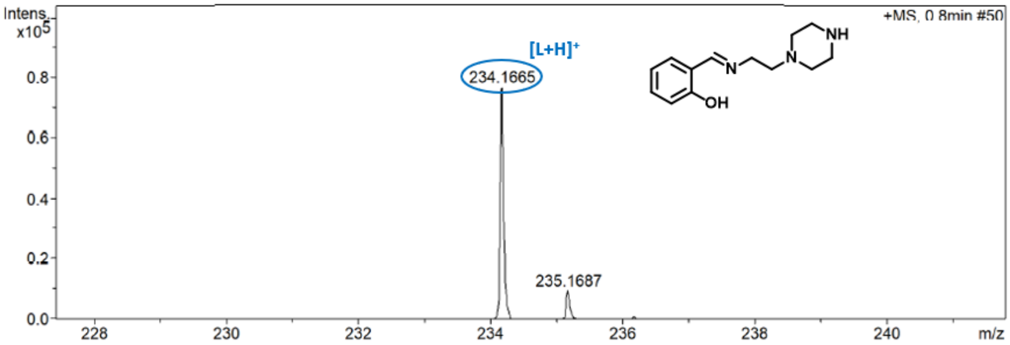


***Figure 4.1:*** *ESI-MS spectrum of Schiff base HL*

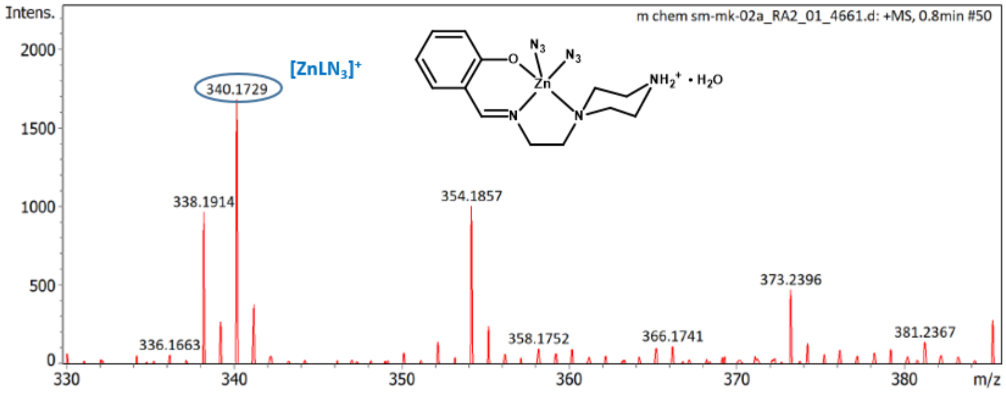


***Figure 4.3:*** *ESI-MS spectrum of [Zn(HL)($N_3$)$_2$]•$H_2O$*

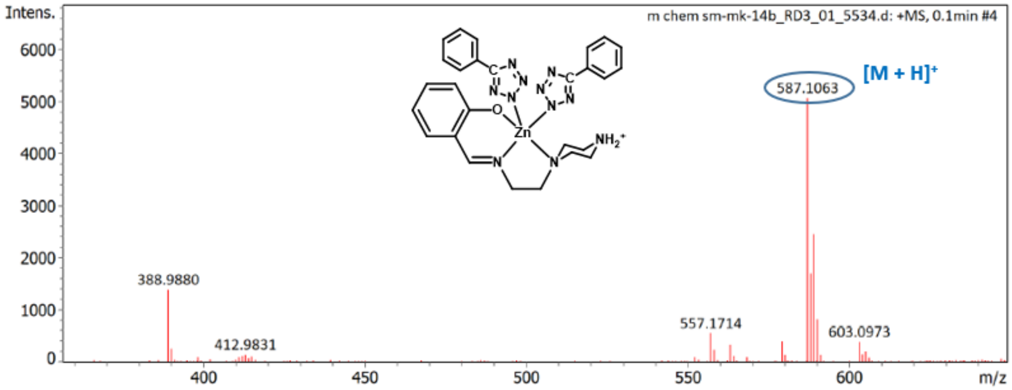


***Figure 4.3:*** *ESI-MS spectrum of [Zn(HL)(5-phenyltetrazolato)$_2$]*

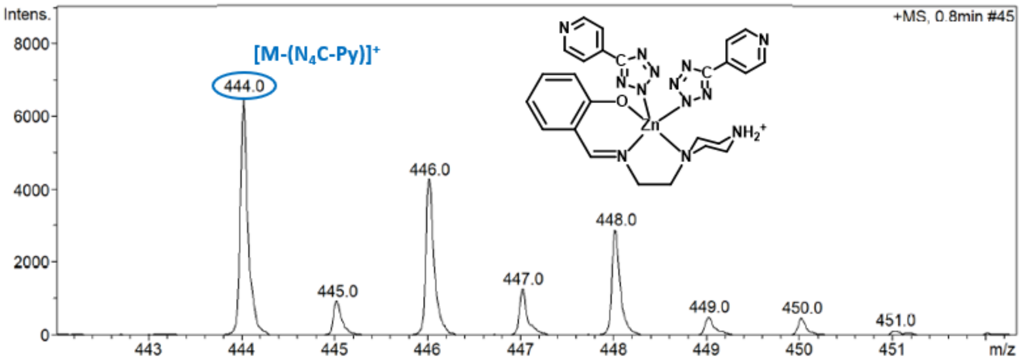


***Figure 4.4:*** *ESI-MS spectrum of [Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$]*

## 4.4 FT-IR Spectra

IR-spectra of Schiff base ligand and complexes showed the characteristic band of various functional groups. The IR data of the complex $[Zn(HL)(N_3)_2]{\bullet}H_2O$ has a band around 2060 $cm^{-1}$ which corresponds to the azide stretching frequency. The stretching frequencies of $[Zn(HL)(N_3)_2]{\bullet}H_2O$ complex are slightly lower than that of Schiff base ligand which indicates binding of $Zn^{2+}$ with the ligand. In the IR spectra of tetrazole complexes, a band at frequency 2060 $cm^{-1}$ is not present which indicates the conversion of azide to tetrazole.

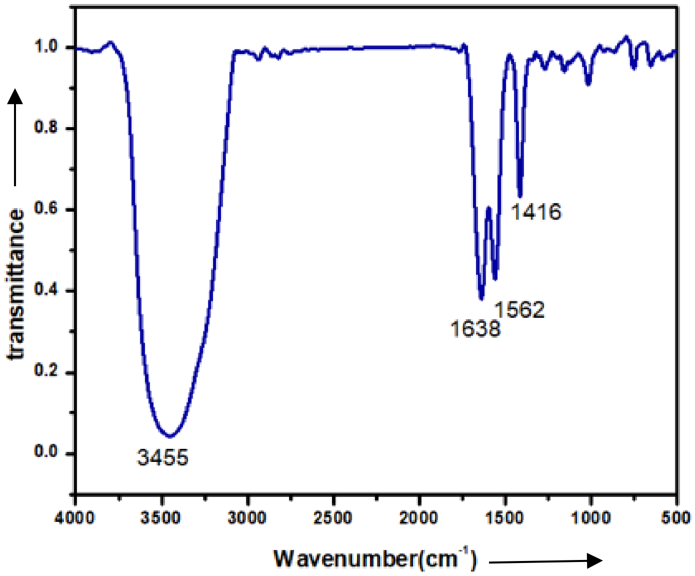


***Figure 4.5:*** *FT-IR spectrum of Schiff base ligand HL*

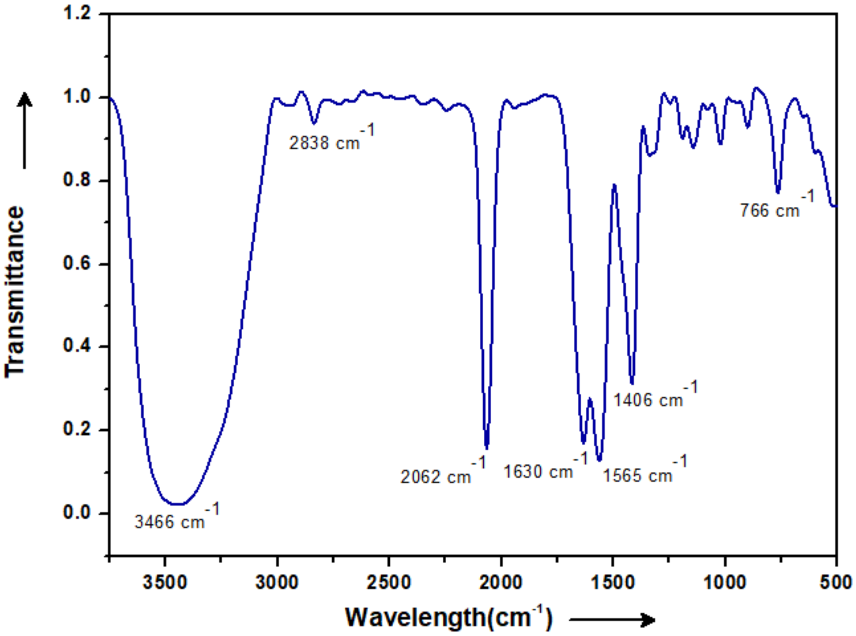


***Figure 4.6:*** *FT-IR spectrum of* $[Zn(HL)(N_3)_2]\cdot H_2O$

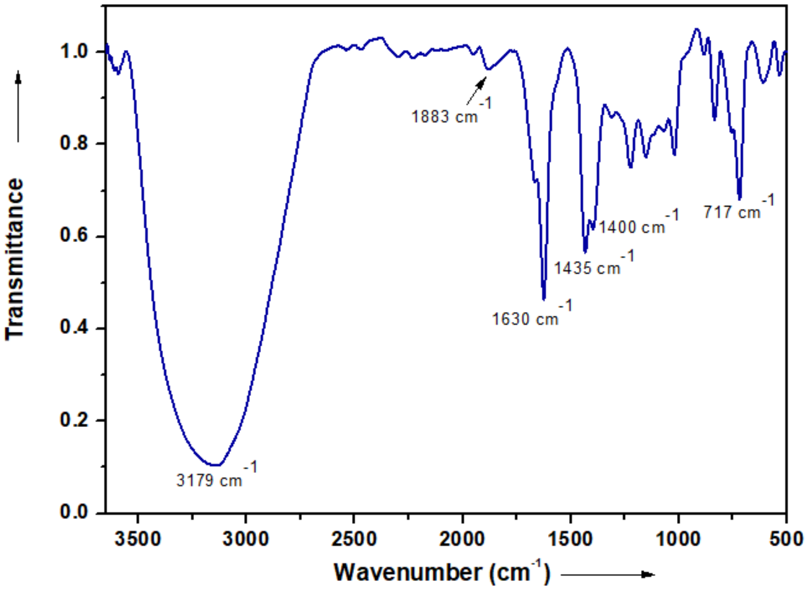


***Figure 4.7:*** *FT-IR spectrum of* $[Zn(HL)(5\text{-phenyltetrazolato})_2]$

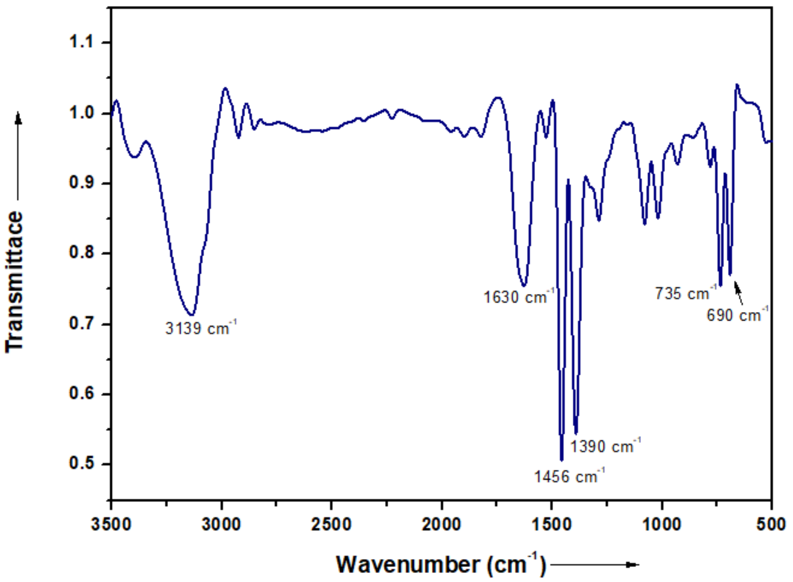


***Figure 4.8:*** *FT-IR spectrum of* ***[****Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$]*

## 4.5 Conductance Measurements

Molar conductance is a physical property that can be used for structure elucidation of metal complexes. It is used to ascertain electrolytic or non-electrolytic nature, geometry and number of ions present in the solution of metal complexes. The molar conductance values of all the three synthesized metal complexes (Table 4.1) when compared with the literature values (Table 4.2) were found to be in the region of non-electrolytes [73]. This means that all the complexes are non-electrolytes i.e., do not have any charge on the coordination sphere and thus do not consists of any counter anion outside the coordination sphere.

***Table 4.1:*** *Conductance measurements*

| **S. No** | **Complex** | **Molar Conductance $\lambda_o$ ($Scm^2mol^{-1}$)** |
|---|---|---|
| 1 | $[Zn(HL)(N_3)_2]\cdot H_2O$ | 21.7 |
| 2 | $[Zn(HL)(5\text{-phenyltetrazolato})_2]$ | 22.5 |
| 3 | $[Zn(HL)\{5\text{-(4-pyridyl)-tetrazolato}\}_2]$ | 44.1 |

***Table 4.2:*** *Reported values of conductance of different electrolytes* [73]

| **S. No** | **Solvent** | **Type of electrolyte** | **Molar Conductance $\lambda_o$ ($Scm^2mol^{-1}$)** |
|---|---|---|---|
| 1. | DMSO | Non-electrolyte | < 50 |
| 2. | DMSO | 1:1 electrolyte | 50-90 |

## 4.6 NMR Spectra

The NMR spectra of Schiff base ligand HL and $[Zn(HL)(N_3)_2]\bullet H_2O$ were found to be in accordance with the predicted structures. NMR of ligand was done in $CDCl_3$ and NMR spectra of complex was done in DMSO-$d^6$. The imine hydrogen was found to be in range of 8.3-8.5 ppm. The aromatic protons were observed in the range of 6.5-7.5 ppm and methylene hydrogens were observed in the range of 2.0-4.0 ppm. A downfield shift has been observed in the NMR of complex (Figure 4.10) compared to the ligand (Figure 4.9) which indicates the binding of $Zn^{2+}$ center with the Schiff base ligand.

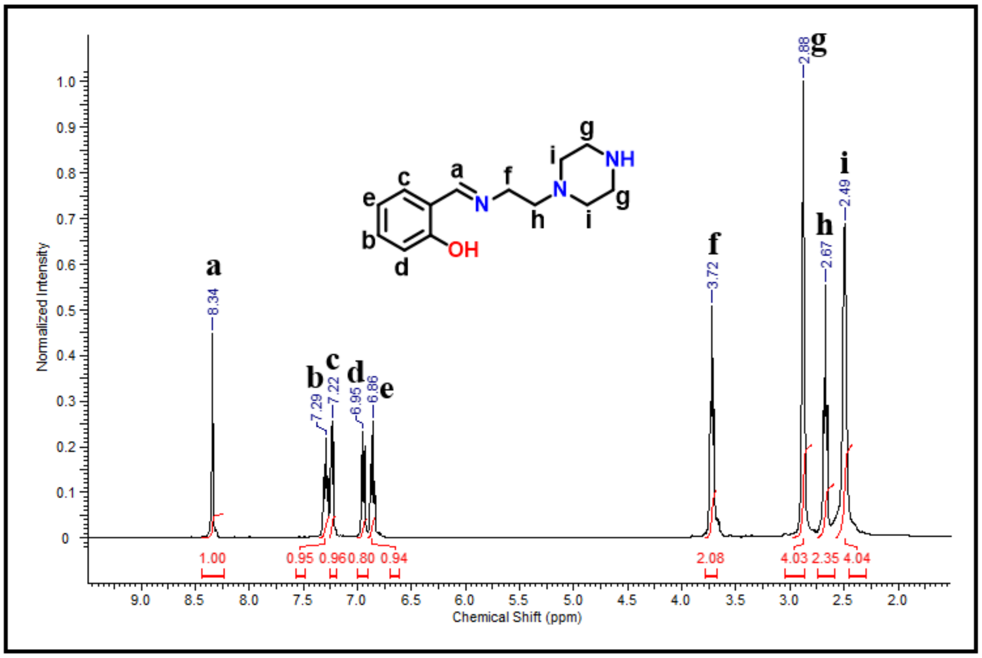


***Figure 4.9:*** *NMR spectrum of Schiff base ligand HL*

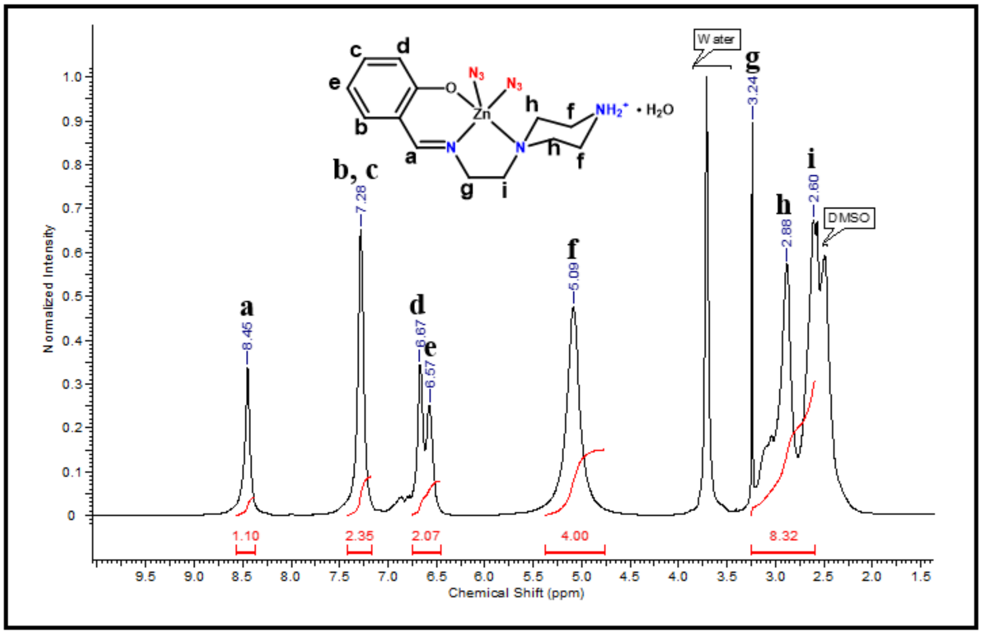


***Figure 4.10:*** *NMR spectrum of [Zn(HL)($N_3$)$_2$]•$H_2O$*

## 4.7 X-Ray Crystallography

The structure of the complex [Zn(HL)($N_3$)$_2$]•$H_2O$ was further confirmed by X-Ray crystallography. The complex shows five coordinated geometry where the ligand is attached in a tridentate fashion with O1 and N2 at axial position and N1 of ligand, N4 and N7 of two azide units at equatorial position of a regular trigonal bipyramid (Figure 4.11).

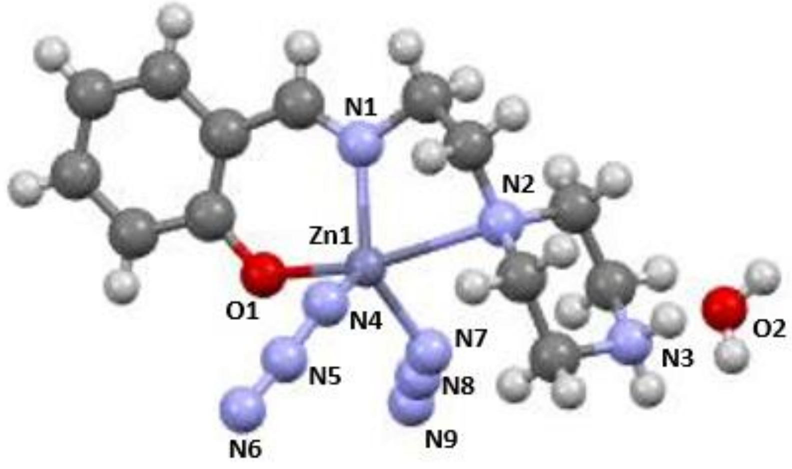


***Figure 4.11:*** *Crystal structure of [Zn(HL)($N_3$)$_2$]•$H_2O$*

## 4.8 Sensing of Anions

To explore the fluorescent nature of complex $[Zn(HL)(N_3)_2]\bullet H_2O$ different solvents were used to check its solubility and the complex was found to be soluble only in DMF and DMSO. The compound was found to be maximum fluorescent in DMSO ($\lambda_{ex}$ = 385 nm) so it was chosen as a solvent for the complex. To check the detection of anions in aqueous systems, water was chosen as a solvent for sodium salts of various anions. For fluorometric analysis first, 1.5 mL of stock solution of complex in DMSO and 1.5 mL of Milli-Q water was taken and fluorescence was recorded. Then, 1.5 mL of stock solution of complex $[Zn(HL)(N_3)_2]\bullet H_2O$ in DMSO was taken to which 1.5 mL of aqueous solution of anion( sodium salts of $Br^-$, $OMe^-$, $SO_3^{2-}$, $I^-$, $NO_3^-$, $SO_4^{2-}$, $CO_3^{2-}$, $NO_2^-$, $HCO_3^-$, $CH_3COO^-$, $S^{2-}$, $Cl^-$, $H_2PO_4^-$ and $SCN^-$) was added to make up the volume 3 mL and fluorescence was recorded. Out of all the ions taken, $H_2PO_4^-$ was successfully able to quench the fluorescence intensity of $[Zn(HL)(N_3)_2]\bullet H_2O$ (Figure 4.12).

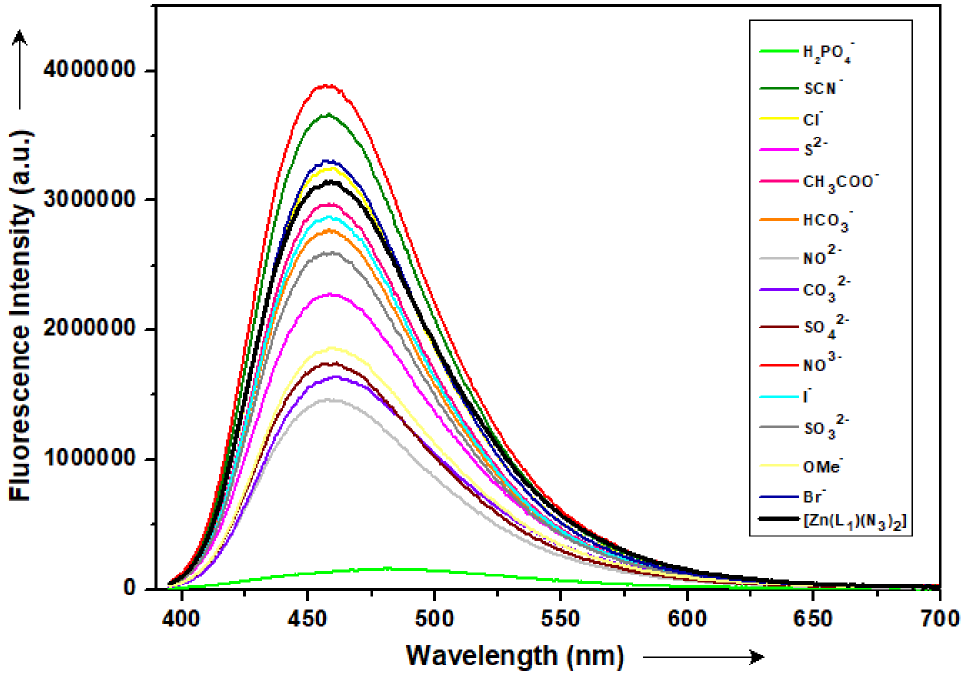


***Figure 4.12:*** *Fluorescence spectra of $[Zn(HL)(N_3)_2]\bullet H_2O$ in presence of different anions and quenching of fluorescence intensity by $H_2PO_4^-$.*

The complex was also checked for whether it senses other phosphates or only $H_2PO_4^-$ ion. When the fluorescence of complex $[Zn(HL)(N_3)_2]\bullet H_2O$ was recorded in presence of different phosphates like $PO_4^{3-}$, $HPO_4^{2-}$ quenching was observed but it was maximum for $H_2PO_4^-$ (Figure 4.13).

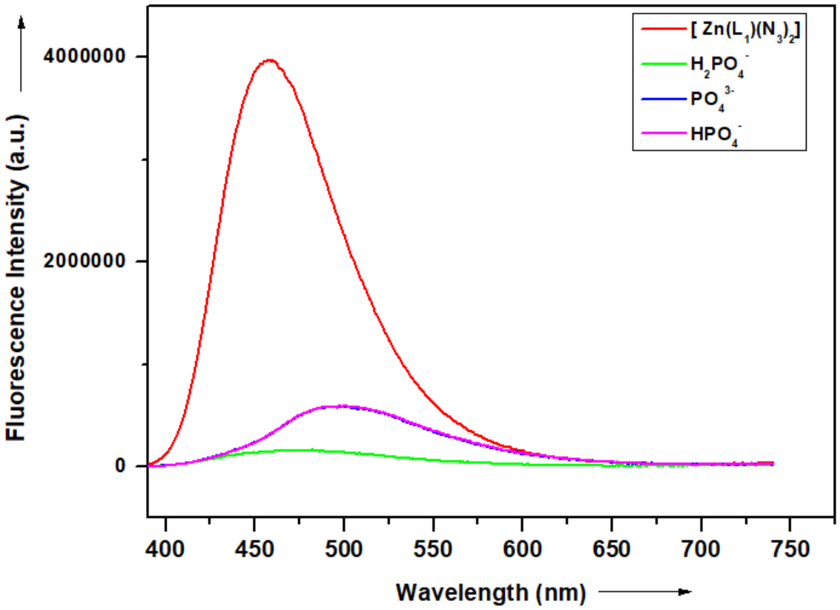


***Figure 4.13:*** *Fluorescence quenching of* $[Zn(HL)(N_3)_2]\bullet H_2O$ *shown by different phosphates*

## 4.8.1 Competitive Sensing Studies

The efficacy of probe for sensing of $H_2PO_4^-$ was checked by taking the fluorescence of the complex $[Zn(HL)(N_3)_2]\bullet H_2O$ in the presence of excess concentration of other anions and in small concentration of $H_2PO_4^-$. It was found that the compound shows very good efficiency in sensing of $H_2PO_4^-$ in the presence of large excess concentration of any other anion. Thus, presence of any other anion in the system do not affects the sensing of $H_2PO_4^-$ by the complex and the fluorescence intensity quenches effectively irrespective of the ion present in the system (Figure 4.14).

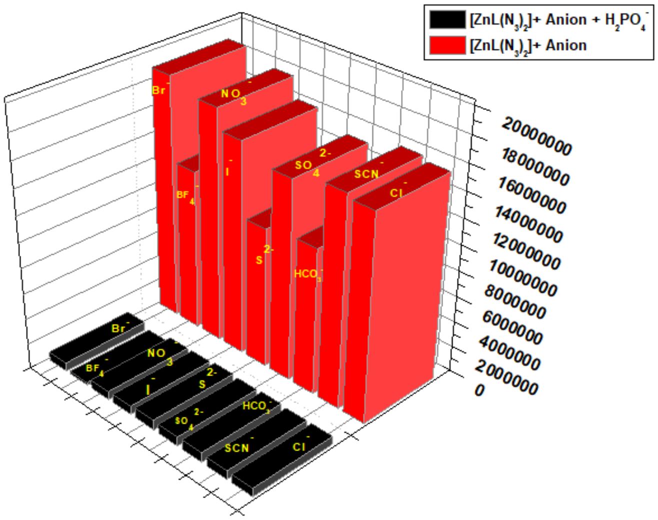


***Figure 4.14:*** *Competitive sensing of $H_2PO_4^-$ by complex $[Zn(HL)(N_3)_2]\bullet H_2O$ in the presence of excess concentration of other anions*

## 4.8.2 <u>Detection Limit Calculation</u>

The detection limit for sensing of $H_2PO_4^-$ was calculated by 3σ/k method where σ = standard deviation of three blank readings and k = slope of curve between fluorescence intensity and concentration plotted by conducting fluorescence titration of the $[Zn(HL)(N_3)_2]\bullet H_2O$ complex in increasing concentration of phosphate.

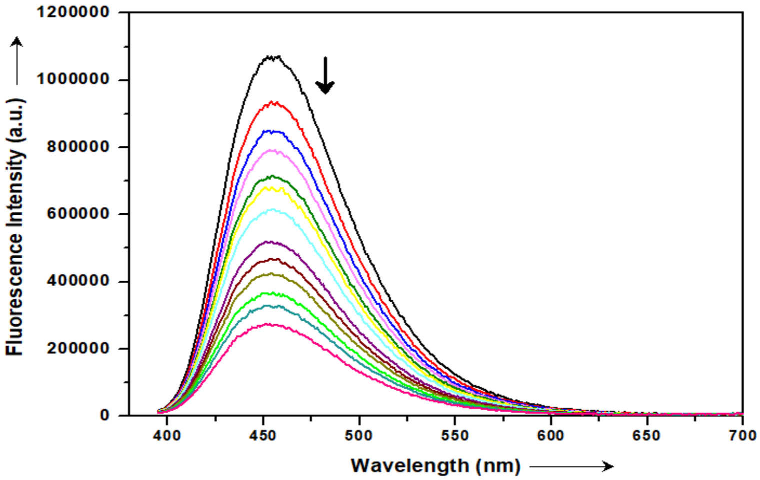


***Figure 4.15:*** *Fluoroscence titration of complex in increasing concentration of phosphates*

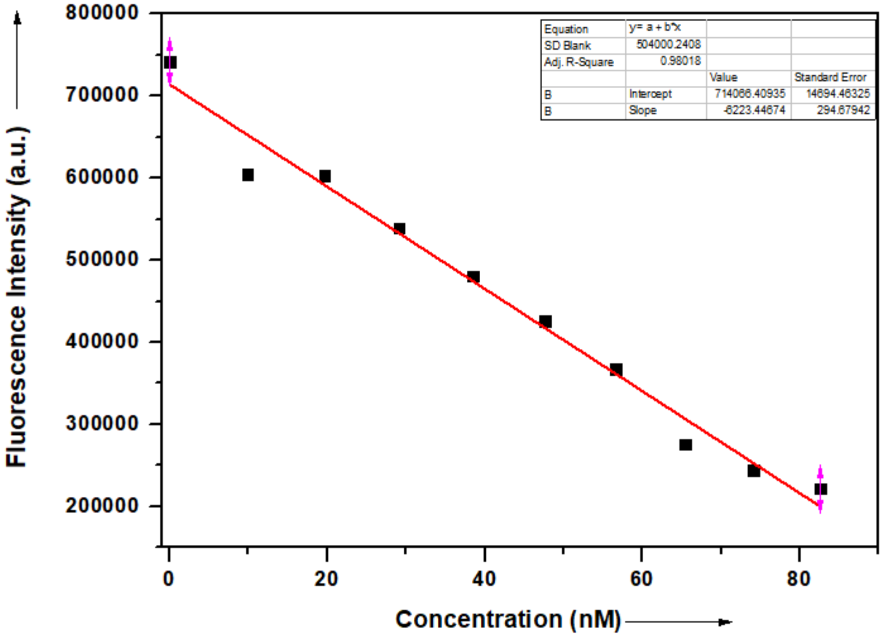


***Figure 4.16:*** *Linear fitting plot to determine slope for calculation of detection limit*

## Calculations of detection limit:

***Table 4.3:*** *Values of standard deviation and slope obtained from the graphs for calculation of detection limit*

| | |
|---|---|
| **Standard deviation (σ)** | 504000.2408 |
| **Slope (k)** | 6223.44674 |

Detection limit = 3σ/k

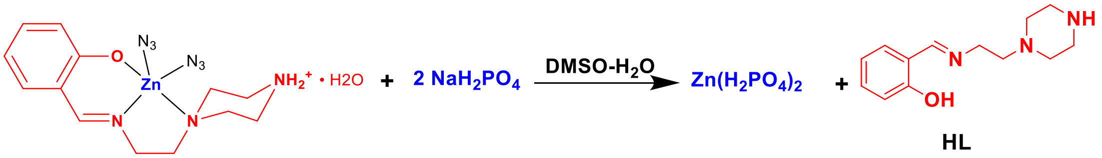
= (3 × 504000.2408)/ 6223.44674 nM

= 243 nM

**Detection limit = 243 nM**

## 4.9 Sensing Mechanism

The sensing mechanism of $H_2PO_4^-$ was identified using electrospray ionization mass spectrometry. To know the underlying mechanism of sensing, solution of complex $[Zn(HL)(N_3)_2]•H_2O$ in DMSO was mixed with the small amount of aqueous solution of $H_2PO_4^-$ ion. On mixing a white precipitate was observed and was isolated, washed and dried. The precipitate obtained and the filtrate were given for ESI-MS to know about the species formed. In the mass spectrum of precipitate a molecular ion peak was observed for $Zn(H_2PO_4)_2$ (with low intensity because of poor solubility of precipitate) and in the mass spectrum of filtrate a molecular ion peak of Schiff base ligand was observed (Figure 4.17). This indicates that $H_2PO_4^-$ ion has very strong affinity for $Zn^{2+}$ ion and thus when the solution of complex is treated with aqueous solution of $H_2PO_4^-$, it sets free the ligand from the complex and itself binds to the $Zn^{2+}$ ion to form $Zn(H_2PO_4)_2$ and thus quenches the fluorescence intensity of the complex and efficiently senses $H_2PO_4^-$ ion [74].



***Scheme 4.5:*** *Scheme for the sensing mechanism of* $H_2PO_4^-$

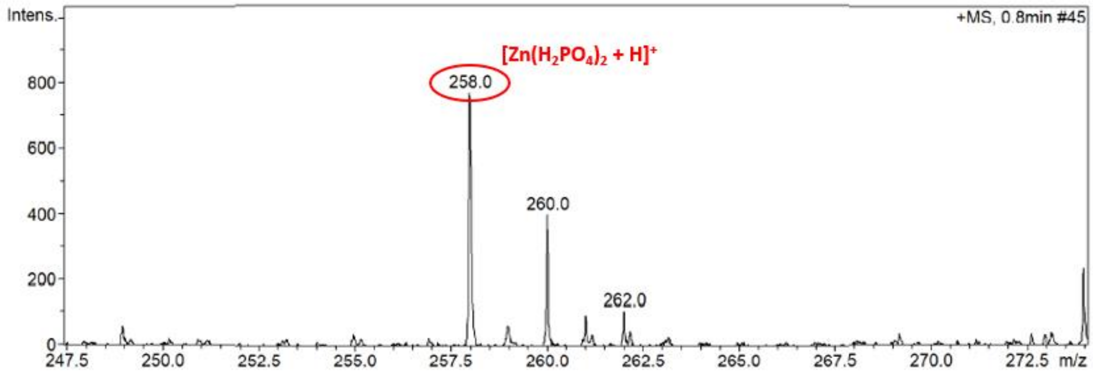


***Figure 4.17:*** *ESI-MS spectrum of the precipitate*

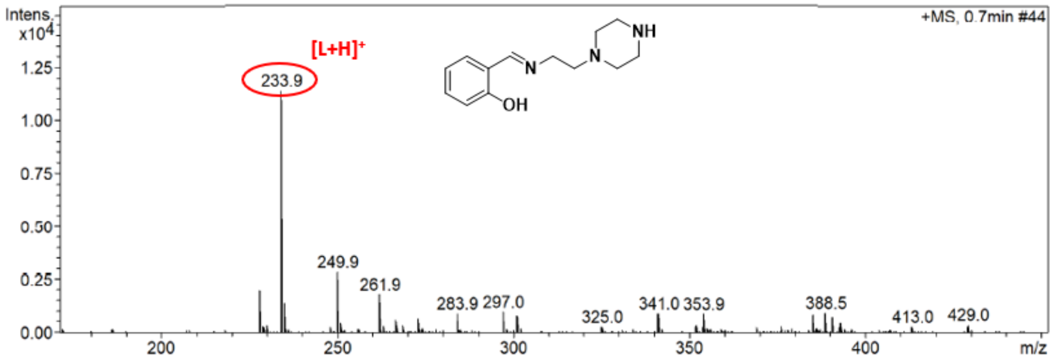
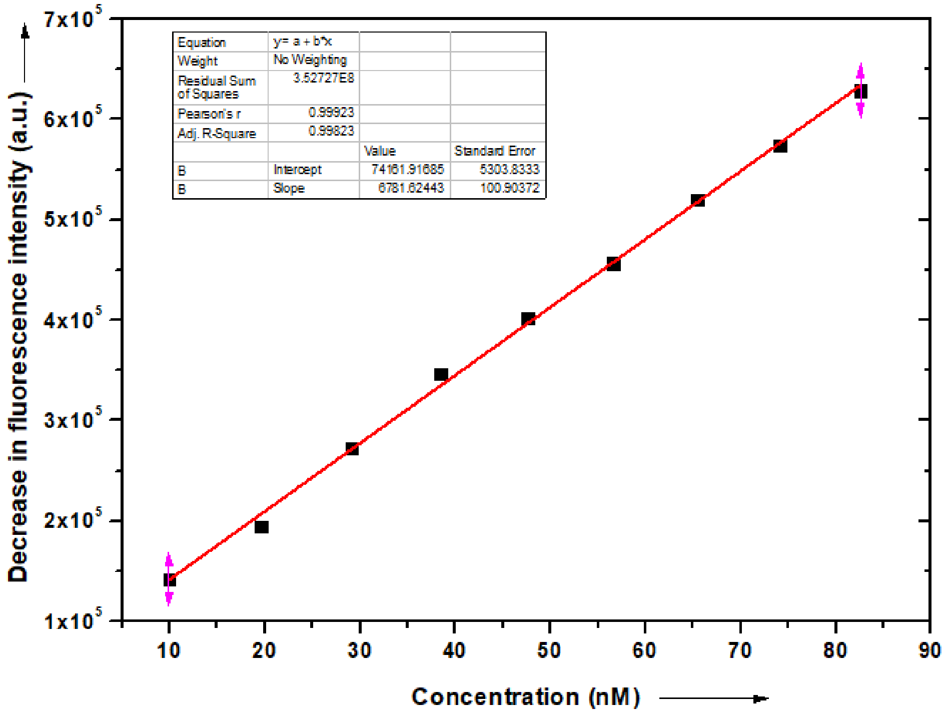


***Figure 4.18:*** *ESI-MS spectrum of the filtrate*

## 4.10 Recovery Analysis

To explore real-life application of the sensor for detection of dihydrogen phosphate ion, the probe was used to detect $H_2PO_4^-$ in real water samples and concentration of the ion in solution was determined using decrease in fluorescence intensity values. For this analysis, known volume of water samples of tap water, distilled water and Simrol pond water were spiked with $H_2PO_4^-$ to make overall concentration of 1 µM. Then, fluorescence titrations were done for the complex with the increasing concentration of $H_2PO_4^-$ and a linear plot between decrease in fluorescence intensity vs. concentration was obtained (Figure 4.19). The complex was then treated with the spiked water samples to observe the values of decrease in fluorescence intensity (Table 4.4) which were used to determine the concentration of dihydrogen phosphate in real water samples. The calculated value was compared with the theoretical values and they were found to be in good agreement with each other (Table 4.5).



| | | | |
|---|---|---|---|
| Equation | y= a + b*x | | |
| Weight | No Weighting | | |
| Residual Sum of Squares | 3.52727E8 | | |
| Pearson's r | 0.99923 | | |
| Adj. R-Square | 0.99823 | | |
| | | Value | Standard Error |
| B | Intercept | 74161.91685 | 5303.8333 |
| B | Slope | 6781.62443 | 100.90372 |

***Figure 4.19:*** *Linear fitting curve obtained from fluorescence titrations*

Linear equation: y = (6781.62443 × x) + 74161.91685

where y = decrease in fluorescence intensity, x = concentration of $H_2PO_4^-$

***Table 4.4:*** *Decrease in fluorescence intensity values of $[Zn(HL)(N_3)_2]{\cdot}H_2O$ for different real life water samples*

| S. No. | Water Sample | Decrease in fluorescence intensity (a.u.) | |
|---|---|---|---|
| | | **Mean** | **Standard deviation** |
| 1. | Distilled water | 402859.82135 | 7.87896E4 |
| 2. | Tap water | 402318.69138 | 7.45874E4 |
| 3. | Simrol pond | 389598.45896 | 8.04878E4 |

***Table 4.5:*** *Comparison of added and detected concentration of* $H_2PO_4^-$

| **S. No.** | **Water Sample** | **Amount of $H_2PO_4^-$ added (µM)** | **Amount detected (µM)** |
|---|---|---|---|
| 1. | Distilled water | 1.0 | 1.018 ± 0.015 |
| 2. | Tap water | 1.0 | 1.016 ± 0.001 |
| 3. | Simrol pond | 1.0 | 0.977 ± 0.020 |

# CHAPTER 5

## Conclusion and Future Scope

In the present work, three new complexes $[Zn(HL)(N_3)_2]\bullet H_2O$, [Zn(HL)(5-phenyltetrazolato)$_2$] and [Zn(HL){5-(4-pyridyl)-tetrazolato}$_2$] have been synthesized. $[Zn(HL)(N_3)_2]\bullet H_2O$ was utilized in anion sensing experiment and it was found to effectively sense phosphates primarily dihydrogen phosphate ion ($H_2PO_4^-$). The detection limit for the dihydrogen phosphate ion was found to be 243 nM. Along with this competitional sensing analysis was performed which revealed good efficiency of $[Zn(HL)(N_3)_2]\bullet H_2O$ for sensing of low concentration of $H_2PO_4^-$ in the presence of large excess concentration of any other anion. Recovery analysis was also performed for checking the applicability of sensor to detect concentration of $H_2PO_4^-$ in real-life water samples and it showed a good agreement between the observed and actual values. These results unfold the possibility to employ this sensor to check the concentration of phosphates in real-life water samples.

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