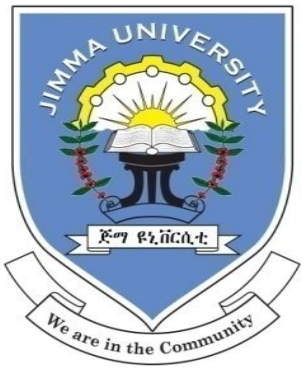

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Synthesis of Schiff's-based Chemosensor for Trace Aluminum ion Detection from Pharmaceutical waste water

A thesis Submitted to the School of Graduate Studies of Jimma University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Materials Science and Engineering

By

Mengistu Jemberu Dagnaw

June, 2019

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Principal Supervisor: - Dr. Prabal Dasgupta (Ph.D.)

Executive Supervisor: - Prof. (Dr.) Chittaranjan Sinha

June, 2019

Jimma, Ethiopia

APPROVAL

The thesis entitled **"Synthesis of Schiff's-based Chemosensor for Trace Aluminum ion Detection from Pharmaceutical wastewater"** submitted by Mengistu Jemberu Dagnaw is approved and accepted as a Partial Fulfillment of the Requirements for the Degree of Masters of Science in Materials Science and Engineering at Jimma Institute of Technology.

Dr. Prabal Dasgupta

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Principal Supervisor	Signature	Date
Prof. Chittaranjan Sinha	Chittaranjan Sinha	15/5/2019
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Executive Supervisor	Signature	Date

As members of the examining board of MSc. thesis, we certify that we have read and evaluated the thesis prepared by Mengistu Jemberu Dagnaw. We recommend that the thesis could be accepted as a Partial Fulfillment of the Requirements for the Degree of Masters of Science in Materials Science and Engineering.

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To Whom It May Concern

This is to certify that Mr. Mengistu Jemberu Dagnaw, M. Sc., School of Graduate Studies of Jimma University, Jimma, Ethiopia with Prof. (Dr.) Prabal Dasgupta, Ph.D., Professor, Jimma University, Ethiopia and Principal Supervisor has executed his Thesis work entitled, **"Synthesis of Schiff's-based Chemosensor for Trace Aluminum ion Detection from Pharmaceutical wastewater"** under the supervision of Prof. (Dr.) Chittaranjan Sinha, Professor & Head, Department of Chemistry, Jadavpur University, Kolkata, India for the period of two months (14/03/2019 - 15/05/19, Challan No. 59274, 14/3/19). Mr. Jemberu has obeyed the rules and regulations of the University related to Project work as a foreign student in the Department of Chemistry, Jadavpur University, Kolkata.

Neither this project nor any of its part is submitted for publication as research article/conference report/in proceedings or for any degree/diploma or other academic award anywhere before.

C. Sinha

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Professor in Chemistry
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DECLARATION

I, the undersigned, declare that this thesis is my original work performed under the supervision of my principal research advisor Dr. Prabal Dasgupta as well as executive supervisor Prof. (Dr.) Chittaranjan Sinha, Department of Chemistry, Jadavpur University, Kolkata – 700 032, West Bengal, India. This research has not been presented as a thesis for a degree in any other university. All sources of materials used for this thesis have been duly acknowledged.

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ABSTRACT

The probe, 1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazol-2-yl)naphthalene-2-ol has been synthesized by the reaction of 2-hydroxy-1-naphthaldehyde and N^1-(Phenyl(pyridine-2-yl)methyl)benzene-1,2-diamine in 1:1 molar ratio. The probe has been characterized by spectroscopic techniques (FT-IR, UV-Vis, ^{1}H NMR) and is non-emissive. The sensing mechanism was based on Excited state intramolecular proton transfer and upon binding with the analyte restriction of intramolecular rotation has been happening. The probe selectively binds Al^{3+} and upon irradiation at 400 nm in presence of a large number of cations shows high emission (λ_{em}, 502 nm) in 4:1 H_2O/MeOH (v/v) solution and serves as a "turn-on" fluorescence chemosensor. The limit of detection (LOD) for Al^{3+} is 3.3 nM (3σ method) which is more than 200 times sensitive than that is recommended by the World Health Organization (WHO), 7.4 mM. The probe is stable in the pH range, 2–12 and maximum turn-on response to Al^{3+} is observed at pH, 6. Formation of the 1:1 metal-to-ligand complex has been ascertained by Mass spectra, Job's plot and Benesi-Hildebrand plot (Binding or Association Constant K_a, $2.3\times10^4\ M^{-1}$). The effluent collected from the pharmaceutical industry has 0.381 mM of Aluminum ion concentration which is trace quantity. A separate in vitro experiment shows that the probe can specifically perceive Al^{3+} ion in (Human liver cancer) cell line. This work aims to find a fluorogenic sensor for Al^{3+} ions, which has been decided by WHO (World Health Organization) as a potential food and drinking water pollutant and found to be detrimental for human health. We devised a sensor which is capable of detecting 3.3 nM of Al^{3+} ion which is by far less than the limit stipulated by WHO.

***Keywords**: Al^{3+}sensor; Binding constant; Live cell imaging; Limit of detection; Naphthaldehyde based probe*

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Last, but not least, I would also like to appreciate my friends and family for giving me the morale and knowledge support for the success of the project.

(Mengistu Jemberu Dagnaw)

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LIST OF ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AFM	Atomic Force Microscopy
ARBD	Aluminum Related Bone Disease
CHEF	Chelation Enhanced Fluorescence
DMEM	Dulbecco's Modified Eagle's Medium
DMSO-D6	Dimethyl Sulfoxide Deuterated
ESI-HRMS	Electro Spray Ionization High-Resolution Mass Spectroscopy
ESIPT	Excited State Intramolecular Proton Transfer
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FT-IR	Fourier Transform Infrared Spectroscopy
HepG2	Hepatits liver cancer cell
HL	Potential Proton of Ligand
HOMO	Highest Occupied Molecular Orbital
HpNAP	H. pylori Neutrophil-Activating Protein
ICPMS	Inductively Coupled Plasma Mass Spectrometry
ICT	Internal Charge Transfer
L	1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazol-2-yl)naphthalene-2-ol
LUMO	Lower Unoccupied Molecular Orbital
MeOH	Methanol
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]
METEC	Metals Engineering Corporation
NMR	Nuclear Magnetic Resonance Spectroscopy
PBS	Phosphate-Buffered Saline
PET	Photoinduced Electron Transfer

PPM	Parts Per Million
TMS	Tetramethyl Silane
UV-Vis	Ultra violate - visible Spectroscopy
WI38	Human lung fibroblast cells

1. INTRODUCTION

1.1 Background

The highest amount of metallic element on the earth's crust is Aluminum (8.3 % of total mass). High level of Al enters in the environment mainly from mining and processing of ores or the production of aluminum metal, alloys, and compounds. Small amounts of aluminum are released into the environment from coal-fired power plants and incinerators. Aluminum cannot be destroyed or biodegraded in the environment. Aluminum particulates in the air settle to the ground or are washed out of the air by rain. Aluminum compounds may be added during the processing of foods, such as flour, baking powder, coloring agents, anticaking agents, etc. People generally consume little aluminum from drinking water. Water is sometimes treated with aluminum salts (in the form of Alum, $Al_2(SO_4)3K_2SO_4.24H_2O$) for drinking purpose.[1]

Exposure of Al^{3+} ion *via* food chain damages the kidneys, the immune system of the human body, affects the central nervous system leads to Alzheimer's disease, Parkinson's disease, dementia, encephalopathy, mainly affects the bone causing osteoporosis, Colic, rickets. Gastrointestinal problems, interference with the metabolism of calcium and breast cancer are additional hazards associated with Al^{3+} contamination. Moreover, 40% of the world's acidic soils are contaminated by the accumulation of Al^{3+} ion, totally hampering the agricultural productive performance.[2]

Commercially, aluminum is used in Ethiopia for construction of the building, transportation, food packaging, beverage cans, cooking utensils, and water purification. Aluminum products are increasing in use because of its corrosion resistance with good strength, recyclable and low density compared to steel. However, a large quantity of aluminum enters into ecosystem and finally into humans and organisms.

Aluminum is not a biological metal or has a direct impact on the toxicity of biochemical reactions. But Al^{3+} forms insoluble or sparingly soluble carbonates, hydroxides, phosphates, sulfates, etc. These salts are deposited in the tissues where nerve cells are working. Deposition of insoluble Al-salts will inhibit or disturb nerve cell transmission. Thus, biological communication, information transfer, etc. in human life is severely disturbed and initiate Al-related bone disease (ARBD), encephalopathy, myopathy and various neurodegenerative diseases such as Alzheimer's disease, Parkinsonism dementia, etc. in the human body. It may also damage plant roots with the increasing application of aluminum. It is of public concern to know the amount of Al present in the directly consumed products or secondary materials. World

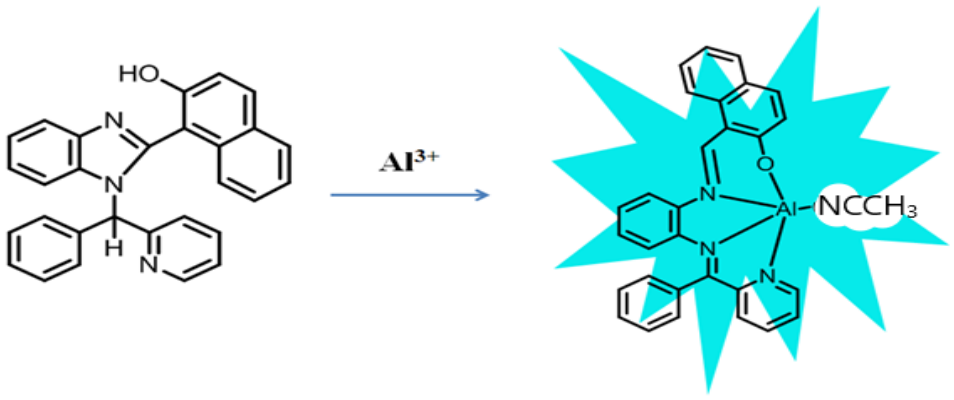
Health Organization (WHO) has assigned Al^{+3} as prime food pollutants with limiting concentration 200 μg/liter (7.41 mM) and has recommended that tolerable weekly dietary human intake is 7.0 mg/kg body weight. Thus, detection of Al^{+3} in water is of urgent importance for monitoring human health.[1-3]

Schiff base ligands have gained recent interest as fluorescent sensors for metal ions including Al^{+3} due to their relatively easy one-step synthesis [4-10]. However, sensing of aluminum in the aqueous medium has been rarely reported [11, 12], mainly due to sparingly soluble or insolubility of organic probe in aqueous media. Therefore, it is a challenging task today to develop new sensors for selectively detecting Al^{+3} in aqueous solutions.

Besides, emissive Al^{+3} complex is serving as a secondary sensor or anion senor like $H_2PO_4^-$, AsO_3^{3-}, PO_4^{3-} etc., especially to F^- [13-16]. Among the entire range of biologically useful anions, F^- possesses significant potential in the prevention of dental caries and treatment for osteoporosis [17, 18].

Conversely, presence of an excess of fluoride in the human body may be dumped as fluorapatite in the bones and teeth leading to osteoporosis, osteosclerosis, dental fluorosis, and skeletal fluorosis. Thus, it is quite important to develop an efficient F^- sensor. The design of dual sensor which is capable to detect sequentially Al^{+3} and F^- by fluorescence ON-OFF signaling response is highly effective.[18-20].

In this work, we have designed and synthesized the probe, 1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazol-2-yl)naphthalene-2-ol for the selective, sensitive detection of Al^{+3} in the semi-aqueous medium. There is no proton transfer during complexation with CH_3CN. The fluorescence sensing properties of HL in the presence of different anions was also investigated and also intracellular detection of Al^{3+} ion in Hep G2 cell.



Ligand [Ligand-Al^{3+}]$^+$

Figure 1.1 The proposed sensing mechanism of Al^{3+} selective sensor ligand.

1.2 Problem Statement

Nowadays, one of the great challenges in the world as well as in Ethiopian industry is waste minimization, less exposure to Al^{3+}. With this background and for the environmental health of the Aluminum industry, I have taken a challenge to design sensitive low-cost fluorogenic sensor selective to Al^{3+} so as to perfectly detect the amount of Al^{3+} ion in industrial effluent.

In Ethiopian industries such as Metals Engineering Corporation (METEC) manufacture many metal objects through conventional techniques such as casting, forging, machining and so on and there are a lot of chips wasted through machining and mostly these chips are not used but wasted as garbage. Some industry in Ethiopia recycle some of the chips using conventional recycling method which is very difficult and causes material loss, this is due to the high surface area to volume ratio of chips, contamination with oxides, especially in case of thin chips; the material loss can reach to 50%. The wasted and dumped chips/scraps cause leaching of toxic substances into the environment as well as causing visual pollution, hazardous to safety issues and causing degradation of wildlife habitats. They generally cause a severe environmental impact and this is the main problem and that is always happening in Ethiopian industry. By simply not using Al chips the other problem raised is exploiting primary resources and consuming energy for producing them. The presence of aluminum ion in the industrial effluent has the following problems:

- Aluminum ion in wastewater cause pollution to drinking water.
- Use of aluminum utensils, cosmetics, antiperspirants, pharmaceuticals such as antacids and buffered aspirin, etc. allow Al^{3+} to enter the human body.

Therefore, it is very important to develop a chemosensor in order to check and measure the concentration of Aluminum ion in industrial effluent as it is harmful to environment.

1.3 Objectives

1.3.1 General objective

Designing of fluorescent chemosensor and investigation of detection towards trace Al^{+3} ions from Pharmaceutical waste water.

1.3.2 Specific objectives

1. To design fluorogenic molecules sensitive for Al^{3+} only.
2. To characterize the probe by spectroscopic techniques and structural analysis.
3. To use emissive Al^{3+} complex for detection of F^-, HF_2^-, PO_4^{3-}, $H_2PO_4^-$, HPO_4^2

4. To quantitatively determine Al^{3+} ion in an industrial wastewater sample.
5. To measure the concentration of Al^{3+} in a living cell using fluorochrome.

1.4 Research Question/Hypothesis

1. What kind of fluorogenic molecules can be used to detect only Al^{3+}?
2. How the probe is characterized by spectroscopic methods and structural study?
3. How the emissive Al^{3+} complex is used to characterize F^-, HF_2^-, P^V (different phosphates)?
4. Does the probe detect Al^{3+} ion in an industrial effluent sample?
5. How the concentration of Al^{3+} is measured in a living cell?

1.5 Significance of the study

A huge number of Al-based industry is now flourishing in Ethiopia. Because of industrial and other human activities Al enters human being and causes different types of neurodegenerative diseases. On considering public health and social progress of Ethiopia, it is important to develop an online low-cost analysis tool for Aluminum. At the phase of sustainable development of the globe, we should inform our people about the health index of the country.

Besides, the results of this study are expected to become valuable up to date information for the Government to develop or invest fund for public health and private or Government Companies for the treatment of wastes before discharging. In addition, precautionary measures may assist to be taken by the Government of Ethiopia for public health because every year huge budget is allocated for public health and one of them is pollution and health. As well as the investigation improves sustainable development and contributes environmental impact. This research may help identify how far we move towards sustainable development with present industrial infrastructure.

2. LITERATURE REVIEW

Aluminum has both technological and industrial value and is useful in the manufacturing of household utensils, water purification instruments, electrical wirings, etc. A good quantity of Al is consumed in cosmetics, antacids, and thus enters biology. It is a non-essential biological element and its availability on the earth's crust is 8.3 % of total mass [21].

Brain and bone disease caused by high levels of aluminum in the body have been seen in children with kidney disease. Bone disease has also been seen in children taking some medicines containing aluminum. In these children, the bone damage is caused by aluminum. In the stomach Al^{3+}prevents the absorption of phosphate, a chemical compound required for healthy bones. Aluminum is found in breast milk, but only a small amount of this aluminum will enter the infant's body through breastfeeding. Typical aluminum concentrations in human breast milk range from 0.0092 to 0.049 mg/L. Aluminum is also found in soy-based infant formula (0.46–0.93 mg/L) and milk-based infant formula (0.058–0.15 mg/L) [21].

Exposure to the levels of aluminum that are naturally present in food and water and the forms of aluminum that are present in dirt and aluminum pots and pans are also harmful. Eating large amounts of processed food containing aluminum additives or frequently cooking acidic foods in aluminum pots may expose a person to higher levels of aluminum. Workers who breathe large amounts of aluminum dust can have lung problems, such as coughing or changes that show up in chest X-rays. Some workers who breathe aluminum-containing dust or aluminum fumes have decreased performance in some tests that measure functions of the nervous system. The Al-related bone disease (ARBD), encephalopathy, myopathy and various neurodegenerative diseases such as Alzheimer's disease, Parkinsonism, dementia, etc. in the human body are due to Al toxicity. Al can also damage plant roots [26-29].

World Health Organization (WHO) has assigned Al^{3+} as prime food pollutants with limiting concentration 200 μg/liter (7.41 mM) and has recommended that tolerable weekly dietary human intake is 7.0 mg/kg body weight [30-32].

2.1 Design of Sensors

A tremendous effort has now been invested in the design of fluorescence chemosensors particularly for selective and sensitive probing of biologically and environmentally important metal ions. Chemosensors can selectively recognize and signal the presence of specific ions/molecules through the naked eye and through spectroscopic instruments. The optical

responses of chemosensors have received significant attention for the last two decades [21-23]. Because of high sensitivity, selectivity, simplicity, real-time analysis, cost-effective, rapid and facile process the spectroscopic tools become applicable as analytical apparatus in the environmental, medical, and biochemical fields as well as in industry [24].

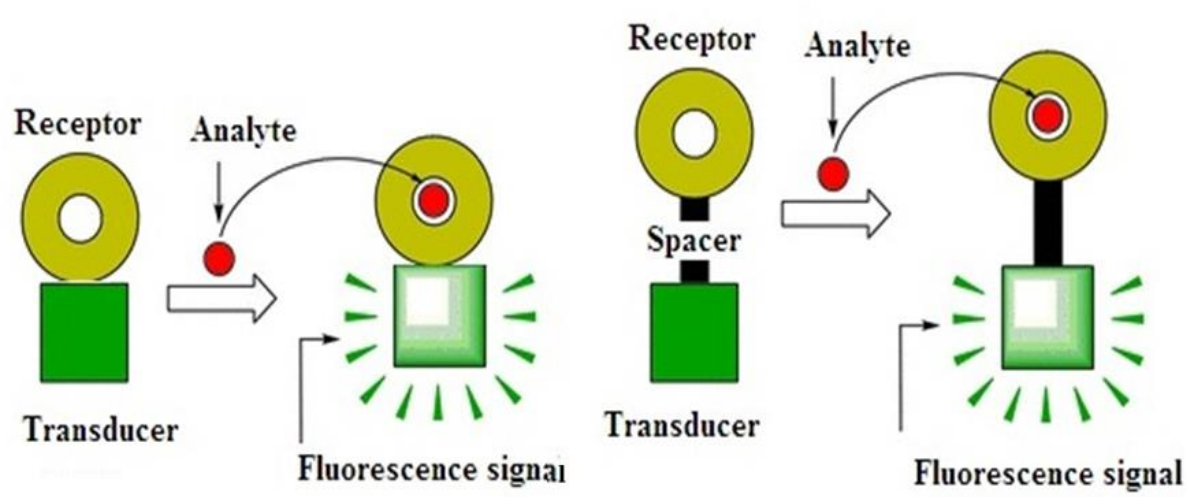


Figure 2.1 Fluorescent sensor cartoon with transducer (signaling unit), receptor (binding unit or ligand), integrated (a) or spacer (b) and analyte (ion/molecule) for recognition

The receptor/ligand unit can bind the ion/molecule with a concomitant change in one or more properties of the system, such as color (colorimetric probes) or fluorescence intensity (fluorescence probes), that arise from the complexation, hydrolysis/substitution, or oxidation/ring transformation. In order to achieve high selectivity, the receptor/ligand is required to have the strongest affinity with the ion of interest. The fluorescent chemosensors have attracted considerable attention due to convenient use and real application in biological systems [25,26].

Metal-specific fluorescence probes are of increasing importance in understanding the neurobiology and general cell biology of metal ions. In fact, a great number of chemosensors have been designed for monitoring heavy toxic metal ions [27]. Schiff's bases, a particular class of chelating molecules, have been widely used as chemical probes due to their facile synthesis, stability in wide pH range, solubility in polar and mixed polar media, and good photophysical properties [28-33].

The Schiff bases serve as N, O bidentate monoanionic chelating agent and have been extensively investigated for more than a century and employed in different aspects including magneto-chemistry, non-linear optics, photophysical studies, catalysis, materials chemistry, chemical analysis, bioinorganic chemistry. Use of iminephenolato function as a chemosensor for ions

(cations and anions) has been explored recently [28-33] for chemometric identification of several ions by absorption and fluorescence spectroscopic routes. Majority of chemosensors are single-ion selective while multi-ion responsive systems are of great interest because they can differentiate and detect an analyte of interest in the presence of interfering ions [34, 35] and are essentially important in the developing of multifaceted molecular logic gates, switching devices, etc. [36, 37].

However, challenges exist in developing such complex molecular systems, especially in detecting ions of biological interest. Among the cations, special attention is focused to develop chemosensors for Zinc because of its extensive bioinorganic chemistry [38, 39] and for Aluminum because of its toxicity [40].

2.2 Chemical sensors

The need for selective and sensitive sensors for real-time monitoring of analytes of biological, clinical, and environmental interest is a challenging task now [43-45]. Of the various kinds of chemosensors, luminescence-based chemosensors present many advantages, since the process is highly sensitive, specific, cheap and versatile. In this contest, special interest has been devoted to the development of luminescent chemosensors for ions and small molecules.

A sensor is a device that converts an input signal from a stimulus into a readable output signal [43]. The input signal can be any measurable characteristic such as quantity or physical variation, while the output is ultimately an electrical signal. Indeed, the development of new sensor technology faces the dilemma of trying to create sensors that are both increasingly sensitive and increasingly robust. Chemical sensors respond to the chemical environment (i.e., interactions with molecular species), rather than the physical environment (e.g., temperature or pressure) [43,44]. Chemical sensors can, therefore, be categorized into two major groups: those that discriminate among analytes based on physical properties (e.g., molecular weight, vapor pressure, etc.) and those that measure chemical properties (e.g., reactivity, redox potential, acid-base interactions, etc.). Chemical sensors can also be grouped by their signal transduction methods into three classes: (i) electrical and electrochemical, (ii) thermometric, and (iii) optical. We will focus on this last class of sensors as array components.

2.2.1 Optical sensors

Optical sensors use light commonly, visible or ultraviolet, to interrogate sensors for analysis [45]. These may be represented as a wavelength selectable light source, the sensor material itself interacting with analytes, and a light detector. What the detector monitors vary by technique (e.g.

refractive index, scattering, diffraction, absorbance, reflectance, photoluminescence, chemiluminescence, etc.), can cover different regions of the electromagnetic spectrum, and can allow measurement of multiple properties (e.g. intensity of light, lifetime, polarization, etc.). Colorimetry (i.e., quantitative measurement of absorbance or reflectance spectra) is one of the oldest of analytical techniques [41] and is a straightforward ‘‘naked-eye’’ quantitative technique. Colorimetric detection is a reasonably simple technique, and the advent of universal digital imaging has given it new and stimulating possibilities. Fluorometry can provide excellent sensitivity and fluorescent sensors are 105 times more sensitive than common absorption spectroscopic technique and much cheaper than Atomic Spectroscopy, (AAS, AES, AFM), Mass spectral studies (ICPMS, etc.**)** [46-48].

Fluorescence-based approaches and fluorescence parameters such as Stokes shift, fluorescence intensity, and anisotropy, emission and excitation spectra, and fluorescence lifetime can provide substantial flexibility as an analytical approach for qualitative identification and quantitative estimation of H^+, cations, anion, and molecules [48,49].

2.2.2 Cation Sensor

Fluorescent sensors consist of a fluorophore linked to a receptor (Fig.1). In the design of such sensors [49], attention should be paid to both receptor and reporter or signaling moieties. The signaling moiety is a signal transducer, i.e. it converts the information (recognition event) into an optical signal expressed as the changes in the photophysical characteristics of the fluorophore. Different cation sensors based on various fluorogenic activities such as Fluorescent PET (photoinduced electron transfer), Fluorescent PCT (photoinduced charge transfer), Excimer-based, CHEF (Chelation Enhancement of Fluorescence), CHEQ (Chelation Enhancement of Quenching), AIEE (Aggregation Induced Enhancement of Emission), ESIPT (Excited State Intramolecular Proton Transfer) and other emission enhancement or quenching technique have been adopted in the sensing [41-50].

The concept of the soft-hard acid-base principle of interaction of ionic centers is the fundamental bases of selection followed by the principle of coordination theory, size of the chelate ring, space in the chelate cavity, etc. monitor the specificity of the sensing activity. Receptors are designed based on the coordination chemistry principle and integrated or attached through a spacer with the transducer unit. Binding of cation to the receptor has developed a strain over the transducer unit which would be responsible for fluorogenic activity enhancement or quenching. Design of receptor may be acyclic or cyclic/macrocyclic with specific binding or chelating centers. The cationic complex of fluoroionophore may interact with external anions which may selectively

extract cation from the binding pocket which depends on relative binding strength and may be responsible for a specific change in fluorescence intensity. This would help to indirect sensitivity of anions and also logic operation may be demonstrated. Chelating and macrocyclic ligands such as crown ethers, cryptands, etc. and their size specific binding of metal ions are useful means for their use as chemosensor in fluorescence technique. Because of the biological and environmental importance of metal ions, numerous sensory reports are available for different metal ions with diverse mechanisms [51].

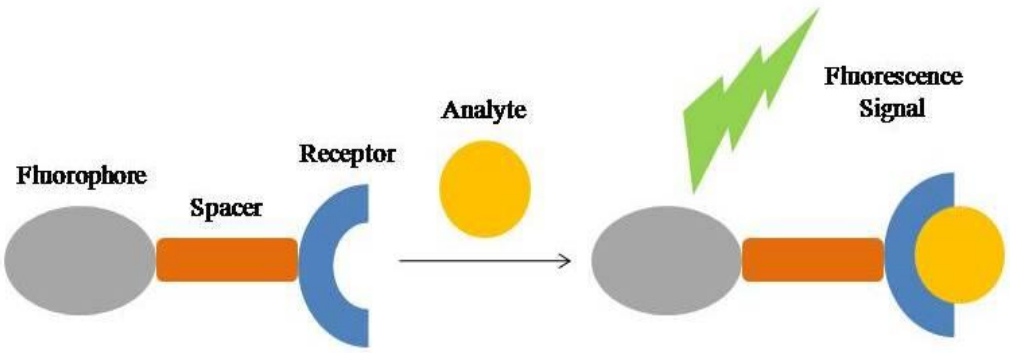


Figure 2.2 Main aspects of fluorescent molecular sensors for cation recognition.

Chemosensors based fluorescence technique is one of the most suitable sensing processes in terms of sensitivity, selectivity, response time and local observation. The sensing mechanism is either enhancement or quenching of emission intensity of the transducer by receiving cations or anions based on Photoinduced Electron Transfer (PET), Internal Charge Transfer (ICT), Chelation Enhanced Fluorescence (CHEF) and Deprotonation mechanisms. The PET [52] has exhibited changes of emission intensities with some or no spectral shifts, whereas ICT [53] is caused both intensity changes and spectral shifts, and CHEF [54] is also provided Fluorescence enhancements with or without any spectral changes. Therefore, the development of highly selective Fluorescence probes for specific ions in the presence of a variety of other metal ions has received great interest.

Design of sensors for selective and sensitive identification at very low concentration in living cells or intracellular fluid is a challenging task [55]. For example, the specific identification by using chromogenic or fluorogenic sensors of Cr^{3+} [56], Mn^{2+} [57], Fe^{3+} [58], Co^{2+} [59], Ni^{2+} [60], Cu^{2+} [61], Zn^{2+} [62] are reported in literature. Non-transition metal ions identification by fluorogenic sensor is a scope of intensive research and reports available for Cd^{2+} [63], Hg^{2+} [64], Pb^{2+} [65], Al^{3+} [66], Ag^{+} [67] etc.

Classical complexometric indicators (many of which are natural products also used as histological stains and some of which date back to the early 1800s) may have greater or lesser degrees of specificity: for example, calcein and Eriochrome Black T are used to detect Ca^{2+}, Mg^{2+}, and Al^{3+}; hematoxylin for Fe^{3+} and Al^{3+}; murexide for Ca^{2+}, Cu^{2+}, Ni^{2+}, and Transition Metal Ions.[31] The design of a sensor for f-block metal ions are a healing task and are approaching fast [68].

2.2.3 Anion sensors

The coordination chemistry of anions was a long-overlooked area of inorganic chemistry. The biological, environmental and medical importance of many anions, from the simple (Cl^-, F^- $HPO4^{2-,}$ $PO4^{3-,}$ etc.) to the multifaceted (ATP, lipid anions, nucleic acids, etc.), has received attention in recent years [69]. The use of dyes for the detection of anions has been an active area of research and has been extensively studied recently [64-74].

There are, however, unique challenges to these studies because anion complexation is quite different from that of metal cations, largely because of the relatively large size of anions and the omnipresence of protons in aqueous media. Anion receptors can be neutral or positively charged and in general anion-receptor interactions are dominated by electrostatics and hydrogen bonding. It is common practice to link a chromogenic or fluorescent reporter moiety to a specific chelating receptor, but one may also use fluorescent Lewis acids directly. Dyes with urea, thiourea, or naphthalimide sites, or metal ion containing dyes have been used heavily as anion binding sites for both colorimetric and luminescent detection. The electrostatic attraction brings anion and sensor very close and then selectivity of the interaction is directed by choice of soft-acid base property, hydrogen bonding efficiency, size of the cavity or binding site, the efficiency of anion-π interaction, etc. These interactions associated with collisional effect may enhance or quench the emissivity of the sensor. Different sensors for identification of CO_3^{2-} [70], HSO_4^- [71], NO_2^- [72], CN^- [73], S^{2-} [74], F^- [75], I^- [76], PO_4^{3-} [77], $H_2PO_4^-$ [78] acetate [79] etc. are well reported in recent literature.

2.2.4 Molecular Sensor

The ion sensors are more prominent than molecular sensors. Design of molecular sensors is based on the events found in living organisms; natural systems are the original form of molecular sensors. Modern molecular sensors are essential parts of many emerging medical diagnostic devices playing significant roles in global health, reducing healthcare costs. Prominent areas of molecular sensor research are the detection of biomolecules for disease diagnosis, the detection

of volatile components for air pollutant characterization, and the detection of chemical analytes for evaluation of the physiological activity.[81]

Small molecules like environmental pollutant (CO, HCN, H_2S, SO_2, NOx), Green House Gases (CO_2, CH_4, vapor, CFC, Cl_2 etc.), Amino acids, Alcohols, low molecular weight Carboxylic Acids, Aldehydes, thiols, aromatic amines, pesticides, herbicides, etc. have been required to be detected at very low concentration for analytical, environmental and health reasons. Many dyes (permitted or nonpermitted) are used as food colors and most of them are colorant restricted or not permitted by FDA; quality of food control for health and safety and very accurate concentration measurement are most urgent. The design and development of highly sensitive and selective fluorescent probes for the detection of the small molecule are thus important. For example, thiols in biological systems are of great interest to current researchers [80-82].

Among the many biologically important thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione , glutathione is well-known as the most important and abundant antioxidant in plants, animals, fungi, and some bacteria and archaea [83, 84]. Many groups are tirelessly working for the design of molecular sensor for Alcohols [85], pesticides and herbicides [86], NOx [87], H_2S [88], Glutathione [89], Galactyl [90] and so on.

2.3 Selective Al^{3+} sensor

Exploration of selective and sensitive chemosensor for the detection of Al^{3+} ions in solution has been of considerable attention with biological and environmental interest [91]. Schiff 's base ligands have gained recent interest as fluorescent sensors for metal ions including Al^{+3} due to their relatively easy one-step synthesis [91-100]. However, sensing of aluminum in the aqueous medium has been rarely reported [101, 102], mainly due to sparingly soluble or insolubility of organic probe in aqueous media. Therefore, it is a challenging task today to develop new sensors for selectively detecting Al^{+3} in aqueous solutions.

Besides, emissive Al^{+3} complexes are serving as anion sensor, especially to F^- [103-106]. Among the entire range of biologically useful anions, F^- possesses significant potential in the prevention of dental caries and treatment for osteoporosis [107, 108]. Conversely, presence of an excess of fluoride in the human body may be dumped as fluorapatite in the bones and teeth leading to osteoporosis, osteosclerosis, dental fluorosis, and skeletal fluorosis[93-95].

Thus, it is quite important to develop an efficient F^- sensor. The design of dual sensor which is capable to detect sequentially Al^{+3} and F^- by fluorescence ON-OFF signaling response is highly

effective. Recently, coumarin-based molecular switch for the sequential detection of Al^{+3} followed by its use for F^- sensing is reported [107, 106]. Besides, rhodamine-based [106] and quinaldine appended [106] chemosensors are also available for the detection of Al^{+3} and F^- [106]. However, the problem appears about the solubility of probes in an aqueous medium.

3. MATERIALS AND METHODS

All reagents and solvents used for synthesis were purchased from commercial sources and used as received. All aqueous solutions were prepared using Milli-Q water (Millipore). 2-hydroxy-1-naphthaldehyde and $Al(NO_3)_3.9H_2O$ was obtained from E. Merck, Germany. N^1-(Phenyl(pyridine-2-yl)methyl)benzene-1,2-diamine was prepared following reported procedure [109].

All other solvents and chemicals were purchased from Merck and Final (AR grade) and used without further purification. UV-vis spectra were recorded on Perkin Elmer Lambda 25 spectrophotometer and fluorescence spectra were obtained using a Perkin Elmer spectrofluorometer model LS55, FT-IR spectra (KBr disk, 4000–400 cm^{-1}) from a Perkin Elmer LX-1FTIR spectrophotometer. NMR spectra were obtained on a Bruker (AC) 500 MHz, NMR spectrometer using TMS as an internal standard. ESI mass spectra were recorded from a Water HRMS model XEVO-G2QTOF#YCA351 spectrometer. All of the measurements were conducted at room temperature. The melting point was determined on a hot-plate melting point apparatus in an open-mouth capillary.

3.1 Experiments

3.1.1 Synthetic procedures and formulation of the probe

The chemosensor was synthesized by the condensation reaction of N^1-(Phenyl(pyridine-2-yl)methyl)benzene-1, 2-diamine (0.273 g, 1.0 mmol) and 2-hydroxy-1-naphthaldehyde (0.172 g, 1.0 mmol) under stirring condition in MeOH (15 ml) for 12 hr. at room temperature synthesizes the probe 1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazole-2-yl)naphthaldehyde-2-ol (Figure 3.1). After ensuring that there was no excess amine left and allowing slow evaporation of MeOH solution of the probe, the solvent has been removed. Eventually, the crude product obtained was purified by paper chromatography and needle-shaped red crystals were obtained.

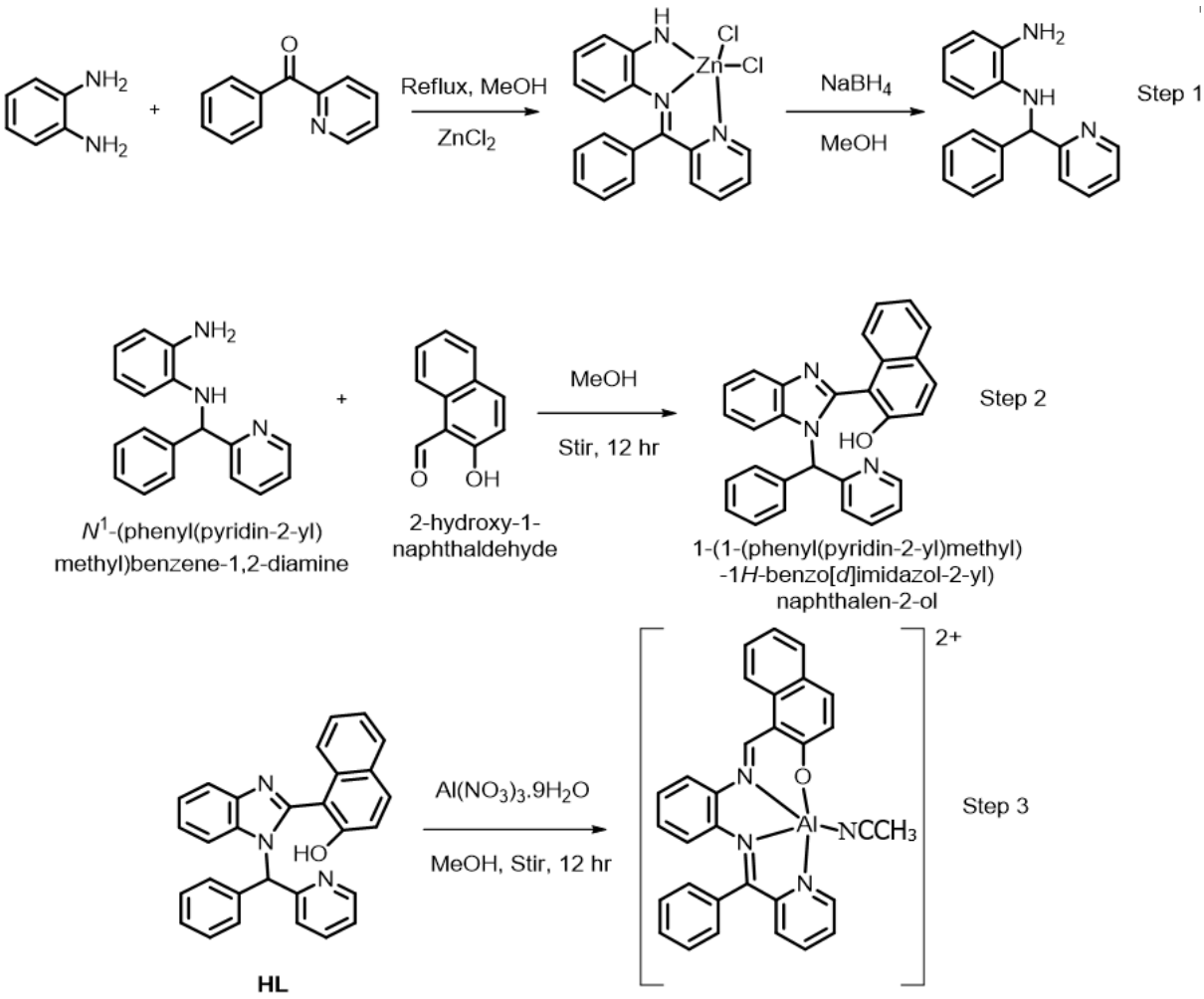


Figure 3.1 Synthesis of HL and its Al-complex

The molecular ion peak obtained using mass spectrometry is higher than expected. But this can be best explained if we take into account the complexation of solvent namely acetonitrile (CH_3CN) with this Al^{3+} ions.

3.1.2 Synthesis of Al-complex

The Al^{3+} complex of the chemosensor was synthesized by adding MeOH (10 ml) solution of the chemosensor (1 mmol, 0.427 g) into a refluxing MeOH (10 ml) solution of $Al(NO_3)_3.9H_2O$ (0.375 g, 1 mmol) and the whole mixture was stirred for 12 hr. in order to yield an orange solution. The solution was allowed to evaporate and upon evaporation, the solvent has been removed from the solution. Finally, an orange-colored solid mass was obtained.

3.2 Spectroscopic Studies

3.2.1 A general method for UV–vis and Fluorescence studies

The probe of (2.14 mg, 0.001 mmol) was dissolved in MeOH (5 ml) and 100 μL of HL solution diluted using 2 ml MeOH-H_2O (v/v 1:4) to make the solution with total volume 2.1 ml. Al $(NO_3)_3.9H_2O$ (3.75 mg, 0.001 mmol) was dissolved in water (10 ml). The Al^{3+} solution (100 μL) were transferred to HL solution prepared above. This procedure for sample solution preparation can also be maintained for other cations. After mixing spectra were recorded at room temperature. For fluorescence study excitation wavelength used was 400 nm (excitation slit = 10.0 and emission slit = 7.0).

3.2.2 Determination of fluorescence Quantum Yield (Φ) of the chemosensor itself and after complexation with Al^{3+}

The fluorescence quantum yield of the chemosensor and the metal-complex were obtained by the following equation given below,[109-111]

$$\Phi_{sample} = (OD_{std} \times A_{sample}) / (OD_{sample} \times A_{std}) \times \Phi_{std}$$

Here, A_{sample} and A_{std} represent the areas under the fluorescence spectral curves of the sample and standard sample, respectively. The optical densities of the sample and standard are designated as OD_{sample} and OD_{std} respectively at the excitation wavelength. Here, acidic quinine sulfate was taken as the standard (Φ_{std} = 0.54) for the quantum yield calculation of ligand and aluminum complex[112].

3.3 Live Cell Imaging

3.3.1 Cell line culture

Human liver cancer cell line Hep G2 and human lung fibroblast cells, WI38 were obtained from National Center for Cell Science (NCCS) Pune, India. The cells were grown in DMEM with 10% FBS (Fetal Bovine Serum), penicillin/streptomycin (100 units/ml) at 37ºC and 5% CO_2. All the required treatments were conducted at 37ºC and at a cell density allowing exponential growth.

3.3.2 Cell Imaging

The Hep G2 cells were grown on coverslips for 24 hrs. Then the cells were either mock-treated or treated with 5 μM of ligand and 10 μM Al^{3+} salt and incubated for 24 hrs. at 37ºC. The cells were washed with 1×PBS and then they were mounted on a glass slide and detected under a fluorescence microscope (Leica).

3.3.3 Cell survivability assay

Cell survivability of the probe was studied for human lung fibroblast cells, WI38 following reported procedure.[110,111] In brief, the viability of WI38 cells after exposure to various concentrations of ligand was assessed by MTT assay. The cells were seeded in 96-well plates at 1×10^4 cells per well and exposed to the probe at different concentrations of 0 µM, 20 µM, 40 µM, 60 µM, 80 µM, 100 µM for 24 hrs. After incubation cells were washed with 1×PBS twice and incubated with MTT solution (450 µg/ml) for 3-4 hrs. at 37ºC. The resulting formazan crystals were dissolved in MTT solubilization buffer and the absorbance was measured at 570 nm by using a spectrophotometer (BioTek) and the value was compared with the control cells

4. RESULTS AND DISCUSSION

4.1 Synthesis and formulation of the probe

The condensation of N^1-(Phenyl(pyridine-2-yl)methyl)benzene-1,2-diamine and 2-hydroxy-1-naphthaldehyde synthesized 1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazol-2-yl) naphthalene-2-ol in good yield of 84% and the melting point was 190 ℃ . It has been characterized by spectroscopic measurements (FTIR, ESI-MS, NMR).

Molecular ion peak of $(HL+H)^+$ was 428.1641 and the calculated molecular weight is 428.1685) supports the molecular identity. The weak band at 3360 cm^{-1} and 3070 cm^{-1} refer to ν(phenolic-OH) and ν(-NH) respectively. Strong stretches at 1620 cm^{-1} are assigned to ν(-CH=N-) stretching. The ^1H NMR spectrum of HL (500 MHz, DMSO-d_6) demonstrates singlet at 15.12 ppm corresponds to δ(phenolic-OH); benzylic-H (CH-N) at 9.73 ppm. Other aromatic protons appear at 5.90-8.64 ppm.

4.1.1 Microanalytical data

The microanalytical data of the probe ($C_{29}H_{21}N_3O$) with the Calculated and Experimentally found values are C, 81.48 (81.11) %; H, 4.95 (5.05) %; N, 9.83 (9.81) %, respectively. ^1H NMR (500 MHz, DMSO-d_6): 15.12 (s, 1H, OH), 9.73 (s, 1H, benzylic-H), 5.90-8.64 (aromatic-H), (Figure 4.1); IR: 3360 cm^{-1} (Phenolic-OH), 3070 cm^{-1} (-NH). No doubt this is N-H because Nitrogen has quadrapole moment and that would give rise to broadening, 1620 cm^{-1} (imine –CH=N-) (Figure 4.2). ESI-MS shows Molecular peak at 428.1641 for $[HL+H]^+$(Mol.wt.(calculated), 428.1685) and at 450.0438 for $[HL+Na]^+$ (Mol.wt. Calculated=450.17) (Figure 4.3).

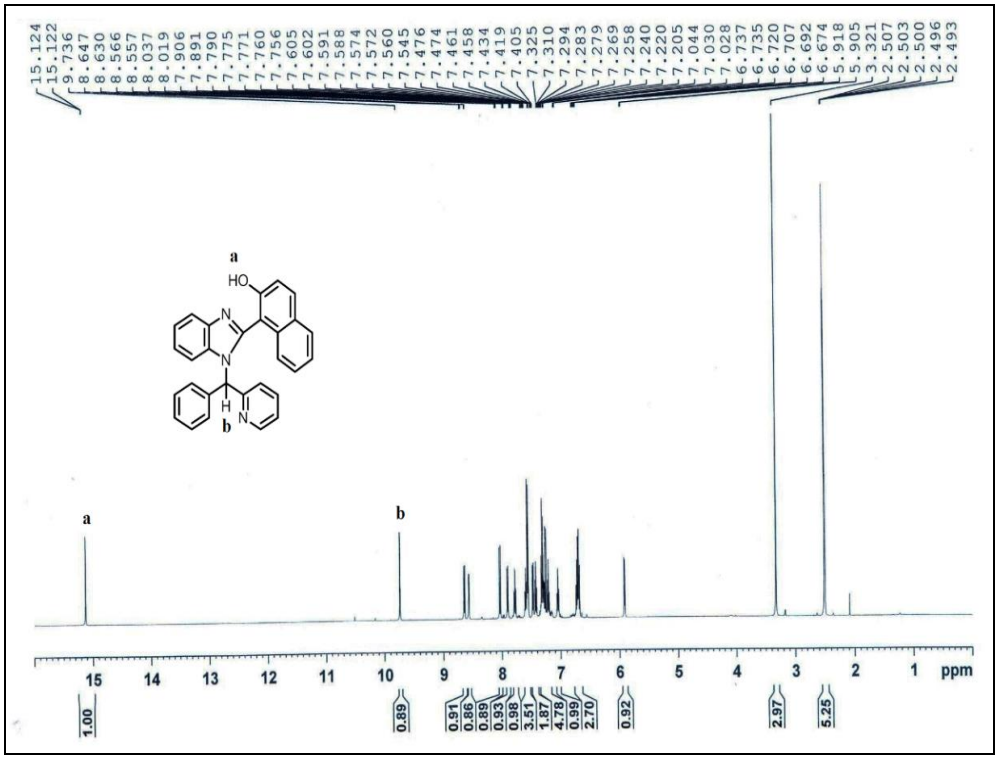


Figure 4.1 ^{1}H NMR Spectrum of HL in DMSO-d6

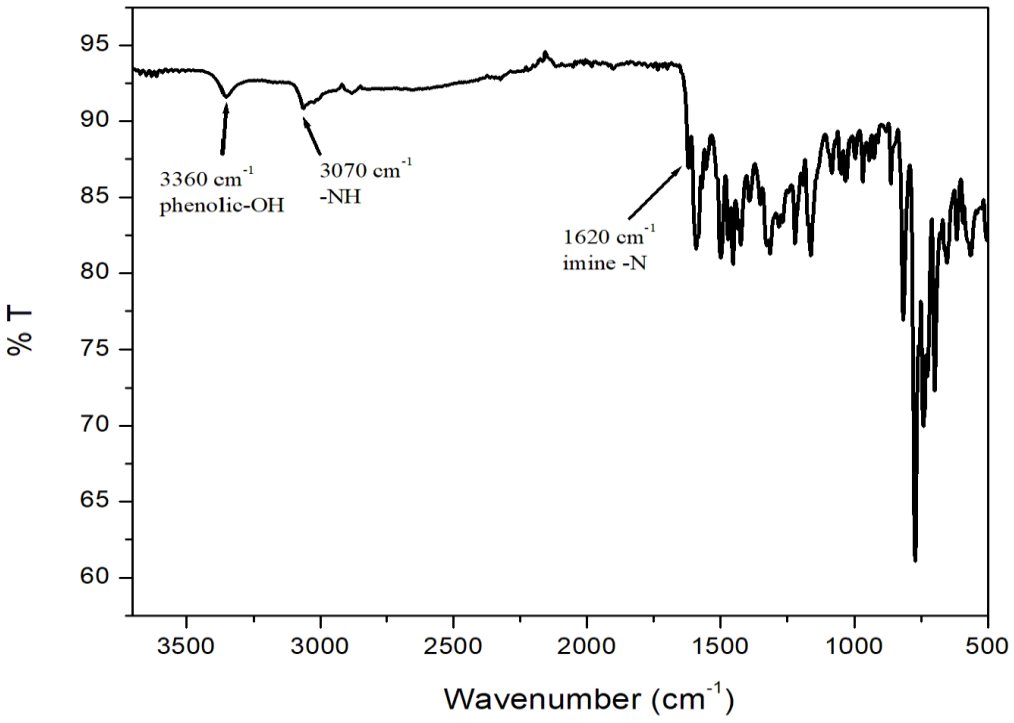


Figure 4.2 IR Spectrum of HL in solid state

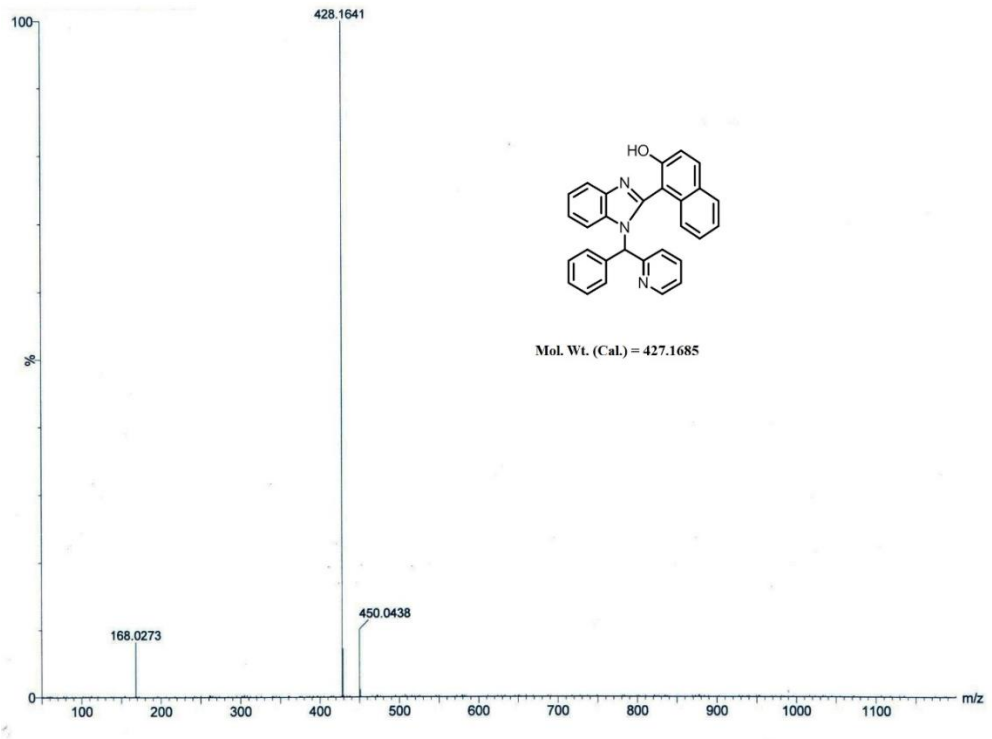


Figure 4.3 HRMS of HL

4.2 X-ray structure of the probe

The red X-ray diffractive crystal of belongs to the Monoclinic system of space group P 21/c (Figure 4.4 and Table 4.1) and it shows the acyclic structure of the probe. The reaction of HL with $Al(NO_3)_3.9H_2O$ in methanol has isolated mononuclear Al-complex, $[Al\mathbf{L}(CH_3CN)]^{2+}$.

The complex has shown no broad peak at 3360 and 3070 cm^{-1} corresponds to the vanishing of -OH and -NH stretching and ν(-CH=N-) is observed at 1625 cm^{-1} which is shifted to higher energy compared to HL. The mass spectrum shows a molecular ion peak at 495.16 which may be due to $[Al\mathbf{L}(CH_3CN) + H^+]$. The absence of δ(phenolic-OH) and -NH support ionization of probe and its binding with Al^{3+} during synthesis. ^{1}H-NMR titration experiment also shows the proposed mechanism for sensing of Al^{3+} (Figure 4.5)

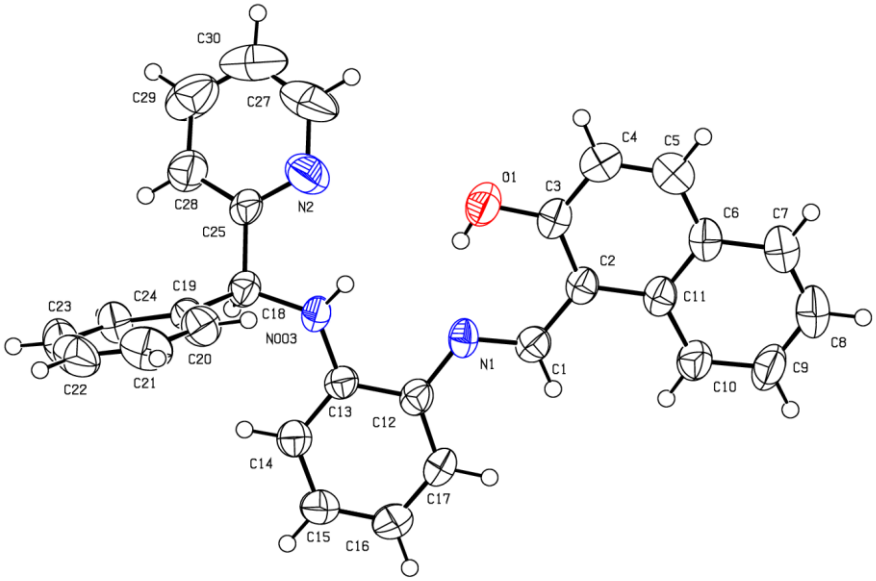


Figure 4.4 X-ray structure (acyclic) of the ligand

Table 4.1 Crystallographic data and structure refinement parameters of the ligand

Formula	HL (ligand)
Empirical formula	$C_{29}H_{21}N_3O$
Formula weight	429.50
Crystal System	Monoclinic
Space group	P $2_1/c$
a (Å)	22.180(3)
b (Å)	5.9098(7)
c (Å)	16.938(2)
α= γ /°	90
β/°	94.383(4)
V $(Å)^3$	2213.7(5)
Z	4
Dc/g cm^{-3}	1.289
μ/mm^{-1}	0.079
λ(Å)	0.71073
data [$I > 2\sigma(I)$]/params	3882/299

GOF[c]	1.138
Final R indices [I >2σ(I)] [a, b]	R1 = 0.0913 wR2 = 0.1730

[a]$\mathbf{R_1}$ = $\Sigma||F_o|-|F_c||/\Sigma|F_o|$; [b]$\mathbf{wR_2}$ = $\{\Sigma[w(F_o^2-F_c^2)^2] / \Sigma[w(F^2_o)^2]\}^{1/2}$; **W** = $[\sigma^2(F_o)^2 + (0.1003P)^2 + 4.9693P]^{-1}$ $(F_o^2 + 2F_c^2)/3$; [c] Goodness-of-fit

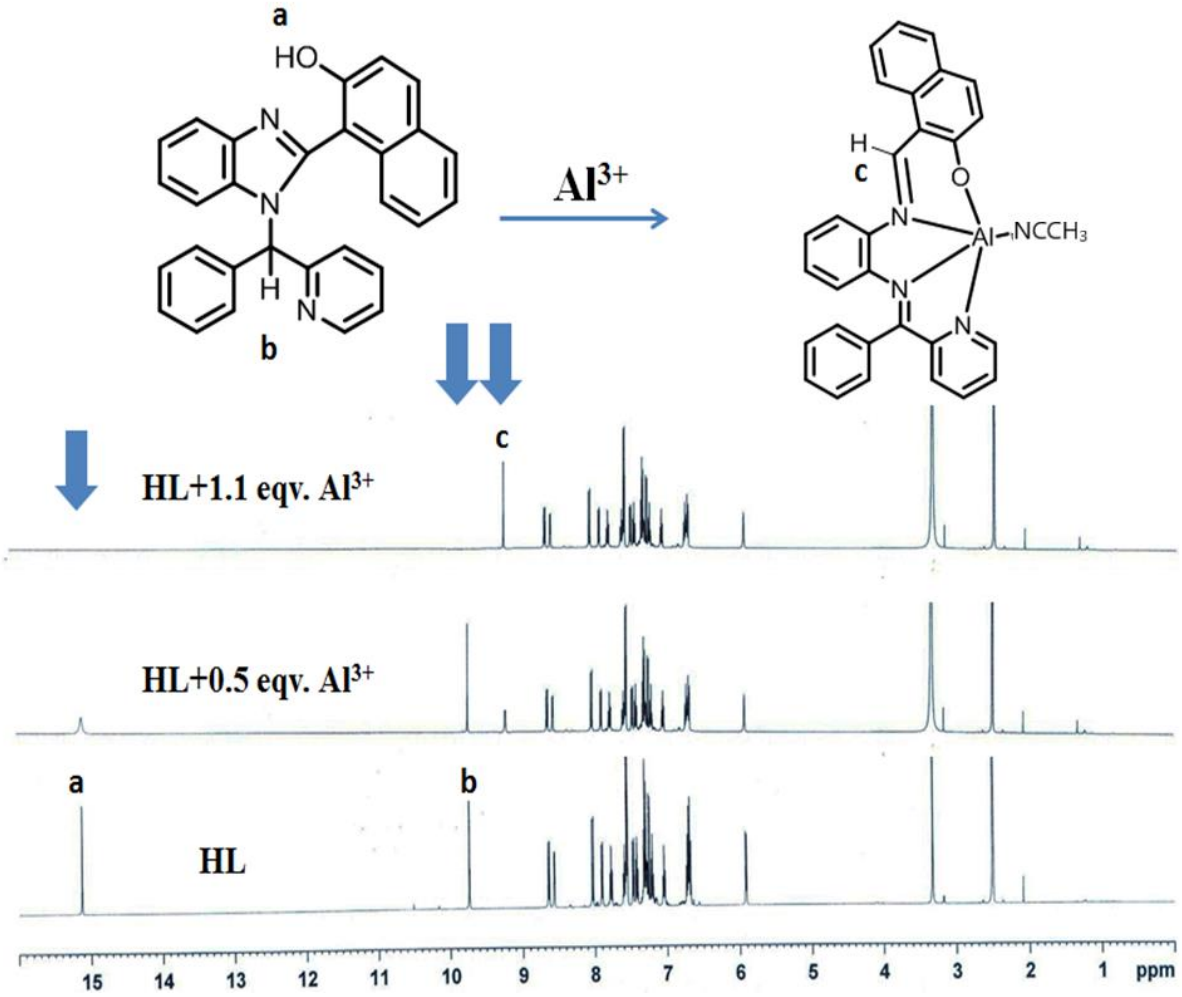


Figure 4.5 ^{1}H NMR titration for the probe with Al^{3+}

4.3 Synthesis of Al-complex

4.3.1 Microanalytical Data

The microanalytical data of the Al^{3+} complex ($C_{31}H_{23}AlN_4O_1$) with the Calculated values (%) C, 75.29; H, 4.69; N, 11.33; IR: 1625 cm^{-1} (imine -CH=N-), (Figure 4.6). The mass spectrum shows a high intense peak at 495.19 for [AlL $(CH_3CN)]^{2+}$ + H^+, M.Wt. calculated = 495.16] (Figure 4.7)

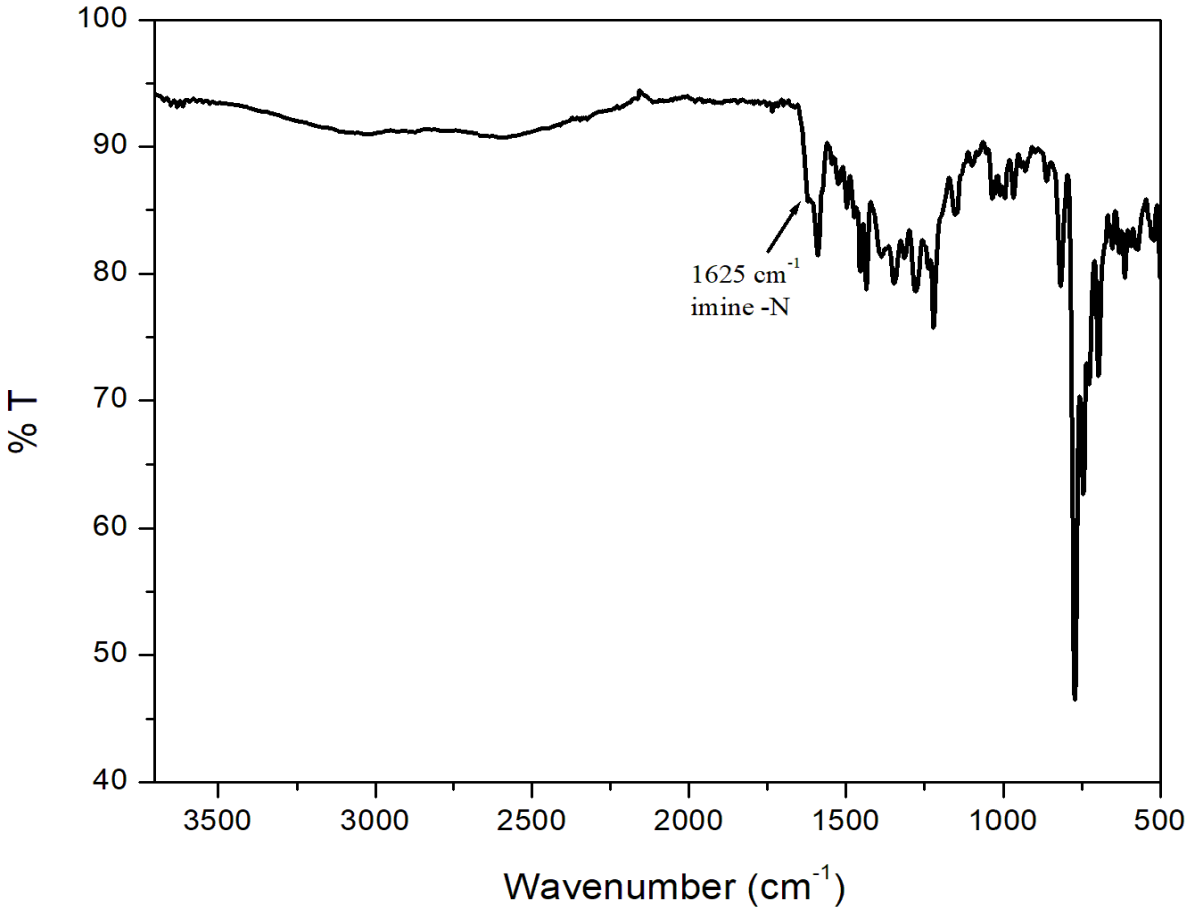


Figure 4.6 IR Spectrum of Al-complex

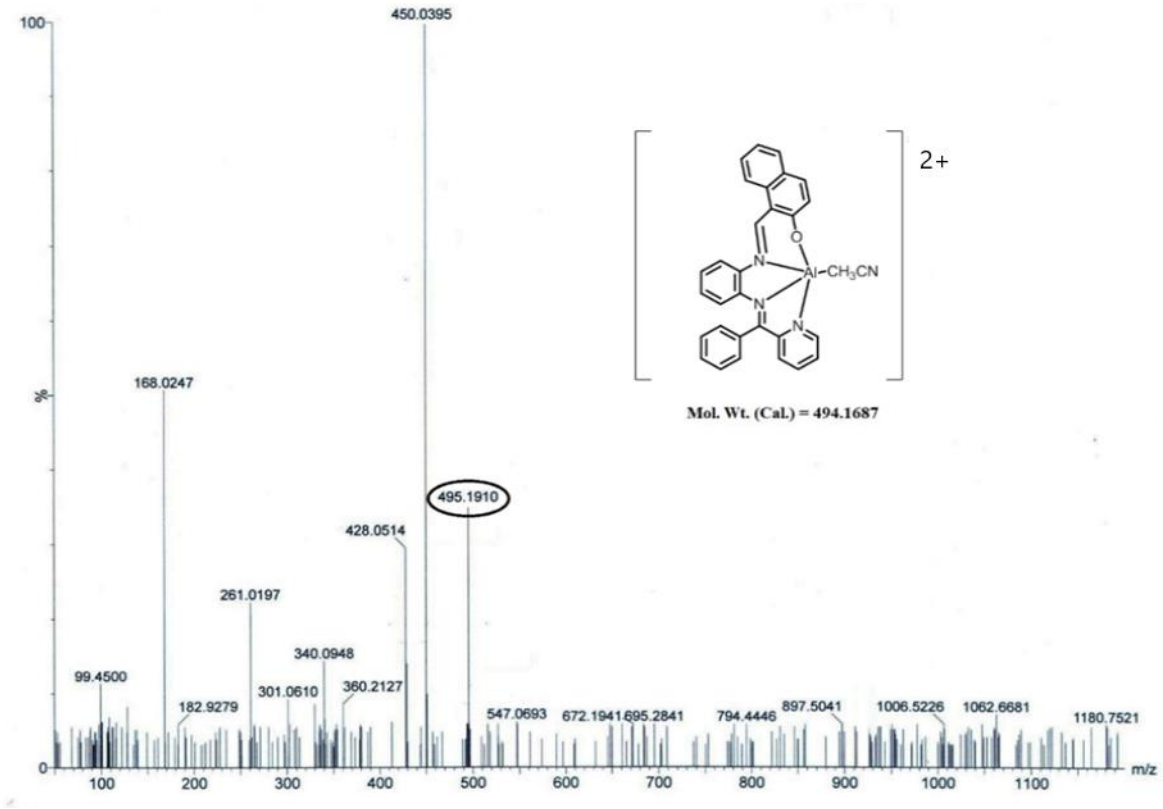


Figure 4.7 HRMS of [AlL (CH_3CN)]$^{2+}$

4.4 UV-vis spectroscopic studies

The interaction of HL with Al^{3+} has been examined by spectrophotometric titration of HL with the incremental addition of Al^{3+} in 1:4 MeOH/H_2O (v/v) at 25ºC. Initially, the probe has absorption maxima at 428 nm, but upon coordination with Al^{3+} ion there is an enhancement of absorption with a small redshift of the maxima to 438 nm (Figure 4.8) which suggests that the reaction is clean and straightforward. The red-shifting of the bands of HL upon Al^{3+} addition is attributed to an intramolecular charge transfer (ICT) through the chelation. The change of absorbance is linear until the molar ratio [Al^{3+}]: [HL] reaches 1: 1, and no longer changes with an increase in [Al^{3+}]. It suggests that the stoichiometry between HL and Al^{3+} is 1: 1.

In order to establish the binding stoichiometry of HL and Al^{3+}, the Job's plot has been generated by plotting absorbance against different mole fractions of Al^{3+} while volume of solution has remained fixed and the molar fraction maxima has been obtained at around 0.5 mole fraction, which indeed supports 1: 1 complex formation of HL and Al^{3+}(Figure 4.9).

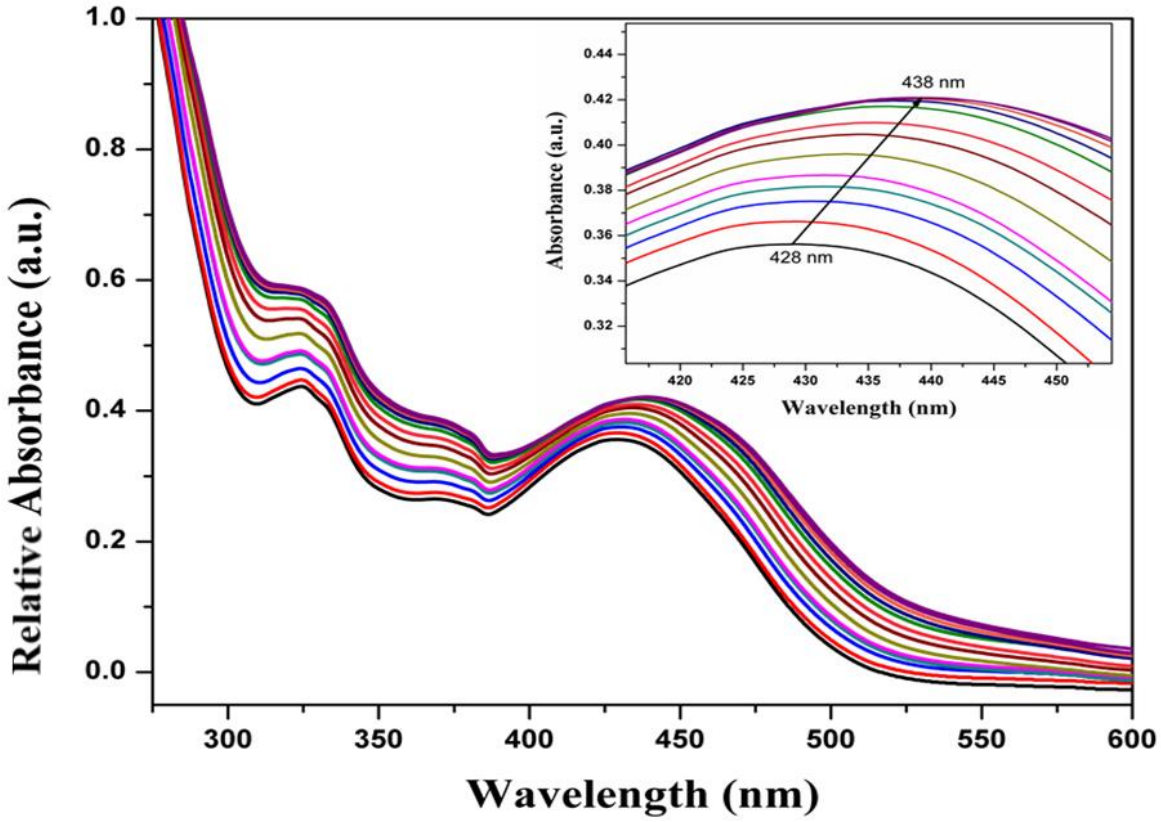


Figure 4.8 Change in absorption spectrum of HL (50 μM) upon gradual addition of Al^{3+} ions (5 μM each) in MeOH/H_2O (v/v 1:4); inset: zoom image at wavelength 415-455 nm.

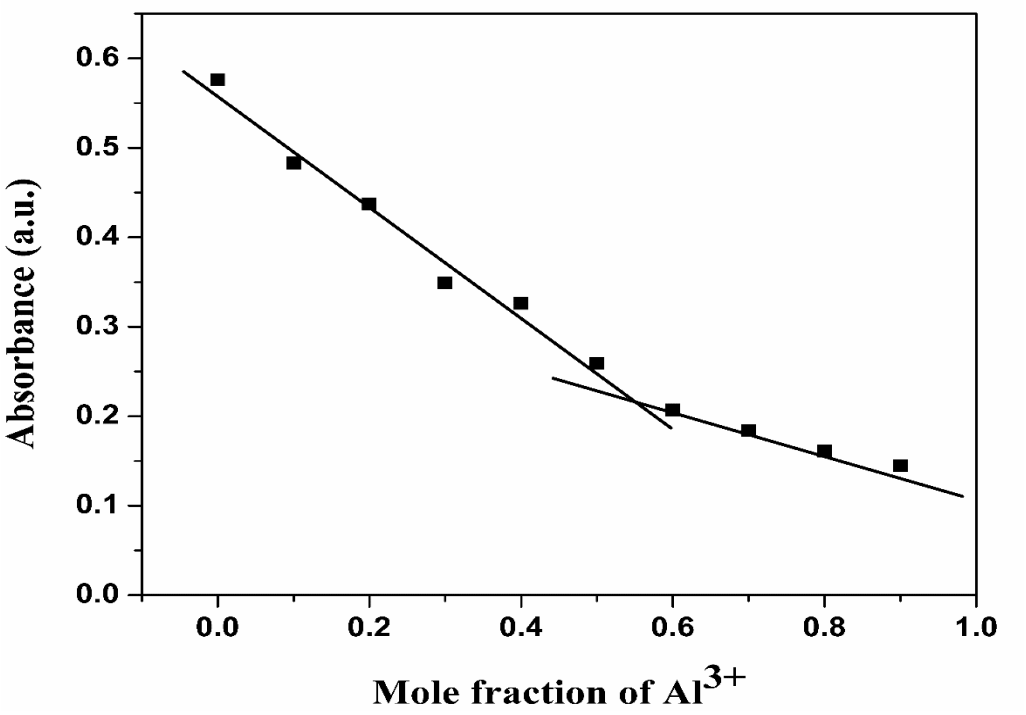


Figure 4.9 Job's plot for the reaction between HL and Al^{3+} in MeOH/H_2O (v/v 1:4)

4.5 Fluorescence sensing for Al^{3+}

The fluorescence quantum yield has been determined using quinine sulfate as a reference with a known quantum yield, ϕ_R = 0.54 [113-115] in MeOH/H_2O (v/v 1:4). Upon excitation of the probe at 400 nm emission has not been observed. The ligand and quinine sulfate ($C_{40}H_{50}N_4O_8S$**)** have been excited at the same wavelength, i.e., at 502 nm maintaining almost the same absorbance. So, the quantum yield of the ligand obtained is (Φ_{ligand}, 0.0186) and after chelation with Al^{3+}, the high intense emission band is observed at 502 nm. As a result, the quantum yield value for the Al^{3+}-complex obtained is ($\Phi_{complex}$, 0.2748). Therefore, fluorescence quantum yield increases as a result of metal complexation.

The fluorescence emission of HL with other cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Pd^{2+}, Cd^{2+}, Hg^{2+}, Cu^{2+}, Ba^{2+} and Pb^{2+}) in MeOH/H_2O (v/v 1:4) is insignificant. Thus, the probe is selectively showing "turn on" emission to Al^{3+} under the identical experimental condition (Figure 4.10).

On incremental addition of Al^{3+} to the solution of the probe the fluorescence intensity increases and becomes saturated when reached at 1: 1 molar ratio which results. The emission intensity of the mixture does not change on excess addition of Al^{3+} (Figure 4.11).

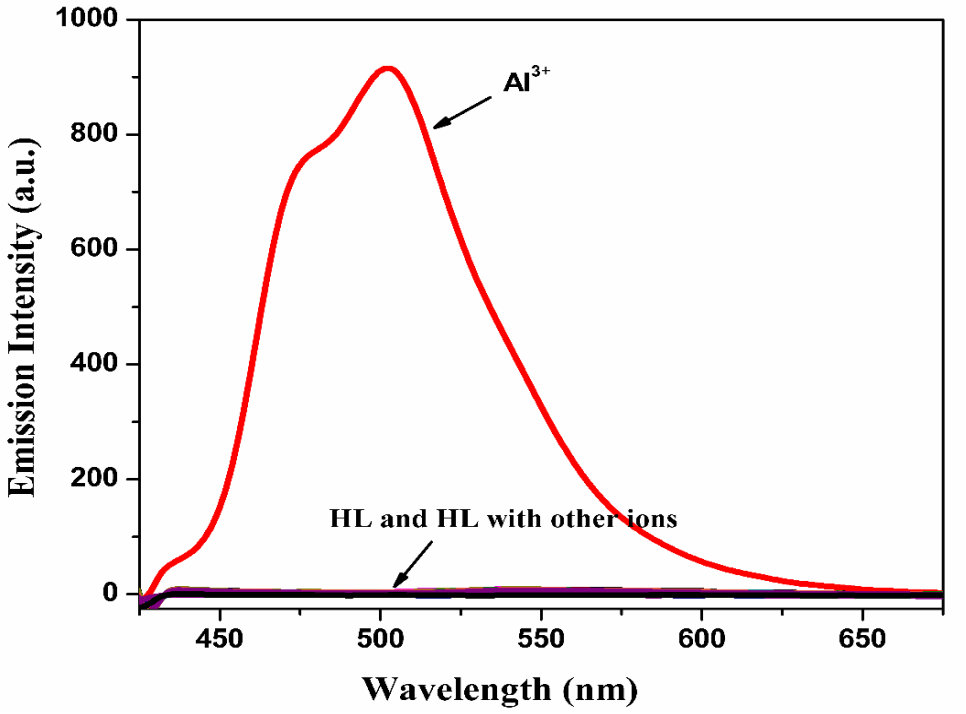


Figure 4.10 Change in emission spectrum of HL (50 μM) upon gradual addition of different metal ions (100 μM each) in MeOH/H_2O (v/v 1:4)

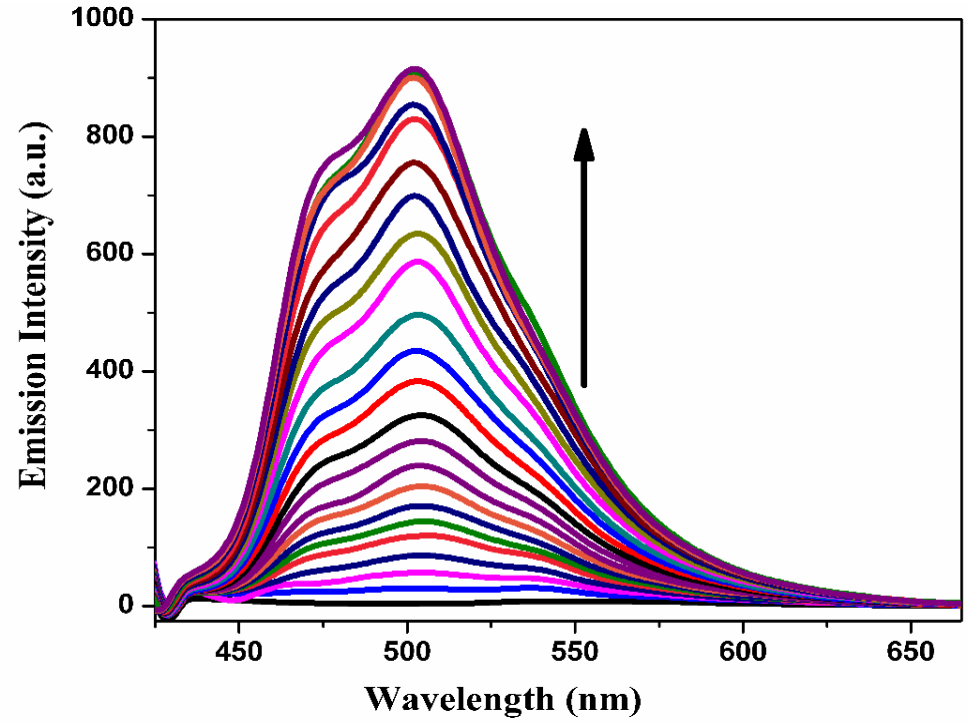


Figure 4.11 Change in emission spectrum of HL (50μM) upon gradual addition of Al^{3+} ions (5μM each) in MeOH/H_2O (v/v 1:4)

4.6 Drawing of Job's Plot by fluorescence spectroscopy method

The augmentation in fluorescence intensity for HL+Al^{3+} may arise from the elimination of ESIPT in free HL and chelation enhancement effect (CHEF) through the coordination of -O and -N donor site of the ligand with the metal ion.

The binding constant value of Al^{3+} with the probe has been determined from the emission intensity data following the modified Benesi-Hildebrand equation, $1/\Delta F = 1/\Delta F_{max} + (1/K[C])\ (1/\Delta F_{max})$. Here, $\Delta F = F-F_0$ and $\Delta F_{max} = F_{max}-F_0$, where F_0, F, and F_{max} are the fluorescence emission intensities of the probe considered in the absence of Al^{3+}, at an intermediate Al^{3+} concentration, and at a concentration of complete saturation where K is the binding constant and [C] is the Al^{3+} concentration respectively. From the plot of $(F_{max}-F_0)/(F-F_0)$ against $[C]^{-1}$ for the probe the value of K has been determined from the slope.

In order to get further insight about the complexation reaction the fluorometric titration has been done and $[(F_{max} - F_0)/(F - F_0)]$ vs. $1/[Al^{3+}]$ has been plotted following Benesi-Hildebrand equation (Figure 4.12) and the association constant (K_a) as determined by fluorescence titration method

for ligand with Al^{3+} is found to be 2.3×10^4 M^{-1}. The limit of detection (LOD) of Al^{3+} has been calculated as 3.3 nM following the 3σ method (Figure 4.13).

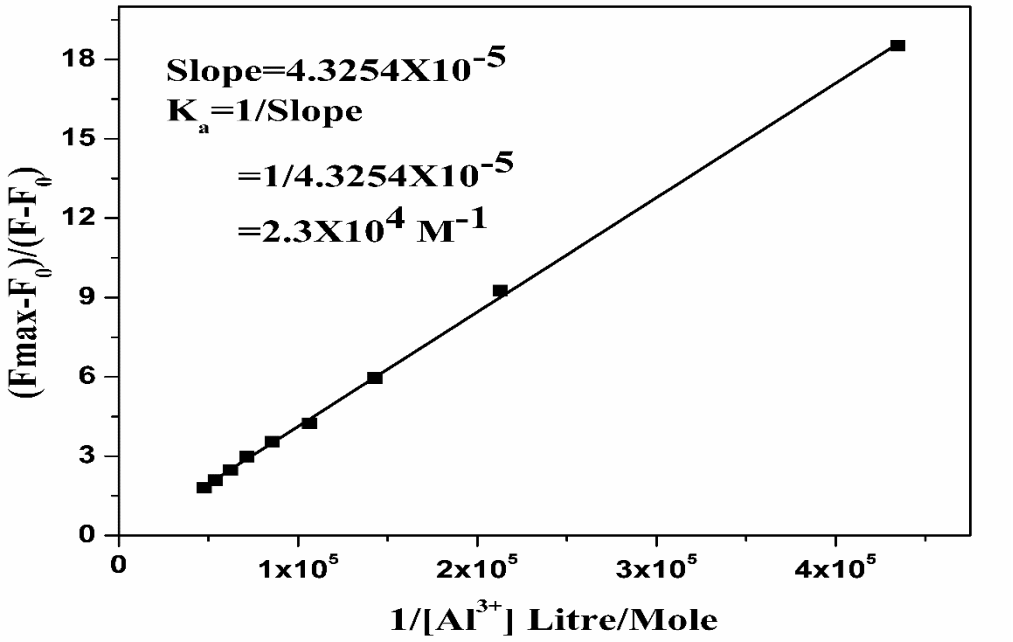


Figure 4.12 Benesi-Hildebrand plot of {(Fmax-Fo)/(F-Fo)} vs. 1/ [Al^{3+}] by fluorescence spectroscopy- fluorescence titration curve of the ligand with Al^{3+}

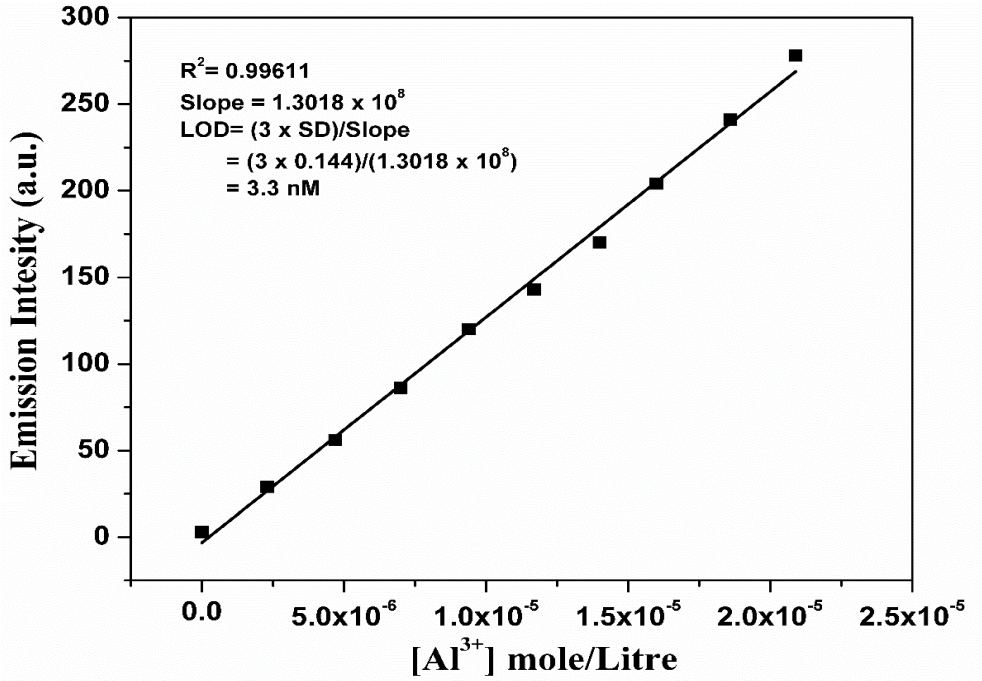


Figure 4.13 The linear dynamic response of HL for $Al^{3+}$and the determination of the detection of limit (LOD) for Al^{3+}

Effect of pH variation on fluorescence intensity of HL and HL-Al^{3+} complex has been studied; it has been observed that there is no significant fluorescence emission of HL at the pH range 2 to 12 and in presence of Al^{3+} the ligand emits in the pH range between 6.0 to 12 (Figure 4.14). The probe is stable in the pH range, 2–12 and maximum turn-on response to Al^{3+} is observed at pH 6. After pH 7 there is a sudden decrease in emission intensity; this may be due to the interaction of bases with a metal ion (Al^{3+}) and lower the ligand-metal interaction. This indicates that the HL is useful for detection of Al^{3+} in the biological pH at a much lower concentration than that of WHO recommended value (7.41 mM) in drinking water [116-117].

For the detection of Al^{3+}, intervention on fluorescence by various cations has been studied (Figure 4.15) by HL and it shows that no significant interference is observed.

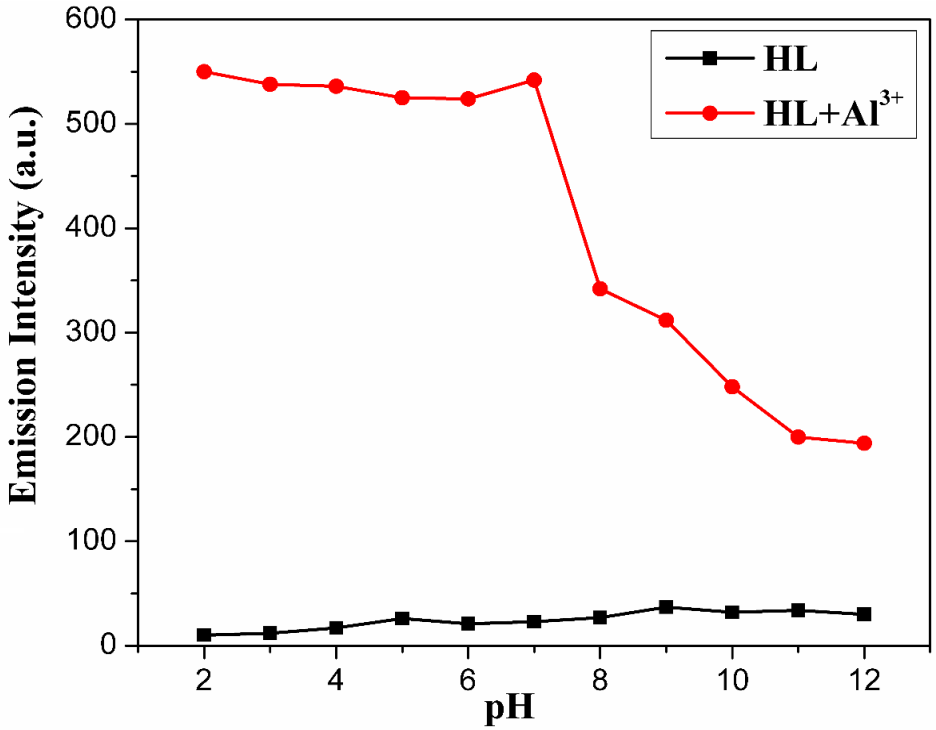


Figure 4.14 Effect of pH on fluorescence intensity of receptor HL and HL-Al^{3+} complex

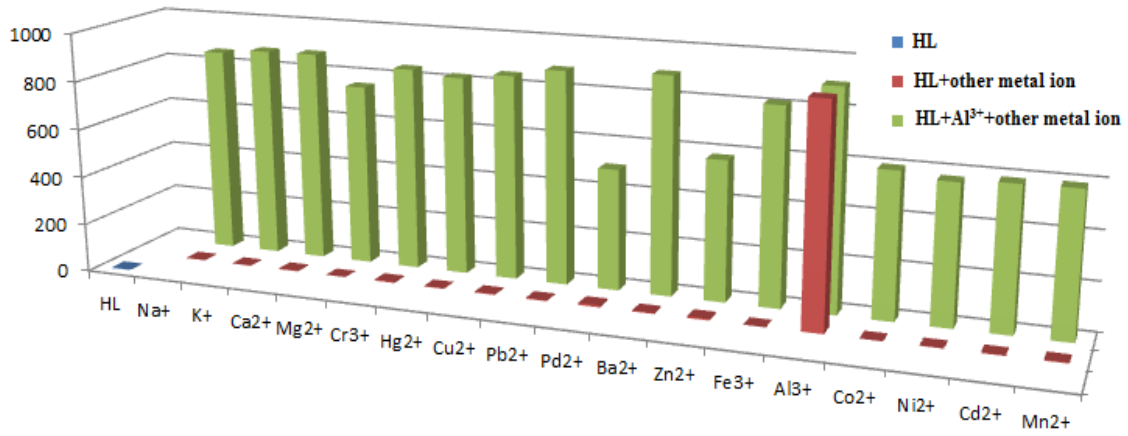


Figure 4.15 Interference of other metal ions towards Al^{3+} ion sensitivity

The stability of the excited probe and its aluminum complex has been checked by fluorescence lifetime measurement. The fluorescence decay profile shows bi-exponential (τ_{av}, 0.3290 ns) and mono-exponential (τ, 3.4637 ns) decay for ligand and its aluminum complex respectively (Figure 4.16). The higher lifetime of the complex with respect to ligand may be due to greater stability of the complex in an excited state.

Here, we checked the sensitivity of highly emissive aluminum complex with different anions like $S_2O_3^{2-}$, SCN^-, PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, I^-, OAc^-, ClO_4^-, SO_4^{2-}, HSO_4^-, Cl^-, F^-, HF_2^-, NO_3^-, Br^-, NO_2^-, CN^-, N_3^-, AsO_4^{3-}, AsO_2^- and we found that some of the anions (PO_4^{3-}, $H_2PO_4^-$, HPO_4^{2-}, F^- and HF_2^-) quench the emission i.e. it is not specific for a single anion (Figure 4.17).

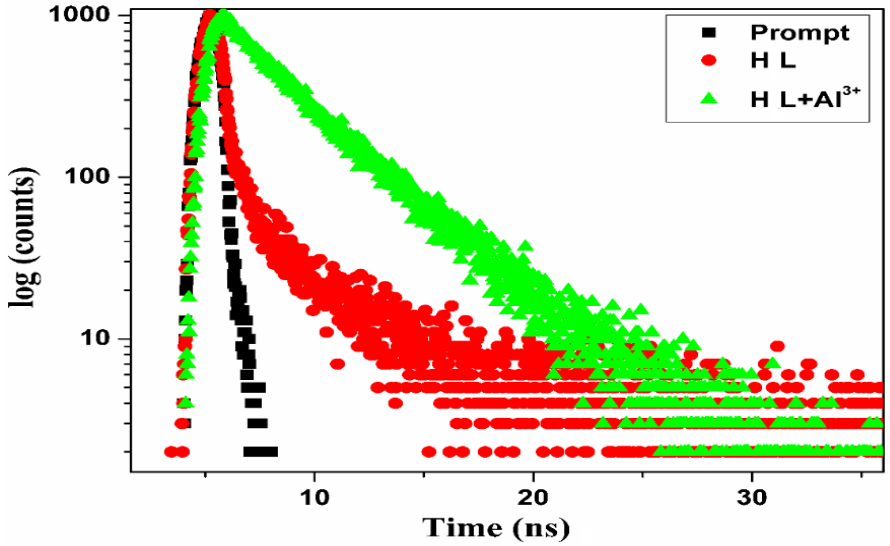


Figure 4.16 Fluorescence lifetime plot of the probe and Aluminum complex in MeOH/H_2O (v/v 1:4)

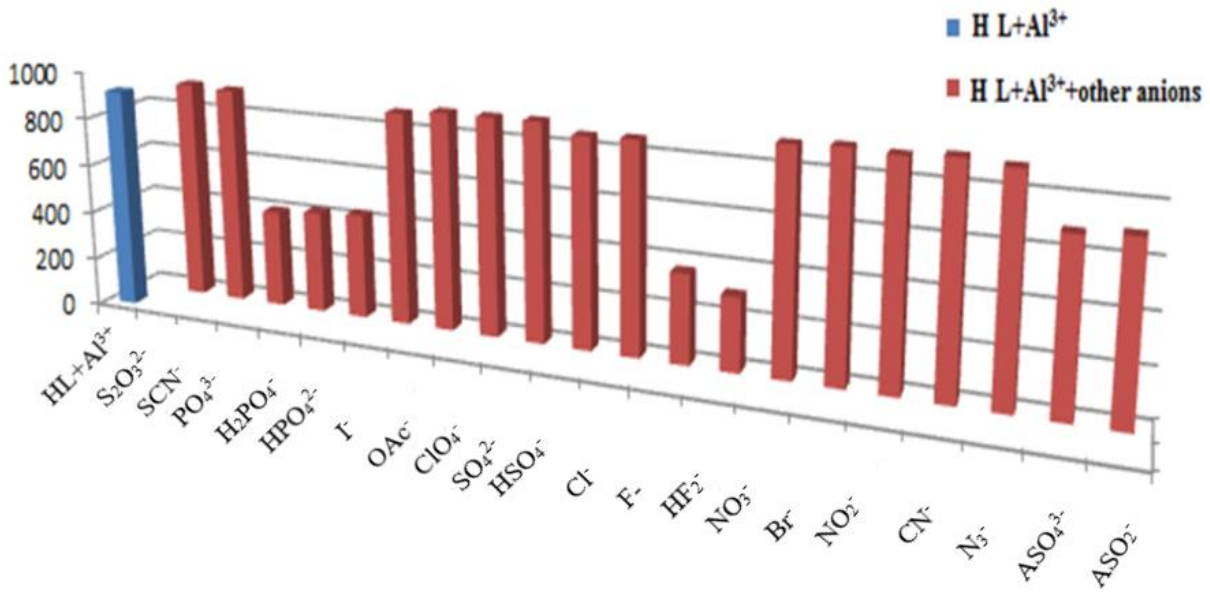


Figure 4.17 Change in emission intensity of the emissive Al-complex with different anions

4.7 Detection of Al^{3+} ion in an Industrial wastewater

A direct application of the 200 ml of the chemosensor for the detection of Al^{3+} ion in an industrial wastewater sample has been studied. The fluorescence intensity is measured at 502 nm emission on excitation at 400 nm to construct calibration plot by volume of analyte (Al^{3+}) and emission intensity (a.u.) in 1:4 MeOH/H_2O (v/v) medium (Figure 4.18). The 200 ml wastewater samples were collected in a clean and specially designed PVC bottle from the Pharmaceutical industry, Kolkata, West Bengal, India. The unknown concentration of Al^{3+} in the collected wastewater sample is measured from the calibration plot and the amount of Al^{3+} are given in Table 2.

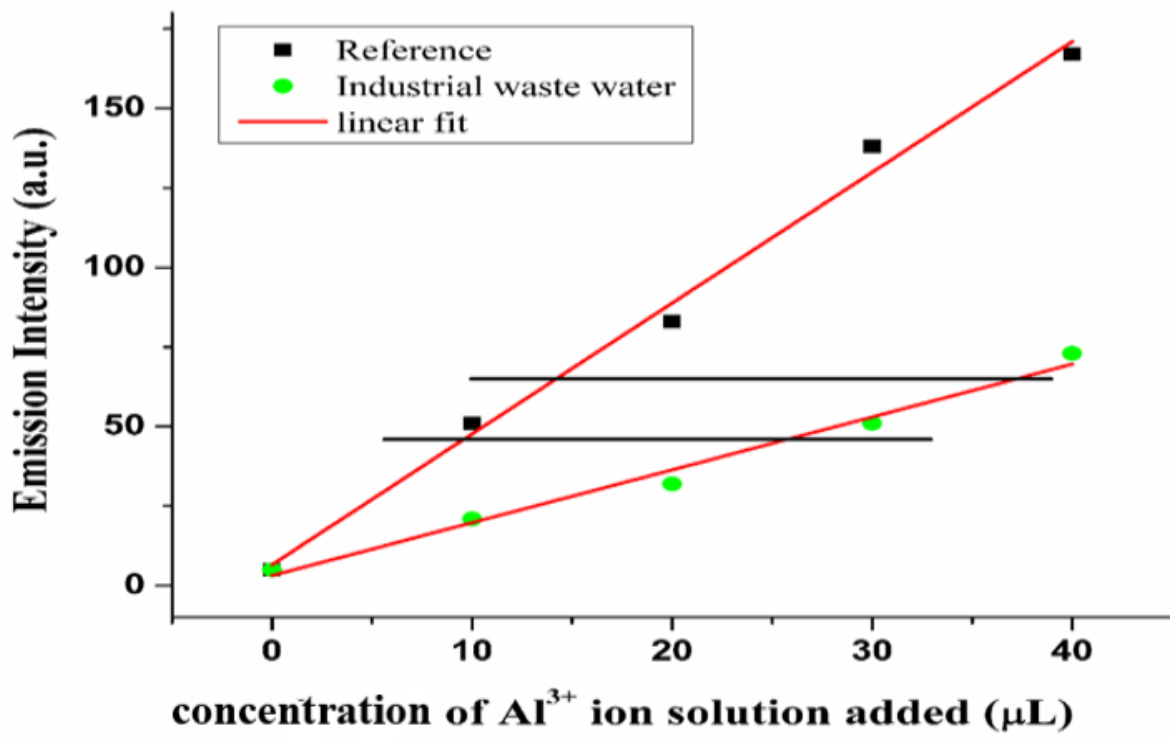


Figure 4.18 The emission intensity of the probe (Reference) at 502 nm vs. volume of Al^{3+} containing a solution for the analysis of Al^{3+} detection in an industrial wastewater sample

It is evident from the plot that there is linear change of emission intensity with concentration of Al^{3+} ion containing effluent was added and no regression was done.

Table 4.2 Estimation of the amount of Al^{3+} ions in industrial wastewater using HL

Sample	S.No.	Emission intensity at 502 nm (a.u.)	The concentration of reference required (mM)	The concentration of industrial wastewater required(mM)	How much times the reference is more concentrated than a sample	Amount of Al^{3+} ions (mM)
Industrial wastewater	1	64	70.12	26.75	2.621	0.381
	2	45	103.73	38.65	2.683	0.372

4.8 Living Cell Imaging

4.8.1 Cell survivability assay

The in vitro cytotoxicity of the probe is estimated for checking the biocompatibility on WI38 cell line. The cells were treated with five different concentrations (20 µM, 40 µM, 60 µM, 80 µM, and 100 µM) of ligand for 24 hrs., and followed by MTT assay. It is observed that the ligand exhibited no significant toxicities even at the highest concentration of 100 µM. Therefore, the ligand is a good biocompatible and is beneficial for biological applications (Figure 4.19).

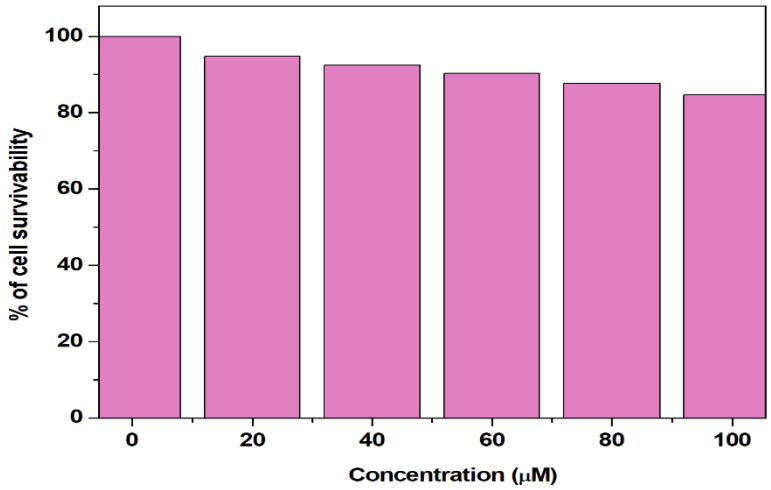


Figure 4.19 Cell survivability study of WI38 cells exposed to the probe

4.8.2 Cell imaging study

The fluorescence microscopic study is performed to envisage the cellular uptake of the probe (5 µM) and Al^{3+} salt (10 µM**)**. A prominent blue fluorescent signal is observed under the microscope (Figure 4.20). From this observation, we can conclude that the cells readily uptake the probe and the Al^{3+} salt which results in blue fluorescent signal. Hence, the probe has had successfully been applied to the detection of intracellular Al^{3+} in HePG2.

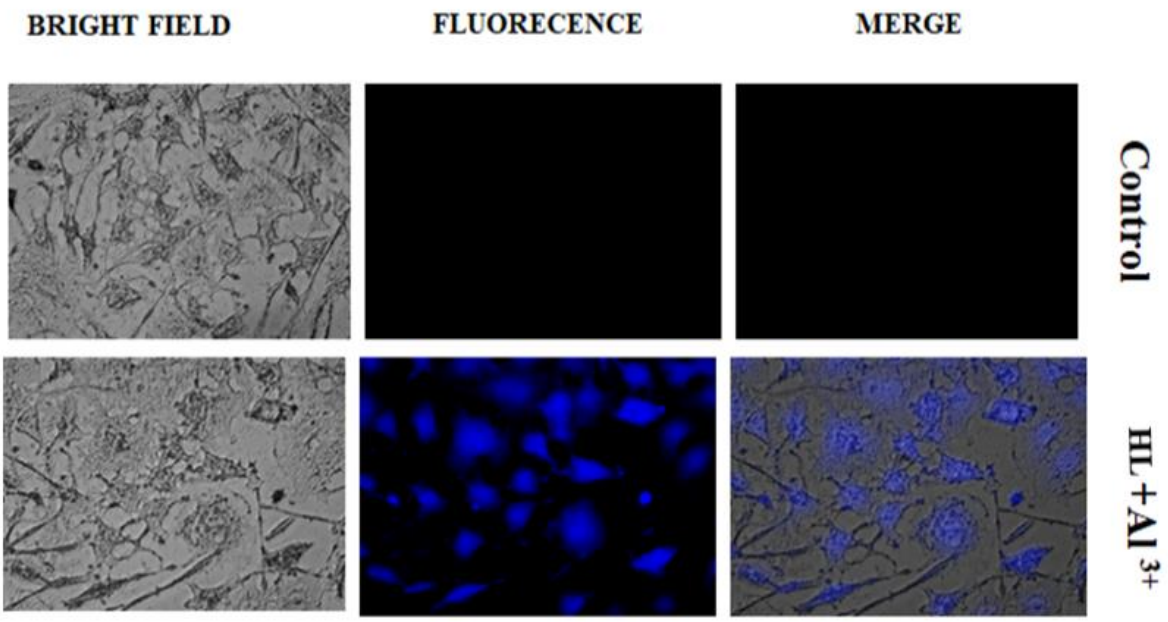


Figure 4.20 Bright field, fluorescence and merged microscopic images of untreated HepG2 (Control), cells treated with HL (5 μM) + Al^{3+} (10 μM)

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The 1-(1-(Phenyl(pyridine-2-yl)methyl)-1H-benzo[d]imidazol-2-yl)naphthalene-2-ol has been synthesized by the condensation reaction of N^1-(Phenyl(pyridine-2-yl)methyl)benzene-1,2-diamine (0.273 g, 1.0 mmol) and 2-hydroxy-1-naphthaldehyde (0.172 g, 1.0 mmol) under stirring condition in MeOH (15 ml) for 12 hr. at room temperature. The probe has successfully been used as "turn-on" fluorescence chemosensor to Al^{3+} ion in presence of a large number of other metal ions upon irradiation at 400 nm and shows high intense blue emission (λ_{em}, 502 nm). The limit of detection (LOD) of the probe for Al^{3+} is 3.3 nM. The 1:1 metal-to-ligand complex has been ascertained by Mass spectral measurement, Job's plot and ^1H NMR measurement. The effluent collected from the pharmaceutical industry has 0.38 mM of aluminum ion concentration at 64 emission intensity which is by far less than that stipulated by WHO (World Health Organization) and World Health Organization prescribed limit is 7.41mM. The probe is also applied to detect Al^{3+} ion in the live cell (Hep G2). HL becomes a versatile sensor for identification of intracellular Al^{3+} in Human liver cancer cell line Hep G2 and human lung fibroblast cells cell lines by fluorescence cell imaging processes and the no toxicity of the probe has been checked by MTT assay up to 100µM. All measurements were performed at room temperature.

5.2 Recommendation

The following recommendations of the research have been clearly identified and should be done in the future work.

- A further detailed investigation has to be carried out on a number of additional samples from different industries to disseminate reliable data of the presence Aluminum in the industrial effluent.
- The crystal structure of the ligand and the complex has to be studied in detail including their HOMO-LUMO transitions with DFT energy computation of the probe and the metal complex.
- The crystal structure of the complex is very important and should be analyzed quite extensively by taking into account the coordination efficiency of the metal and the ligand.
- As long as the chemical shift value of the aromatic compounds is very close it is not so easy to distinguish the proton NMR signal peaks of it. Therefore, the proton signal peaks should be expanded and distinguished clearly.
- The solid-state fluorescence experiment should be done in order for the probe to be used as light emitting diodes.

REFERENCES

1. G. Berthon, *Coord. Chem. Rev*., 228 (2002) 319–341

2. Z. Krejpcio and R. W. Wojciak, Pol. J. *Environ. Stud*., 11 (2002) 251–254

3. J. Barcelo and C. Poschenrieder, *Environ. Exp.* Bot., 48 (2002) 75–92

4. S.K. Shoora, A.K. Jain, V.K. Gupta, *Sensors and Actuators* B 216 (2015) 86–104

5. M. Mukherjee, S. Pal, S. Lohar, B. Sen, S. Banerjee, S. Banerjee, and P. Chattopadhyay, *Analys*t, 139 (2014) 4828–4835

6. A. Barba-Bon, A.M. Costero, S. Gil, M. Parra, J. Soto, R. Martinez-Manez, F. Sancenon, *Chem.Commun*. 48 (2012) 3000–3002

7. S. Das, A. Sahana, A. Banerjee, S. Lohar, D.A. Safin, M.G. Babashkina, M. Bolte, M.Y. Garcia, I. Hauli, S.K. Mukhopadhyay, D. Das, *Dalton Trans*. 42 (2013) 4757–4763

8. R. Patil, A. Moirangthem, R. Butcher, N. Singh, A. Basu, K. Tayade, U. Fegade, D. Hundiwale, A. Kuwar, *Dalton Trans*. 43 (2014) 2895–2899

9. S. Sen, T. Mukherjee, B. Chattopadhyay, A. Moirangthem, A. Basu, J. Marek, P.Chattopadhyay, *Analyst* 137 (2012) 3975–3981

10. W.H. Ding, W. Cao, X.J. Zheng, D.C. Fang, W.T. Wong, L.P. Jin, *Inorg. Chem.* 52 (2013) 7320–7322

11. C.Y. Lu, Y.W. Liu, P.J. Hung, C.F. Wan, A.T. Wu, *Inorg. Chem. Commun*. 35 (2013)273–275

12. S.A. Lee, G.R. You, Y.W. Choi, H.Y. Jo, A.R. Kim, I. Noh, S.J. Kim, Y. Kim, C. Kim, *Dalton Trans*. 43 (2014) 6650–6659

13. S. Guha, S. Lohar, A. Sahana, A. Banerjee, D.A. Safin, M.G. Babashkina, M.P.Mitoraj, M. Bolte, Y. Garcia, S.K. Mukhopadhyay, D. Das, *Dalton Trans*. 42 (2013) 10198–10207

14. Yunqiang Tang, Jinguo Sun, Bingzhu Yin, *Anal. Chim.* Acta 942 (2016) 104-111

15. S. Mukherjee, P. Mal, HelenStoeckli-Evans, J. *Lumin.*, 172(2016)124–130

16. D. Sarkar, P. Ghosh, S. Gharami, T. K. Mondal, N. Murmu, *Sensors and Actuators* B 242 (2017) 338–346

17. Y.-Sheng Mi, D.-Ming Liang, Y.-Ting Chen, X.-Biao Luo, J.-Nan Xiang, *RSC Adv*. 4 (2014) 42337–42345

18. B. Sen, M. Mukherjee, S. Banerjee, S. Pal, and P. Chattopadhyay, *Dalton Trans*., 44 (2015) 8708 - 8717

19. G.J. Park, H.Y. Jo, K.Y. Ryu, C. Kim, *RSC Adv*. 4 (2014) 63882–63890

20. Amalraj A, Pius A. J, *Fluor Chem*. 178 (2015) 73-78

21. Bao YY, Liu B, Wang H, Tian J, Bai RK. *Chem Commun*, 47 (2011)3957 - 3959

22. B. Wang, Eric V. Anslyn (Eds), *Chemosensors: Principles, Strategies, and Applications*, John Wiley & Sons, Inc., 2011

23. A. W. Czarnik (Ed), *Fluorescent Chemosensors for Ion and Molecule Recognition*, Vol. 538, ACS Symposium Series; American Chemical Society: Washington, DC, 1993

24. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Ed., Springer, 2006

25. J. W. Birks, J. R. Poulsen, C. L. Shellum, In D. Eastwood, L. J. Cline Love (Eds.), *Progress in Analytical Luminescence,* American Society for Testing Materials, Philadelphia (1988) pp. 26–40

26. P. P. Mondal and A. Diaspro, *Fundamentals of Fluorescence Microscopy: Exploring Life with Light*, Springer, 2014

27. A. D. Arulraj, R. Devasenathipathy, S. Chen, V. Vasantha, S. Wang, *Highly selective and sensitive fluorescent chemosensor for femtomolar detection of silver ion in an aqueous medium*, Sens. Bio-Sens. Res. 6 (2015) 19-24

28. L. N. Neupane, E. T. Oh, H. J. Park, K. H. Lee, *Detection of Heavy Metal Ions in 100% Aqueous Solution and Cells with a Fluorescence Chemosensor Based on Peptide Using Aggregation-Induced Emission,* Anal Chem. 88 (2016) 3333-3340

29. C. Chen, D. Liao, C. Wan, and A. Wu, *A turn-on and reversible Schiff base fluorescence sensor for* Al^{3+} *ion,* Analyst.138 (2013) 2527-2530

29. Y. Choi, G. Park, Y. Na, H. Jo, S. Lee, G. Yu, C. Kim, *A single Schiff base molecule for recognizing multiple metal ions: A fluorescence sensor for Zn(II) and Al(III) and a colorimetric sensor for Fe(II) and Fe(III),* Sensors and Actuators B: Chemical. 194 (2014) 343-352

30. A. Kumar, A. Kumar, and D. Pandey, *N, N-diethylamine appended binuclear Zn (II) complexes: highly selective and sensitive fluorescent chemosensors for picric acid*, Dalton Trans. 45 (2016) 8475-8484

31. K. Aich, S. Goswami, S. Das, and C. Das Mukhopadhyay, *A new ICT and CHEF based visible light excitable fluorescent probe easily detects in vivo* Zn^{2+}, RSC Adv. 5 (2015) 31189-31194

32. A. Pradhan, S. Mandal, S. Banerjee, A. Mukherjee, S. Das, A. R. Khuda Bukhsh, A. Saha, *A highly selective fluorescent sensor for zinc ion based on quinoline platform with potential applications for cell imaging studies,* Polyhedron. 94 (2015) 75-82

33. K. Kaur, V. K. Bhardwaj, N. Kaur, N. Singh, *Fluorescent primary sensor for zinc and resultant complex as secondary sensor towards phosphorylated biomolecules: INHIBIT logic gate, Inorg.* Chim. Acta. 399 (2013) 1-5

34. A. Kumar, A. Kumar, and D. S. Pandey, *N, N-diethylamine appended binuclear Zn(II) complexes: highly selective and sensitive fluorescent chemosensors for picric acid,* Dalton Trans. 45 (2016) 8475-8484

35. P.S. Hariharan, S. P. Anthony, *Selective turn-on fluorescence for* Zn^{2+} *and* Zn^{2+} + Cd^{2+} *metal ions by single Schiff base chemosensor,* Anal. Chim. Acta. 848 (2014) 74-79

36. M. Suresh, A. Ghosh, A. Das, *A simple chemosensor for* Hg^{2+} *and* Cu^{2+} *that works as a molecular keypad lock,* Chem. Commun. (2008) 3906-3908

37. V. Bhalla, V. Vij, M. Kumar, P. R. Sharma, T. Kaur, *Recognition of Adenosine Monophosphate and* H_2PO_4 *using Zinc Ensemble of New Hexaphenylbenzene Derivative: Potential Bio-probe and Multichannel Keypad System,* Org. Lett. 14 (2012) 1012-1015

38. S. Rahul-Clermont, M. F. Dunn, *The biological chemistry of zinc in Copper and Zinc in Inflammatory and Degenerative Diseases*, Springer Netherlands, 1998

39. I. Bertini, H. B. Gray, S. J. Lippard, J.S. Valentine, *Bioinorganic Chemistry*, University Science Books, Mill Valley, California, 1994

40. PF Zatta, A-C Alfrey (Eds), *Aluminium Toxicity in Infants' Health and Disease*, World Scientific, 1997

41. J. Mendham, R.C. Denney, J. D. Barnes, M.J.K. Thomas, *Vogel's Quantitative Chemical*

42. J. Janata, *Principles of Chemical Sensors*, Springer Sciences, USA, 2009

43. P. Gründler, *Chemical Sensors: An Introduction for Scientists and Engineers*, Springer-Verlag Berlin Heidelberg, 2007

44. G. Orellana, M. C. Moreno-Bondi (Eds.), Frontiers in Chemical Sensors: *Novel Principles and Techniques*, Springer-Verlag Berlin Heidelberg, 2005

45. R. Narayana Swamy, S. Otto (Eds.), *Optical Sensors*: *Industrial Environmental and Diagnostic Applications*, Springer-Verlag Berlin Heidelberg, 2004

46. V. C. Yang, T. T. Ngo (Eds), *Biosensors and Their Applications*, Springer, USA, 2000

47. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 3rd Edn, Springer, USA, 2006

48. B. Valeur, *Molecular Fluorescence Principles and Applications*, Wiley-VCH Verlag GmbH, 2001

49. B. Valeur, I. Leray, Coord. Chem. Rev., 205 (2000) 3–40. B. Valeur, *Probe design and chemical sensing*, in: J.R. Lakowicz (Ed.), *Topics in Fluorescence Spectroscopy*, 4 (1994) 21, Plenum, New York. L. Prodi, F. Bolletta, M. Montalti, N. Zaccheroni, Coord. Chem. Rev, 205 (2000) 59–83

50. D. Parker, Coord. Chem. Rev., *specifically devoted to 'Luminescent Sensors'* (a special issue), (2000)

51. V. Amendola, L. Fabbrizzi, F. Forti, M. Licchelli, C. Mangano, P. Pallavicini, A. Poggi, D.Sacchi and A. Taglieti, *Coord. Chem*. Rev., 2006, 250, 273

52. M. Sauer, Angew. *Chem.*, Int. Ed., 2003, 42, 1790

53. K. Hanaoka, Y. Muramatsu, Y. Urano, T. Terai and T. Nagano, *Chem*.–Eur. J., 2010, 16, 568

54. D. A. Pearce, N. Jotterand, I. S. Carrico and B. Imperiali, J.Am. *Chem.* Soc., 2001, 123

55. D. Udhayakumari, S. Suganya, S. Velmathi, J. Luminen. 141 (2013) 48-52

56. D. Li, C. –Y. Li, H. –R. Qi, K.-Y. Tan, Y.-F. Li, *Sensors and Actuators*, 223 (2016) 705712

57. Y. J. Lee, C. Lim, H. Suh, E. J. Song, C. Kim, *Sensors and Actuators*, 201 (2014) 535-544.

58. G. J. Park, G. R. You, Y. W. Choi, C. Kim, *Sensors and Actuators, B*, 229 (2016) 257-

59. Y. Zhou, D. Huang, W. Wang, Q. Chao, Nanjing Daxue Xuebao, Ziran Kexue, 28 (1992) 255-60

60. Sheel C. Dodani, Qiwen He, and Christopher J. Chang, J. Am. *Chem. Soc*., 131 (2009) 18020–18021. T. Poltue, R. Rangkupan, S. T. Dubas, L. Dubas, *Mater. Let*., 65 (2011) 2231-2234

61. L. Liu, A. Wang, G. Wang, J. Li, Y. Zhou, *Sensors and Actuators B*, 215 (2015) 388-395

62. C. Patra, A. K. Bhanja, C. Sen, D. Ojha, D. Chattopadhyay, A. Mahapatra,C. Sinha, *Sensors and Actuators B*, 228 (2016) 287-294. A. K. Bhanja, C. Patra, S. Mondal, D. Ojha, D. Chattopadhyay and C Sinha, RSC Adv., 5 (2015) 48997–49005

63. Y. Zhang, J. Shen, H. Yang, Y. Yang, Z. Zhou, S. Yang, *Sensors and Actuators* B, 207 (2015) 887-892

64. G. Chen, Z. Guo, G. Zeng and L. Tanga, *Analyst*, 2015, 140, 5400–5443.

65. J. Prabhu, K. Velmurugan, R. Nandhakumar, *Spectrochim.* Acta Part A, 144 (2015) 23–28. H. Sun, L. Yu, H. Chen, J. Xiang, X Zhang, Y. Shi, Q Yang, A. Guan, Q. Li, Y. Tang, Talanta, 136 (2015) 210-214. S. Zhan, Y. Wu, Y. Luo, L. Liu, L. He, H. Xing, P. Zhou, *Analytical Biochemistry* 462 (2014) 19–25

66. S. Mondal, A. K. Bhanja, D. Ojha, T. K. Mondal, D. Chattopadhyay and C. Sinha, *RSC Adv*., 5 (2015) 73626–73638.

67. L.-Q. Li, L.-J. Gao, *Spectrochim. Acta*-Part A, 152 (2016) 426-430. C. Zheng, A. Yuan, Z. Zhang, H. Shen, S. Bai, H. Wang. J. *Fluor.* 23 (2013) 785-791

68. L.-L. Wu, J. Zhao, H. Wang and J. Wang, CrystEngComm, 2016, DOI: 10.1039/c5ce02444g

69. P. A. Gale and C. Caltagirone, *Anion sensing by small molecules and molecular ensembles*, Chem. Soc. Rev., 2015, 44, 4212—4227

70. J. Vaněk, P. Lubal, P. Hermann, P. Jr. Anzenbacher, J *Fluoresc*., 23 (2013) 57-69

71. D. Sain, C. Kumari, A. Kumar, S. Dey, Supramol. *Chem.*, 28 (2016) 239-248. M. Aleskovic, N. Basaric, N. Doslic, V. Tomasic, K. Mlinaric-Majerski, *Supramolecular Chem*., 26 (2014) 850-855

72. K. C. Rout, B. Mondal, *Inorg. Chim.* Acta 437 (2015) 54-58. M. Bianchi, M. Bağlan, S. Atılgan *Sensors and Actuators B*, 222 (2016) 315 – 319. R. Gao, F. Wang, Y. Zhu, G. Li, Supramol. *Chem.*, 28 (2016) 204-211. K. Tang, Y. Zheng, W. Zhang, Q. Wang, *Science of Advanced Materials*, 6 (2014) 612-616

73. L. G. Nandi, C. R. Nicoletti, I. C. Bellettini, and V. G. Machado, Anal. Chem., 86 (2014) 4653–4656. J. -J. Li, W. Wei, X.-L. Qi, X. Xu, Y. -C. Liu, Q. -H. Lin, W. Dong, *Spectrochimica* Acta - Part A: 152 (2016) 288-293. C. Cheng, H.-Y. Chen, C.-S. Wu, J. S. Meena, T. Simon, F. -H. Ko, *Sensors and Actuators B*, 227 (2016) 283-290

74. S. Erdemir, B. Tabakci, M. Tabakci, *Sensors and Actuators*, B: 228 (2016) 109-116

75. A. Sarkar, S. Bhattacharyya, A. Mukherjee, Dalton Trans., 45 (2016) 1166-1175. S. Ghosh, *Supramol. Chem*., (2016) 1-7. Y. Jo, C. Yunhee, C. Nagesh, D. –G. Cho, J. Org. Chem., 79 (2014) 9418-9422. A. K. Bhoi, P. K. Sahu, G. Jha and M. Sarkar, *RSC Adv*., 5 (2015) 61258–61269. S. Sharma, M. S. Hundal, G. Hundal, Tet. Lett. 54 (2013) 2423-2427

76. R. Indumathy, P. S. Parameswaran, C. V. Aiswarya, B. U. Nair, Polyhedron, 75 (2014) 22-29

77. M. N. Abbas, A. L. A. Radwan, N. M. Nooredeen, M. A. A. El-Ghaffar, J. *Solid State Electrochem.* (2016) 1-14. O. Karagollu, M. Gorur, F. Gode, B. Sennik, F. Yilmaz, *Sensors and Actuators* ,193(2014) 788-798

78. A. Hens, P. Mondal, K. K. Rajak, *Polyhedron*, 85 (2015) 255-266

79. M. Devi, A. Dhir and C. P. Pradeep, New J. *Chem.*, 40 (2016) 1269-1277. D. Sharma, S. K A. Kumar, S. K. Sahoo, Tet. Lett., 55 (2014) 927-930. L. Zhao, W. Yun, K. Guang, J. H Chen, Z. Hua, M. Li, B. Liu, J. F. Zhang, Q. H. Zhao, Y. Zhou, J. *Fluorine Chem*., 158 (2014) 53-59
80. L.M. Hyman, K. J. Franz, *Coord. Chem*. Rev. 256 (2012), 2333-2356

81. H.T. Zhang, R.C. Liu, J. Liu, L. Li, P. Wang, S.Q. Yao, Z.T. Xu, H.Y. Sun,*Chem. Sci*., 7 (2016) 256-260

82. Y.W. Men, Z.F. Li, J. Zhang, Z.Z.Tong, Z. Xi, X.B. Qiu, L. Yi, Tet. Lett., 56 (2015) 5781-5786

83. F.B. Yu, P. Li, B.S. Wang, K.L. Han, J. Am. *Chem. Soc.*, 135 (2013) 7674-7680

84. F. Xie, H. Tan, Z.B. Li, H.B. Yang, *Anal. Methods*. 6 (2014) 6990-6996. J. X.J. Zhang, K. Hoshino, *Mol. Sensors and Nanodevices*, (2014) 1–42

85. A. D. Naik, K. Robeyns, C. F. Meunier, A. F. Leonard, A. Rotaru, B. Tinant, Y. Filinchuk, B. L. Su, Y. Garcia, *Inorg. Chem*., 53 (2014) 1263-1265

86. F. Delattre, F. Cazier, F. Cazier, A. Tine, Current Anal. Chem., 5 (2009) 48-52

87. A. Afzal, N. Cioffi, L. Sabbatini, L. Torsi, *Sensors and Actuators B*, 171–172(2012) 25-42; H. Dacres, R. Narayanaswamy, *Sensors and Actuators B*, 107 (2005) 14-23

88. A. I. Ayesh, A. F.S. Abu-Hani, S. T. Mahmoud, Y. Haik, *Sensors and Actuators* B, 231 (2016) 593-600

89. C. Yang, W. Deng, H. Liu, S. Ge, M. Yan, *Sensors and Actuators B*, 216 (2015) 286-292

90. K. Singhal, B. Ramanujam, V. Mariappanadar, C. P. Rao, Org. Lett., 8 (2006) 35

91. M. Taki, M. Desaki, A. Ojeda, S. Doshi, T. Hirayama, I. Hamachi, Y. Yamamoto, J. Am. *Chem. Soc*., 130 (2008) 12564–12565

92. S.K. Shoora, A.K. Jain, V.K. Gupta, *Sensors and Actuators* B 216 (2015) 86104

93. M. Mukherjee, S. Pal, S. Lohar, B. Sen, S. Banerjee, S. Banerjee, and P. Chattopadhyay, *Analyst*, 139 (2014) 4828–4835

94. A. Barba-Bon, A.M. Costero, S. Gil, M. Parra, J. Soto, R. Martinez-Manez, F. Sancenon, *Chem.Commun*. 48 (2012) 3000–3002

95. S. Das, A. Sahana, A. Banerjee, S. Lohar, D.A. Safin, M.G. Babashkina, M. Bolte, M.Y. Garcia, I. Hauli, S.K. Mukhopadhyay, D. Das, *Dalton Trans*. 42 (2013)4757–4763

96. R. Patil, A. Moirangthem, R. Butcher, N. Singh, A. Basu, K. Tayade, U. Fegade, D. Hundiwale, A. Kuwar, *Dalton Trans*. 43 (2014)2895–2899

97. S. Sen, T. Mukherjee, B. Chattopadhyay, A. Moirangthem, A. Basu, J. Marek, P. Chattopadhyay, *Analyst* 137(2012) 3975–3981

98. W.H. Ding, W. Cao, X.J. Zheng, D.C. Fang, W.T. Wong, L.P. Jin, *Inorg. Chem*. 52 (2013) 7320–7322

99. C.Y. Lu, Y.W. Liu, P.J. Hung, C.F. Wan, A.T. Wu, *Inorg. Chem. Commun*. 35 (2013)273–275

100. S.A. Lee, G.R. You, Y.W. Choi, H.Y. Jo, A.R. Kim, I. Noh, S.J. Kim, Y. Kim, C. Kim, *Dalton Trans*. 43 (2014) 6650–6659

101. S. Guha, S. Lohar, A. Sahana, A. Banerjee, D.A. Safin, M.G. Babashkina, M.P.Mitoraj, M. Bolte, Y. Garcia, S.K. Mukhopadhyay, D. Das, *Dalton Trans*. 42 (2013) 10198–10207

102. Yunqiang Tang, Jinguo Sun, Bingzhu Yin, *Anal. Chim.* Acta 942 (2016) 104-111

103. S. Mukherjee, P. Mal, HelenStoeckli-Evans, J. *Lumin*., 172(2016)124–130

104. D. Sarkar, P. Ghosh, S. Gharami, T. K. Mondal, N. Murmu, *Sensors and Actuators B* 242 (2017) 338–346

105. Y.-Sheng Mi, D.-Ming Liang, Y.-Ting Chen, X.-Biao Luo, J.-Nan Xiang, RSC Adv. 4 (2014) 42337–42345

106. B. Sen, M. Mukherjee, S. Banerjee, S. Pal, and P. Chattopadhyay, *Dalton Trans*., 44 (2015) 8708 – 8717

107. G.J. Park, H.Y. Jo, K.Y. Ryu, C. Kim, RSC Adv. 4 (2014) 63882–63890

108. *Analysis,* 6th Edn, 2009, Prentice Hall

109. S. Chaudhuri, S. Bera, M. K. Biswas, A. S. Roy, T. Weyhermüller, P. Ghosh *Inorg. Chem.* Front., 2014, 1, 331-341

110. A. Samui, K. Pal, P. Karmakar, S. K. Sahu, *Materials Science and Engineering C* (2019)98, 772-781

111. K. Pal, S. Roy, P. K. Parida, A. Dutta, S. Bardhan, S. Das, K. Jana, P. Karmakar, *Materials Science and Engineering C*, (2019) 95, 204–216

112. D. Maiti, A. S. M. Islam, M. Sasmal, C. Prodhan, and M. Ali *Photochem. Photobiol. Sci*., 2018, 17, 1213-1221

113. D. W. Ellis, *Fluorescence and Phosphorescence Analysis* (Edited by D. M. Hercules)

114. R. Romanowicz and A. C. Testa, J. *Phys. Chem.*72,793 (1968)

115. J. W. Eastman, Photochem. Photobiol.6,55 (1967). SO. *Interscience*, New York

116. G. Berthon, Coord. *Chem. Rev*., 228 (2002) 319–341

117. Z. Krejpcio and R. W. Wojciak, Pol. J. *Environ. Stud*., 11 (2002) 251–254