

# **PROJECT REPORT ON**

## ***Rhodamine based Fluorometric and Colorimetric Chemosensor for the detection of $\text{Cu}^{2+}$ ion.***

Submitted in partial fulfillment of the Requirement for the degree of

**MASTERS OF SCIENCE IN CHEMISTRY-2023**

Submitted by

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Date – 31/07/2023



***Dedicated To My Parents***



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**HALDIA GOVERNMENT COLLEGE**

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I hereby forward this project work entitled ***Rhodamine based Fluorometric and Colorimetric Chemosensor for the detection of  $\text{Cu}^{2+}$  ion*** by GOBINDA GIRI 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 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 or approve any statement made, opinion expressed or conclusion drawn therein the thesis only for the purpose for which it is submitted.

**BOARD OF EXAMINER**

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## Acknowledgement

A moment comes which comes but rarely in a student's life, when with utmost pleasure and satisfaction, I myself, **GOBINDA GIRI**, submit my project on ***Rhodamine based Fluorometric and Colorimetric Chemosensor for the detection of Cu<sup>2+</sup> 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.

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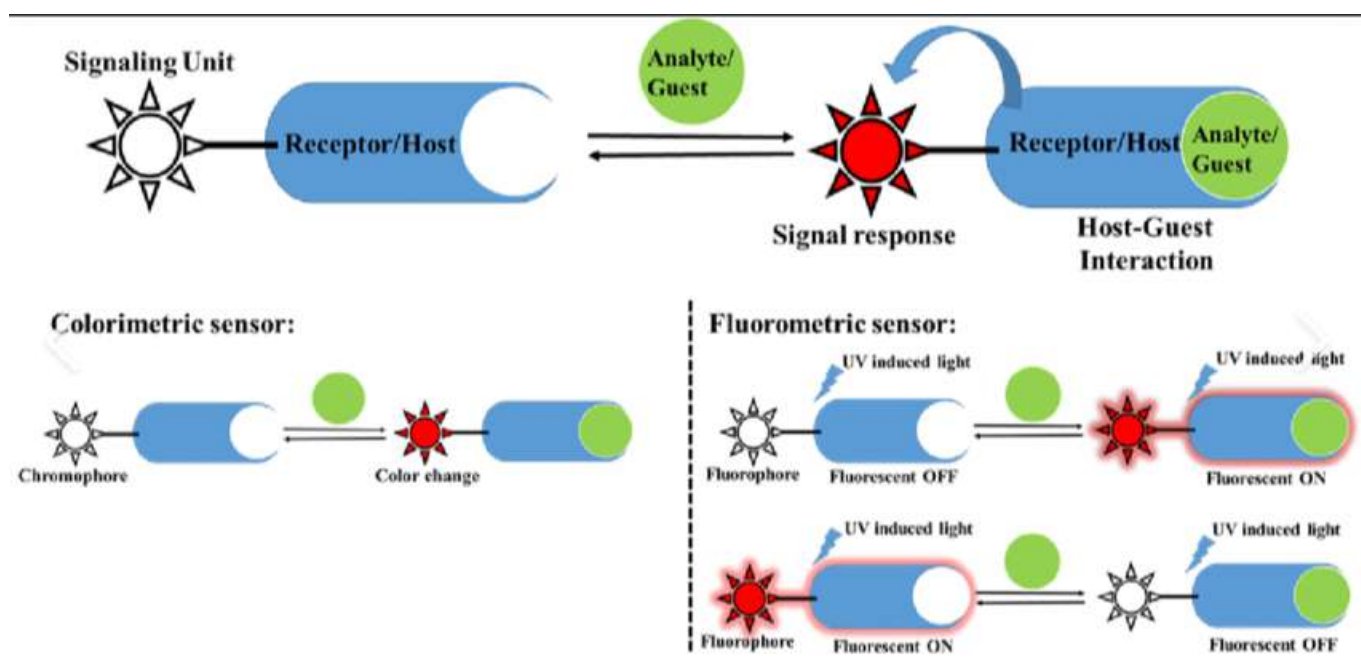


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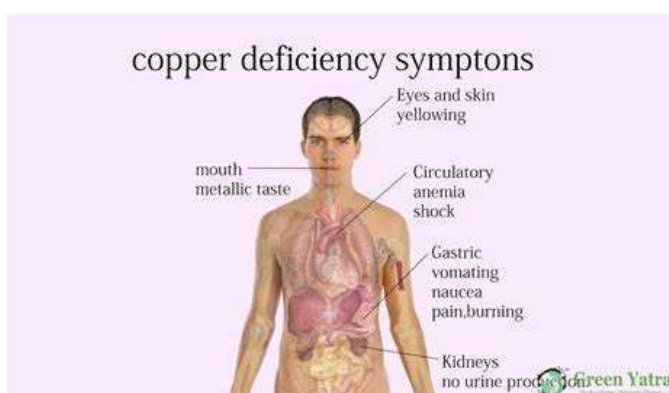
## Introduction:

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 Goppelsröder, 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.



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 ( $\text{Cu}^{2+}$ ) is an essential trace element for many biological processes and systems. However, at high concentrations, it can become an environmental pollutant. Therefore, there is a need to identify and quantify  $\text{Cu}^{2+}$  ions in various samples. This is why the development of efficient  $\text{Cu}^{2+}$  optical chemosensors, such as the rhodamine derivative described in the cited paper, is of great interest. One disadvantage of  $\text{Cu}^{2+}$  is that it can become an environmental pollutant at high concentrations. Copper ( $\text{Cu}^{2+}$ ) can pollute the environment through various sources and activities. One of the main sources of  $\text{Cu}^{2+}$  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  $\text{Cu}^{2+}$  pollution. When these substances are applied to crops or soil, they can leach into groundwater into nearby water bodies, causing contamination.





## Previous work:

1. A rhodamine-based fluorogenic probe bearing the quinaldine unit (L1) was developed by Huang et.al (2011)<sup>[1]</sup> as a turn-on fluorescent chemosensor for  $\text{Cu}^{2+}$ . Upon binding with  $\text{Cu}^{2+}$ , 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  $\text{Cu}^{2+}$ . Importantly, L1 can selectively respond to  $\text{Cu}^{2+}$  over other commonly coexistent metal ions (such as  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cr}^{3+}$ ) in aqueous media with a rapid response time .

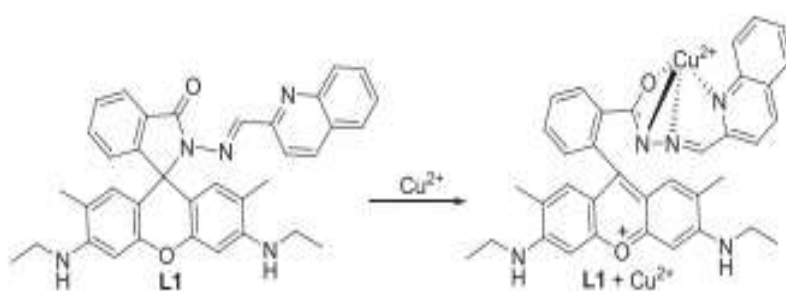
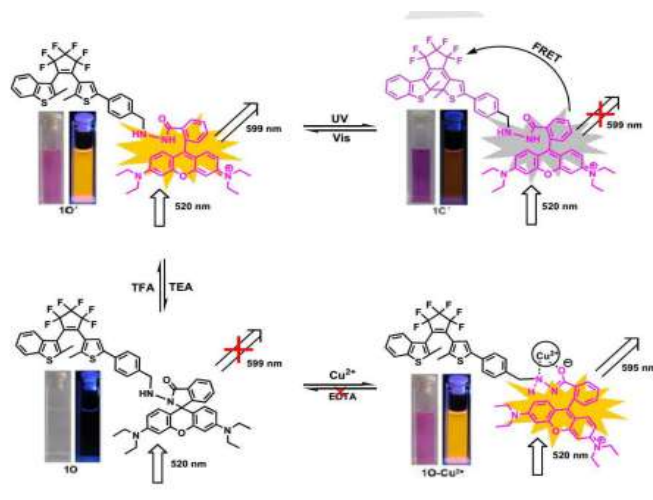
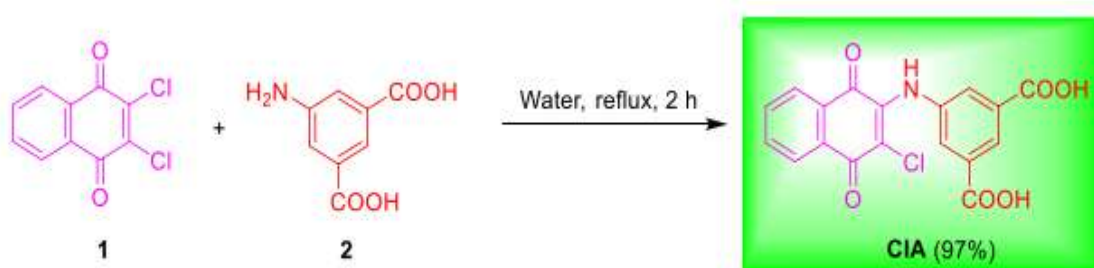


Fig. 4. Probable Complexation Mechanism of L1 with  $\text{Cu}^{2+}$ .

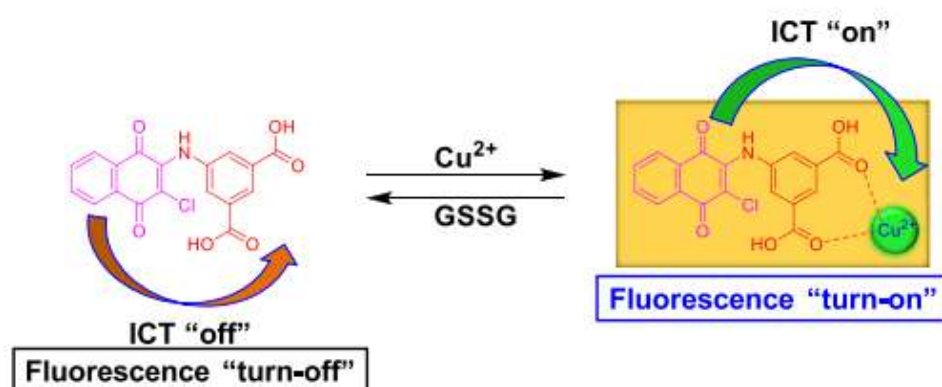
2. A new photochromic diarylethene with a phenyl-linked rhodamine B unit was synthesized and its photochemical properties were systematically studied by Shouzhi Pu et.al (2014)<sup>[2]</sup>. The diarylethene could be used as a multiple fluorescence switch by sequential stimulations with protons and 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  $\text{Cu}^{2+}$  in aqueous acetonitrile. When triggered by  $\text{Cu}^{2+}$ , 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.



3. Numerous chemosensors have been developed by Ravichandiran et.al (2021)<sup>[3]</sup> for next-generation detection systems because of their ease of use and promising characteristics to distinguish signals between various analytes binding. In this work, a simple, naphthoquinone-based mitochondria-targeting chemosensor (CIA) has been fabricated for the simultaneous detection of  $\text{Cu}^{2+}$  and GSSG (glutathione oxidized) through an “on-off” mode in a buffered semi-aqueous solution. Significantly, the CIA chemosensor showed a sensitive detection response towards  $\text{Cu}^{2+}$  and GSSG with low detection limits (0.309 mM, and 0.226 mM, respectively). These findings indicate that the chemosensor CIA can discriminate human cancer cells from normal cells. The CIA was also confirmed to possess the ability to target mitochondria. More importantly, the present CIA chemosensor detected  $\text{Cu}^{2+}$  in zebrafish larvae, indicating the probe has tissue penetration ability.



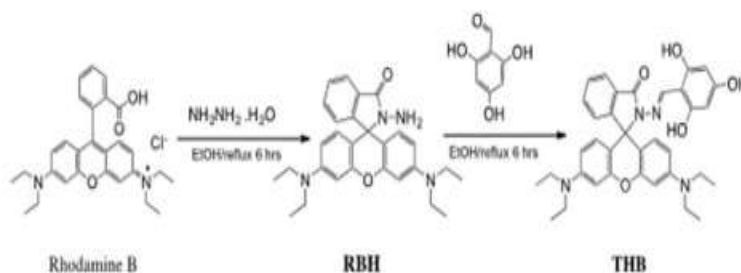
Scheme 1. Synthesis of the CIA chemosensor.



Scheme 2. The plausible detection mechanism of CIA towards  $\text{Cu}^{2+}$  and GSSG.

4. A new colorimetric “off-on” rhodamine based chemosensor for metal ions (2,4,6-trihydroxybenzaldehyde rhodamine B hydrazone, THB) was synthesized via Schiff base chemistry between rhodamine B hydrazide and 2,4,6-trihydroxybenzaldehyde by Wei Cheah et.al (2021)<sup>[4]</sup>. THB selectively turned pink in the presence of  $\text{Cu}^{2+}$ , and its

sensitivity was evaluated. Moreover, detection of  $\text{Cu}^{2+}$  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  $\text{Cu}^{2+}$  was verified. Additionally, THB can be reversibly used for up to three times with administration of EDTA and the coordination of THB with  $\text{Cu}^{2+}$  was corroborated by the shifting of carbonyl amide peak in the FT-IR spectra. The potential of THB as a simple and feasible sensor in multiple practical applications.

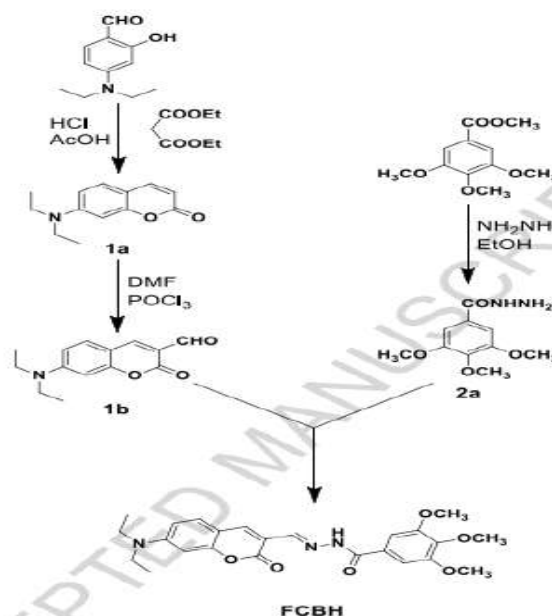


**Scheme 1.** Schematic representation for synthesis of RBH from Rhodamine B, and subsequently THB with addition of 2,4,6-trihydroxybenzaldehyde.

5. A novel coumarin-based receptor bearing a benzohydrazide (FCBH) was developed by Mergu et.al (2017)<sup>[5]</sup> as a fluorescent chemosensor with high selectivity toward  $\text{Cu}^{2+}$ . The sensor was successfully applied to the monitoring of  $\text{Cu}^{2+}$  in aqueous solution. After the addition of  $\text{Cu}^{2+}$  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  $\text{Cu}^{2+}$ . Upon the addition of  $\text{Cu}^{2+}$ , 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  $\text{Cu}^{2+}$ . Additionally, the quenched fluorescence of the FCBH-  $\text{Cu}^{2+}$  complex was restored upon the addition of  $\text{CN}^-$  ions. The possible sensing mechanism of FCBH toward  $\text{Cu}^{2+}$  was derived from experimental and theoretical examinations.

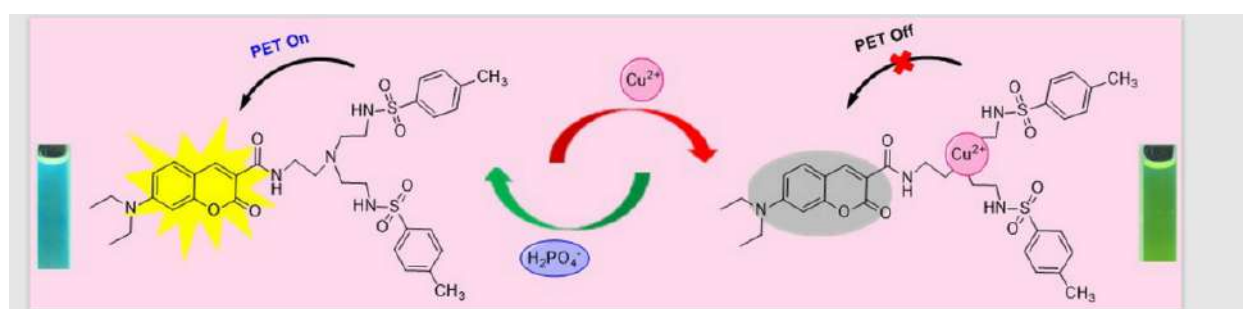


**Fig. 1.** Naked-eye and fluorescence images of FCBH (20  $\mu\text{M}$ , DMSO/water, 1:1) upon addition of various metal ions (1 equiv.).



**Scheme 1.** Synthesis of (E)-N'-((7-(diethylamino)-2-oxo-2H-chromen-3-yl)methylene)-3,4,5-trimethoxybenzohydrazide (FCBH).

6. In this study, a highly selective fluorescence chemosensor N-(2-(bis(2-((4-methylphenyl)sulfonamido)ethyl)amino)ethyl)-7-(diethylamino)-2-oxo-2H-chromene-3-carboxamide(1) was synthesized and characterized by Meng et.al (2018)<sup>[6]</sup>. This tren-based chemosensor 1 showed an “on-off-on” fluorescence response toward Cu<sup>2+</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> with a 1:1 binding stoichiometry in CH<sub>3</sub>CN/HEPES buffer medium (9:1 v/v) by UV/vis and fluorescence spectroscopies.. The stable pH range for Cu<sup>2+</sup> and H<sub>2</sub>PO<sub>4</sub><sup>-</sup> determination was from 4 to 7.5.



## 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  $\text{Cu}^{2+}$  ion.

#### ❖ Property of $\text{Cu}^{2+}$ :

Copper ( $\text{Cu}^{2+}$ ) has several properties that make it unique and useful in various applications:

Copper can exist in different oxidation states, with  $\text{Cu}^{2+}$  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.

#### ❖ Harmful effects caused by $\text{Cu}^{2+}$

Copper ( $\text{Cu}^{2+}$ ) can pollute the environment through various sources and activities. One of the main sources of  $\text{Cu}^{2+}$  pollution is industrial activities, such as mining, smelting, and manufacturing processes. The use of copper-based pesticides and fertilizers can also contribute to  $\text{Cu}^{2+}$  pollution. When these substances are applied to crops or soil, they can leach into groundwater. When electronic waste is not properly managed, it can release copper and other toxic substances into the soil and water. Copper can also accumulate in sediments and bioaccumulate in organisms, leading to toxic effects in higher trophic levels of the food chain.

#### ❖ 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. Colorimetric detection relies on changes in absorbance, while fluorometric detection measures changes in fluorescence intensity.

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

## 2. Experimental:

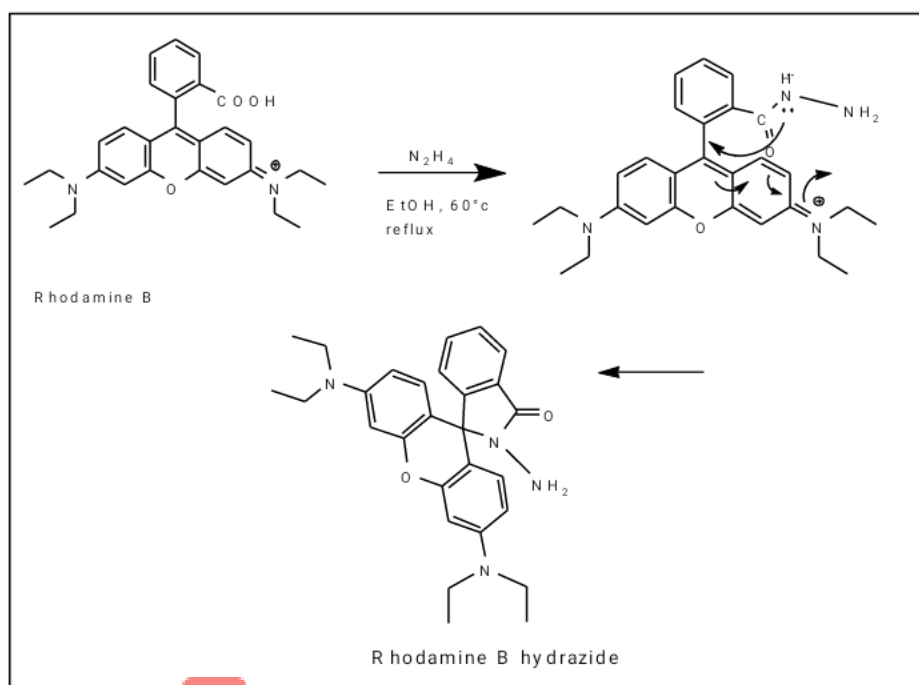
**2.1. Materials and equipment:** General methods unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Column chromatography was carried out on silica gel, also fluorescence and UV light was use.

### 2.2.1 Synthesis of Chemosensor (Rhodamine B derivatives L) :

#### ❖ 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.

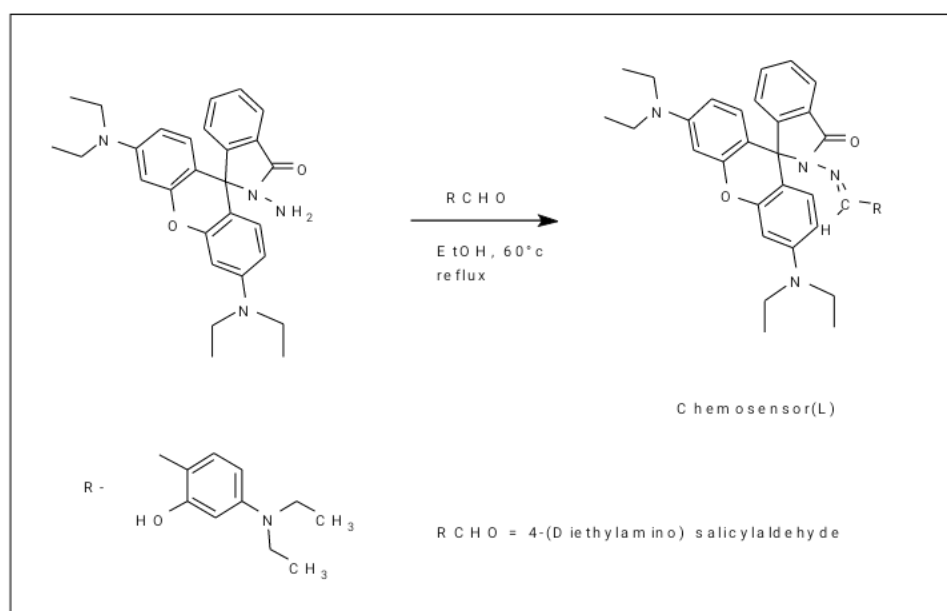
Reaction Scheme:



❖ 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 Persian orange solid.

Reaction Scheme:



❖ Preparation of metal ion solutions for fluorescent study:

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

Stock solutions of probes (chemosensor L) 1 mM were also prepared in EtOH. The test solution were prepared by placing 30  $\mu\text{L}$  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.

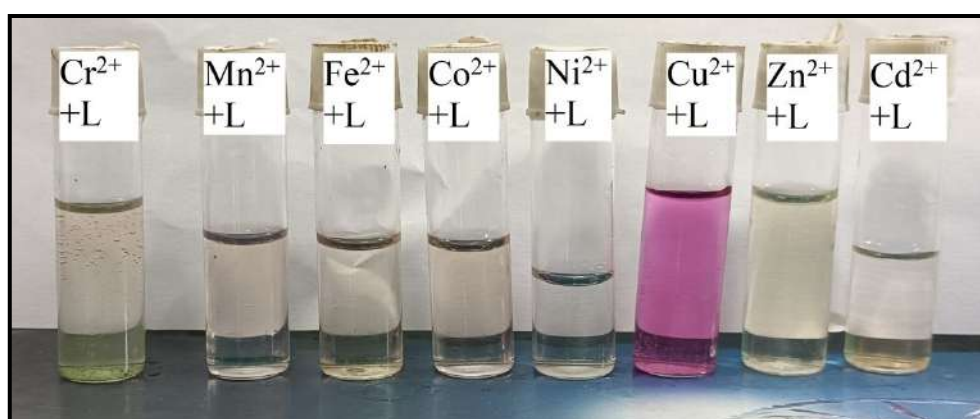
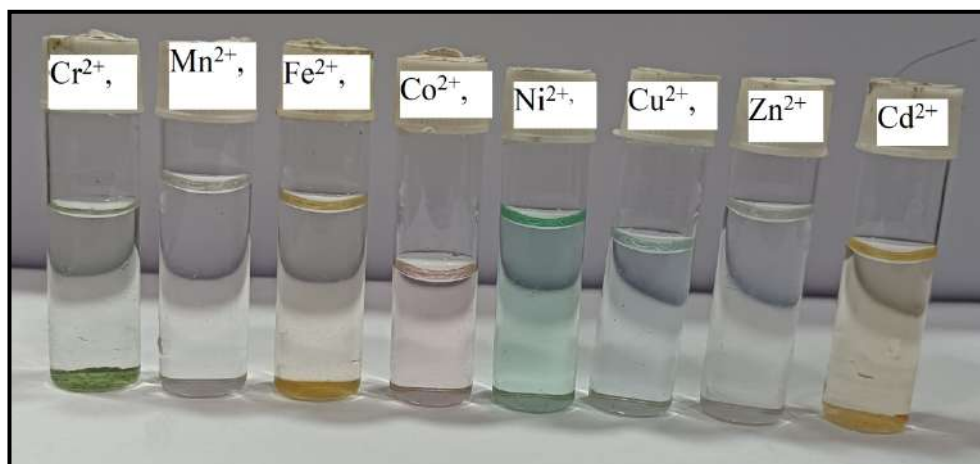
### 3. Results and discussion:

#### ❖ Colorimetric and fluorometric change:

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







Colorimetric change of metal cations in stock solutions in presence of Chemosensor (L)

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



Fluoremetric change of metal cations in stock solutions and Chemosensor (L) in presence of Fluorescence light.



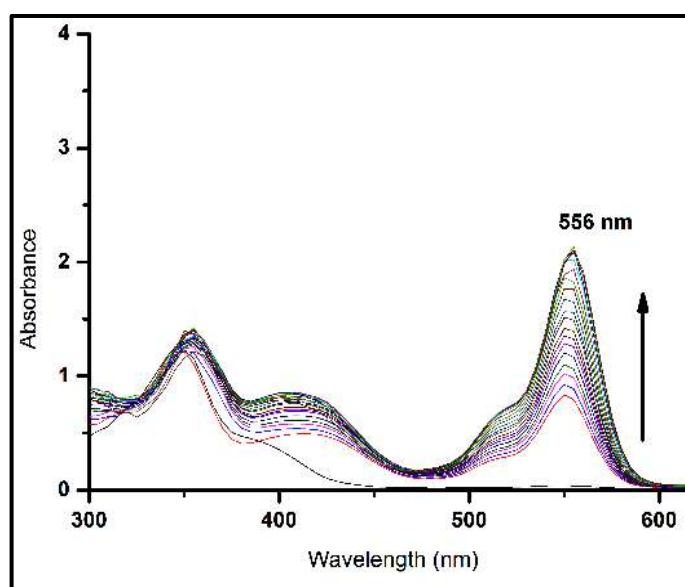
Chemosensor(L) in visible light  
presence of



Chemosensor(L) in  
Fluorescence light

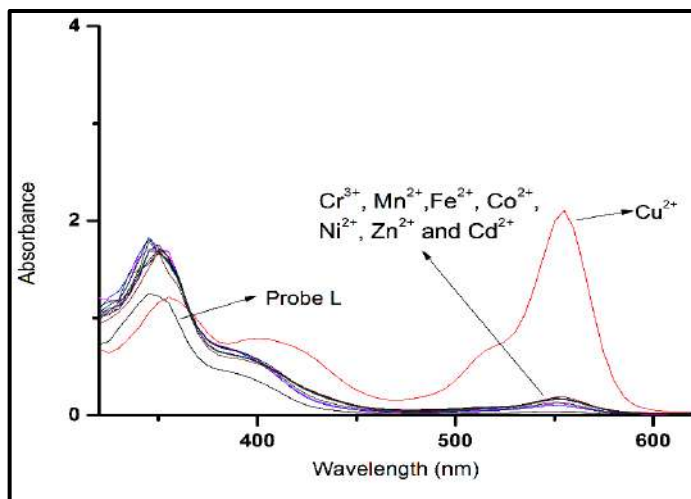
### ❖ Spectral studies of L in presence of ions :

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  $\text{Cu}^{2+}$  ion , the peak intensity at 556 nm increase.



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

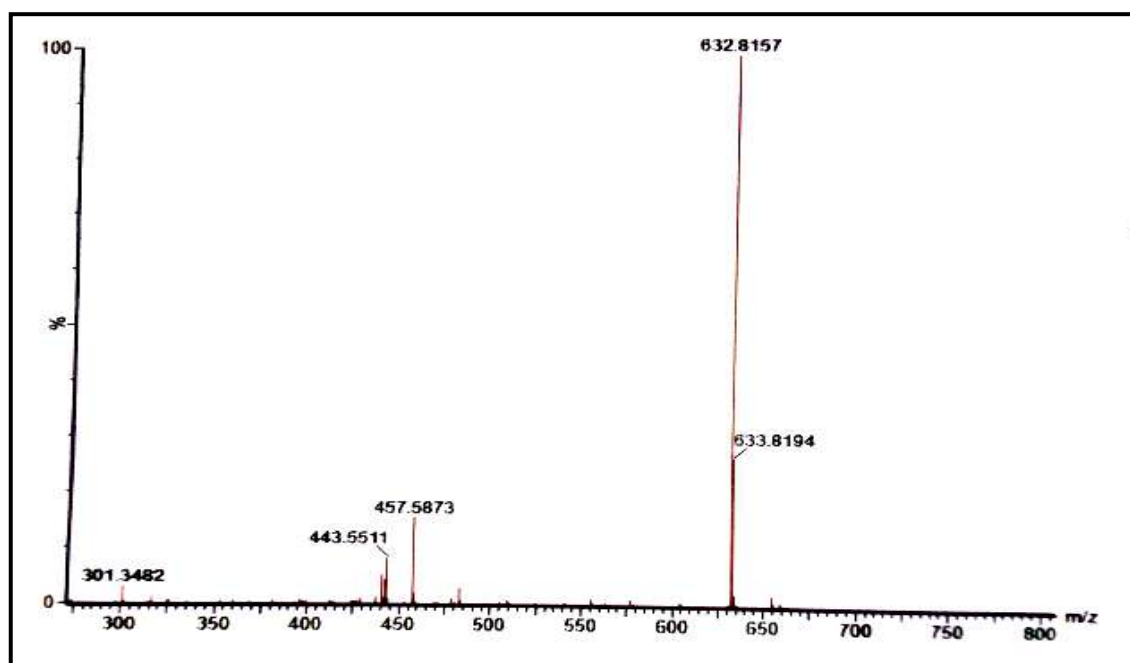
But in the presence of other metal ions ( like-  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cd}^{2+}$ ) Changes in the absorption spectra of L at 556 nm doesnot occurred as shown in figure 2.



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

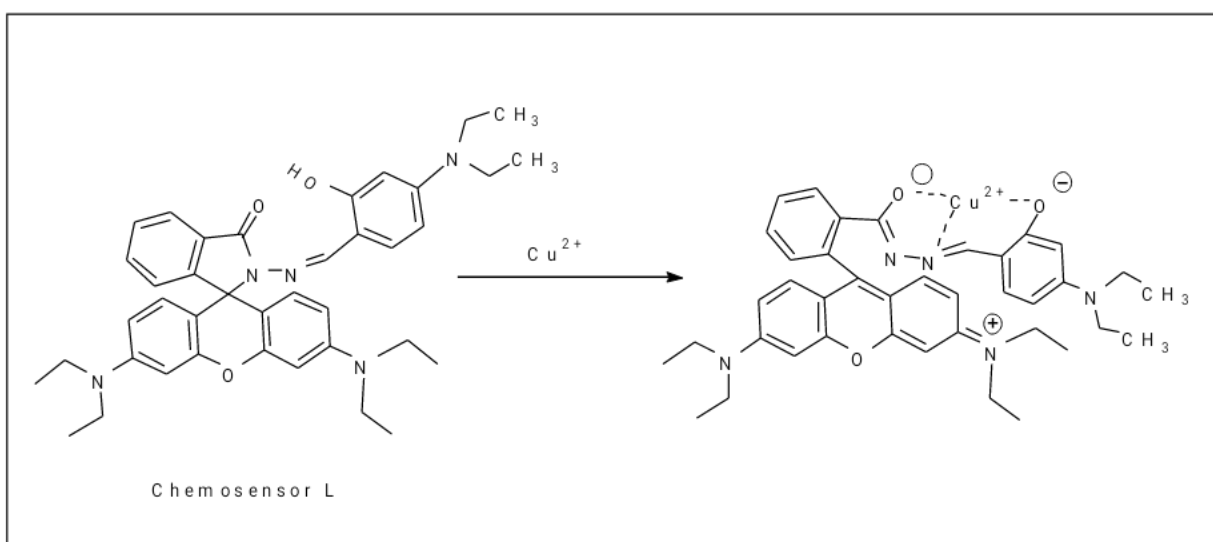
### ❖ Mass spectrum of probe L:

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



### Sensing Mechanism:

- ❖ Spirolactum ring opening occurred in Chemosensor due to the presence of the  $\text{Cu}^{2+}$  ion. Upon addition of  $\text{Cu}^{2+}$  ion in Chemosensor (L), the colour of Chemosensor changes from Persian orange to pink colour.



### Conclusion:

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

## ❖ The future plan of work:

- The final spectroscopic characterization by  $^1\text{H}$  NMR,  $^{13}\text{C}$  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  $\text{Cu}^{2+}$  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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