PROJECT REPORT ON

Rhodamine Based Fluorometric And Colorimetric Chemosensor For The Detection Of Cu^{2+} Ion.

Submitted in partial fulfillment of the Requirement for the degree of

MASTERS OF SCIENCE IN CHEMISTRY-2023

Submitted by

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DEDICATED TO MY PARENTS

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Debhog , Haldia-721657

I hereby forward this project work entitled ***Rhodamine based Fluorometric and Colorimetric Chemosensor for the detection of Cu^{2+} ion*** by **SOUMEN HAZRA** in partial fulfillment of the 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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CERTIFICATE 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 war ant 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 or conclusion drawn therein the thesis only for the purpose for which it is submitted.

BOARD OF EXAMINER

Acknowledgement

A moment comes which comes but rarely in a student's life, when with utmost pleasure and satisfaction, I myself, **SOUMEN HAZRA** , submit my project on ***Rhodamine based Fluorometric and Colorimetric Chemosensor for the detection of Cu^{2+} ion*** **.** 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 of our department.

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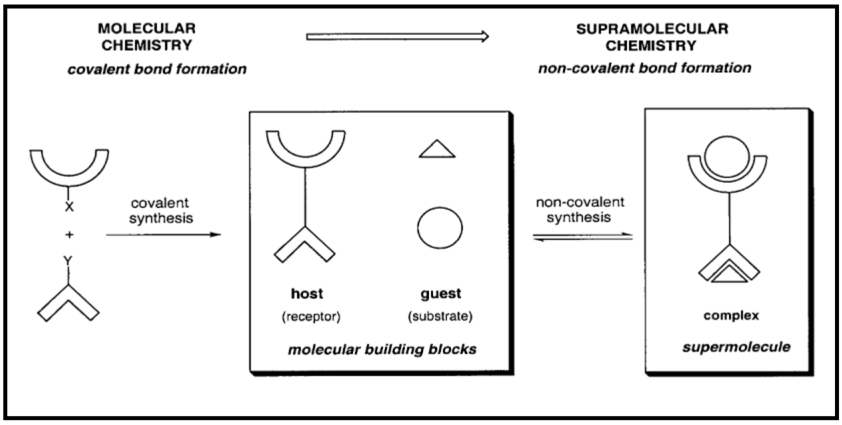
Content :

Introduction:

A molecular sensor or chemosensor is a molecular structure (organic or inorganic complexes) that is used for sensing of an analyte, to produce a detectable change or a signal. The action of a chemosensor, relies on an interaction occurring at the molecular level. The application of chemosensors is referred to as chemosensing, which is a form of molecular recognition. All chemosensors are designed to contain a signalling moiety and a recognition moiety , that is connected either directly to each other or through a some kind of connector or a spacer. Chemosensors were first defined in the 1980's, the first example of such a fluorescent chemosensor can be documented to be that of Friedrich Goppelsroder, who in 1867, developed a method for the determination/sensing of aluminium ion, using fluorescent ligand/chelate. This and subsequent work by others, gave birth to what is considered as modern analytical chemistry.



CHEMOSENSOR

Analyte detection by a fluorescent chemosensor is usually achieved through one or more common photophysical mechanism, including chelation induced enhanced fluorescence (CHEF), intramolecular charge transfer (ICT), photoinduced electron transfer (PET), aggregation induced emission (AIE) and the number of approaches is still expending. Chemosensors are used in everyday life and have been applied to various areas such as in chemistry, biochemistry, immunology, physiology, etc. and within medicine in general, such as in critical care analysis of blood samples. Chemosensors can be designed to detect/signal a single analyte or a mixture of such species in solution.

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, causing contamination[1].

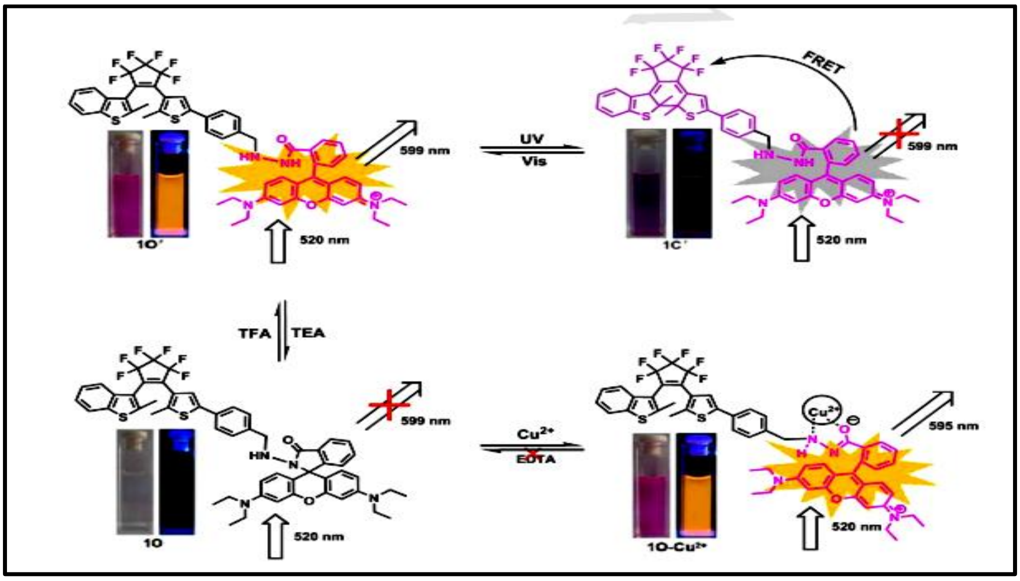
Therefore, there is a need to identify and quantify Cu^{2+} ions in various samples. This is why the development of efficient Cu^{2+} optical chemosensors, such as the rhodamine derivative described in the cited paper, is of great interest.

Previous work:

1) A new photochromic diarylethene with a phenyl-linked rhodamine B unit was synthesized by Shouzhi Pu et al. (2014)[2] and its photochemcial 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.

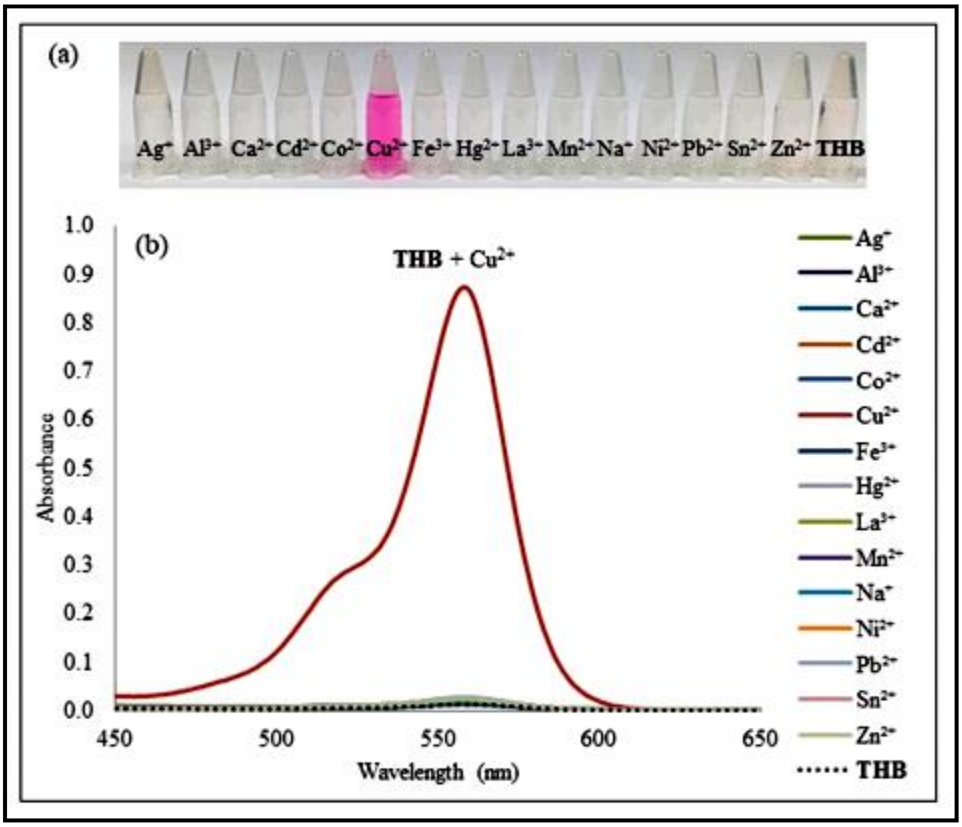
Additionally, the diarylethene could act as a naked-eye chemosensor for the recognition of Cu^{2+} in aqueous acetonitrile. When triggered by Cu^{2+}, the fluorescence intensity of the diarylethene at 594 nm was dramatically enhanced by 133-fold with a significant fluorescence change from dark to bright 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) [3] 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 Cu^{2+}, 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.

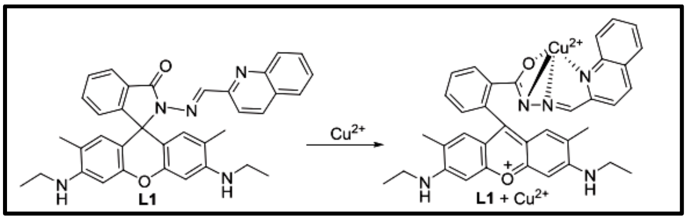
The selectivity of THB towards different metal ions was evaluated in the presence and absence of various metal ions (Ag^{+}, Al^{3+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, La^{3+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, and Zn^{2+}) by observing colour changes, as well as UV–vis 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). This observation was further reflected by its UV–vis spectra.



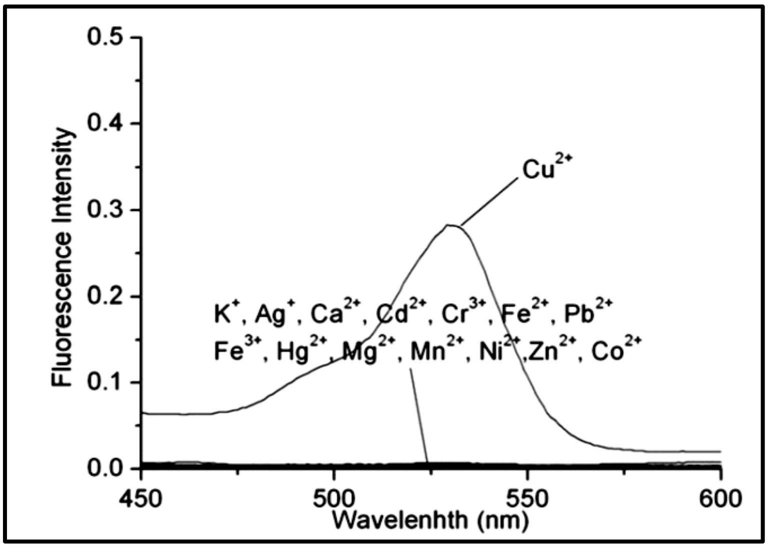
(a)Solution of THB turned pink in the presence of Cu2+ ion, but remain colourless with addition of other cations in 30% acetonitrile solution. (b) UV–vis spectra of THB (10 µM) in the presence of various cations (10 μM) in 30% acetonitrile solution at room temperature. Addition of Cu2+ ion led to emergence 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+}byLiang Huang et al. (2011) [4].

   Upon binding with Cu^{2+}, comparable amplifications of absorption and fluorescence signals were observed, which suggest that chemosener 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 L1with Cu^{2+}.

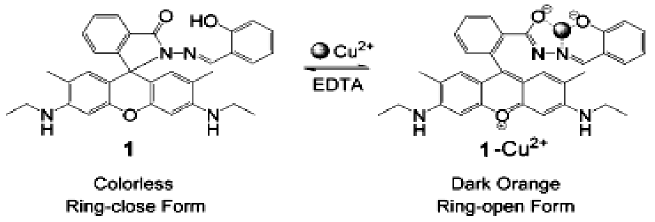


Changes in the absorption spectra of L1 (2×10^{-5} mol L^{-1}) in the presence of different metal ions (1×10^{-4} mol L^{-1}) in water/CH_3CN (1:1 v/v).

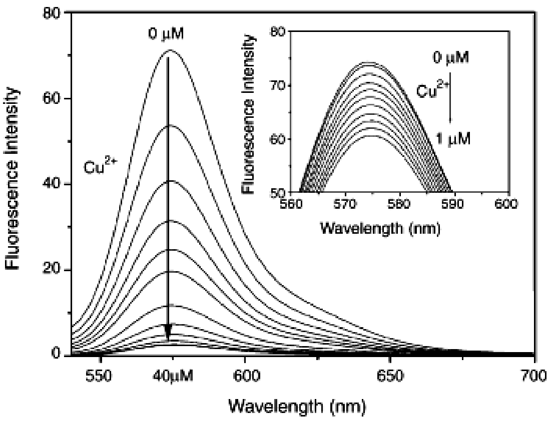
4) A highly selective and sensitive rhodamine-based colorimetric chemosensor (1) for quantification of divalent copper in aqueous solution has been investigated by Yu Xiang et al. (2008) [5].

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. 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+}.



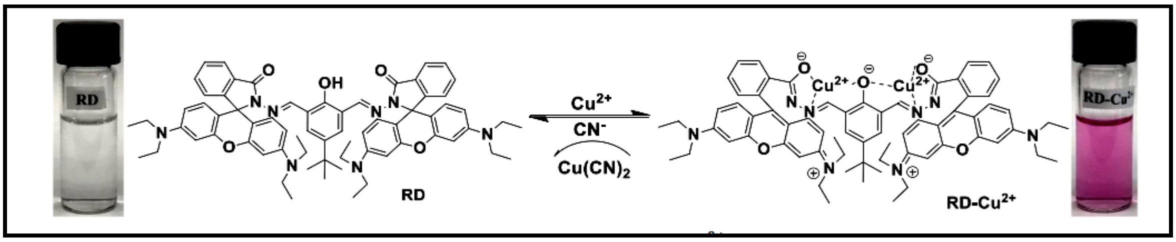
Fluorescence emission spectra of 50µM 1 in the presence of 1µM RB upon the addition of Cu^{2+} in 50% (v/v) water/ethanol buffered by 10 mM NaAc–HAc at pH 7.0. Excitation was performed at 529 nm. Inset: fluorescence emission spectra of 10µM 1 and 1µM RB in the presence of 0-1µM Cu^{2+}.

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)[6], using microwave irradiation.

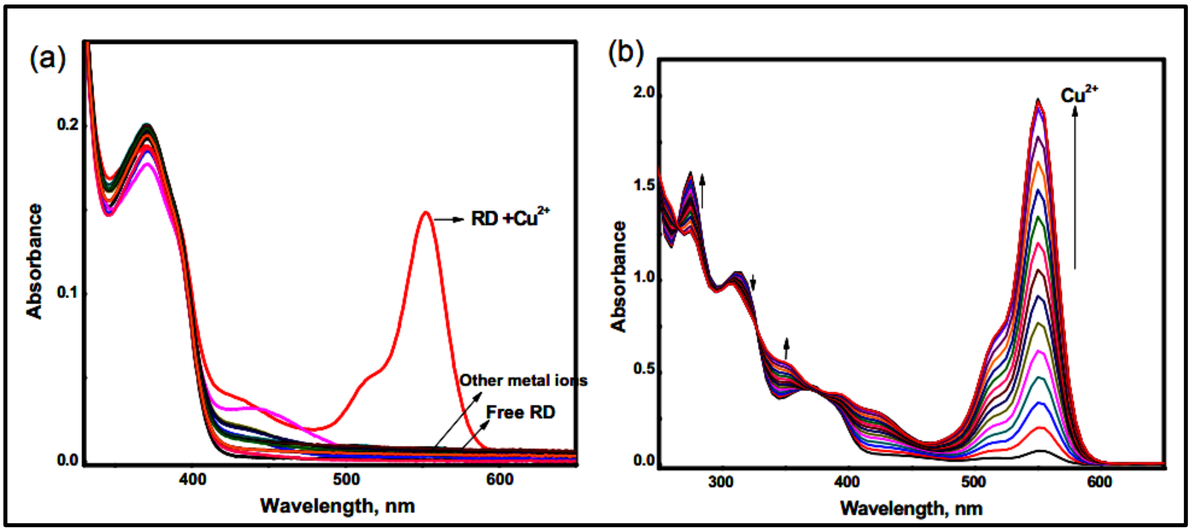
The sensor allows selective detection of Cu^{2+} by forming absorptive complex and trigger the formation of highly colored ring-open spirolactam. The recognition ability of the sensor was investigated by absorbance, Job’s plot, nuclear magnetic resonance (NMR), infrared (IR) and time dependent-density 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 color of the solution changes from colorless “off” to pink red color “on”, allowing “naked-eye” detection.



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

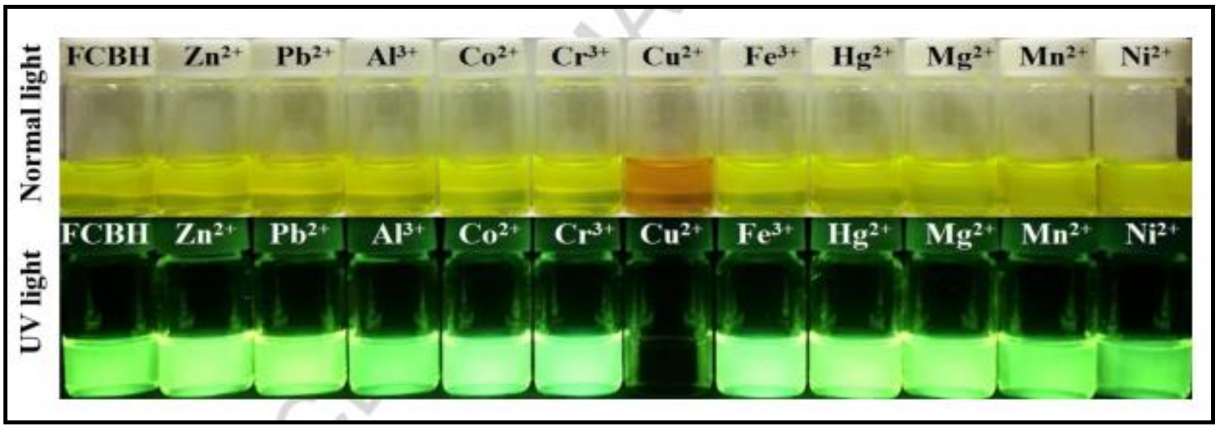


(a) UV -vis spectra of RD (10 µM) with metal ions in CH_3CN/H_2O (7:3 v/v) solution. (b) UV-vis spectra of RD (10 µM) with Cu^{2+} (0–20 µM) in CH_3CN/H_2O (7:3 v/v) solution.

6) A novel coumarin-based receptor bearing a benzohydrazide (FCBH) was developed by Mergu et.al (2017)[7] as a fluorescent chemosensor with high selectivity toward Cu^{2+}.

The sensor was successfully applied to the monitoring of Cu2+ in aqueous solution. After the addition of Cu^{2+} to FCBH, the color of the solution changed from greenish-yellow to red, and the absorption band at 457 nm red-shifted to 517 nm.

The fluorescent green color of FCBH disappeared and the fluorescence emission was completely quenched in the presence of Cu^{2+} . Upon the addition of Cu^{2+} , deprotonation of FCBH occurred, and a 1:1 metal-ligand complex formed. DFT theoretical investigation was carried out to understand the behavior of the sensing probe toward Cu^{2+} . Additionally, the quenched fluorescence of the FCBH-Cu^{2+} complex was restored upon the addition of CN− ions. The possible sensing mechanism of FCBH toward Cu^{2+} was derived from experimental and theoretical examinations.



Naked-eye and fluorescence images of FCBH (20 µM, DMSO/water, 1:1) upon addition of various metal ions (1 equiv.).

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, diarrhea. 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 Fluoremetric changes were observed for Rhodamine B Chemosensor in presence of Cu^{2+}.

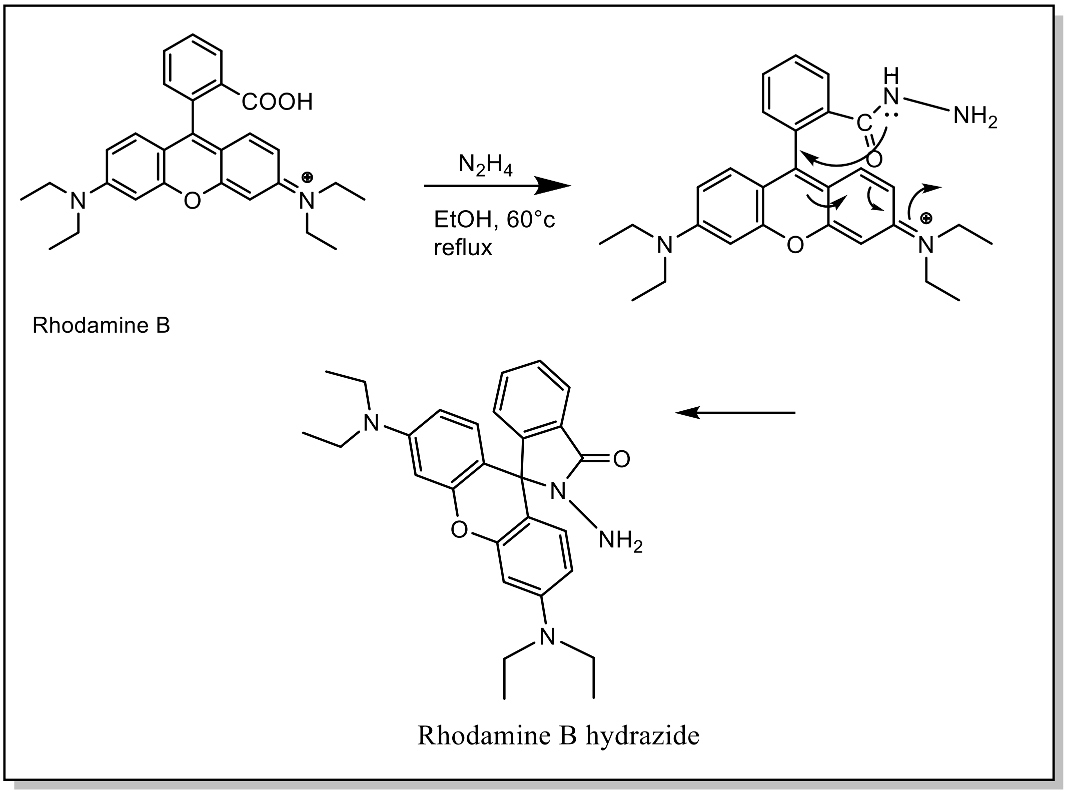
2) *Experimental:*

❖ *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.

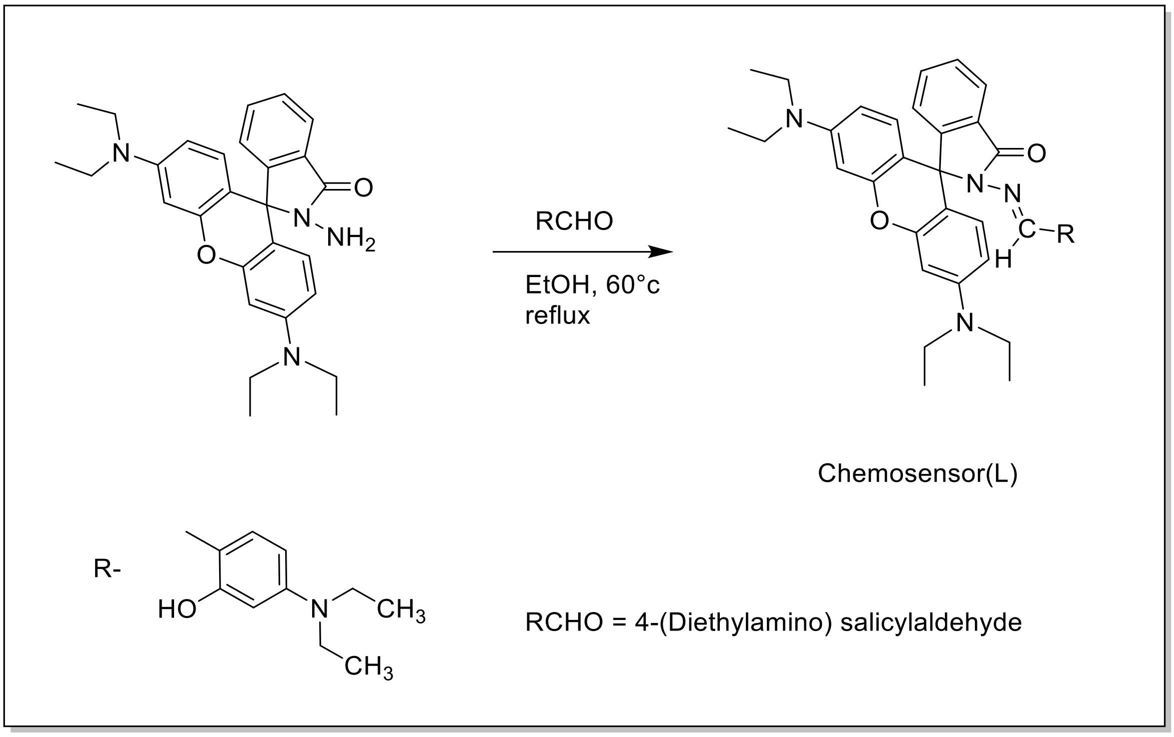
❖ *Synthesis Rhodamine B hydrazide:*

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 was 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.



❖ *Synthesis of Chemosensor (L):*

4-(Diethylamino) salicylaldehyde (17 mg) was added to 38mg Rhodemine B hydrazide , then 10 ml Ethyl alcohol added. After prepation 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 red solid.



❖ ***Preparation of metal ion solutions for fluorescent study*:**

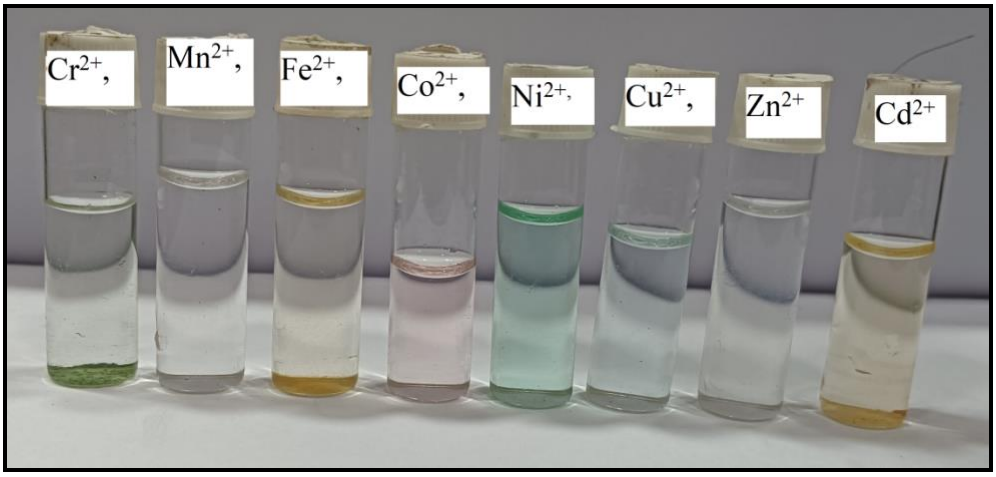
Stock solution (10mM) of the salts of Cu^{2+}, Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+} ions in distilled water were prepared.

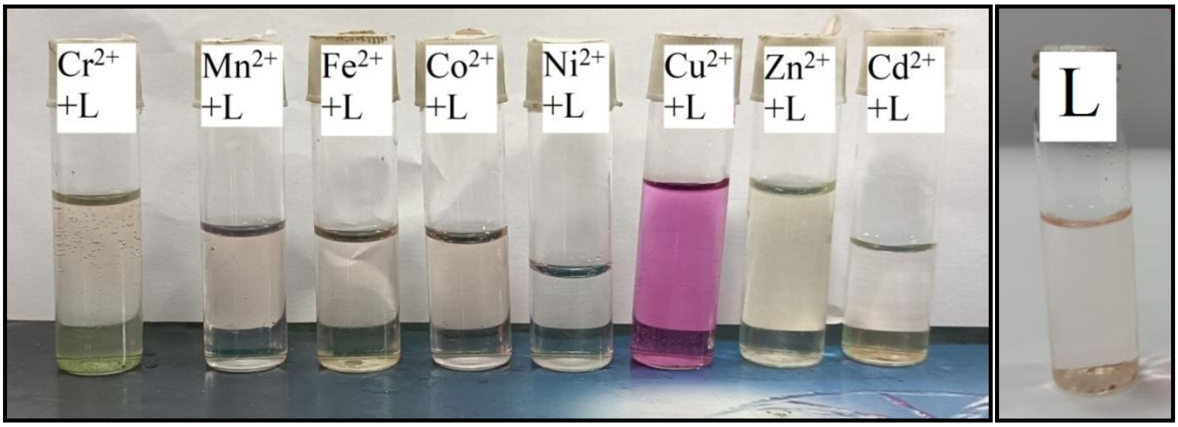
Stock solutions of probes (chemosensor L) 1 mM were also prepare in EtOH. The test solution were prepared by placing 30 µL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 3 ml with distilled water and EtOH.

Results and discussion:

❖ ***Colorimetric change:***

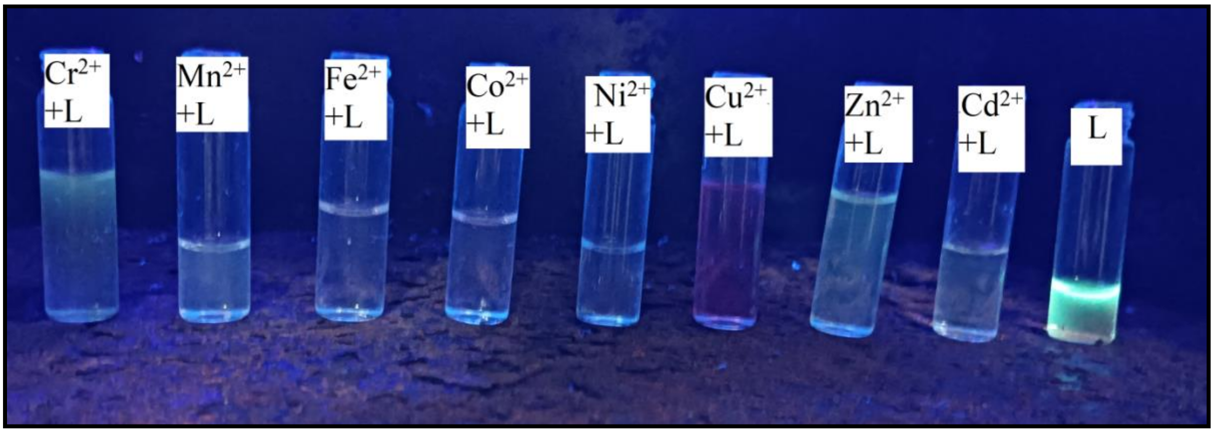
It is observed that when colorless solution of Cu^{2+} added with the solution of probe (Chemosensor L) then its turns to deep pink in color over the any other prepared solution of metal ions (Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+}) that is shown below.





❖ ***Fluorometric change:***

In presence of fluorescence light chemosensor solutions and chemosensor (L) with Cu^{2+} solutions is shown below.



Under UV lamp (λ_{max}=365nm)

❖ ***Spectral studies of L in presence* of ions :**

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

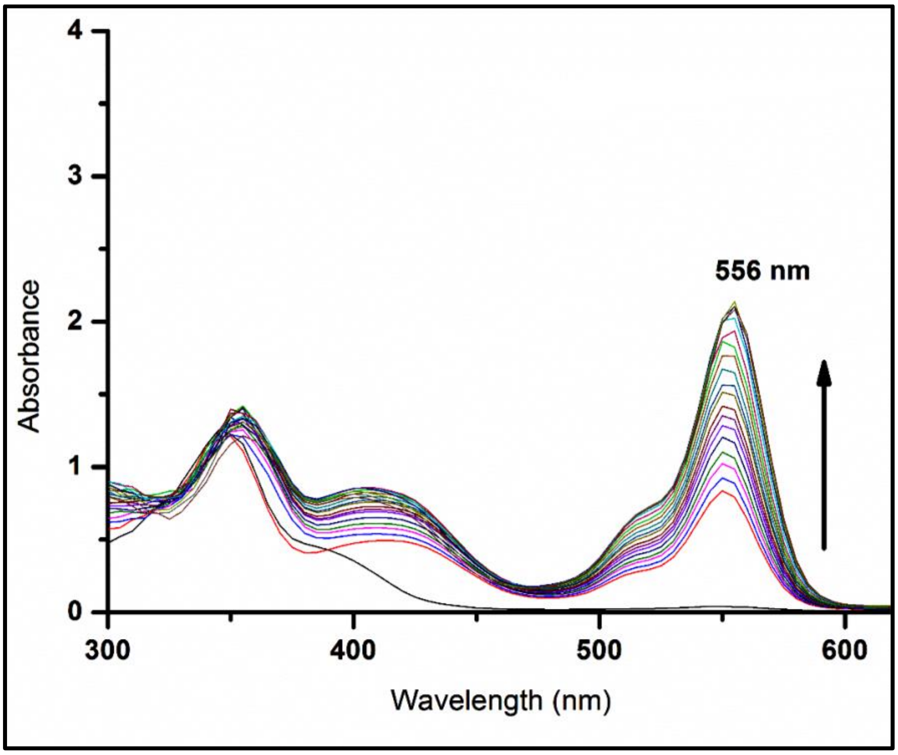


Figure 1- UV-Vis absorption spectra of probe **L** in presence of Cu^{2+} solution.

But in the presence of other metal ions (like- Fe^{2+}, Co^{2+}, $Ni^{2+,}$ Cr^{2+}, Mn^{2+}, Zn^{2+} and Cd^{2+}) Changes in the absorption spectra of L in 556 nm doesnot occurred as shown in figure 2.

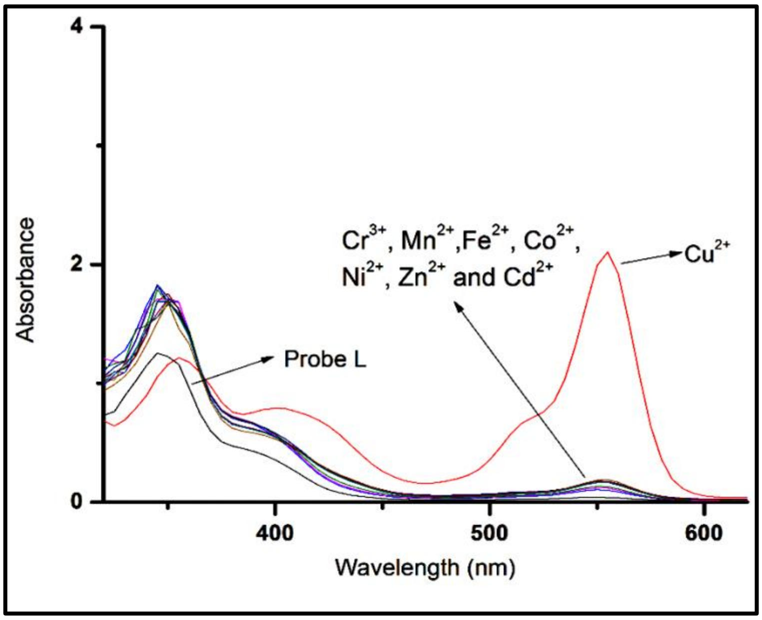
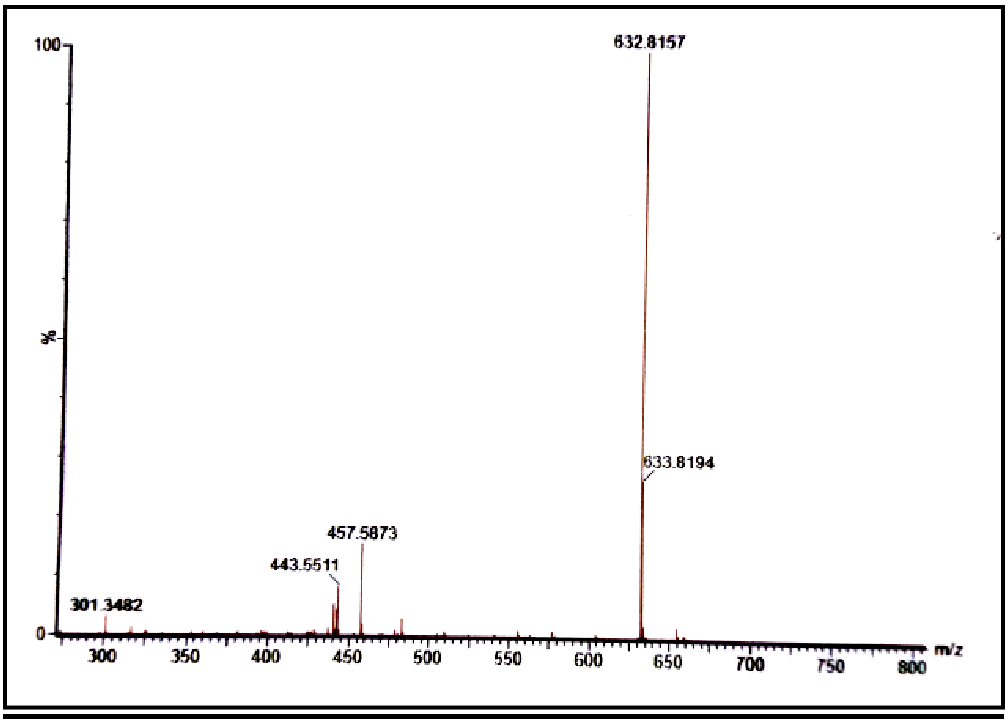


Figure 2- UV-Vis absorption spectra of probe **L** in presence of various cations.

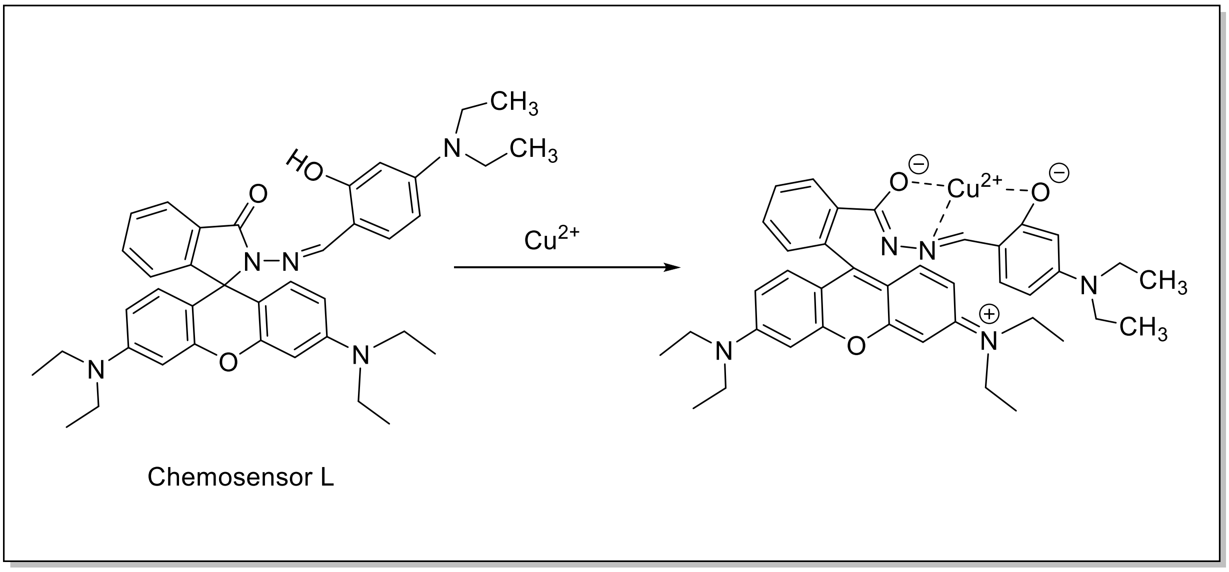
❖ ***Mass spectrum of probe L:***

`A highly intense peak was observed at 632 g/mole , indicates the formation of the chemosensor L.



❖ **Sensing Mechanism:**

Spirolactum ring opening occurred due to the presence of the chemosensor L and Cu^{2+} .



Conclusion:

In conclusion, we have reported a new rhodamine derivative-based colorimetric and fluorescent chemosensor for the detection of cu^{2+} in aqueous solution with high selectivity and sensitivity. The colorimetric and fluorometric changes are occured due to the chelation of cu^{2+} ion in rhodamine derivative (L) through spirolactum ring opening.

The future plan of work:

- The final spectroscopic characterization by ^1H NMR, ^{13}C NMR, and analysis of the advanced intermediate are in progressed.
- Further modifications and structural variations of rhodamine derivative to enhance the selectivity, sensitivity, and stability of the chemosensors for Cu^{2+} detection.
- Validation of the performance of the chemosensor in real-world samples, such as environmental water samples, industrial wastewater, and biological samples, to assess their practicality and reliability. Are left for the future plan of work.

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