Egypt. J. Chem. **Vol. 68,** No. 10 pp. 201 - 225 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Rhodamine-based Colorimetric Chemosensor for Metal Cation Detection

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Abstract

Colorimetric chemosensors and fluorescent probes have gained popularity in recent years as a quick, cost-effective, and simple method for detecting and monitoring toxic species such as metal cations, anions, and toxic gases. This approach offers a more straightforward alternative to complex and time-consuming analytical techniques. Heavy metal ions pose a significant environmental threat due to their potential risks to ecosystems and human health. This review focuses on the latest advancements in colorimetric chemosensors using rhodamine derivatives for the detection of metal cations. The review discusses the synthesis, detection limit, sensitivity, and binding mechanism of rhodamine-based chemosensors with metal ions from 2010 to 2025. While these sensors have shown promise, there is still room for improvement in terms of repeatability and sensitivity. The goal of this review is to inspire researchers to explore new possibilities for enhancing rhodamine-based chemosensors in the future.

Keywords: Rhodamine; Colorimetric chemosensor; Fluorescent probe; Metal Cations; synthesis and bio-imaging.

1. Introduction

Metal ions play a vital role in various essential biological processes within the human body [1-7]. They are involved in regulating osmotic control, metabolism, biomineralization, and signaling. Elevated concentrations of specific metal ions such as Hg^{2+} and Pb^{2+} can have detrimental effects on health. Conversely, maintaining appropriate levels of essential metal ions like Cu^{2+}, Ca^{2+}, Fe^{3+}, and Zn^{2+} is crucial for normal metabolic activities. **Figure 1** illustrates the influence of variations in metal ion levels on various physiological functions in the body. [6, 8]. Thus, a major research question emerged: how to monitor and quantify these metal ions in a quick, simple, and inexpensive way even at ultra-low concentrations [9-18]. Colorimetric (or chromogenic) chemosensors are materials that change color or fluorescence when they bind to a specific chemical. This color change provides a visual indication that can be used in sensing systems. Colorimetric chemosensors are particularly useful for detecting the presence of anions, cations, and changes in pH, making them of great interest in various applications [15, 19-27]. Colorimetric chemosensors generally work according to one of two main principles [28, 29]. As shown at the top mechanism in **Figure 2**, the chemosensor molecule consists of a receptor subunit and another indicator (signalling) subunit that is modified after a binding occurs at the receptor component. The two subunits in this method are joined by a covalent bond, and the binding of the gust molecule causes the electronic structure of the created molecule to change, giving it new color characteristics.

The chemosensors receptor is connected to an indicator that changes color or fluorescence when specific stimuli are detected. This change occurs when the targeted guest interacts with the receptor, as shown in the mechanism at the bottom of **Figure 2**. This interaction involves molecular assemblies binding at specific sites on the molecule, resulting in the release of the indicator and a visible alteration in its optical characteristics. Lately, various techniques have been used for the detection of toxic species, including physical, chemical and biological methods. Complicated instruments such as spectroscopic apparatus (mass spectrometry, atomic absorption, and atomic emission spectroscopy), high-performance liquid chroma-tography (HPLC), and electrochemical sensors are used for detecting heavy metals in solutions.

Also pH changes can be detected by microelectrodes, acid-base indicator titration, and potentiometric titration. Nevertheless, most of these methods are characterized by complicated procedure and sometimes high cost process. Thus, improve of susceptibility, fast and low cost method for detection of toxic species is a major challenge nowadays. Therefore, colorimetric chemosensor and fluorescence probes provide a reliable alternative with a simple application in monitoring the analytes by naked eyes instead of using complicated instruments with high sensitivity levels and low coast [13, 28, 30-37]. Many studies being conducted today focus on developing novel colorimetric chemosensors and fluorescence probes that are sensitive, selective, and work at very low concentrations to monitor dangerous species. This obvious priority is anticipated given its important role in environmental preservation and health care, [28] which has been demonstrated in several beneficial applications and prevented danger in various fields [13, 38-41]. There are a wide variety of chromophores and fluorophores used in sensors to detect different analytes. These include coumarin, Schiff base, triphenylamines, indolo- quinoxaline, 1,8-naphthalamide, phenothiazine, xanthene derivatives, and many more [42-53], Thus this review presents the recent advances

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Receive Date: 29 December 2024, Revise Date: 10 February 2025, Accept Date: 16 February 2025
DOI: 10.21608/ejchem.2025.348625.11070

of the colorimetric chemosensor based on rhodamine moiety as a spiroring-opening system for detection of metal cations concerning with the synthesis and binding mechanism with metal cations and detection limit .

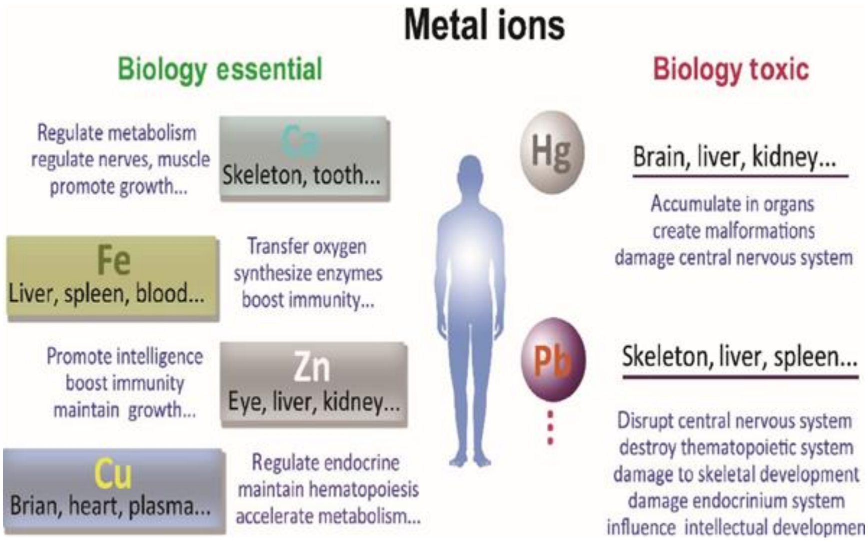


Figure 1: *Effect of metal ions on essential life processes [8].*

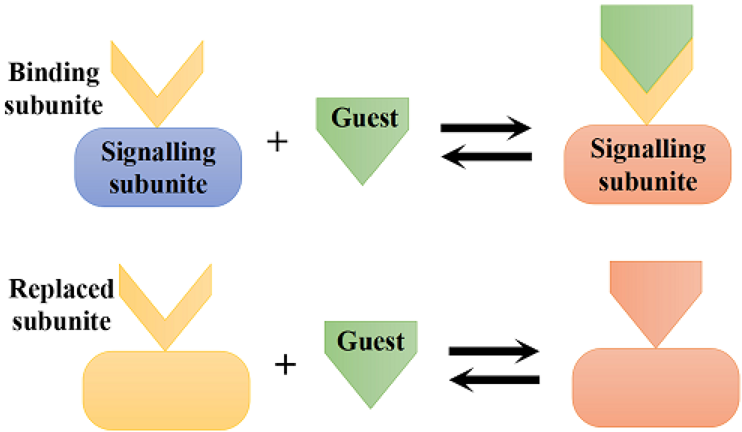


Figure 2: *Mechanisms of colorimetric chemosensor operation [29].*

2. Chemistry of Rhodamine

Rhodamine is a fluorophore that belongs to the xanthene family, along with fluorescein. The general structures of xanthene chromophore and rhodamine dyes are shown in **Figure 3**. Rhodamine has a distinct structural identity and finds wide applications in designing fluorescent sensors. In aqueous solutions, these dyes exist in two forms in equilibrium, a non-fluorescent ring-closed lactone form and a fluorescent ring-opened zwitterion form. The equilibrium between these two forms enables fluorescence to be switched on and off by analytes with high sensitivity [54, 55]. To create various rhodamine conjugates, a typical approach involves reacting a nucleophilic group in the target molecule with a 4'- or 5'-activated rhodamine derivative (shown in Figure 3B where G = activated ester, acyl chloride, sulfonyl chloride, or isothiocyanate). There are numerous activated rhodamine dyes available commercially, but they may contain a mix of isomers or be too costly if pure.

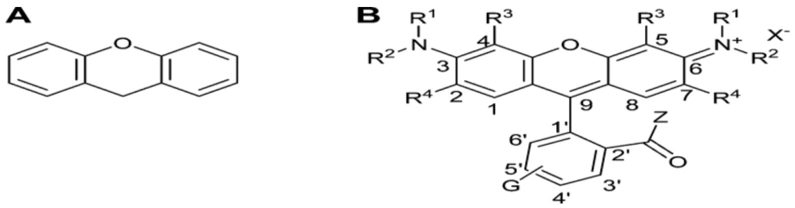
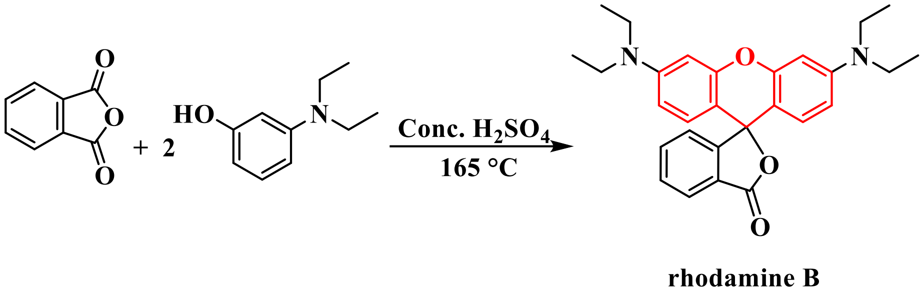


Figure 3: *Molecular structures of xanthene (A) and rhodamine dyes (B).*

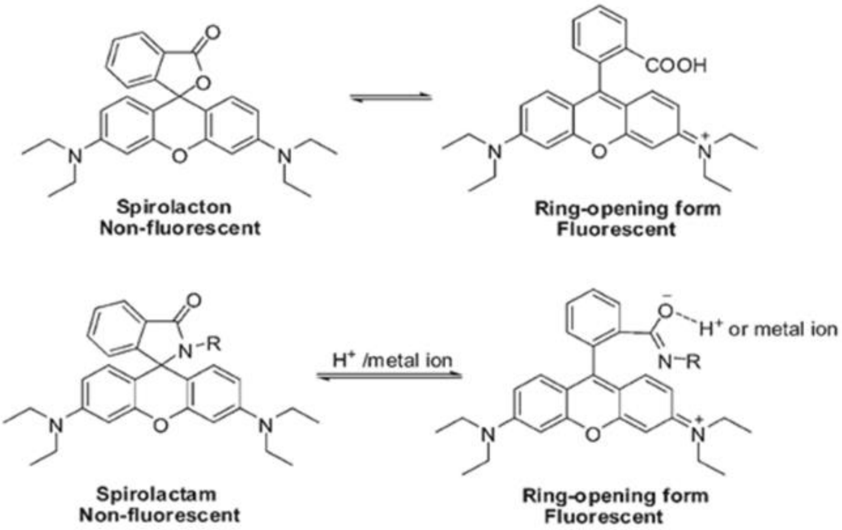
2.1. Synthesis of Rhodamine

Noelting and Dziewonsky were the first group synthesized rhodamine in 1905 [56] as shown in **Scheme 1**. It was carried out by direct condensation of phthalic anhydride with 3-(diethylamino) phenol in concentrated sulphuric acid and the mixture was heated for 48 hours at high temperature (165 °C).



Scheme 1: Synthesis of rhodamine B.

However, the discovery of the rhodamine spirolactam ring opening mechanism was only mentioned in 1997 by Dujols. It was used for the first time as a chemosensor for copper detection, making it an interesting compound for researchers [57]. The closed rhodamine spirolactam structure does not exhibit any emission spectra and is colorless. Upon opening of the spirolactam ring, the emission spectra of the opened ring significantly increases and a pink color appears, as shown in **Scheme 2**. Rhodamine B spirolactam/ spirolactone rings can be reconfigured and opened by various external factors. For example, in acidic conditions, the spirolactam ring of rhodamine B can be opened, resulting in a red color. Additionally, heavy metal ions can coordinate with carbonyl and amide groups, leading to an amplification of emission and a color change.



Scheme 2: *Ring-opening of spirocyclic rhodamine B.*

2.2. Rhodamine as Colorimetric Chemosensor

Rhodamine's sensitivity, selectivity, and quick signalling make it an attractive chemosensor for a variety of metal ions. Rhodamine-based chemosensors undergo structural changes upon ion binding, leading to detectable color or fluorescence signals that can be quantified for sensitive detection and monitoring of metal cations even in nano-scale concentration [58-60]. The concept behind rhodamine-based chemosensors for cations is to add metal chelating groups to rhodamine by changing its structure. The rhodamine dye acts as a fluorescence indicator by releasing light, and these metal-binding components function as receptors that attach selectively to the desired metal ion. The chemosensor modifies the fluorescence intensity or wavelength by causing a structural change in the rhodamine unit such as opening the spirolactam ring after connecting with the metal ion. Spectroscopic methods such as fluorescence and time-resolved fluorescence spectroscopy, along with fluorescence microscopy for intracellular monitoring, can be used to identify and quantify the change in emission. Rhodamine-based chemosensors have become important instruments in chemical sensing applications, providing advantages for diagnostics, environmental monitoring, and biological research. Due to its unique optical characteristics, which include significant Stokes shift, strong photostability, and tunable fluorescence, rhodamine dyes are a good choice for developing sensitive and specific chemosensors. Recent advances in synthetic chemistry and molecular design have increased the variety of rhodamine-based chemosensors that can identify different materials, such as organic molecules, metal ions, and biomolecules. These chemosensors can be used with various analytical platforms, like wearable sensors, paper-based sensors, and microfluidic devices, to satisfy particular sensing requirements. Rhodamine-based chemosensors are promising instruments for future sensing technologies because of their adaptability and versatility, which allow them to solve a variety of issues in various areas. **Figure 4 (I-A)** presents the first report of a rhodamine-based chemosensor, [57] while **(I-B)** show cases an early sensor with two rhodamine units in the scaffold [61]. **Figure 4 (I-C)** represents one of the earliest Schiff base type sensors[62]. Among the several rhodamine moieties, rhodamine 6G or B are frequently utilized (**Figure 4-II**). **Figure 4-III** depicts the typical sensing mechanism, where the presence of metal ions can cause the spirolactam ring to open. Ring opening is not always seen, however; some observations have indicated that the spirolactam ring does not open even after being exposed to metal ions. For monitoring the potential of the mitochondrial membrane, rhodamine 123 is one of the additional rhodamine sensors [63] and rhodamine 101 for DNA binding (Figure 4-IV) [64]. The sulforhodamine assay is used to determine cell density by measuring cellular protein content [65, 66]. Si-rhodamine is used for brain imaging and monitoring hydrogen sulfide (H_2S) levels in brain cells [67].

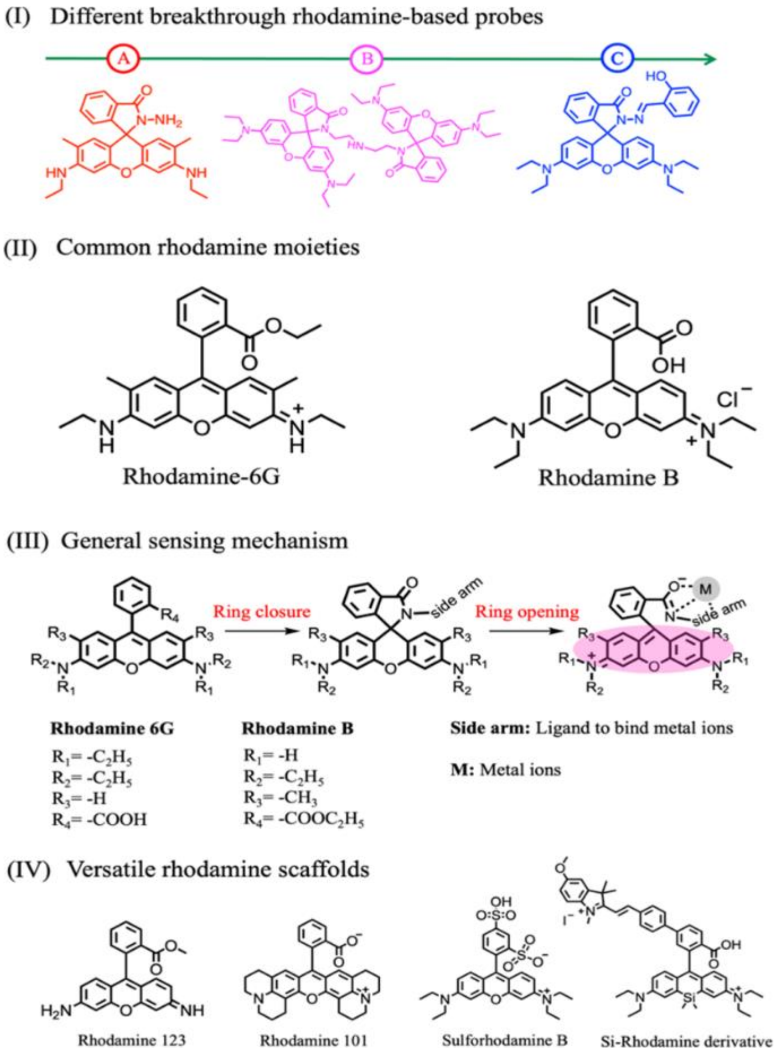


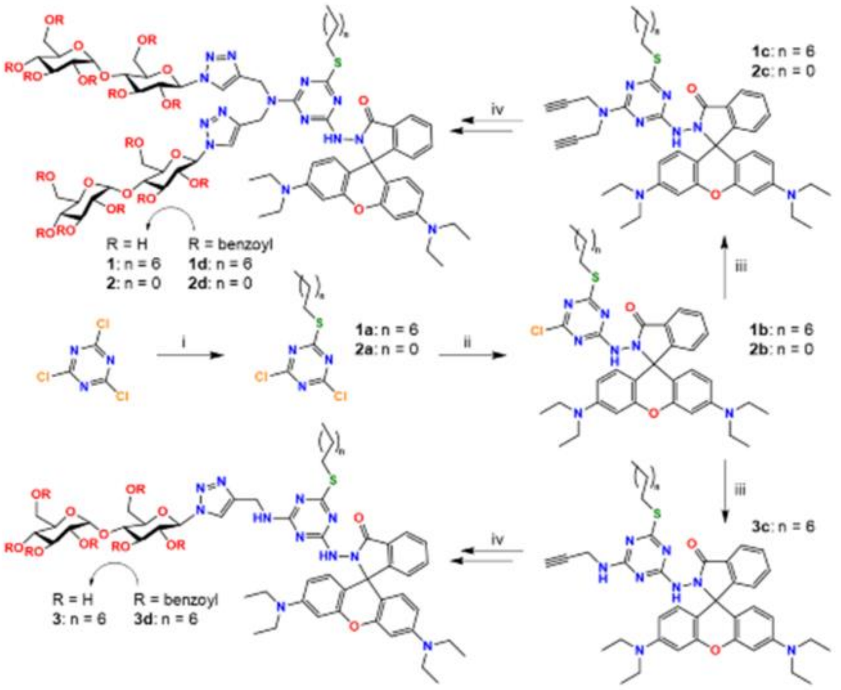
Figure 4: *Rhodamine based chemosensor and its ring opening mechanism.*

3. Colorimetric Chemosensors Based on Rhodamine moiety and related derivatives for metal cation recognition

3.1. Rhodamine based colorimetric chemosensor for Copper ion (Cu^{2+})

Cu^{2+} ion is one of the most fundamental heavy metal which became after iron and zinc [68], Cu^{2+} is a fundamental trace element that is involved in several biological processes [69, 70]. Many proteins and metalloenzymes, including dopamine-hydroxylase, tyrosinase, cytochrome c oxidase, lysyl oxidase, and superoxide dismutase, can contain copper ions as catalytic cofactors [71]. Copper is essential for various biological functions, such as electron transfer, oxygen metabolism, and iron metabolism through ceruloplasmin which oxidizes iron before it is transported in the blood to tissues [72]. Copper plays a crucial role in enzymes that scavenge free radicals and is essential for hormone biosynthesis. It is also involved in the biosynthesis of collagen and elastin, which are vital structural components of the skin, tendons, and extracellular matrix [73]. The increasing of the concentration limit of copper could be a serious risk for human body [74]. Overconsumption of copper can cause a number of health problems, including dyslexia, headaches, nausea, dizziness, vomiting, diarrhea, and irritation of the mouth, nose, and eyes. It can also lessen water systems' capacity to purify themselves and interfere with the body's normal detoxification processes. On the other hand, low copper levels can cause illnesses like Menkes disease, anemia, hypothermia, impaired immune system, and reduced organ function [75-77]. Thus the easy, fast and accurate detection and monitoring of copper cation in aqueous media for environmental and biological samples is a preferable to avoid its toxicity problems [78].

Jeong et al. developed and prepared three water-soluble colorimetric fluorescent sensors based on 1, 3, 5-triazine core and rhodamine B hydrazine as presented in **Scheme 3** [79]. The most successful sensor for detecting Cu^+ in aqueous solutions that change from colorless to pink in daylight and emit a strong yellow fluorescence was sensor 1, which had an octyl chain and a branched maltose group. These sensors were modified with a hydrophobic alkyl chain, a hydrophilic maltose, and a rhodamine B hydrazine fluorophore. The prepared sensor 1 had a limit of detection of 20 nM and a dissociation constant of 1.1 × 10−12 M.



Scheme 3: *Synthesis scheme of probes 1–3. Reagents and conditions: i) K_2CO_3, alkyl thiol, THF, RT or 0 ℃, 2 hr; ii) rhodamine B hydrazide, THF, reflux, overnight; iii) DIPEA, propargyl or dipropargyl amine, THF, reflux, overnight; iv) perbenzoylated maltosyl azide, $Cu(SO_4)_2{\cdot}5H_2O$, sodium ascorbate, THF, water, RT, overnight; NaOMe, DCM, MeOH, RT [79].*

Sensor 1 was utilized to differentiate the intracellular Cu^+ levels in cancer cells compared to normal cells. This indicates the potential of the probe for Cu^+ bioimaging and disease detection, as shown in **Figure 5** [79].

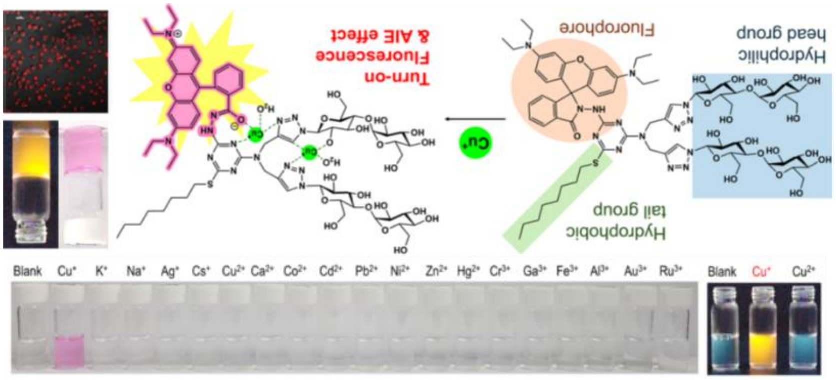
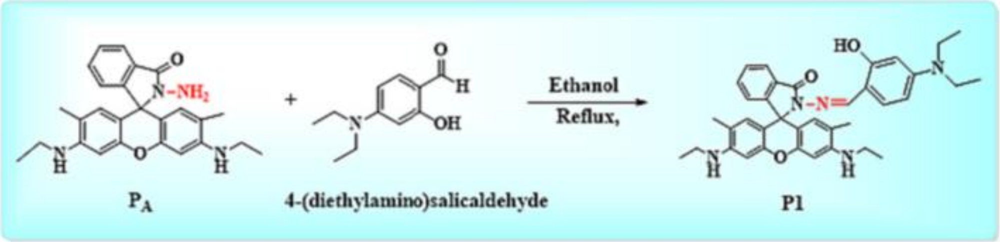


Figure 5: *Binding mechanism of the prepared rhodamine based sensor (sensor1) and its selectivity with metal cations in aqueous media and the bio-image application [79].*

Mulimani et al. prepared rhodamine-based Schiff base **P1**[2-((4-(diethyl amino)-2-hydroxybenzylidene) amino)-3′, 6′-bis (ethyl amino)-2′, 7′ dimethyl Spiro [isoindoline-1, 9′-xanthen]-3-one] by a condensation reaction of 4 (diethylamino) salicylaldehyde with rhodamine 6G hydrazide as presented in **Scheme 4** [80].



Scheme 4: *Synthetic rout of synthesized sensor P1 [80].*

P1 has shown impressive ability to detect Fe^{3+} and Cu^{2+} ions and the color of solution changed from colorless to pink for Fe^{3+} and orange for Cu^{2+} ions as shown in **Figure 6**. This color change occurred due to a charge transfer mechanism with a 1:1 stoichiometry between the metal ions and **P1**. The limit of detection for **P1** with Fe^{3+} and Cu^{2+} ions was found to be 1.09µM (0.06 ppm) and 7.7µM (0.48 ppm), respectively [80].

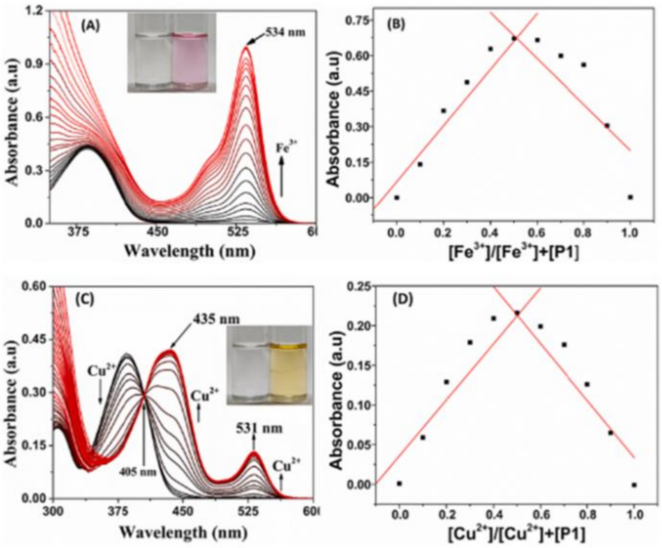


Figure 6*: (A) UV–visible titration spectral changes of **P1** with Fe^{3+}ions (B) Job's plot for **P1** with Fe^{3+} ions, (C) UV–visible titration spectral changes of **P1** with Cu^{2+} ions, and (D) Job's plot for **P1** with Cu^{2+} ions [80].*

The high sensitivity of **P1** in detecting metal ions at low concentrations has led to the creation of a straightforward, selective, and affordable paper strip device and swab kit, as shown in **Figure 7** [80].

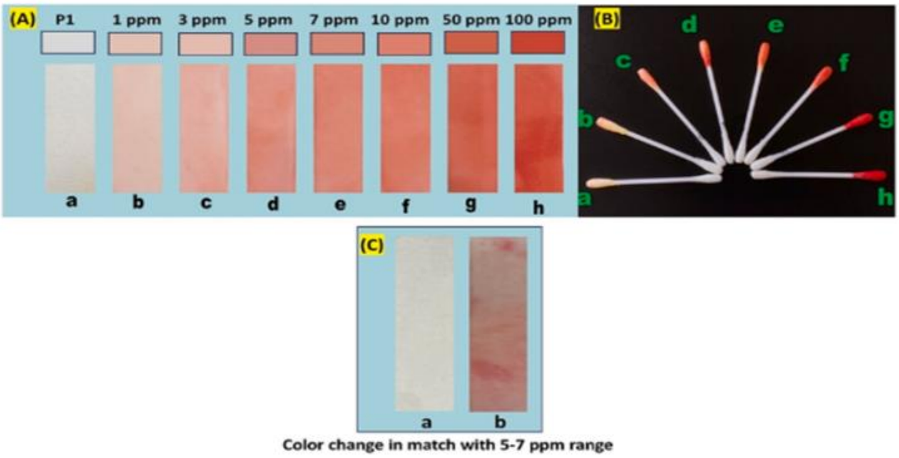
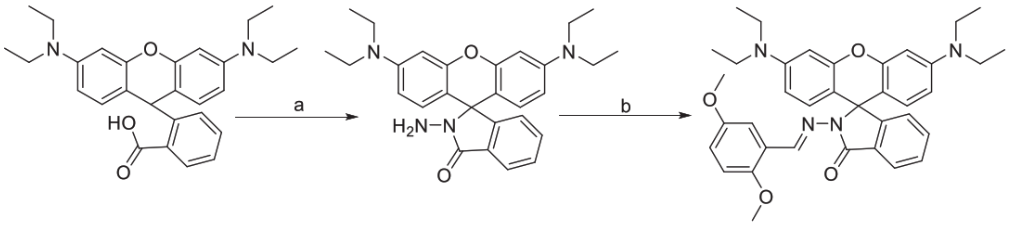


Figure 7: *Change in the color of **P1** coated (A) test paper, (B) swab sticks, after the addition of Cu^{2+} ions, and (C) Change in the color of **P1** coated with test paper after addition of (a) Blank **P1**, (b) Cu industrial effluent [80].*

Segura et al. reported colorimetric and fluorescence probe(E)-3′,6′-bis(diethylamino)-2-((2,5-dimethoxy benzylidene)amino)spiro[isoindoline-1,9′-xanthen]-3-one (**WGB**) for copper cation detection based on rhodamine derivative which prepared by a two-step reaction of rhodamine B (I) with hydrazine hydrate in methanol as shown in **Scheme 5** [81].



I **II** **WGB**

Scheme 5: *Synthetic route for Rhodamine derivative (**WGB**). Reagents and conditions: a) hydrazine hydrate, MeOH, reflux; b) 2, 4-dimethoxy benzaldehyde, r.t. 24 h. [81].*

The prepared sensor **WGB** shows very high sensitivity towards copper cation detection forming 1: 2 complex which record detection limit 6.76 × 10-8 M as well as high selectivity without any significant interference with other metals and the color changed from yellow to pink, with a significant increase in fluorescence due to the ring opening of the rhodamine spirolactam with charge transfer to one of the ethylamino groups of rhodamine. Furthermore, **WGB** was applied in bioimaging for monitoring SH-SY5Y cells as in **Figure 8** [81].

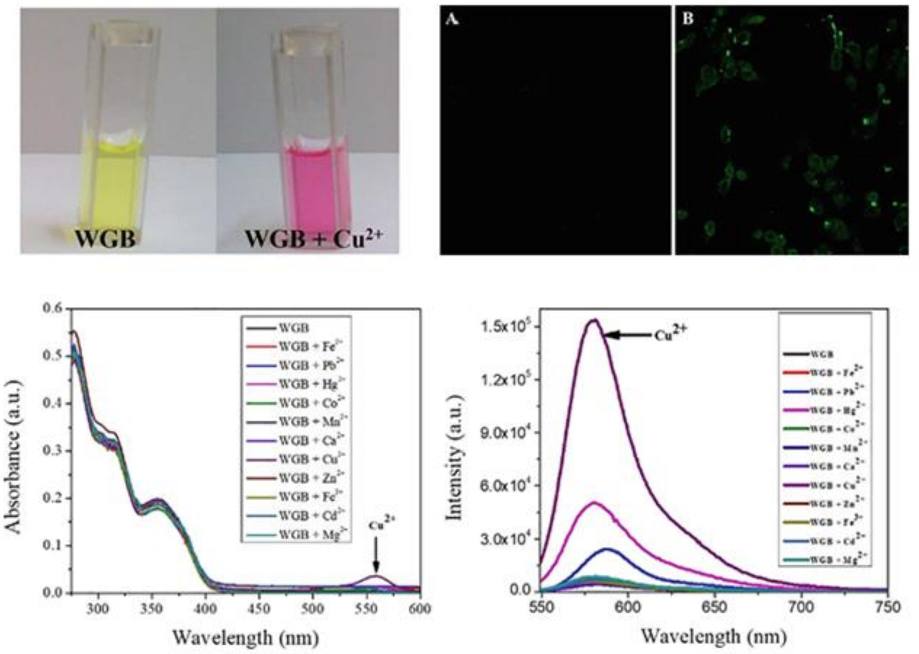
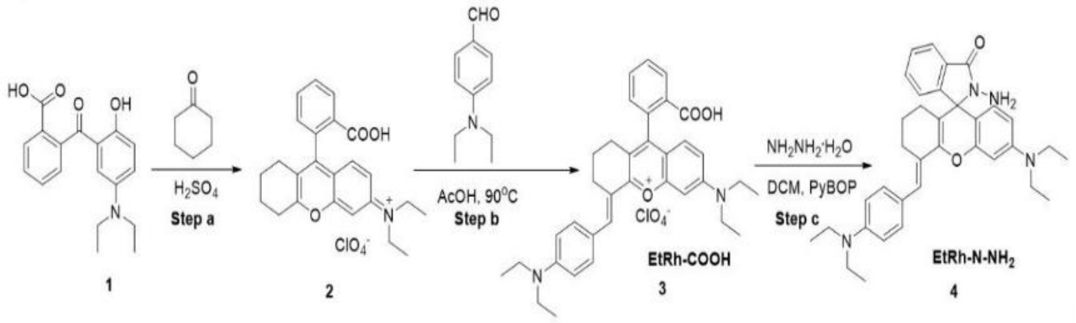


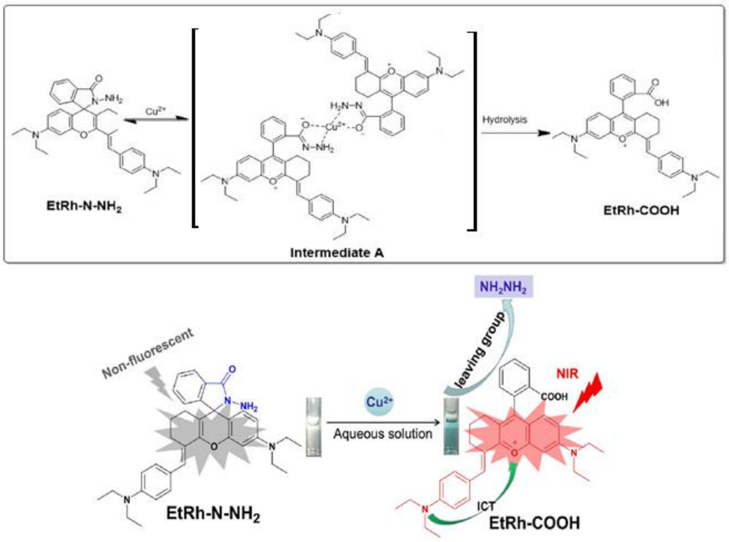
Figure 8: *The colorimetric change and fluorescen for the rhodamine derivative (**WGB**) in the presence of Cu^{2+} and different metal cations as well as the bioimaging application for SH-SY5Y cell [81].*

Hu et al. have reported a near-infrared (NIR) fluorescence chemosensor **EtRh-N-NH$_2$** for the detection of Cu (II). **EtRh-N-NH$_2$** was prepared through a three steps benzoic acid. Firstly, the reaction of compound **1** and cyclohexanone generates the three-membered ring intermediate **2** in the presence of concentrated sulfuric acid as shown in **Scheme 6** [82]. Then compound starting with 2-(5-(diethylamino)-2-hydroxyl) **2** was condensed with 4-diethylamin obenzaldehyde to form fluorophores **EtRh-COOH**. Finally, a ring-closure action was done on the carboxyl moiety of the fluorophore to produce the aminohydrazide probe **EtRh-N-NH$_2$**.



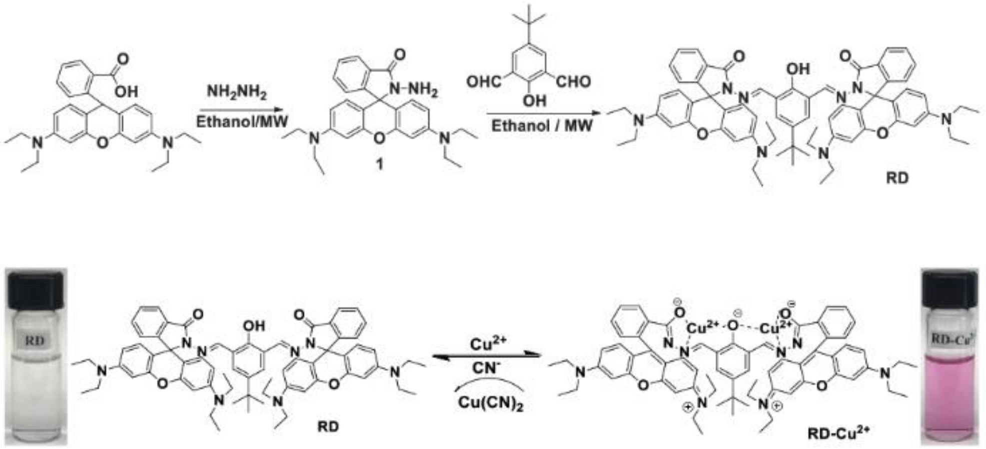
Scheme 6: *synthesis of* ***EtRh-N-NH$_2$*** *sensor [82].*

Scheme 7 illustrates how Cu^{2+} interacts with the sensor EtRh-N-NH2 by forming a link and chelating with its carbonyl and amino groups. This interaction leads to the hydrolysis of the hydrazide, producing the highly fluorescent EtRh-COOH. The sensor has a high detection limit of 6 nM. The resulting product, EtRh-COOH, emits light at a wavelength of 762 nm in the near-infrared (NIR) region, with a Stokes shift of up to 75 nm [82].

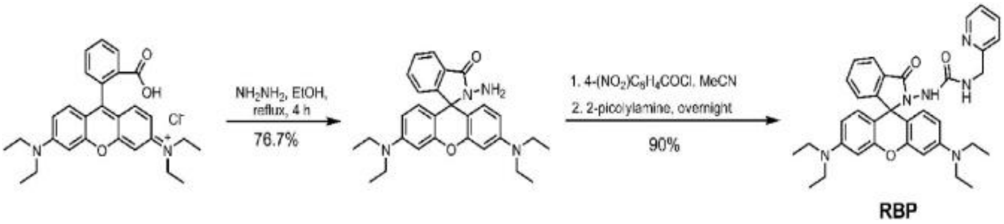


Scheme 7: *Proposed mechanism of* ***EtRh-N-NH$_2$*** *sensor for binding and fluorescent turning-on process [82].*

Abebe et al. designed and synthesized a novel sensor (**RD**) bearing rhodamine B and 4-tert-butyl phenol unit using microwave irradiation as shown in **Scheme 8**. The sensor shows high selective detection of Cu^{2+} by forming 1: 2 complex and the color changed from colorless to pink which was observed with naked eye due to ring-opening of spirolactam as in **Scheme 8**. As well as the prepared sensor (**RD**) showed high sensitivity toward copper cation detection which record detection limit 0.26µM [83].



Scheme 8: *Synthetic routs and proposed binding mechanism for rhodamine derivative sensor* ***RD*** *[83].*



Scheme 9: *Synthesis of **RBP** sensor [55].*

Wechakorn et al. synthesized a colorimetric and fluorescent sensor, selective for Cu^{2+} ion based on rhodamine semicarbazide conjugated picolyl- amine (**RBP**) as presented in **Scheme 9** [55]. The synthesized sensor **RBP** shows high selectivity for Cu^{2+} ions compared to other metal ions. This selectivity is confirmed by the color change from colorless to pink and significant fluorescence emission. **RBP** also demonstrates high sensitivity to Cu^{2+} ions, with a rapid reaction time of one minute and a low detection limit of 5.8 nM. In the presence of Cu^{2+} ions, the ring-opening mechanism of **RBP** triggers the fluorescence response, which is subsequently followed by a hydrolysis reaction.

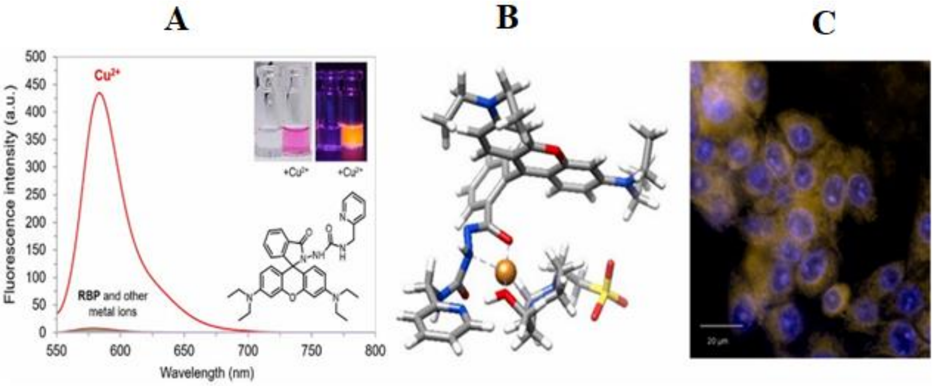


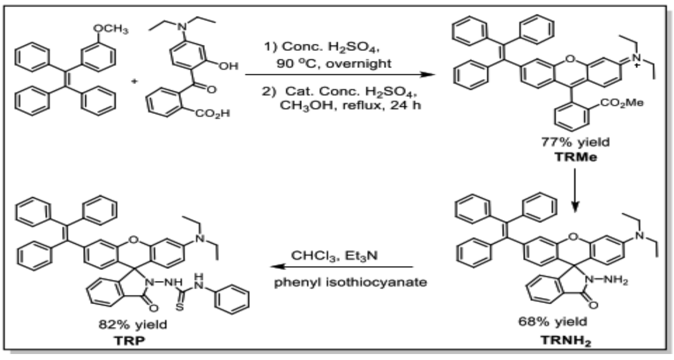
Figure 9: *Emission spectra (A), DFT study (B) and bioimaging (C) of the prepared **RBP** sensor* [55].

The photophysical properties of **RBP** were investigated in MeCN/HEPES buffer, including UV–Vis absorption and fluorescence emission. Additionally, the **RBP** sensor was successfully utilized for detecting Cu^{2+} ions in real water samples (A) and fluorescence imaging in HepG2 cells (C) as presented in **Figure 9**. The binding mechanism with copper was investigated by Job's plot and DFT study (B) which confirming the formation of 1:1 complex between the semicarbazide-picolylamine moiety of **RBP** and copper ion.

3.2. Rhodamine based colorimetric chemosensor for Mercury ion (Hg^{2+})

Mercury and its ionic form are highly toxic to the environment and human health [84, 85]. The dangerous effect of mercury, even at very low concentrations, is caused by its easy conversion into highly toxic organo-mercury compounds by bacteria in the environment. These compounds then bio-accumulate through the food chain [86, 87]. Mercury, even at very low concentrations of over 2 ppb in the human body, can cause serious diseases and problems. It has the ability to easily and quickly penetrate the outer skin, lungs, and cell membranes, resulting in DNA damage, impairment of mitosis, and permanent damage to the central nervous system [88, 89].

Li et al. designed and synthesized a tetraphenyl ethylene functionalized rhodamine chemosensor (**TRP**) as shown in **Scheme 10** [90]. The synthesized **TRP** was showing a ratiometric sensing performance for detection of Hg^{2+} which showing a significant colorimetric change from colorless to purple-red color while no significant effect was observed with other metal cations.



Scheme 10: *Synthetic procedure for the synthesis **TRP** chemosensor* [90].

The synthesized **TRP** was showing a ratiometric sensing performance for detection of Hg^{2+} which showing a significant colorimetric change from colorless to purple-red color while no significant effect was observed with other metal cations. Additionally a ratiometric emission was turned on and a significant emission was recorded at 463, 649 nm as presented in **Figure 10**.

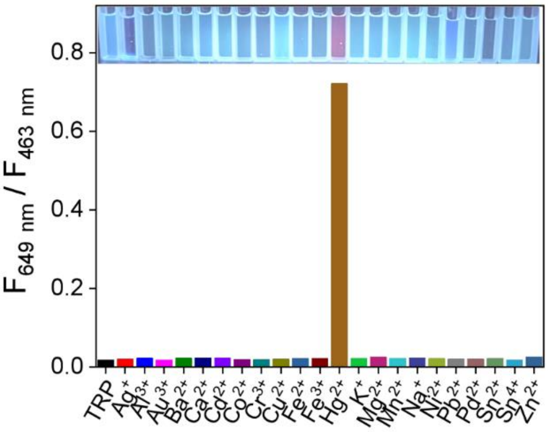
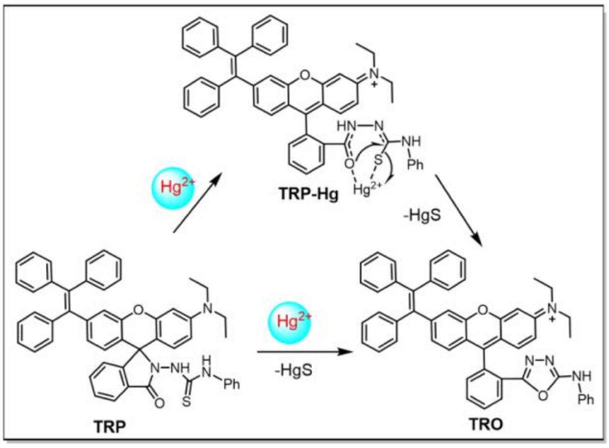


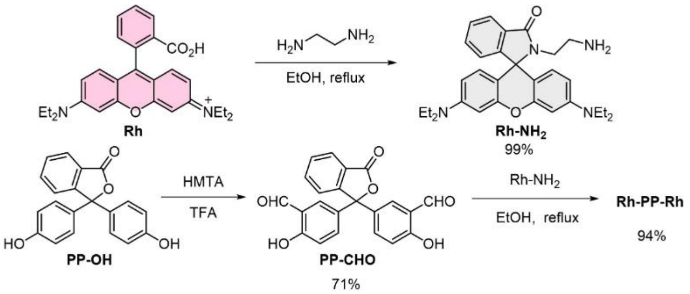
Figure 10: *Emission spectra of the prepared* ***TRP*** *after binding with* Hg^{2+} *[90].*



Scheme 11: *The binding mechanism of* ***TRP*** *with* Hg^{2+} *[90].*

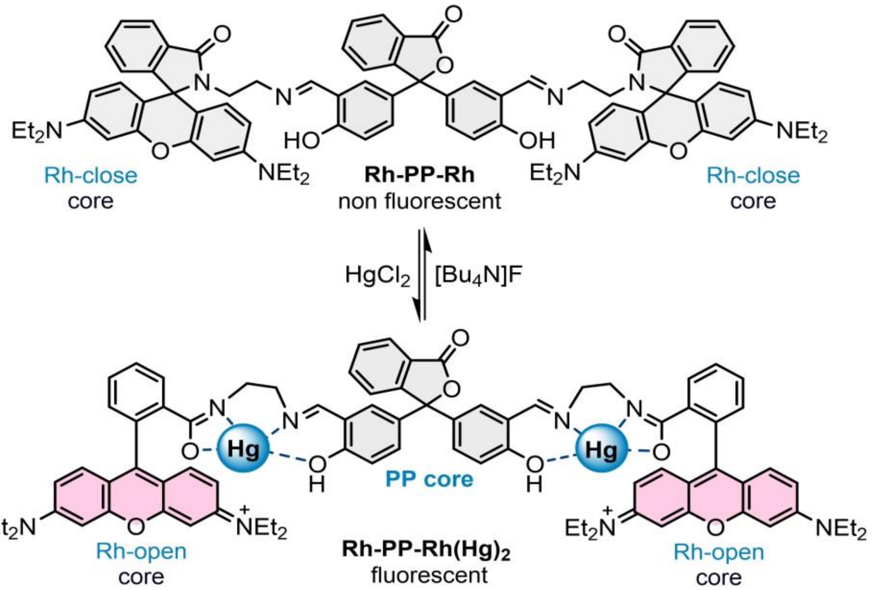
The prepared sensor showing high sensitivity to mercury which record 10 nM as limit of detection (LOD). The binding mechanism was elucidated as shown in **Scheme 11**. It demonstrated that a mercury-promoted desulfurization reaction, followed by cyclization, produced the 1, 3, 4-oxadiazole molecule **TRO**. **TRO** has a much larger conjugate system compared to **TRP**. This example of sensor was applied for bioimaging of HeLa cells using laser confocal microscope.

Hussein et al. described and synthesized a new rhodamine based sensor for mercury detection which synthesized by the reaction of rhodamine B with ethylenediamine by reflux in ethanol forming N-ethylamine rhodamine derivative (Rh-NH_2) which formed in excellent yield (99%). The prepared Rh-NH2 was refluxed with 5,5'-(3-oxo-1,3-dihydro isobenzofuran-1,1diyl)bis(2-hydroxybenzaldehyde) **PP-CHO** forming the target sensor **Rh-PP-Rh** as two rhodamine moiety connected by phenolphthalein link as presented in **Scheme 12** [91].



Scheme 12: *Synthetic procedure for* ***Rh-PP-Rh*** *[91].*

The prepared sensor has a good selectivity for Hg^{2+} cation with high sensitivity (LOD) 334 nM in aqueous medium. The binding mechanism with Hg^{2+} was described in **Scheme 13** which showing the formation of 1:2 complex with ring opening of both rhodamine moieties.



Scheme 13: *The binding mechanism for suggested interaction between Hg^{2+} and* ***Rh-PP-Rh*** *[91].*

The colorimetric study was showing a color change from colorless to pink color and new absorption band at 558 nm corresponding to the formed complex and turn on fluorescence with emission band at 582 nm. A solid-state colorimetric study was evaluated for **Rh-PP-Rh** as optical probe for detecting Hg^{2+} using colorimetric solvents and pre-treated filter paper which showing the color change to orange color on filter paper and the emission was also observed under UV lamp as presented in **Figure 11**.

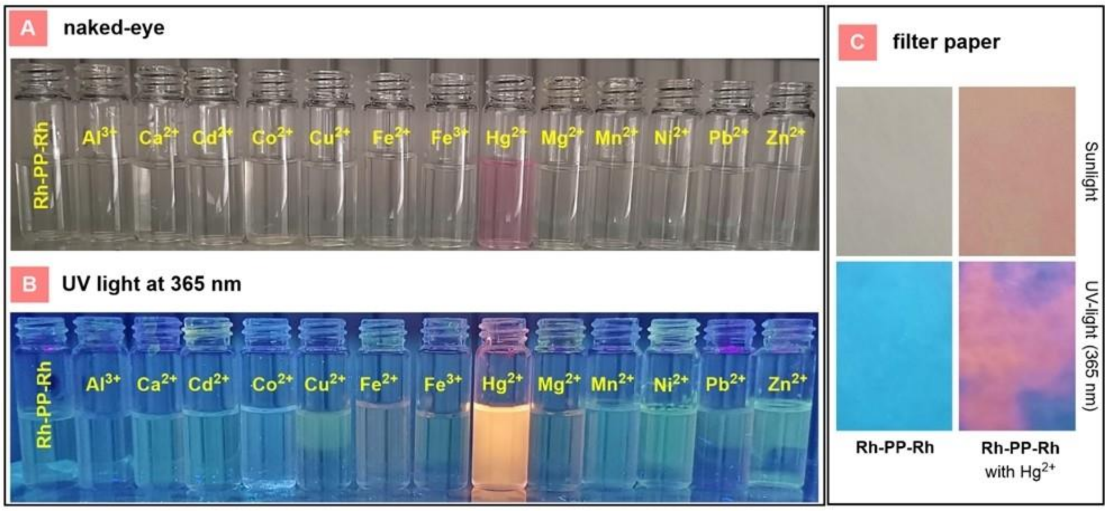
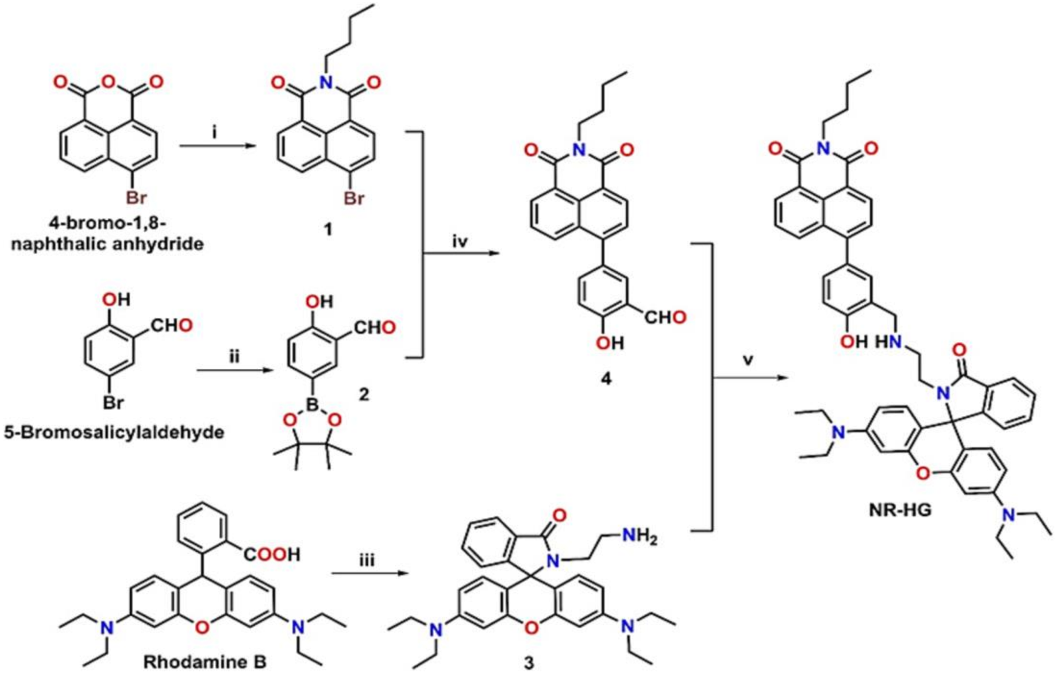


Figure 11: *The colorimetric change under visible light (A) and fluorescence change of* ***Rh-PP-Rh*** *(B) the color change under visible and UV light on filter paper (C)* [91].

Naphthalimide based rhodamine colorimetric and fluorescence probe sensor (**NR-HG**) was synthesized by Mishra et al. [92]. The presented **NR-HG** was developed by incorporating a modified 1, 8-naphthalimide moiety with rhodamine B using a suitable ethylenediamine spacer. The **NR-HG** sensor was prepared through multi step reaction as presented in **Scheme 14** which starts by the reaction of naphthalic anhydride derivative with an alkyl amine to form a naphthalimide derivative (1) this was followed by the Suzuki-Miyaura cross-coupling reaction to produce compound (4). The prepared *N*-ethylene- diamine derivative of rhodamine (3) was then condensed with 4-salicylaldehyde naphthalamide derivative (4) forming **NR-HG.**



Scheme 14: *The synthetic rout for* ***NR****-HG, Reagent and conditions: (i) n-butylamine, AcOH, 90 ◦C, 5 h; (ii)* B_2P *in 2,* $Pd(PPh_3)2Cl2$*, NaOAc, PEG-600, 80 ◦C, 3 h; (iii) ethylenediamine, reflux, 6 h; (iv)*$Pd(OAc)_2$*,* Cs_2CO_3*, xantphos, toluene, 90 ◦C, 5 h; (v) cat AcOH,* $NaBH(OAc)_3$*, DCE (dichlo- roethane), rt, 5 h [92].*

The **NR-HG** sensor showing a high selectivity characters for Hg^{2+} and detection limit was calculated 491 nM as presented in **Figure 12**. The sensing performance of **NR-HG** is demonstrated by a color change from colorless to pink due to ring opening and chelation with Hg^{2+}, forming a 1:1 complex. The fluorescence of the complex is detected through a chelation-assisted FRET process. FRET is a phenomenon where excitation energy is transferred from a donor fluorophore to an acceptor fluorophore without emitting a photon. In order For FRET-based chemosensors to work there must be an overlap between the emission of the donor and the absorption of the acceptor [93-95].

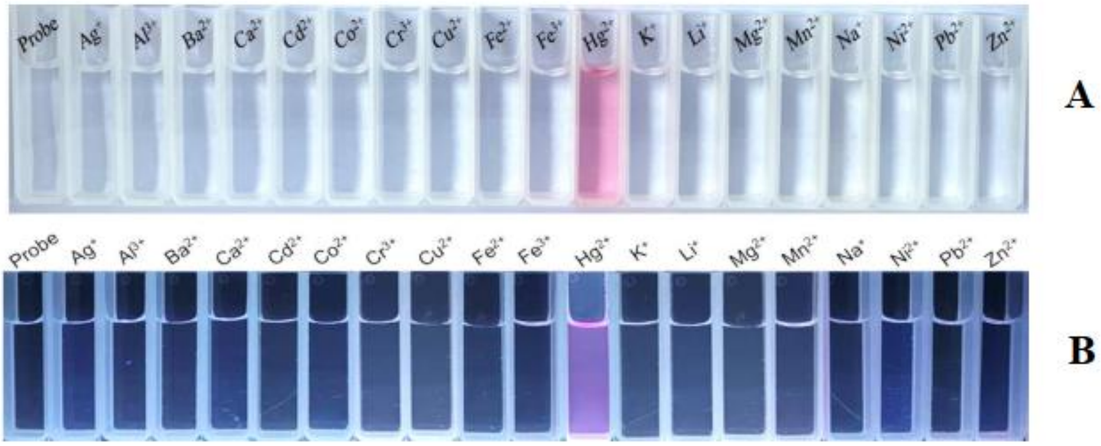
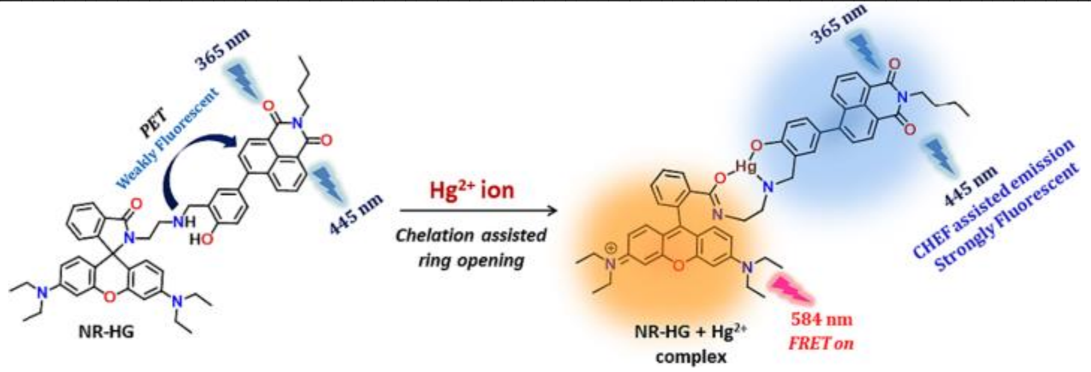


Figure 12: *The colorimetric change (A) and emission (B) for the detection of* Hg^{2+} *by* ***NR-HG*** *[92].*

The binding mode of the **NR-HG** sensor with Hg^{2+} is illustrated in **Scheme 15**. It shows that both the carbonyl oxygen "O" and the -NH- nitrogen "N" of the rhodamine amine moiety, as well as the "O" from the -OH group of the naphthalimide-salicylaldehyde moiety, are involved in the interaction with Hg^{2+}, resulting in the formation of a 1:1 complex. This interaction leads to a color change and turn-on emission [92].



Scheme 15: *Sensing mechanism by FRET for **NR-HG** sensor [92]*.

FRET-based sensors produce signals both in the absence and presence of the guest analyte to minimize interference and accurately detect the probe. The designed probe initially has weak emission due to the PET process. When Hg^{2+} binds to the probe, it inhibits the PET process, triggering CHEF and subsequently initiating FRET. Therefore, the presented sensor probe operates through the CHEF-assisted FRET pathway as shown in **Scheme 15**. The **NR-HG** sensor was used for bio-image application for SiHa cells as shown in **Figure 13**.

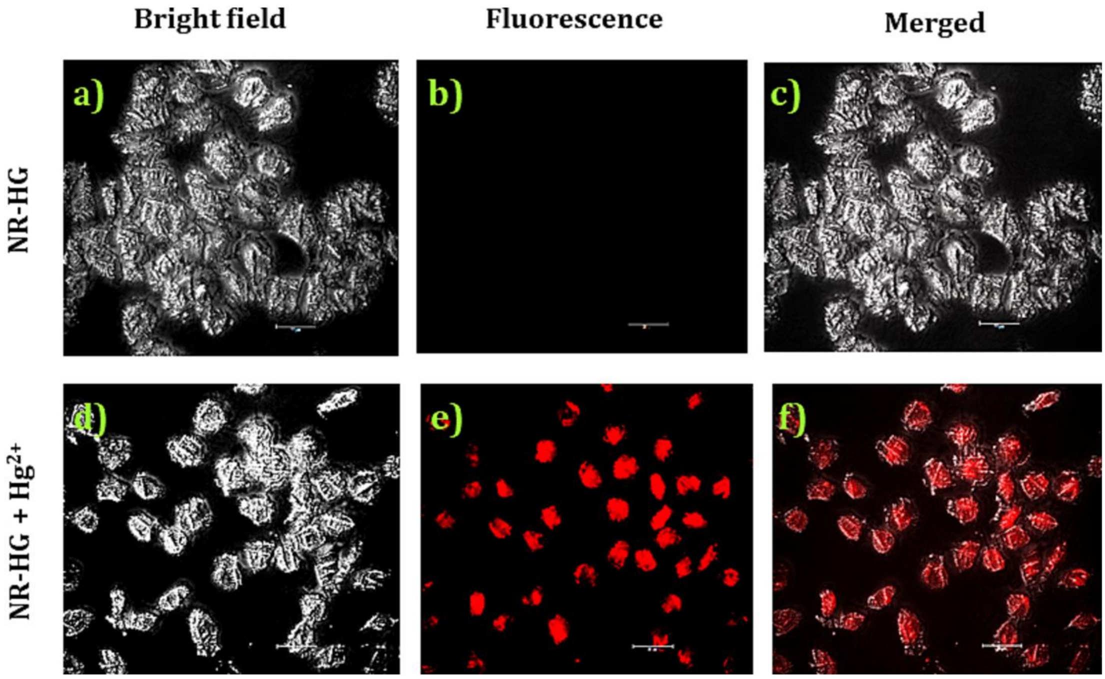
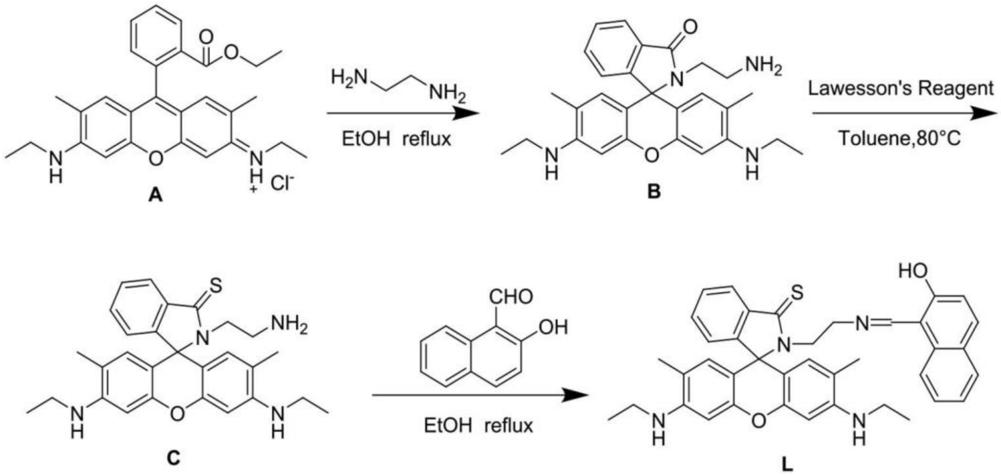


Figure 13: *The bio-image of SiHa cell using **NR-HG**. **NR-HG** in bright field (a), fluorescence field (b) and merge image (c), **NR-HG** in presence of Hg^{2+} in bright field (d), florescence (e), and merged image (f) [92].*

Bai et al. prepared and designed fluorescent and colorimetric chemosensor by two steps reaction based on rhodamine 6G. The target sensor (**L**) was prepared by the reaction of rhodamine 6G (A) with ethylenediamine to form *N*-ethylamine rhodamine derivative (B) followed by reaction with Lawesson's reagent to form rhodamine derivative (C) which react by condensation reaction with 2-hydroxy-1-naphthaldehyde to obtain the sensor (**L**) as shown in **Scheme 16** [96]. The colorimetric chemosensor **L** was found to effectively detect Hg^{2+} ions. The interaction between the chemosensor and Hg^{2+} was confirmed using various analytical techniques such as ultraviolet-visible spectrophotometry, fluorescence spectroscopy, electrospray ionization-mass spectrometry, Fourier-transform infrared spectroscopy, and frontier molecular orbital calculations. Furthermore, the chemosensor **L** was successfully incorporated into test strips and silica gel plates, demonstrating outstanding selectivity and high sensitivity in detecting Hg^{2+} ions.



Scheme 16: *The synthetic procedure of sensor **L** [96].*

The binding mechanism with the sensor showed interesting results. When the ratio of Hg^{2+} to sensor was 1:1, no color change was detected due to desulfurization and no ring opening was observed. However, when the ratio of Hg^{2+} increased than 1, a pink color change and significant emission were observed. Additionally, new absorption and emission bands at 526 nm and 550 nm, respectively, were recorded, indicating the formation of the LO-Hg^{2+} complex, [96] as shown in **Figure 14**.

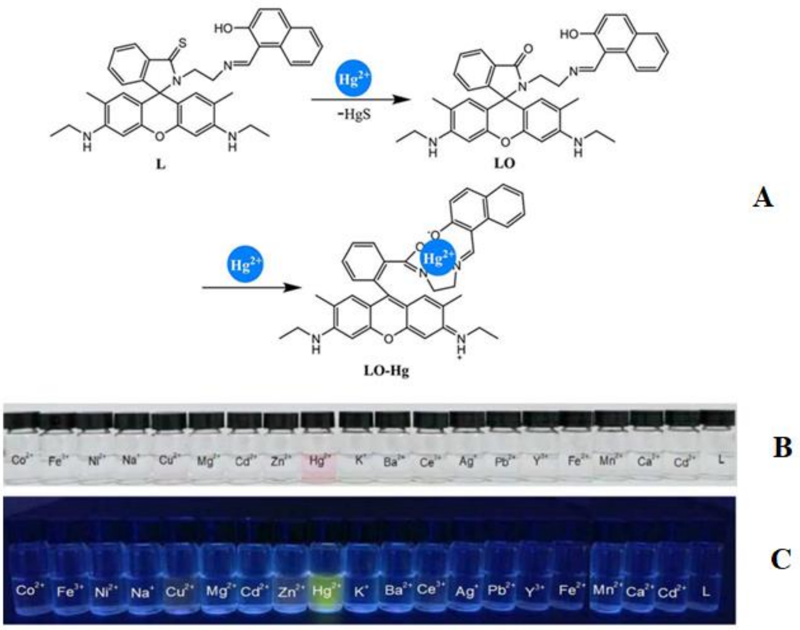
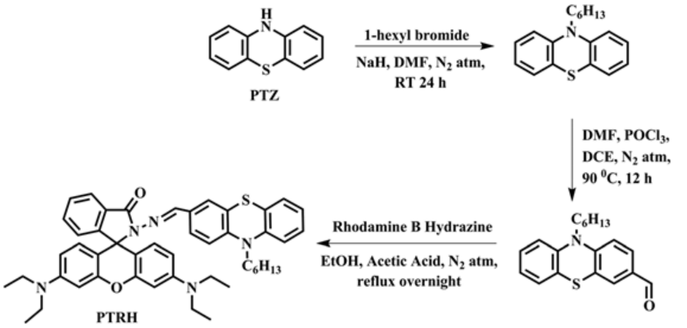


Figure 14: *The binding mechanism(A) and the colorimetric change (B)as well as the turn on emission(C) of the prepared sensor **L** [96].*

3.3. Rhodamine based colorimetric chemosensor for zinc ion (Zn^{2+})

Zinc is one of the important transition element which its abundance fall in the second position after iron. Zinc ions is essential as microelements for human life, zinc is one of the basic element for many biological activities such as gene code, DNA recombination or recognition, neural signal transmission and cellular metabolism [97-100]. The abnormal change of the zinc concentration in the human body can be the reason of seriously problem for the human health and may cause physiological diseases [101-103]. Therefore, the monitoring of zinc ion especially in aqueous media which have a great value. Colorimetric and fluorescent probe for Zn^{2+} monitoring have been created and improved as a widely used technique because of the advantage such as convenience, sensitivity and celerity [97, 104-107].

Phenothiazine based rhodamine derivative sensor (**PTRH**) for recognition of zinc cation was synthesized by Karmegam et al. The synthetic procedure was described in **Scheme 17** which started by alkylation of phenothiazine followed by formylation reaction (Vilsmeier-Haack formylation reaction). The carboxaldehyde derivative of alkylated phenothiazine reacted with rhodamine B hydrazine forming the sensor **PTRH** [108].



Scheme 17: *Synthetic pathway for the preparation of **PTRH** sensor [108].*

The remarkable selectivity for Zn^{2+} ions exhibited by the **PTRH** probe was confirmed by emission spectrum and electronic studies. When the Zn^{2+} ion bound to **PTRH**, the emission wavelength gets shift to 608 nm was accompanied by color change from a pale yellow to pink color (under visible light) and green to pinkish red fluorescence emission (under UV light) as presented in **Figure 15**.

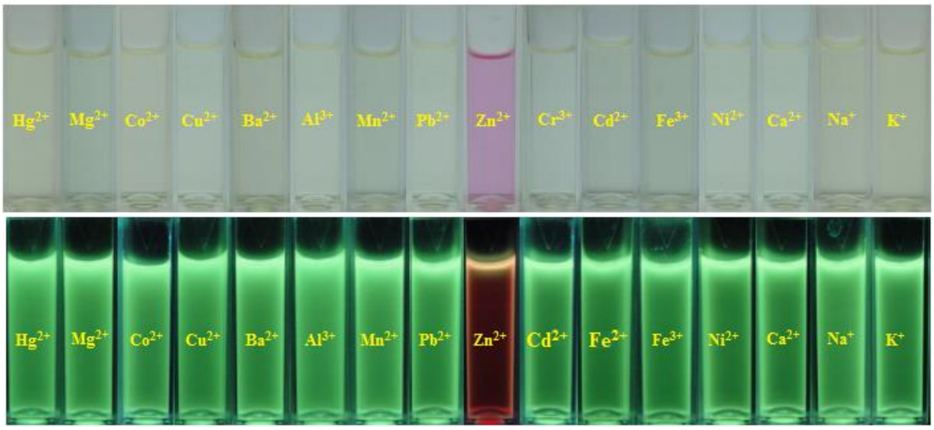
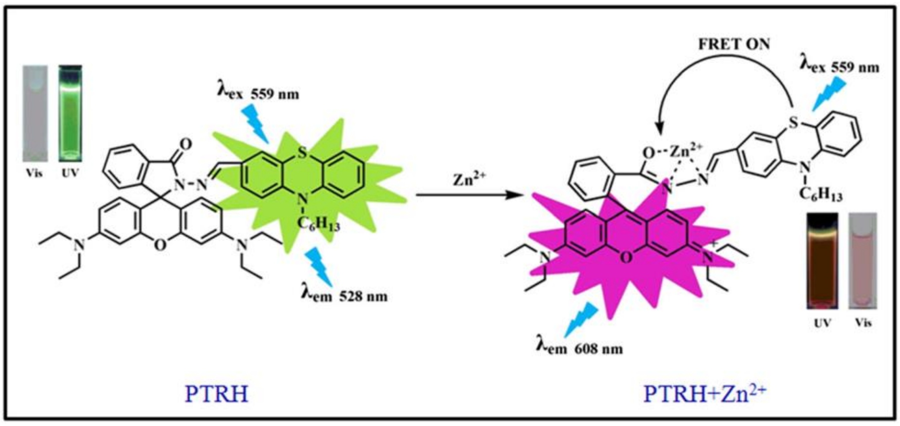


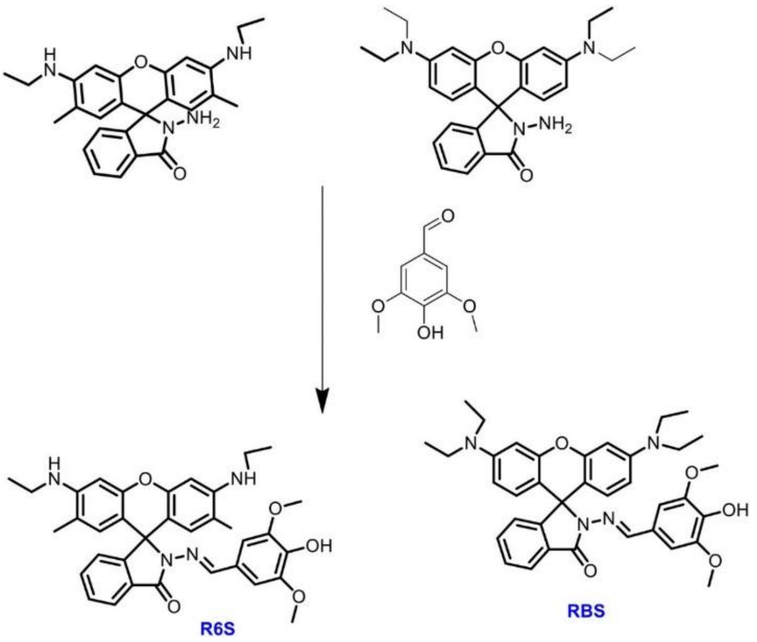
Figure 15: *The selectivity study of **PTRH** sensor in visible and under UV spectra [108].*

The colorimetric and fluorescence change caused by the ring opening of the spirolactam moiety in the **PTRH** ligand. The emission band of the **PTRH** probe at 528 nm confirmed the ring-closed form of **PTRH** as a result of the 1:1 complex formation, as shown in **Scheme 18**. The LOD for zinc ion recognition was recorded 28.9 nM. This sensor was applied in bio-image application for HeLa cell [108].



Scheme 18: *The mechanism for interaction of Zinc ion with **PTRH** sensor [108].*

Two rhodamine based chemosensor for Zinc cation (**R6S** and **RBS**) were presented by Zhang et al. as shown in **Scheme 19** [109]. The presented sensors were prepared by the condensation reaction of the corresponding hydrazide form of rhodamine 6G and rhodamine B with syringaldehyde (4-hydroxy-3, 5- dimethoxybenzaldehyde) in ethanol and catalytic amount of acetic acid.



Scheme 19: *Synthetic procedure of **R6S** and **RBS** sensors for zinc cation detection* [109].

The prepared sensors demonstrated a very specific color change from colorless to pink when Zn^{2+} ions were added. Additionally, the fluorescence was turned on due to the interaction of Zn^{2+} causing a ring-opening. The **RBS** and **R6S** have the capability to identify zinc ions at very low concentrations of 0.18 and 0.19 nM, respectively. The spiro-ring opening of the spirolactam was clearly shown by the affinity of these sensors, **RBS** and **R6S**, for Zn^{2+} ions. ^{1}HNMR titration of the probes with Zn^{2+} ions was investigated for the zinc complex formation. The DFT was used to prove the spirolactame ring opening mechanism due to the interaction of zinc with sensor forming 1:1 complex. The two sensors **RBS** and **R6S** were applied in bio-image applications for HeLa cells as shown in **Figure 16**.

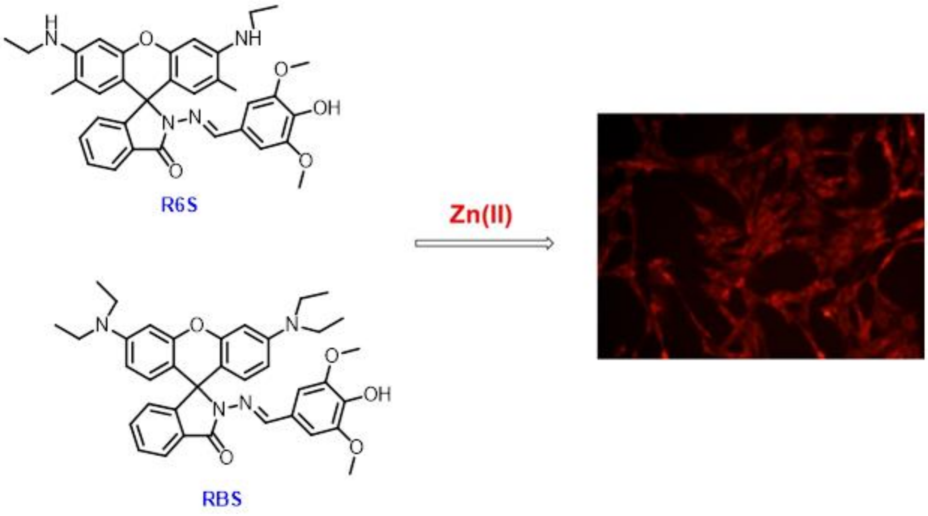


Figure 16: *Bio-image of prepared sensors **R6S** and **RBS** for Hela cells* [109].

3.4. Rhodamine based colorimetric chemosensor for Aluminum ion (Al^{3+})

Aluminum (Al^{3+}) is the third most abundant element on Earth. While it is not essential for the human body, high levels of aluminum can lead to various diseases in plants and human, such as Alzheimer's, dementia, and encephalopathy. The recommended daily intake of aluminum for human is less than 10 mg, with a weekly tolerable intake of less than 7 mg/kg of body weight, as suggested by the World Health Organization (WHO) [110, 111]. Aluminum compounds are commonly used by pharmaceutical industries in the production of analgesics and antacids. However, exposure to aluminum through sources like acid rain, cooking with aluminum foil, and using aluminum utensils can lead to toxicity in human and soil, posing risks to agricultural production and aquatic life. Researchers face challenges in designing aluminum sensors due to the poor solubility of organic compounds in water and the strong hydration ability of aluminum in aqueous environments [112]. Therefore, the development of chemosensors that can detect aluminum ions in aqueous media is crucial for monitoring and mitigating the environmental and health hazards associated with aluminum toxicity [113].

Genc et al. prepared a chromo-fluorogenic sensor, based on rhodamine (**BESN**), for the recognition of Al^{3+} at nanomolar levels [114]. The synthetic procedure is outlined in Scheme 20, involving the reaction of ring opening of rhodamine B with tris(2-aminoethyl)amine (Tren) [115], followed by a condensation reaction with 4-(diethylamino) salicylaldehyde to form the **BESN** sensor in good yield. The binding of **BESN** with Al^{3+} was investigated through absorption and emission spectra, revealing a noticeable colorimetric and fluorogenic change. Studies on the binding mechanism and sensitivity of **BESN** showed high selectivity for interfering metal ions and a strong sensing performance with a detection limit of 130 nM. The binding constant of **BESN** with Al^{3+} was determined to be 3.19×10^{-3} M. Infrared, high-resolution mass, and emission spectroscopic studies confirmed the 1:1 binding of Al^{3+} with BESN, as shown in **Scheme 20**.

Scheme 20: *The interaction of Al^{3+} with synthesized sensor **BESN*** [114].

The **BESN** was applied for detecting of aluminum in food samples such as vegetables, tuna fish, and water samples. It was also utzilied for the development of a test kit and smartphone application for detecting Al^{3+} ions. Additionally, it was employed for monitoring Al^{3+} ions in living cells (A549–cells) using a bio-image application, as represented in **Figure 17**.

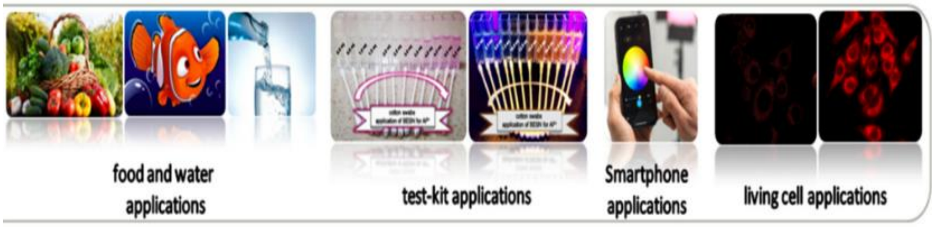


Figure 17: *Application study for the **BESN** sensor* [114].

Ghosh et al have prepared two analog chemosensor for detecting of Al^{3+} based on the reaction of rhodamine 6G with 4-(2-aminoethyl)morpholine and 4-(3-aminopropyl)morpholine forming **L-Et** and **L-Pr** respectively as shown in **Figure 18** [116].

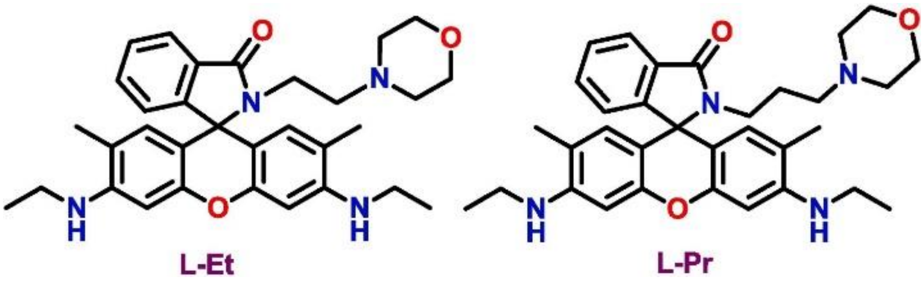


Figure 18: *The chemical structure of the synthesized **L-Et** and **L-Pr*** [116].

Among of different metal ions, only Al^{3+} is detected by both sensors. The detection is achieved through color changes (from colorless to pink), an increase in absorption intensity at 530 nm, and fluorescence intensity at 555 nm as illustrated in **Figure 19**. These changes are a result of the spirolactam ring opening and complexation with Al^{3+} by the chemosensors. Both sensors form a 1:1 complex with a high association constant. The limit of detection values for Al^{3+} with **L-Et** and **L-Pr** are 1.7 nM and 1.1 nM, respectively, indicating high sensitivity. These sensors have been successfully used in C6 cell imaging studies. The introduction of a methylene group does not affect the spectral characteristics of the sensor. Neither of the sensors contains an imine bond, so hydrolysis in the presence of Al^{3+} is not possible.

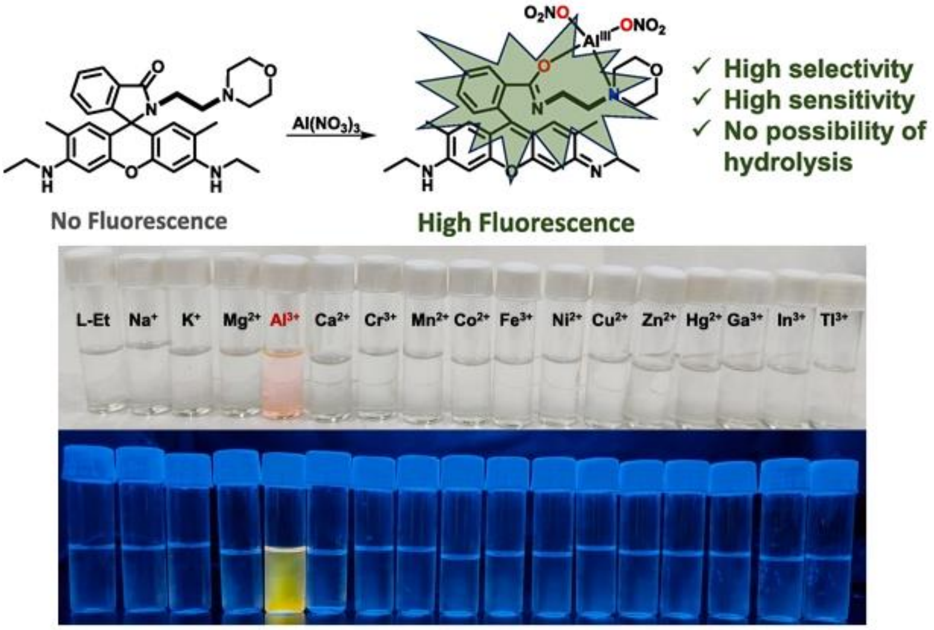
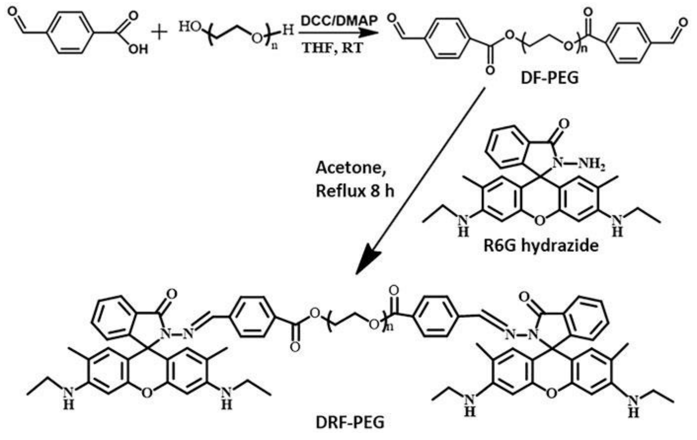


Figure 19: *The colorimetric and emission change after binding of Al^{3+} with **L-Et** sensor* [116].

3.5. Rhodamine based colorimetric chemosensor for iron ion (Fe^{3+})

Iron (Fe^{3+}) is crucial for living organisms as it is a key component of haemoglobin, myoglobin, and various enzymes that aid in vitamin B metabolism [117, 118]. In cellular biochemical reactions, Fe^{3+} is essential for biological signal transmission and material transportation [119-121]. Both deficiency and excess of Fe^{3+} can lead to various dysfunctions in organisms. A lack of Fe^{3+} can impact the synthesis of important enzymes and reduce their activities, affecting immunity, intelligence, and the body's ability to regulate temperature [122-126]. On the other hand, excessive intake of Fe^{3+} can cause oxidative damage to cells and reduce blood circulation to the heart [127, 128]. Therefore, it is vital to develop a rapid and sensitive method to detect the distribution of Fe^{3+} in living organisms. Using a Schiff base reaction between rhodamine 6G hydrazide and benzaldehyde-functionalized polyethylene glycol, Qiu et al. created a water-soluble rhodamine-poly(ethylene glycol) conjugate (DRF-PEG) for the detection of Fe^{3+} with high biocompatibility, as illustrated in **Scheme 21** [129].



Scheme 21: *Synthetic rout for **DRF-PEG** from rhodamine 6G.*

The water solubility of the rhodamine derivatives was significantly improved by adding a **PEG** segment to **DRF-PEG**, [129] enhancing their biosensing capabilities. Upon the addition of Fe^{3+}, a new absorption peak at 532 nm was observed for Rh and **DRF-PEG**. This peak is due to the ring opening of the spirolactam structure in the rhodamine moieties, causing the solution to turn orange as represented in **Figure 20**. The colorimetric sensor had a detection limit of 3.16 μM for Fe^{3+} in pure water at pH 6.5, while the fluorescent sensor had a detection limit of 1.00 μM.

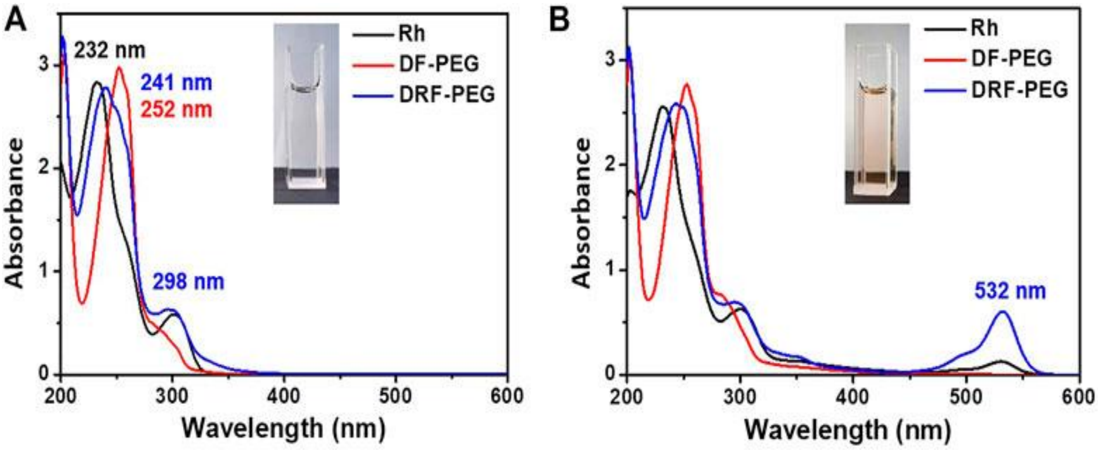
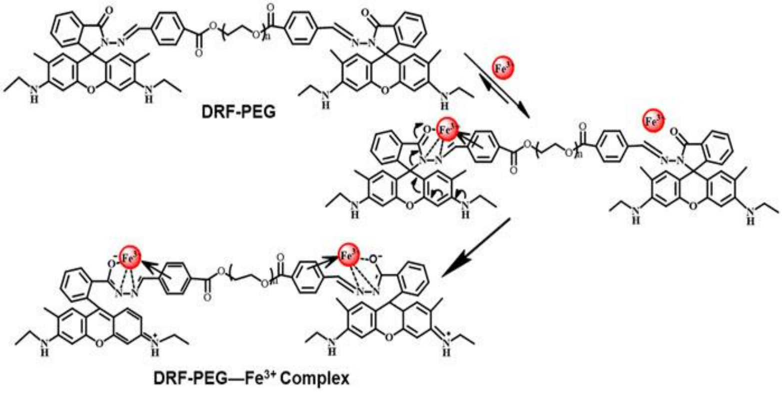


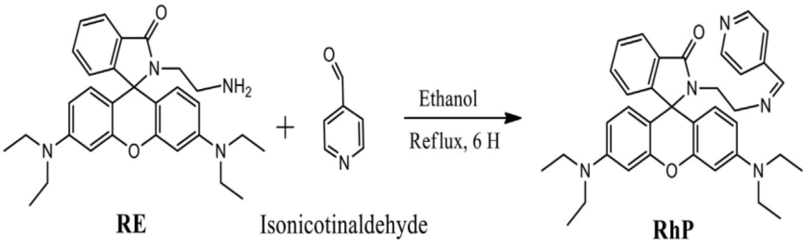
Figure 20: *Absorption spectra of* ***Rh****,* ***DF-PEG****and* ***DRF-PEG*** *(A), Absorption spectra of* ***Rh****,* ***DF-PEG*** *and* ***DRF-PEG*** *in presence of* Fe^{3+} [129].

The unique sensing mechanism of **DRF-PEG** towards Fe^{3+} is based on the intramolecular charge transfer (ICT) process [129]. This process involves the interaction of O and N atoms in the rhodamine moiety and benzene groups from the benzaldehyde-modified **PEG** segment with Fe^{3+} as presented in **Scheme 22**. **DRF-PEG** was used to quantitatively measure Fe^{3+} levels in fetal bovine serum samples and to perform ratiometric imaging of Fe^{3+} in HeLa cells.



Scheme 22: *The binding mechanism and the interaction of* ***DRF-PEG*** *towards* Fe^{3+} [129].

Shellaiah et al. synthesized a new colorimetric chemosensor and fluorescence probe based on rhodamine B derivative for detection of Fe^{3+} by direct condensation reaction of rhodamine B ethylenediamine and isonicotinaldehyde in ethanol forming **RhP** [130] as shown in **Scheme 23**.



Scheme 23: *Synthetic procedure of the* ***RhP*** *sensor for* Fe^{3+} *detection* [130].

The **RhP** exhibited a distinct pale-pink colorimetric and "turn-on" fluorescence response to Fe^{3+} in aqueous conditions (H_2O: DMSO), unlike other interfering ions. Absorption (UV–Vis) and photoluminescence (PL) spectrum measurements revealed new peaks at 561 and 592 nm, respectively, upon Fe^{3+} detection **Figure 21**. The 1:1 stoichiometry and binding sites were confirmed through Job's plot, ESI-mass, and ^1H NMR titrations. The limit of detection (LOD) and binding constant of the **RhP +** $\mathbf{Fe^{3+}}$ complex were determined to be 102.3 nM and 6.265×10^4 M^{-1}, respectively. The reversibility of the **RhP +** $\mathbf{Fe^{3+}}$ complex by EDTA was represented in **Scheme 24** [130].

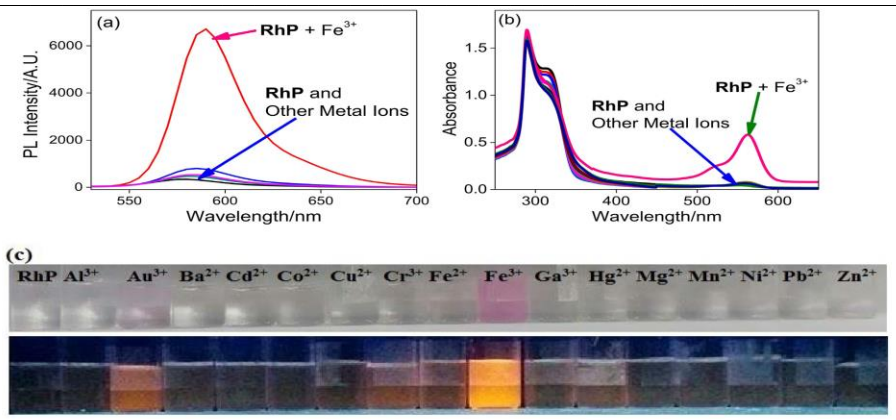
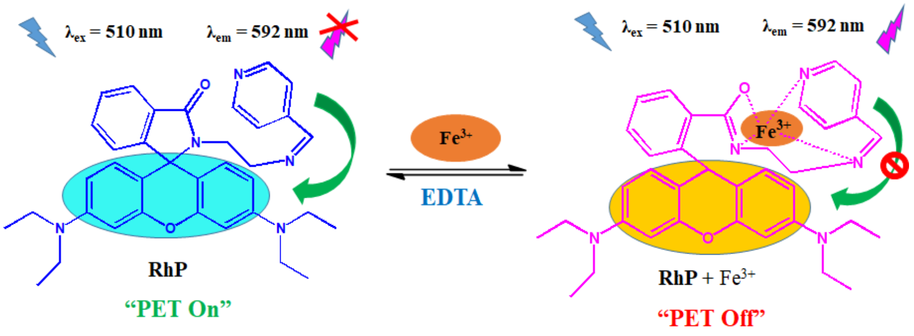


Figure 21: *The colorimetric and fluorescence change of* ***RhP*** *with* Fe^{3+} [130].



Scheme 24: *The interaction and binding mechanism of* Fe^{3+} *with* ***RhP*** [130].

3. Conclusions

Colorimetric chemosensors based on various chromophores and fluorophores are widely available, but creating highly sensitive and selective chemosensors remains a challenge. The increasing research in this field is driven by the need for quick and simple detection of harmful substances in biological and environmental samples. However, there is a lack of multi-functional chemosensors that can detect different analytes with distinct color changes or emissions for each, making the development of such sensors highly desirable. This review focuses on xanthene-based sensors, specifically rhodamine derivatives, for detecting cations such as Cu^{2+}, Hg^{2+}, Zn^{2+}, Al^{3+}, and Fe^{3+}. The modified rhodamine derivatives discussed in the literature demonstrate promising selectivity towards metal ions. Creating new rhodamine derivatives linked to different chromophores can improve sensitivity and fluorescence quantum yield, enhancing sensor development. In the future, there is a growing interest in the advancement of smart materials such as polymeric films, membranes, and textiles for the sensitive and efficient monitoring of toxic species in sensor applications. This review highlights the opportunity for creativity in sensor design and material development to improve detection capabilities.

4. Conflicts of interest

"There are no conflicts to declare".

5. Acknowledgments

We are very grateful for the National Research Centre, Dokki, Egypt for all kind of supports which provided for us.

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