PROJECT REPORT
ON
“Rhodamine based Colorimetric and Fluorometric Chemosensor for the detection of Cu^{2+}”

Submitted in partial fulfilment of the Requirement for the degree of

MASTERS OF SCIENCE IN CHEMISTRY-2023

Submitted by

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Dedicated To My Parents

HALDIA GOVERNMENT COLLEGE,

Debhog, Haldia – 721657

I hereby forward this project work entitled **“Rhodamine based Colorimetric and Fluorometric Chemosensor for the detection of Cu^{2+}”** by **SONALI JANA** in partial fulfilment of requirements for the degree of MASTERS OF SCIENCE IN CHEMISTRY of the Haldia Government College, Debhog, Haldia – 721657.

This review has been completed under my guidance in the Department of Chemistry, Haldia Government College, Debhog.

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CERTICIFATE OF APPROVAL

The foregoing project is hereby approved as a creditable study of a science subject carried out and presented in a manner satisfactory to warrant its acceptance as a prerequisite for the degree for which it has been submitted. It is understood that by this approval the undersigned do not necessarily endorse of approve any statement mode, opinion expressed of conclusion drawn there in the thesis only for the purpose for which it is submitted**.**

BOARD OF EXAMINERS

Acknowledgement

A moment comes which comes but rarely in a student's life, when with utmost pleasure and satisfaction, I myself, Sonali Jana , submit my project on **"Rhodamine based Colorimetric and Fluorometric Chemosensor for the detection of Cu^{2+}".** I take this opportunity to express my gratitude and sincere thanks to my project guide, **Dr. Saikat Kumar Manna** whose motivating personality, constant encouragement and sustained guidance has made this project to come true.

I am also thankful to my teachers Dr. Dipankar Pramanik, Mr. Uday Sankar Midya, Dr. Khokan Samanta, Dr. Sudipta Pathak, Dr. Barnali Jana and Mr. Monotosh Mondal for their continuous inspiration. I also acknowledge all the staff members for our department.

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CONTENT

1. INTRODUCTION

Jean Merie Lean, who coined the term “Supramolecular Chemistry” in 1969, defined the term Supramolecular chemistry as, “Chemistry of the union between molecules through the intramolecular bonds”,” chemistry beyond the molecule” or “chemistry of non-covalent bonding”. This brunch of chemistry involves investigating molecular system in which the most important feature is that, components are held together by intermolecular forces.

Fluorescent chemosensors capable of selectively recognising guest species are receiving considerable attention in supramolecular chemistry because of their potential applications in environmental detection, molecular catalysis and biological fluorescence imaging etc. A fluorescent chemosensor is a molecular system for which the physiochemical properties change upon interaction with a chemical species so that a change in fluorescence is produced.

Analyte detection by a fluorescent chemosensor is usually achieved through one or more common photo-physical mechanism, including chelation induced enhanced fluorescence (CHEF), intra-molecular charge transfer (ICT), photo-induced electron transfer (PET), aggregation induced emission (AIE) and the number of approaches is still expanding.

The construction of a fluorescent game of cells are usually involves 2 integrated components one is signalling fluorophore and another is guest receptor that poses a recognition capability.

The term molecular recognition refers to the specific interaction between molecules through non covalent bonding such as hydrogen bonding, metal coordination, hydrophobic forces, van der Waals forces, π-π interactions, electrostatic and/or electromagnetic effects. The host and guest involved in molecular recognition exhibit molecular complementarity with respect to size, shape and chemical functionality of the host- guest system. Molecular recognition represents the central event at the basis of many fundamental chemical and biological phenomena, such as catalysis, transport and sensing, and it also constitutes a topic of foremost importance in Supramolecular Chemistry.

Chemical sensing allows for the study and control of chemical processes from the laboratory to the industrial scale, and plays an important role in the food industry for the control of food quality and safety.

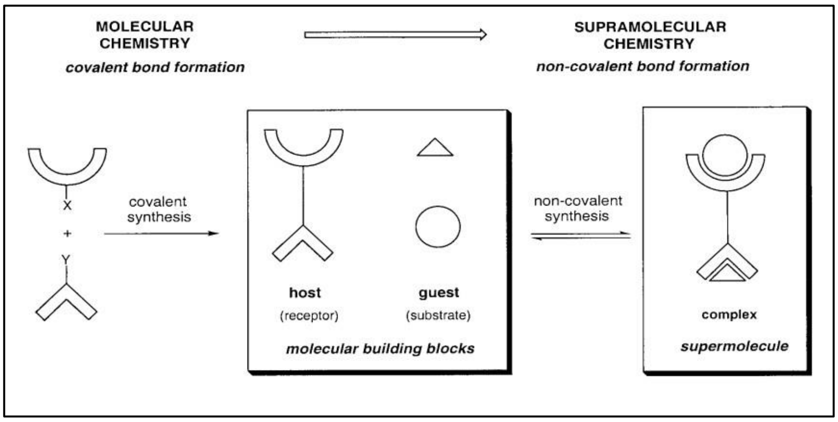


Figure 1. From molecular to supramolecular chemistry: molecules, supermolecules**.**

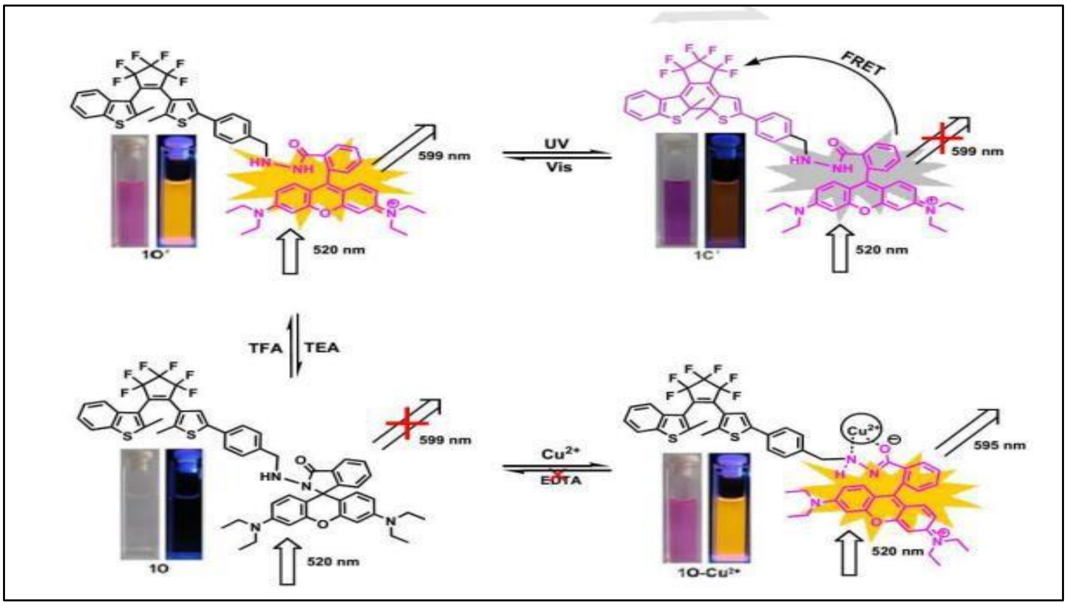
Copper (Cu^{2+}) is an essential trace element for many biological processes and systems. However, at high concentrations, it can become an environmental pollutant.

Copper (Cu^{2+}) can pollute the environment through various sources and activities. One of the main sources of Cu^{2+} pollution is industrial activities, such as mining, smelting, and manufacturing processes. These activities can release copper-containing waste and effluents into water bodies, leading to contamination of aquatic ecosystems. Agricultural practices, such as the use of copper-based pesticides and fertilizers, can also contribute to Cu^{2+} pollution. When these substances are applied to crops or soil, they can leach into groundwater or runoff into nearby water bodies, Caussincontamination[1].

Therefore, there is a need to identify and quantify Cu^{2+} ions in various samples. This is why the development of efficient Cu^{2+} optical chemosensors, such as the rhodamine derivative described in the cited paper, is of great interest.

Previous work:

[1] A new photochromic diarylethene with a phenyl-linked rhodamine B unit was synthesized by Shouzhi Pu et al. (2014)[1] and its photochemical properties were systematically studied. The diarylethene could be used as a multiple fluorescence switch by sequential stimulations with protons and light. As a result, an INHIBIT logic gate was constructed by using the stimulation of trifluoroacetic acid and ultraviolet as inputs and the fluorescence intensity at 599 nm as output. Additionally, the diarylethene could act as a naked-eye chemosensor for the recognition of Cu^{2+} in aqueous acetonitrile. When triggered by Cu^{2+}, the fluorescence intensity of the diarylethene at 594 nm was dramatically enhanced by 133-fold with a significant fluorescence change from dark to bright yellow.



[2] A new colorimetric “off-on” rhodamine based chemosensor for metal ions (2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone, THB) was synthesized by Poh Wei Cheah et al.(2021)[2] via Schiff base chemistry between rhodamine B hydrazide and 2,4,6-trihydroxybenzaldehyde. Of all the metal ions tested, it selectively turned pink in the presence of Cu2+, and its sensitivity was evaluated. Coordination with Cu^{2+} was accompanied by a binding constant of 5.95 × 103 M^{-1} whilst its detection limit is remarkably low (0.48 μM; WHO acceptable limit = 31.5 μM). Moreover, detection of Cu^{2+} with THB was independent of pH as pinkish mixture with high absorbance was observed in a wide range from pH 4–12. Consequently, a 1:1 binding ratio of THB to Cu^{2+} was verified. Additionally, THB can be reversibly used for up to three times with administration of EDTA and the coordination of THB with Cu^{2+} was corroborated by the shifting of carbonyl amide peak in the FT-IR spectra. Then, the prototype of THB as an onsite test kit for detection of Cu^{2+} in 100% aqueous condition was also successfully demonstrated. However, THB is weakly cytotoxic against the colorectal adenocarcinoma cell line HT-29 albeit non-toxic towards the noncancerous colon cell line CCD-18Co (IC50 via MTT: HT-

29 = 39.24 ± 1.59 µM; CCD-18Co > 50.62 µM). Nonetheless, these findings proposed the potential of THB as a simple and feasible sensor in multiple practical applications.

The selectivity of THB towards different metal ions was evaluated in the presence and absence of various metal ions (Ag^+, Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, La^{3+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sn^{2+}, and Zn^{2+}) by observing colour changes, as well as UV–vis absorption and fluorescence spectroscopy. All experiments were carried out in 30% acetonitrile solution, where 1 equivalence of metal ions were introduced into THB (10 µM) solution. Upon addition of the aforementioned metal ions, only Cu^{2+} was able to induce observable colour change (colourless to pink) as seen in Fig. 2a. This observation was further reflected by its UV–vis spectra (Fig. 2b)

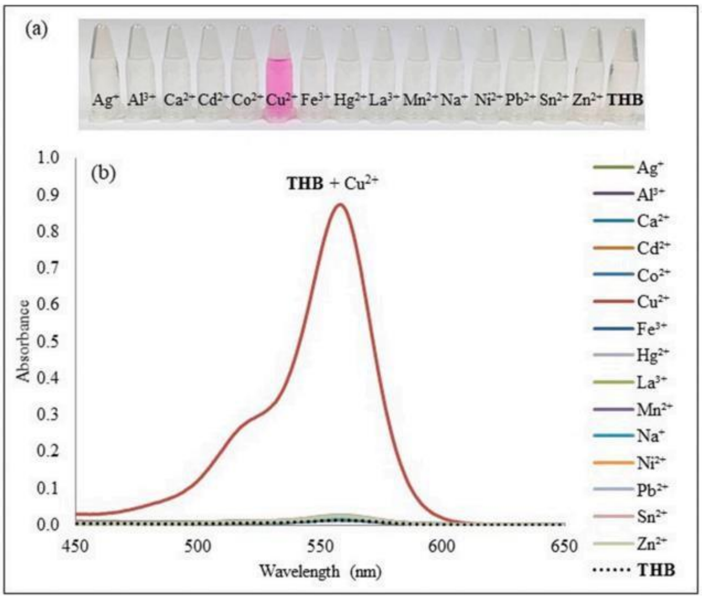
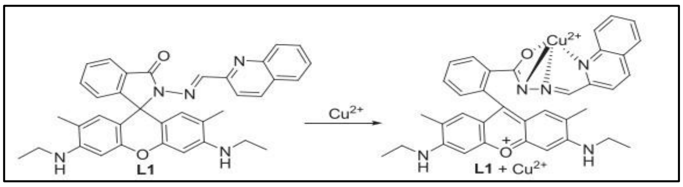


Fig. 2. (a) Solution of **THB** turned pink in the presence of Cu^{2+} ion, but remain colourless with addition of other cations in 30% acetonitrile solution. (b) UV–vis spectra of **THB** (10 µM) in the presence of various cations (10 µM) in 30% acetonitrile solution at room temperature. Addition of Cu^{2+} ion led to emergence of a strong absorption band (red line).

[3] A rhodamine-based fluorogenic probe bearing the quinaldine unit (L1) was developed as a turn-on fluorescent chemosensor for Cu^{2+} by Liang Huang et al. (2011)[3]. Upon binding with Cu^{2+}, comparable amplifications of absorption and fluorescence signals were observed, which suggest that chemosensor L1 effectively avoided the fluorescence quenching caused by the paramagnetic nature of Cu^{2+}. Importantly, L1 can selectively respond to Cu^{2+} over other commonly coexistent metal ions (such as K^+, Ca^{2+}, Mg^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Zn^{2+}, Cr^{3+}) in aqueous media with a rapid response time (<2 min). In addition, biological imaging studies using living cells to monitor Cu^{2+} is successfully demonstrated.



Complexation Mechanism of **L1**with Cu^{2+}.

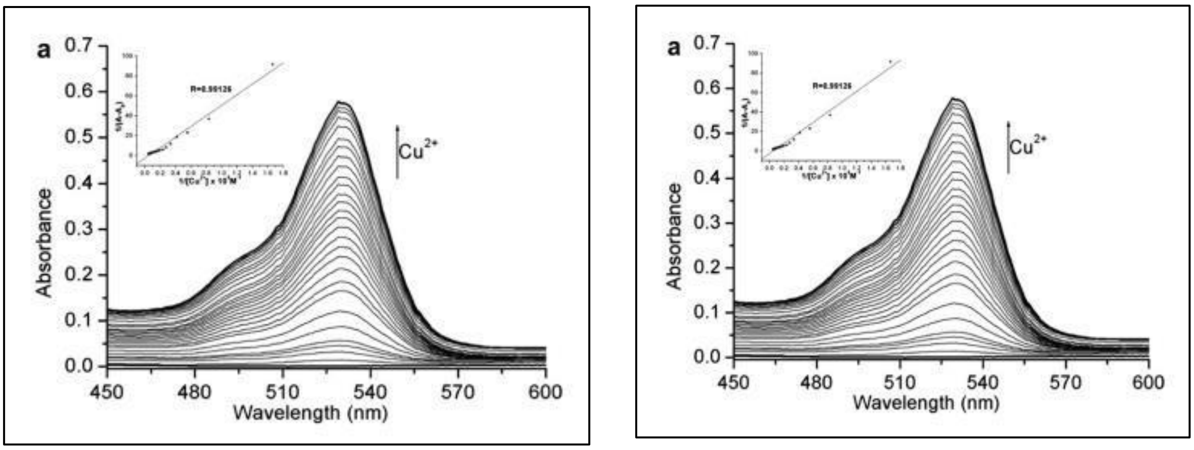
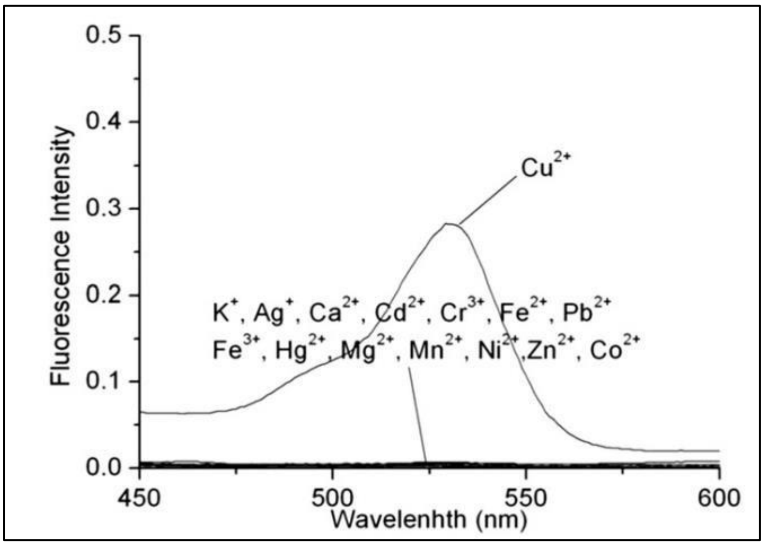


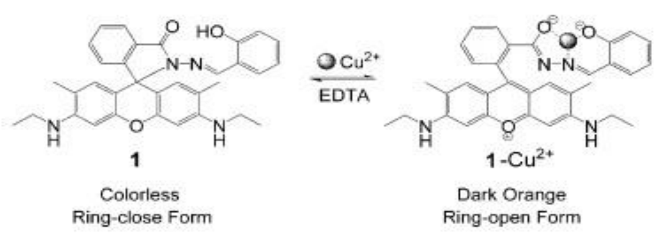
Fig..2. a) Absorption spectra of L1 (2 × 10^{-5} mol L^{-1}) in water/CH_3CN (1:1, v/v) in the presence of different amounts of Cu^{2+} (0e10 equiv). Inset: absorbance at 531 nm as a function of Cu^{2+} concentration b) Fluorescence spectra of L1 (2 ×10^{-5} mol L^{-1}) in water/CH_3CN (1:1, v/v) upon addition of Cu^{2+} (0e2.5 equiv). Inset: Job's plots according to the method for continuous variations, indicating the 1:1 stoichiometry for L1-Cu^{2+}(the total concentration of L1 and Cu^{2+} is 2.5 ×10^{-5} mol L^{-1}). (**λ_{ex}**= 500 nm).



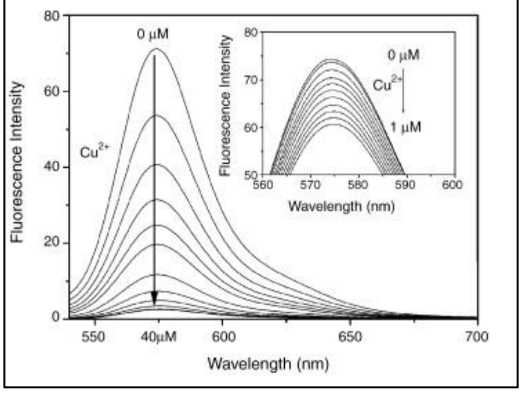
Changes in the absorption spectra of L1 (2× 10^{-5} mol L^{-1}) in the presence of different metal ions (1 ×10^{-4} mol L^{1}) in water/CH_3CN (1:1 v/v).

[4] A highly selective and sensitive rhodamine-based colorimetric chemosensor (1) for quantification of divalent copper in aqueous solution has been investigated by Yu Xiang et al. (2008)[4]. It was designed using salicylaldehyde hydrazone and rhodamine 6G as copper-chelating and signal-reporting groups, respectively. In environmentally friendly media (50% (v/v) water/ethanol and 10 mM NaAc–HAc neutral buffer (pH 7.0)), the sensor exhibited selective absorbance enhancement to Cu^{2+} over other metal ions at 529 nm, with a dynamic working range of 0.05–5.00 µM and a detection limit of 10 nM Cu^{2+}, respectively. To achieve fluorometric determination of Cu^{2+}, the Cu^{2+}-induced absorbance enhancement of 1 was efficiently converted to fluorescence quenching by fluorescence inner filter effects using rhodamine B (RB) as a fluorophore. The selectivity and sensitivity of fluorescence analysis were similar to those of absorptiometric measurement. Both absorptiometric and fluorometric methods were successfully applied to the detection of Cu^{2+} in three water samples.

The selectivity of 1 to Cu^{2+} over other metal ions was also investigated to evaluate whether 1 could serve as a Cu^{2+}- selective colorimetric chemosensor in aqueous condition. In the presence of 1.0 equiv. other metal ions such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Hg^{2+} and Pb^{2+}, no significant enhancement of absorbance could be observed at 529 nm compared to that of Cu^{2+}.



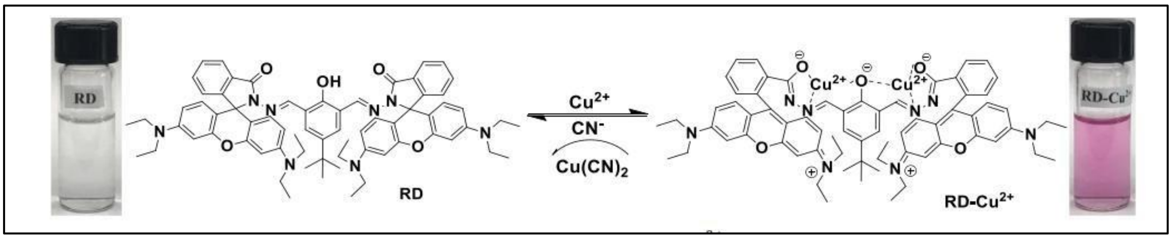
Scheme 1. Reversible binding of **1** and Cu^{2+}.



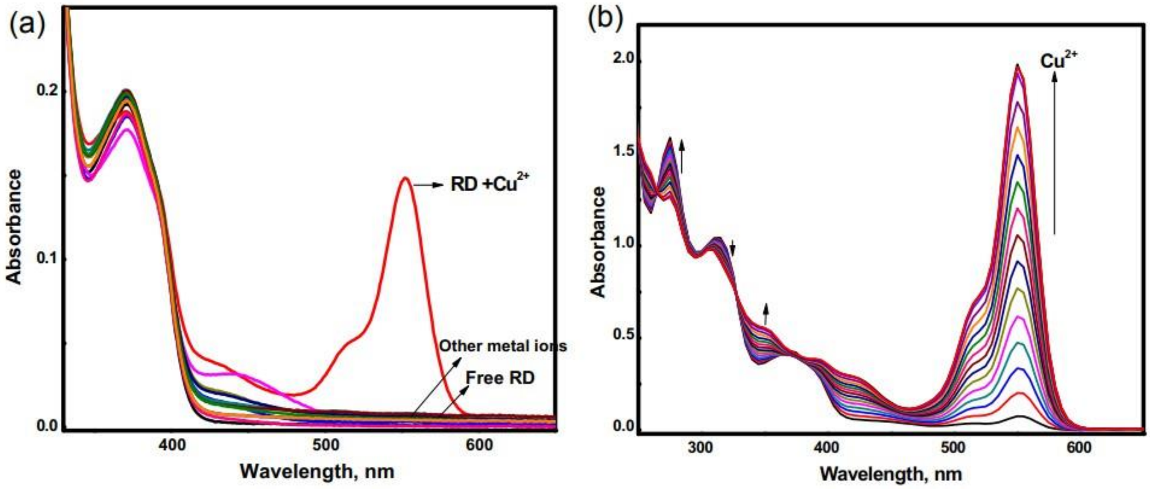
Fluorescence emission spectra of 50µM 1 in the presence of 1µM RB upon the addition of Cu^{2+} in 50% (v/v) water/ethanol buffered by 10 mM NaAc–HAc at pH 7.0. Excitation was performed at 529 nm. Inset: fluorescence emission spectra of 10µM 1 and 1µM RB in the presence of 0-1µM Cu^{2+}.

[5] A novel sensor (RD) bearing rhodamine B and 4-tert-Butyl phenol unit have been designed and synthesized by Fasil Abebe et al. (2020)[5], using microwave irradiation. The sensor allows selective detection of Cu^{2+} by forming absorptive complex and trigger the formation of highly colored ring-open spirolactam. The recognition ability of the sensor was investigated by absorbance, Job's plot, nuclear magnetic resonance (NMR), infrared (IR) and time dependent-density functional theory (TD-DFT) calculations.

The detection limit of RD for Cu^{2+} ion was estimated based on the absorbance titration experiment as 0.26 µM. The selectivity of the sensor for the detection of Cu^{2+} over other interference ions (Na^+, K^+, Mg^{2+}, Ca^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Hg^{2+}, Pb^{2+}, Fe^+, Fe^{3+}, and Cr^{3+}), the absorbance of RD in the separate presence of Cu^{2+} was determined, as well as in the presence of other possible interference ions individually. The absorbance of sensor RD significantly increased in the presence of Cu^{2+}. The colour of the solution changes from colourless "off" to pink red colour "on", allowing "naked-eye" detection.



A possible proposed binding mechanism of sensor RD towards Cu^{2+} in the presence and absence of cyanide ion.



(a) UV -vis spectra of RD (10 µM) with metal ions in CH_3CN/H_2O (7:3 v/v) solution. (b) UV-vis spectra of RD (10 µM) with Cu^{2+} (0–20 µM) in CH_3CN/H_2O (7:3 v/v) solution.

Present work:

1) Aim of work:

The development of fluorescent chemosensor for various ions has received considerable attention, due to their important biological and environmental roles. Among the various fluorophores, Rhodamine B derivatives have been actively used as fluorescent chemosensor for detection of Cu^{2+} ion.

Copper can exist in different oxidation states, with Cu^{2+} being the most common and stable form in aqueous solutions. This redox activity allows copper to participate in electron transfer reactions and play a crucial role in biological processes such as respiration and photosynthesis. Copper is an excellent conductor of electricity, making it widely used in electrical wiring and electronic devices. Its high electrical conductivity is due to the movement of free electrons within its crystal lattice.

Copper (Cu^{2+}) can pollute the environment through various activities. One of the main sources is industrial activities, such as mining, smelting, and manufacturing processes. The use of copper-based pesticides and fertilizers, can also contribute to Cu^{2+} pollution.

Ingesting a high amount of copper, usually in drinking water, can cause vomiting, nausea, abdominal pain, diarrhoea. Ingesting higher than recommended amounts of copper lead to severe illness, such as kidney and liver damage.

Rhodamine-based chemosensors have several advantages over other detection processes for metal ions. Firstly, rhodamine derivatives are widely used as reporting groups in optical analysis due to their low cost. Secondly, rhodamine-based chemosensors can exhibit selective optical response to specific metal ions after chemical modification. Rhodamine-based chemosensors can be designed to work in aqueous solutions with high selectivity and sensitivity. Rhodamine based chemosensors can be used for both colorimetric and fluorometric detection.

In the current study, we report Rhodamine B derivatives (L) as selective fluorescent Chemosensor for detection of Cu^{2+}. Colorimetric and Fluorometric changes were observed for Rhodamine B Chemosensor in presence of Cu^{2+}.

2.Experimental:

2.1. Materials and equipment:

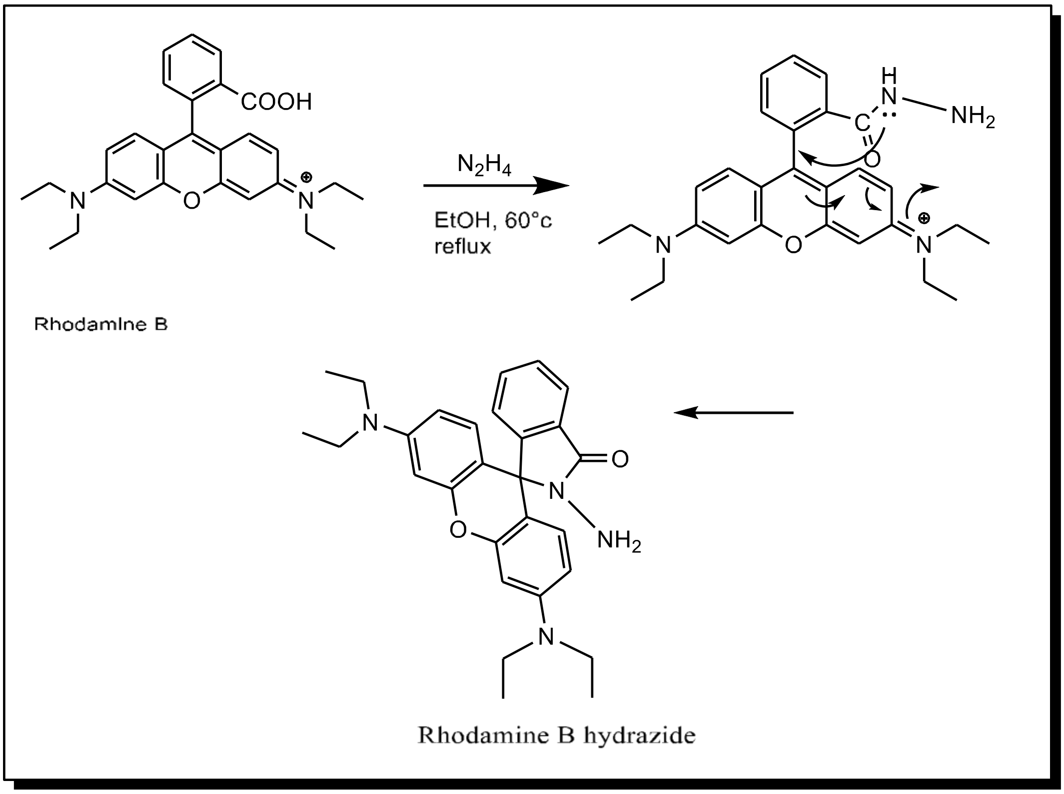
General methods unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Column chromatography was carried out on silica gel, also fluorescence and UV light was use.

2.2 Synthesis of Chemosensor (Rhodamine B derivatives L):

2.2.1 Synthesis Rhodamine B hydrazide:

Rhodamine B 53.8 mg was dissolved in 10 ml Ethyl alcohol then 10 ml Hydrazine added. The reaction mixture was refluxed in a magnetic stirrer for 3 hours at 60°c. After 3 hours the reaction mixture poured in ice water for precipitation. Then precipitation was filtered, and 38 mg (% of yield= 80%) of Rhodamine B hydrazide, as a light pink solid was stored in the refrigerator.

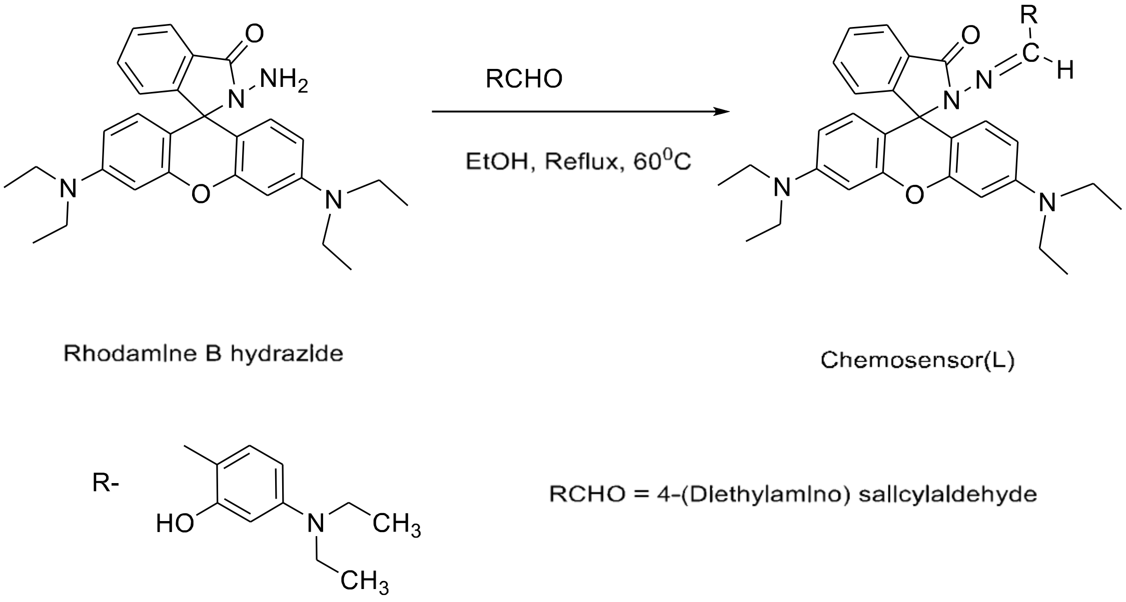
➢Reaction Scheme:



2.2.2 Synthesis of Chemosensor (L):

4-(Diethylamino) salicylaldehyde 17 mg was added to 38mg Rhodamine B hydrazide, then 10 ml ethyl alcohol added. After preparation of reaction mixture, the mixture was refluxed in a magnetic stirrer for 4 hours at 60°C. After 4 hours the product was collected through evaporation to give 27mg (% of yield= 39.26%) of compound (L), as a dark brown solid.

➢ Reaction Scheme:

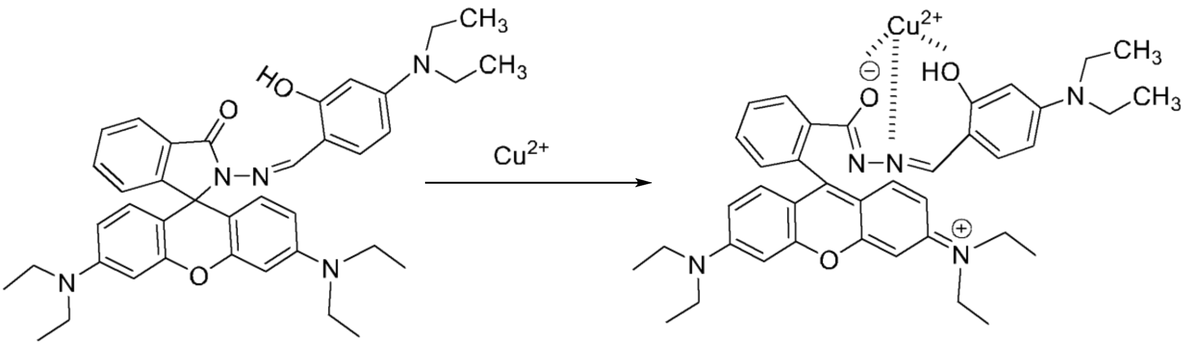


2. 3. Preparation of metal ion solutions for fluorescent study:

Stock solution (10mM) of the salts of Cu^{2+}, Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+} ions in distilled water were prepared.

Stock solutions of probes (chemosensor L) 1 mM were also prepare in EtOH. The test solution were prepared by placing 30 µL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 3 ml with distilled water and EtOH

➢ Chemosensing reaction scheme:



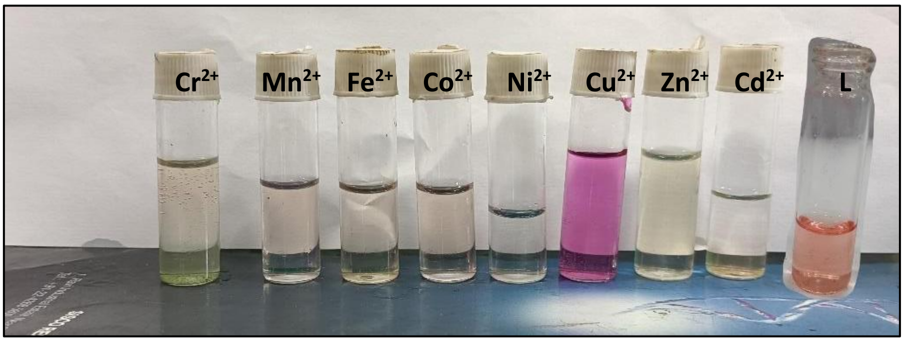
Chemosensor L

Intense pink coloration

3. Results and discussion:

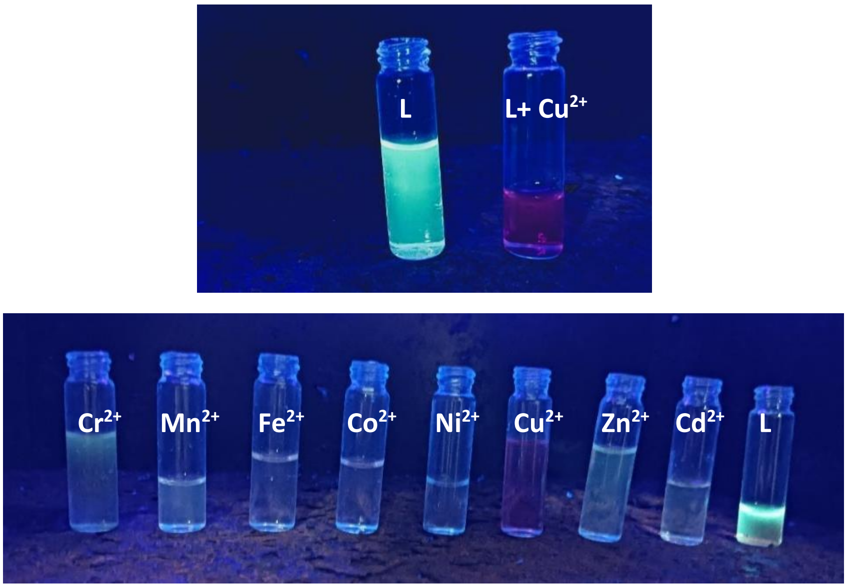
3.1 Colorimetric and fluorometric change:

It is observed that when colourless solution of Cu^{2+} added with the solution of probe (Chemosensor L) then its turns to deep pink in colour over the any other prepared solution of metal ion (Cu^{2+}, Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+}) that is shown below.



Visible colour change of chemosensor in presence of various metal ions

It is observed that in fluorescence light Cu^{2+} ion is fluorescence inactive but in presence of probe (Chemosensor L) showed intense fluorescent colour change over the any other metal ion that is shown below.



Fluorescent colour change of chemosensor- L in presence of various metal ions

3.2. Spectral studies and purpose:

At first the absorption spectra of probe (Chemosensor L) was investigated in methanol at room temperature. As shown in fig. 1, probe L showed absorption peak at 556nm. Upon addition of Cu^{2+}, the peak intensity at 556nm gradually increases.

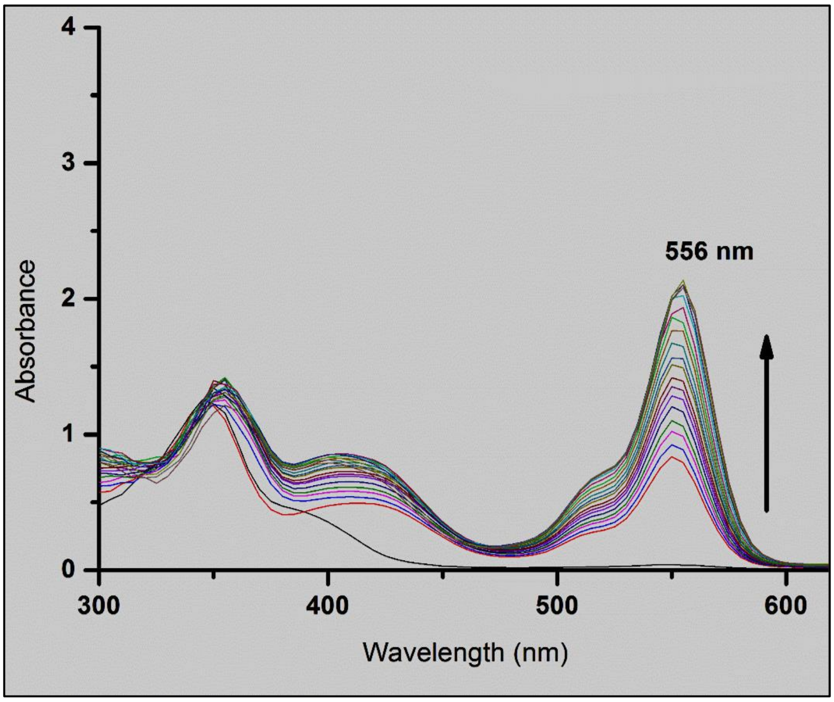


Fig. 1. UV-vis absorption spectra of probe **L** in presence of Cu^{2+} solution.

But in presence of other metal ions (Cu^{2+}, Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+}) the absorption peak intensity at 556nm does not increases as shown in the figure below.

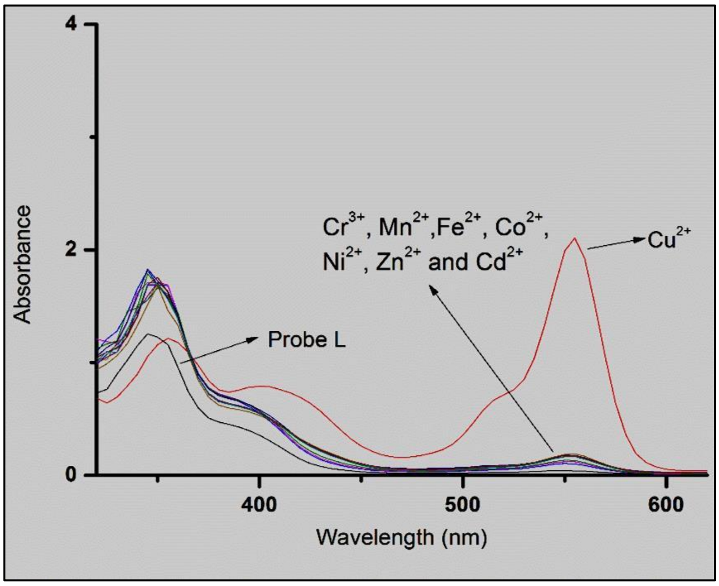
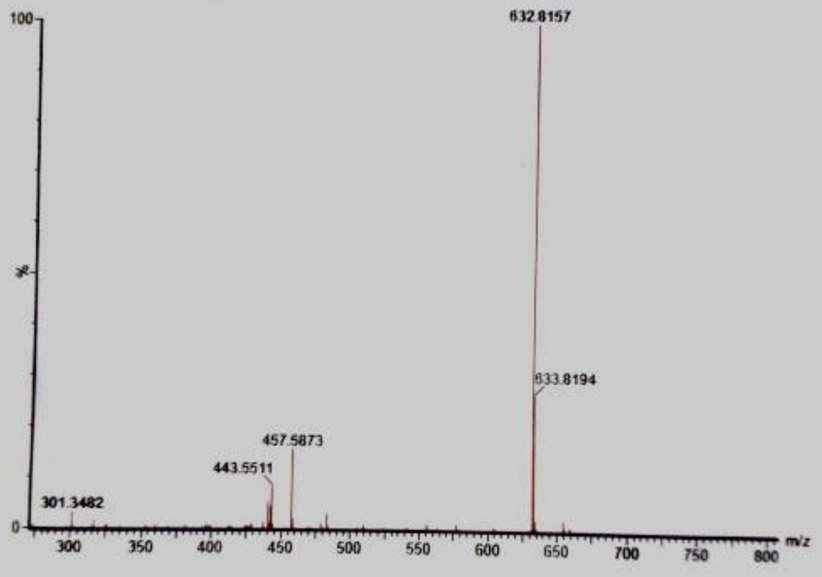


Fig.2. UV-vis absorption spectra of probe **L** in presence of various cations.

Mass spectrum of probe L:

A highly intense peak (base peak) was observed at m/z 632, that indicates the formation of chemosensor –L.



Mass spectrum of probe L.

Conclusion:

In the current study, we synthesized new rhodamine hydrazide derivatives, in which the unique spirolactam ring was clearly presented. Chemosensor – L showed highly selective fluorescence changes with Cu^{2+} among the various metal ions. These selective changes were attributed to the spirolactam ring opening processes and subsequent hydrolysis.

The future plan of work:

The final spectroscopic characterization by ^{1}H NMR, ^{13}C NMR, and analysis of the advanced intermediate are in progressed.

- Further modifications of the receptors if needed for improvement of the binding ability.
- X-ray crystal structure of the compound which will clearly indicate the structure of chemosensor -L.

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