RHODAMINE BASED CHEMOSENSORS FOR COPPER AND IRON

A

Thesis

submitted in partial fulfillment of the requirements

for the award of the degree of

MASTER OF SCIENCE

IN

CHEMISTRY

By

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JULY, 2011

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As this thesis sees the light of day, I find myself at a loss of words to express my thankfulness to almighty, but for whose gracious blessings, this work would not have been conceived, much less completed.

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I thank all my friends who constantly motivated me and supported me throughout the project.

RENU KAMAL

CANDIDATE'S DECLARATION

I hereby declare that the work being presented in the entitled **"Rhodamine Based Chemosensors For Copper and Iron"** in the partial fulfillment of the requirements for the award of the degree of Masters in Chemistry, School of Chemistry and Biochemistry, Thapar University, Patiala, is my own work during the period of Jan 2011 to July 2011, under the supervision of Dr. Kamaldeep Paul, Assistant Professor and Dr. Vijay Luxami, Lecturer, School of Chemistry and Biochemistry, Thapar University, Patiala. I have not submitted the matter embodied in this dissertation for the award of any other degree.

Patiala

Date: 15/07/2011

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This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

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This is to certify that the project entitled **"Rhodamine Based Chemosensors For Copper and Iron"** being submitted by Ms. Renu Kamal, Roll No. 300902016 in the partial fulfilment of the requirement for the award of degree of Master of Science in the School of Chemistry and Biochemistry, Thapar University, Patiala, is a bonifide work carried out under our supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other university.

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Introduction & Review of Literature

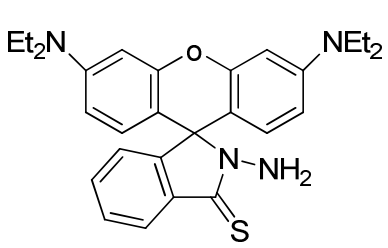
Chemosensor is a molecule that reacts with an analyte to produce colorimetric, fluorometric and potentiometric changes and is also called as molecular sensor. The chemosensor is actually a superamolecule that combine through molecular recognition with some form of reporter so that the presence of the guest can be achieved. The general operating principle of chemosensors is based on the coordinating events[1]. The reaction of the chemosensors with analyte and the accompanying signal changes are reversible[2]. The coordination is a typical reversible chemical reaction in which any change in the concentration of cation or anion determines the relative amount of coordinated and free moiety. The following figure shows the operating protocols of chemosensors.

The most widely used chemosensor, involves the binding site/signalling subunit approach in which two units are covalently bonded to give an optical response following the coordination to selective analyte[3-8]. The other approach is the displacement approach. It involves a binding site and a signal reporter but these two units are not covalently bonded. On addition of the analyte, it coordinates with the binding site and with subsequent release of signaling subunit.

Various complexes can be used as chemosensors e.g. polycylic aromatic hydrocarbons, aromatic heterocycles, sapphyrins and porphyrins, metal complexes based on ruthenium, rhenium, osmium, iridium complexes etc. Dyes can also be used as signaling subunit eg. Fluorescein, Rhodamine, Eosin etc. Out of these Rhodamine is used as a tracer dye within water to determine the rate, direction of flow and transport. Rhodamine dye is fluorescent so it can be determined easily and inexpensively with

instrument called flourometers. The dye is soluble in water but the chlorinated water decomposes the Rhodamine B. Rhodamine is also present in other forms i.e. Rhodamine 6G, Rhodamine 123 but Rhodamine B is most commonly used. Rhodamine dyes belongs to the family of xanthene along with flourescein and eosin dyes. Depending on the substituent present on the Rhodamine it exhibits photophysical properties such as absorption, emission maxima, fluorescence life time and fluorescence quantum yield. The differences in the photophysical properties of Rhodamine dye are defined by non radiative process (internal conversion). On the other hand, Rhodamine dye with two alkyl substituents at each nitrogen shows activated internal conversion consequently the flouresence and lifetime vary with temperature.

The activated process seems to be associated with non fluorescent twisted interamolecular charge transfer state (TICT)[9] characterized by an electron transfer from amino groups to the xanthene moiety followed by rotation between them. The energy of the TICT state is higher than the energy of the first excited singlet state for the dyes without activated processes and lower for those with activated internal conversion. Thus the activated energy dissipiation is explained by the population of the TICT state that is non-emissive and deactivates quickly to the ground state. The lifetime of the lactone ring in Rhodamine B derivative and very low quantum yield results in electron transfer reactions in the excited state that generate the charge transfer excited state and singlet and triplet state of the dye in the zwitterionic form[10].



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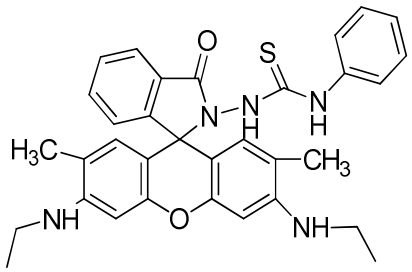
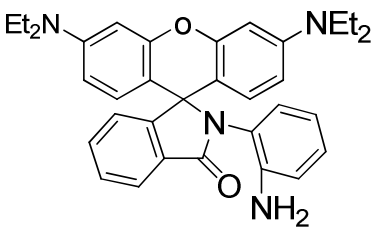


2

During the past few decades, the synthesis of various rhodamine based chemosensors is in progress. Designing chemosensors based on rhodamine spirolactam have several advantages[11-12]: they have not only great absorbance and fluorescence intensity

enhancement toward some specific metal ion but also strong colour development against colourless blank during the sensing event. A rhodamine spirolactam based chemosensor **1** was highly selective for Hg^{2+} via colour/fluorescence changes.

The colourless solution showed almost no absorption peak in the visible wavelength range (>400) in aqueous solution. Upon addition of Hg^{2+} it showed an absorption maximum at wavelength 561 nm. The absorption increases linearly with increasing concentration of Hg^{2+} and it can be detected at least down to 1.0×10^{-7}M, a concentration in the ppb range, when **1** was employed to be 1.0×10^{-5}M. The maximum excitation and emission wavelength for chemosensor **1** was 558 nm and 582 nm respectively. Chemosensor **2** which was found to be highly selective for Hg^{2+}, undergoes oxidiazole formation when thiosemicarbazide moiety was liberated by Hg^{2+} facilitating the ring opening of spirolactam form[13-16]. The chemosensor **2** was studied in water- methanol (80:20 v/v) system at pH 7 which showed that it exists in spirocyclic form predominantly. Addition of Hg^{2+} ion to the solution of **2** caused instantaneous development of pink colour and yellow fluorescence in which the maximum emission shift from 553 nm to 557 nm. The chemosensor **2** is highly selective for Hg^{2+} because the enhancement in the fluorescent intensity resulting from addition of $Hg^{2+}$is not influenced by the addition of other metal ions. So, **2** can be used for the rapid detection of Hg^{2+} ions in aqueous media[17].



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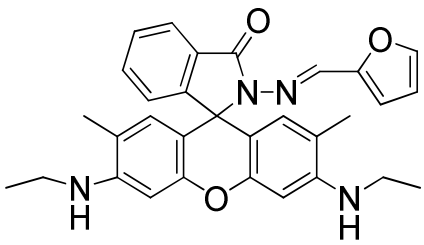
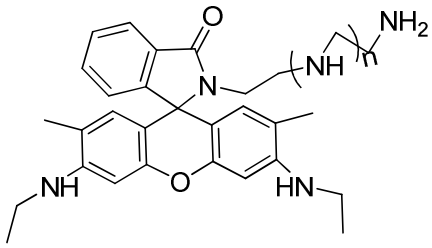


4

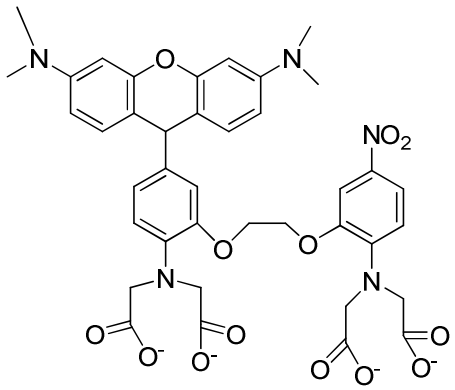
Chemosensor **3** was employed as selective probe for NO molecule in aqueous solution by the ring opening of spirolactam ring in Rhodamine B[18]. The compound exhibited a "turn – on" type chromogenic and fluorogenic behaviour towards NO in aqueous solution with great sensitivity and selectivity. It can be used for the detection of NO

under physiological conditions. The spectral studies were performed in the phosphate buffer water-acetonitrile (80:20 v/v) system at pH 7.40. Compound **3** did not show any absorption or fluorescence peaks in the longer wavelength range. But the addition of NO carrier to **3** at 37˚C caused development of strong absorbance at wavelength at 554 nm and a fluorescence peak at λ_{em} ~574 nm.

This strong colour enhancement was pH dependent which exhibited no fluorescence changes at pH >3, and when pH was greater than 5 then it showed the visible changes. Chemosensor **4** showed the photophysical properties based on the ring opening mechanism of the spirolactam rings. Addition of metal ion leads to the spirocycle opening via coordination resulted in pink color and orange fluorescence. The absorption band was observed at 529 nm upon addition of metal ion and resulted in pink colour. The coordination of **4** with Cu^{2+} was also observed by fluorescence titration in H_2O-CH_3CN solution. On addition of Cu^{2+} a 32 fold enhancement in the fluorescence intensity was observed at 552 nm followed by excitation at 495 nm. Receptor **5-6** showed enhanced response in the presence of Fe^{3+} and Cr^{3+} upon incorporation into the solution[19,20]. The chemosensor **5-6** in the pH range 6.0-8.0 bound to Fe^{3+} & Cr^{3+} in a very weak manner. At pH<5 an enhancement in the fluorescence intensity was observed due to the formation of ring opened state.



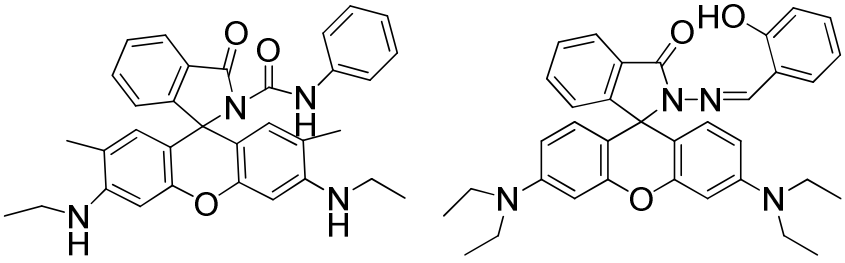
5 n=1
6 n=2



7

The Receptor **7** based on 5N-BAPTA ionophore (BAPTA=1,2 Bis (2-aminophenoxy)ethane-N,N,N',N'-tetracetic acid) exhibited a strong affinity for Cd^{2+} rather than Ca^{2+} ions. The absorption spectra of **7** exhibited several bands in the range of 300 to 640 nm due to the combination of absorption spectrum of 5N-BAPTA and

rhodamine moieties. The emission spectrum of **7** consisted of a single band centred at 576 nm where λ_{exc} = 551 nm which was due to rhodamine fluorescence. The fluorescence in this molecule occurred due to photoinduced electron transfer (PET) process[21] occurring from one of the aromatic amine group of BAPTA to the rhodamine fluorophore. Receptor **8** was used specially for the selective detection of acetate ions in the aqueous media in the presence of complex formed by **8** + Fe^{3+} ion[22].

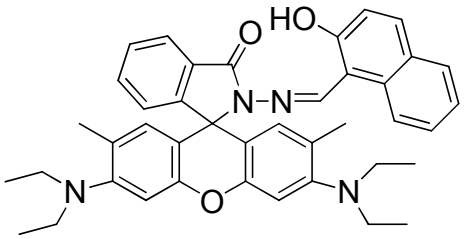


8

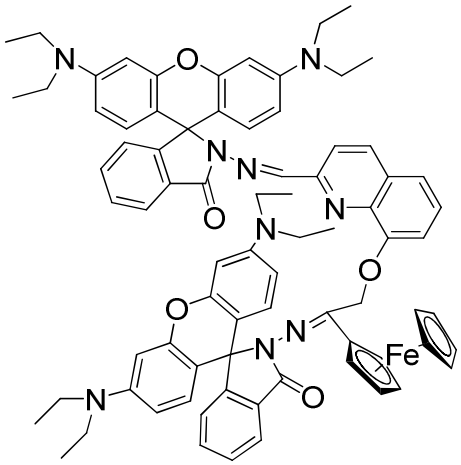
9

Addition of Fe^{3+} caused change in colour from colourless to pink with increase in absorption band from 500 nm to 530 nm and also resulted in remarkably enhanced fluorescence at 556 nm with fluorescence quantum yield 0.86. Then interactions of **8**-Fe^{3+} with anions were investigated firstly by UV- visible spectroscopy, which showed a decrease in the intensity of peak at 556 nm with the addition of AcO^- ions. No significant change was observed including other anions F^-, Cl^-, Br^- etc. The colour of the solution changed from pink to colourless Receptor **8** was the first chemosensor based on metal ion complexes that can detect acetate ion over other ions in the presence of Fe^{3+} and dihydrogen phosphate ions in an aqueous environment. Receptor **9** was investigated for the detection of hypochlorites in tap water[23]. The compound showed an absorption peak centered at 555 nm with the addition of hypochlorites to the Tris-HCl buffer solution of **9**. The receptor **9** was used as a Cu^{2+} sensing molecule which also gave the optical signal in the presence of hypochlorite sensitively & selectively, due to the formation of Cu^{2+} complexes. The colourless solution of **9** remained same in the presence of Cu^{2+} but turned out to be magenta by the addition of hypochlorites to this solution. In order to observe the change with time, the kinetic value of $A_{30\ min}$- $A_{6\ min}$ /A_6

$_{min}$, were calculated where $A_{30\ min}$ and $A_{6\ min}$ were the absorption of the solution at 30 minutes and 6 minutes respectively. The observed values showed that in higher concentrations, the reaction almost completed within 6 minutes and at lower concentrations, the reaction time was longer (30 min) due to the different oxidation capacity of different amount of hypochlorites. The probe **9** was also responsible for the detection of H_2O_2 but not as good as ClO^- ions because the oxidability of H_2O_2 was much weaker than that of ClO^-. To evaluate applicability of the system for the sensing of natural system, tap water was analysed using this hypochlorite sensing system. The experimental results showed that no absorption was observed with the addition of deionised water, addition of tap water resulted in a significant increase in absorption. Receptor **10** showed the very significant photophysical behaviour for the detection of Cu^{2+}. The absorption spectra of **10** were recorded in the buffer solution (pH 5.0) which gives a very weak band at 500 nm, addition of Cu^{2+} ion resulted in a remarkable increase in absorption at 556 nm, which was ascribed to the ring opened tautomer of **10**.



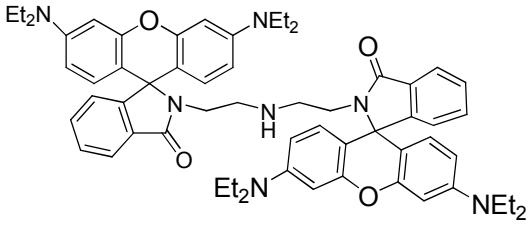
10



11

The reaction between **10** and Cu^{2+} was so rapid that it takes place within seconds. The titration procedure exhibited the visible colour change from colourless to purple in the presence of Cu^{2+} in CH_3CN aqueous media[24]. The detection limit was found to be at nanomolar level via the absorbance enhancement. Receptor **11** showed a "turned ON" fluorescence behaviour in the presence of mercury for efficient bio-imaging. The molecule was cell permeable and can be used as a fluorescent probe for the detection of mercury in living cells. The compound gave an absorption peak at 561 nm by the

addition of Hg^{2+} to the HEPES buffer (pH 7.2) H_2O:CH_3CN solution of **11** clearly resulting in the ring opened amide form of **11** upon Hg^{2+} binding. The complexation of Hg^{2+} with **11** was also investigated by means of fluorescence, which gave the new emission band at 585 nm upon addition of Hg^{2+}. In the presence of 10 equiv. of Hg^{2+} the compound gave an intense red fluorescence with a quantum yield of 0.21 and the fluorescence enhancement factor at 585 nm was 100-fold increase which was due to delocalisation of xanthene moiety in the rhodamine group. The studies were done with laser scanning fluorescence microscopy for the detection of Hg^{2+} in living cells. Thus, the study of this compound provided a useful way for the synthesis and application of chemosensors which can help to detect the metal ions present in the living cells[25].



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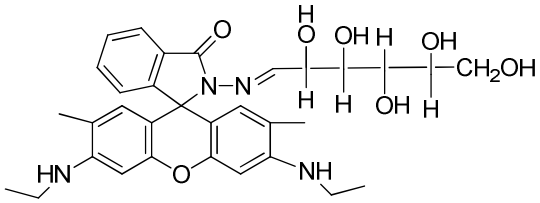
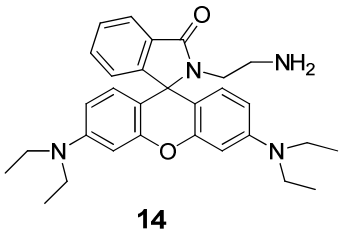


13

Receptor **12** displayed highly selective Fe^{3+} amplified fluorescence in both ethanol and buffered water[26]. Addition of Fe^{3+} ion into the colourless solutions (in both ethanol & buffer) generates a purple colour and orange fluorescence. The characteristic absorption peak was observed at 557 nm after addition of 1 equiv. of Fe^{3+} ion. The fluorescence enhancement was observed at 510 nm. Receptor **13** was specifically used for the detection of Hg^{2+} in the ppb range in ground water[27]. The free molecule **13**, in the aqueous solution exhibited weak fluorescence at 550 nm but upon addition of Hg^{2+} to the solution developed an emission band significantly. It was observed that there is formation of the xanthene moiety in the rhodamine group. The chemosensor **13** bind with Hg^{2+} reversibly and was unaffected in the presence of other metal ions. There was an important point for this chemosensor that the addition of KX to the solution caused negligible influence on the binding event but the addition of an aqueous solution of NaI to the solution of **13** –Hg^{2+} species caused the flouresence intensity to be diminished significantly. Addition of Na_2S caused significant decrease in the fluorescence intensity

thus it can be used as an excellent expellent for the Hg^{2+} ion. Receptors **14-19** were highly selective for the detection of mercury. The absorption spectra of **14-19** in CH_3CN showed no absorption peak in the range 400-700 nm region due to spirolactam conformation in the solution. However addition of metal ion leads to a stronger absorption at ~560 nm and at ~515 nm which is the characteristic peak of rhodamine dyes. The spectral studies were done also in the presence of acetonitrile-water system (1:1 v/v) in the presence of various metal ions. The difference was found that in pure CH_3CN, all the probes showed selectivity with Hg^{2+}, however in case of acetonitrile-water system (1:1 v/v) no selectivity was observed in the probe **14.** Other metal ions such as Pb^{2+} and Fe^{2+} which have induced a small absorption enhancement in all the probes (except **18)** in case of CH_3CN, did not showed such absorption enhancement in CH_3CN-H_2O (1:1 v/v) medium.



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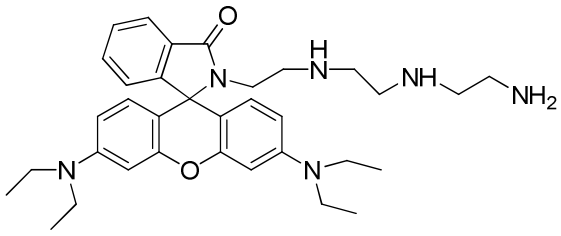
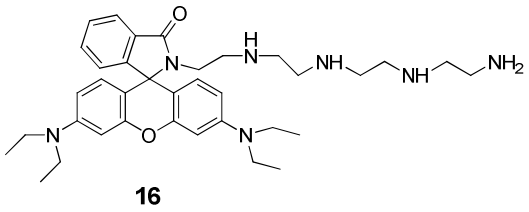


15

The spectral response of each probe was varied with their structural design, which incorporates different binding sites on their receptor unit, exploiting their different binding affinity and different binding modes of complexation. The complexation of **14-19** were found to be reversible with addition of counter ions such as iodide and acetate ions, which shows that these probes are chemosensors for the detection of Hg^{2+}. These chemosensors were very appreciable because these can detect the Hg^{2+} in the detection limit $0.3\text{-}0.5\times10^{-8}$ M[28].



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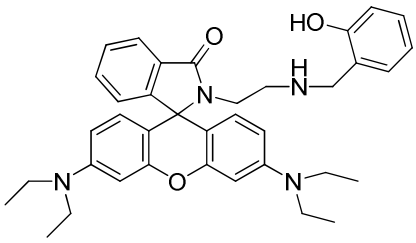

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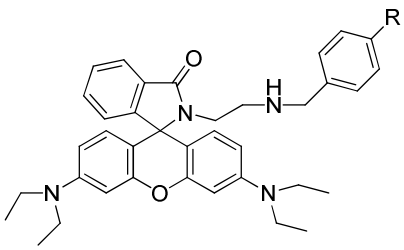
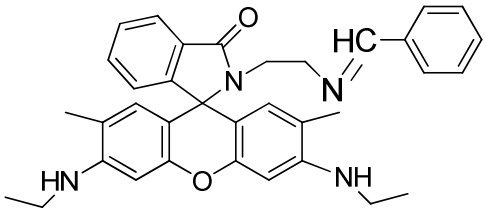


18 R=NEt_2
19 R=$N(EtOH)_2$



20

Recently Jie Mao, Que Hin et.al have developed a new chemosensor **20** which was a rhodamine derived Schiff's-base and used for the detection of mercury at 2 ppb level in drinking water[29]. The spectral studies were done in the Tris-HCl buffer solution. The fluorescence spectra's were obtained by the excitation at 500 nm.

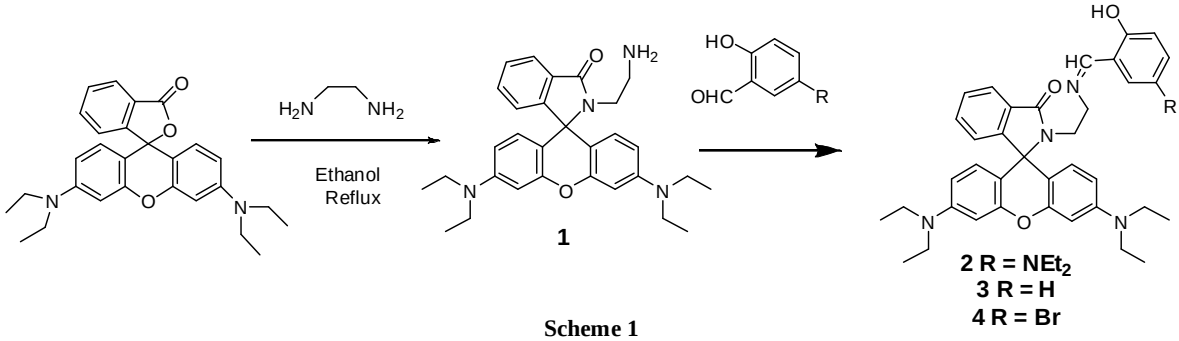
The rhodamine absorbance increased gradually at 527 nm with the addition of Hg^{2+} in maximum five minutes indicating that rhodamine structure is formed in which Hg^{2+} was firstly chelated with ═N=C- Group then with O=C group inducing a structural change in **20**. The fluorescent enhancement was due to the chelation of Hg^{2+} with the nitrogen atom of the imine group and oxygen atom of the amide groups. Which resulted in the ring opening form and was known as the best example of chemosensor for Hg^{2+} ion

Results and Discussion

In the present work, three new chromogenic sensors **2-4** has been synthesized based on Rhodamine B and substituted salicylaldehyde hybrid Schiff base dyads and investigated their photophysical behaviour towards different monovalent and divalent metal ions. The chemosensor **2** showed the selective behaviour towards the Fe^{2+} metal ion whereas chemosensor **3** and **4** showed the differential behaviour towards Fe^{2+} and Cu^{2+} ions.

Synthesis of chemosensors

The probes **2-4** have been synthesized by stirring the solution of **1** and 2-Hydroxybezaldehyde, 5-Diethylamino-2-hydroxybenzaldehyde, 4-Bromo-2-hydroxybezaldehyde in dry ethanol at 60˚ C to get the pure compound **2-4** respectively. The structures of the probes were characterized by ^1H NMR.



Scheme 1

Photophysical Studies

The chemosensor **2** (25µM, CH_3OH) shows an absorption spectrum having λ_{max} at 361 nm (ε= 15000 mol^{-1} cm^{-1}). On addition of different metal ions, *viz*. Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Ag^+ and Pb^{2+}, to solution of **2 (** figure 1**)**, there is no significant change in its UV-vis spectrum except in case of addition of Fe^{2+} ions. On addition of Fe^{2+} ions to the solution of **2**, a new absorption band at 558 nm appeared and showed the visible colour change from colourless to red. Hence the compound can be served as a "SELECTIVE NAKED – EYE" chemosensor for the detection of Fe^{2+} ion.

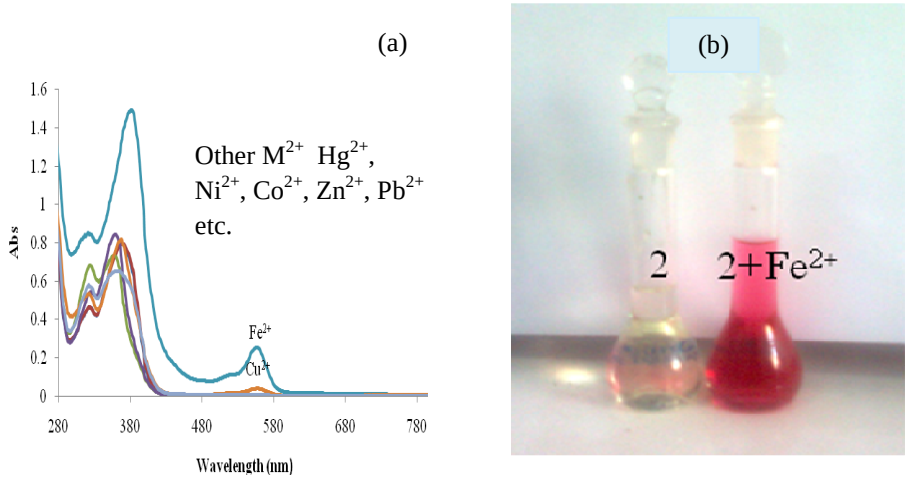


Figure 1: (a) Effect of metal ions on UV spectra of **2** (25µM, CH_3OH) (b) visible colour change of **2** upon addition of Fe^{2+} ions.

In order to evaluate the effect of this metal on the photophysical behaviour of **2**, the absorption titrations of this compound were performed at various concentrations of Fe^{2+} ions. Upon incremental addition of Fe^{2+} ions to the solution of **2** at10µM in CH_3OH the absorption band at 390 nm increases along with formation of new absorption band at 558 nm (figure2). The appearance of new absorption band at 558 nm is responsible for the visible colour change from colourless solution to red which indicates the ring opening state of spirocyclic form in the solution.

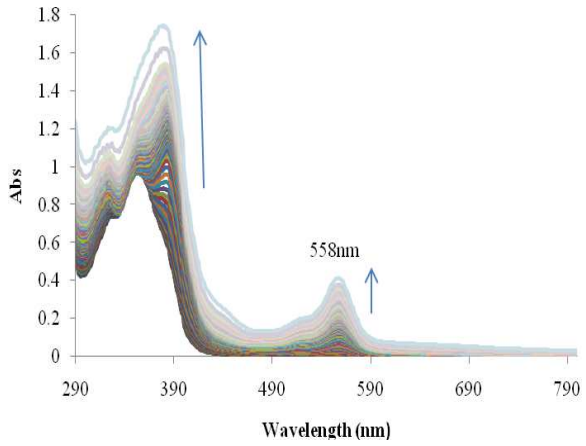


Figure 2: Effect of incremental addition of Fe^{2+} ion on chemosensor **2** (10µM, CH_3OH).

The chemosensor **3** (25 µM, CH_3OH) displayed an absorption spectrum having λ_{max} at 370 nm (ε = 5000 mol^{-1} cm^{-1}). On addition of different metal ions, *viz.* Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Ag^+ and Pb^{2+}, to solution of **3**, there is no significant change in its UV-vis spectrum except in case of addition of Cu^{2+} and Fe^{2+} ions (figure 3). On addition of Cu^{2+} to the solution of **3**, a new broad absorption band at 565 nm appeared and showed the visible colour change from colourless to pink. In case of addition of Fe^{2+} to the solution, a new absorption band at 560 nm appeared and showed the visible colour change from colourless to brown.

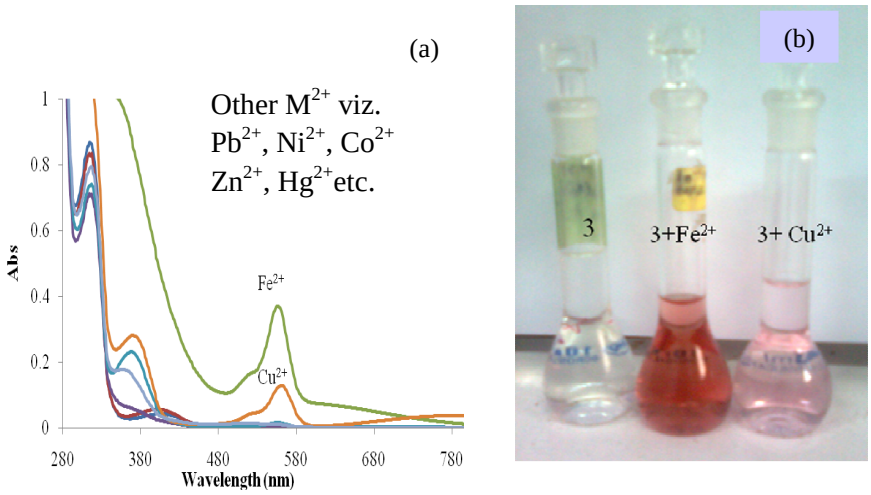


Figure 3: (a) Effect of metal ions on UV- spectra of **3** (25µM, CH_3OH) (b) Visible colour change of **3** upon addition of Fe^{2+} & Cu^{2+} ions.

In order to evaluate the effect of incremental addition of these metal ions on the photophysical behaviour of probe **3**, the absorption titrations of these compounds were performed at various concentrations of Fe^{2+} and Cu^{2+} ions. Upon incremental addition of Fe^{2+} ions to the solution of **3** at 20µM in CH_3OH the absorption band at 378 nm increases along with the formation of new absorption band at 560 nm. This new absorption band at 560 nm is responsible for the appearance of brown colour from colourless. In case of incremental addition of Cu^{2+} ions, the absorption band at 378 nm appeared but there was no absorption band at 500-600 nm (figure 4). This was in accordance with its visible colour change from colourless to pink.

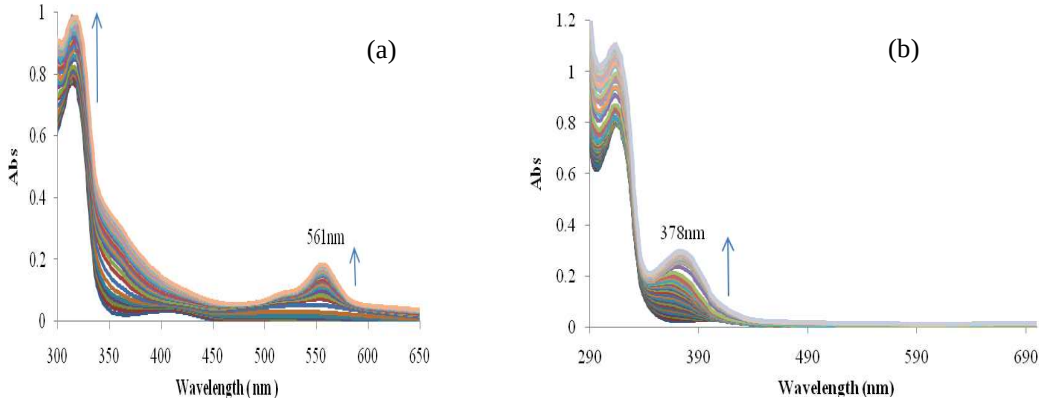


Figure 4: (a)**:** Effect of incremental addition of Fe^{2+} (b) Cu^{2+} ions on chemosensor **3** (20µM, CH_3OH).

The chemosensor **4** (25µM, CH_3OH) shows an absorption spectrum at λ_{max} at 319 nm (ε= 5000 $mol^{-1}cm^{-1}$). On addition of different metal ions, *viz*. Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Cr^{3+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Hg^{2+}, Cd^{2+}, Ag^{+} and Pb^{2+}, to solution of **4**, there is no significant change in its UV-vis spectrum except in case of addition of Fe^{2+} ions (figure 5). On addition of Fe^{2+} to the solution of **4,** a new absorption band at 560 nm appeared and showed visible colour change from colourless to dark brown. In case of addition of Cu^{2+} ions to the solution, a new absorption band at 565 nm appeared with change in the colour of the solution from colourless to magenta.

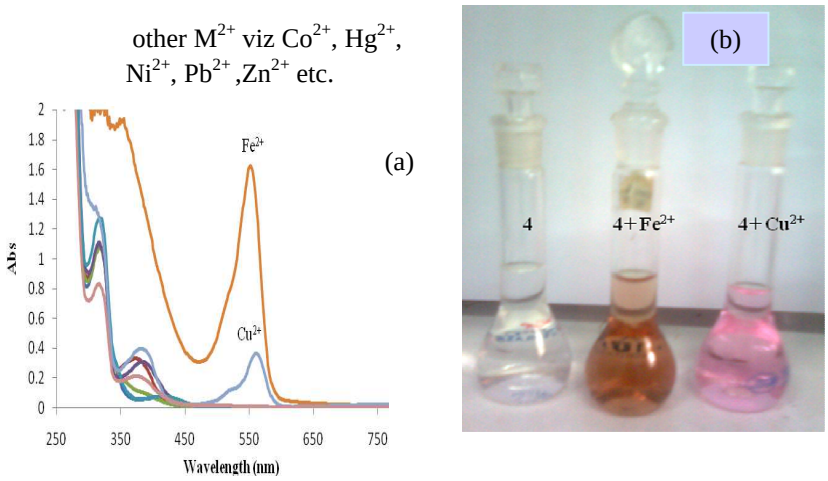


Figure 5: (a) Effect of metal ions on the UV spectra of **4** (25µM, CH_3OH) (b) visible colour change upon addition of Fe^{2+} & Cu^{2+} ions.

In order to evaluate the effect of incremental addition of these metal ions on the photophysical properties of **4**, the absorption titrations of these compounds were performed at various concentrations of Fe^{2+} and Cu^{2+} ions. Upon incremental addition of Fe^{2+} ions to the solution of **4** at 20µM in CH_3OH the absorption band at 309 nm increases along with the formation of new absorption band at 560 nm. This absorption band at 560 nm is responsible for the appearance of brown colour from colourless. In case of incremental addition of Cu^{2+} ions, the absorption band at 320 nm and 385 nm appeared but there was no absorption band at 500-600 nm (figure 6). This was in accordance with visible colour change from colourless to magenta.

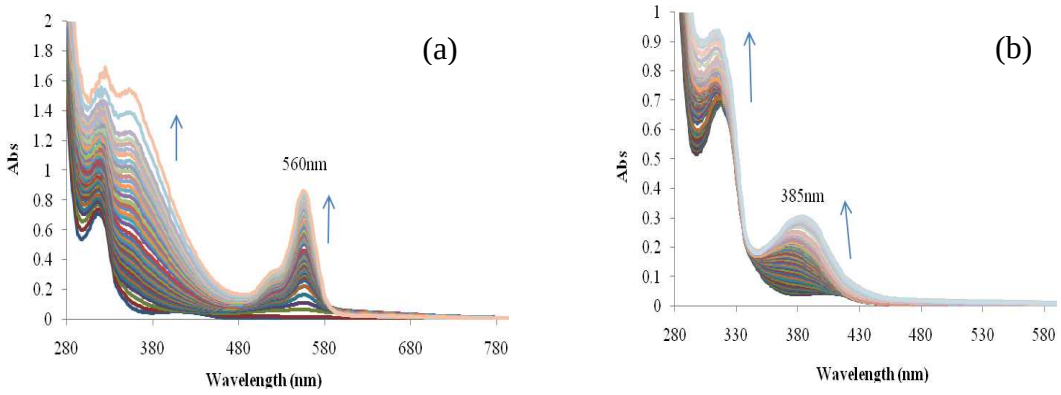
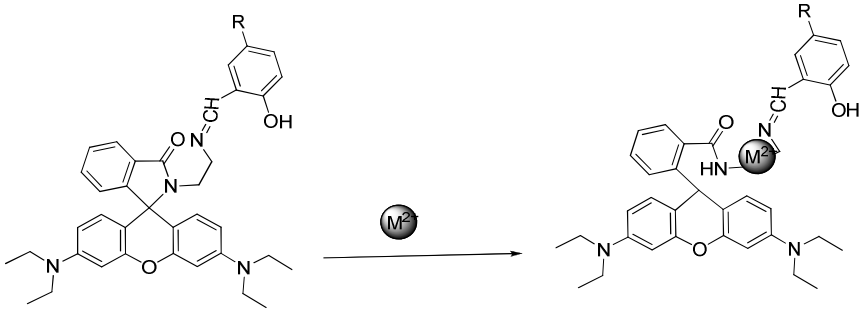


Figure 6 : (a)**:** Effect of incremental addition of Fe^{2+} (b) Cu^{2+} ions on chemosensor **4** (20µM, CH_3OH).

It was significantly observed that the chemosensor **4** shows more predominant visible colour changes on addition of Fe^{2+} and Cu^{2+}. This is due to the electron withdrawing effect of Br group at the para position of chemosensor **4**.



In the pure methanol system, the Rhodamine B derivatives **2-4** forms nearly colourless solution indicating that the spirocyclic form exists predominantly (Proposed mechanism). However, the addition of Fe^{2+} ions to the solution of **2-4** results in the change in the colour of the solutions from colourless to reddish brown and also the change in the colour takes place during the addition of Cu^{2+} ions from colourless to pink. The change in the colour of the solution indicates the ring opened state of the spirocyclic form in the presence of metal ions. Hence the chemosensor **2** can be served as a "NAKED –EYE" sensor for the detection of Fe^{2+} ions. The chemosensor **3-4** can be used as differential sensor for the detection of Fe^{2+} and Cu^{2+} ions.

Experimental

Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on JEOL 300 MHz NMR spectrometer using $CDCl_3$ as solvent. Chemical shifts are given in ppm with TMS as an internal reference. *J* values are given in Hertz. Signals are abbreviated as singlet, s; doublet, d; double-doublet, dd; triplet, t; multiplet, m. Chromatography was performed with silica 100-200 mesh and reactions were monitored by thin layer chromatography (TLC) with silica plates coated with silica gel HF-254. All the chemicals viz. Rhodamine B, Ethylenediamine, 2-Hydroxybenzaldehyde, 5-Diethylamino-2-hydroxybenzaldehyde and 4-Bromo-2-hydroxybenzaldehyde were purchased from Aldrich, Lobachem and Spectrochem were used without further purification.

General procedure for spectral detection

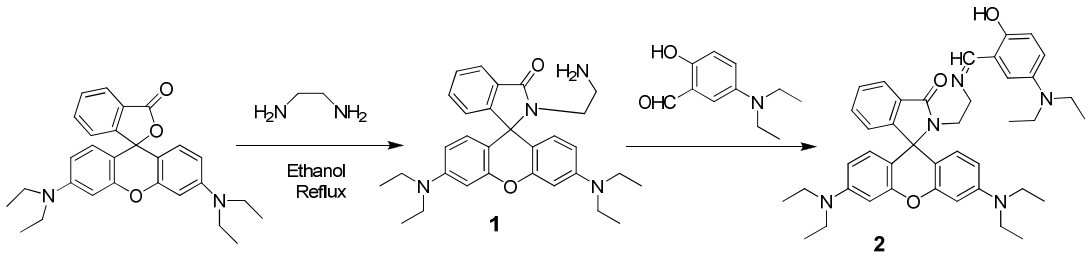
All the solvents were of analytical grade and used after distillation. All UV-Visible spectrum were recorded at Analytic Jena SPECORD 205 UV-visible spectrophotometer. UV-Vis Spectrophotometer by using slit widths of 1.0 cm and matched quartz cells. All absorption scans were saved as ACS II files and further processed in Excel™ to produce all graphs shown. The stock solutions of chemosensor **2-4** (1 mM) were prepared in CH_3OH. The appropriate amount of aliquot was transfered to measuring flask and solutions were diluted with doubly distilled methanol to get desired solution of the chemosensor. The addition of different concentration of $M(ClO_4)_2$ were carried out with a micropipette in aliquots of 1.5-6.0 µl in the same cell and each time the solution was allowed to stand for 3 min before recording the UV-vis spectrum.

Synthesis of chemosensors 2-4

i) Synthesis of N-(Rhodamine B)lactam-ethylenediamine: The N-(Rhodamine B) lactam-ethylenediamine was synthesized as reported in literature[30]. Rhodamine B (958mg, 2mmol) was dissolved in 20 mL hot ethanol, followed by addition of ethylenediamine (1mL, 15mmol). The reaction mixture was refluxed for 4 h till the fluorescence of the solution has been disappeared. The reaction was cooled to room

temperature and solid was washed with absolute ethanol for three times to obtain the pure product **1** with 97.8% yield.

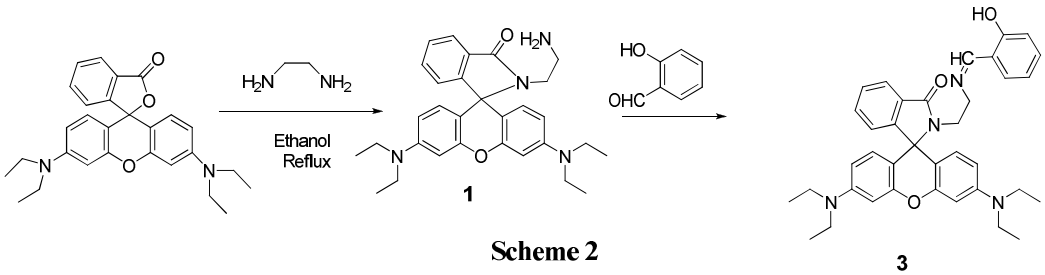
ii) Synthesis of 3',6'-bis(diethylamino)-2-(2-(5-diethylamino)-2-hydroxybenzyledeneamino)ethyl) spiro[isoindoline-1,9'-xanthen]-3-one (2):



Scheme 1

N-(Rhodamine B) lactam- ethylenediamine (200 mg, 0.38 mmol) was dissolved in 5ml THF, followed by the addition of 5-Diethylamino-2-hydroxybenzaldehyde (0.0734 gm, 0.36 mmol). The reaction mixture was stirred for 24 h. The solvent was evaporated and the product was filtered and solid was washed with ether. The pure product **2** was obtained with 81.9% yield; m.pt : 180-190 ˚C; ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.89-7.91 (dd, 1H, ^1J = 2.4Hz, ^2J = 5.4Hz), 7.74 (s, 1H), 7.39-7.45 (dd, 2H, ^1J = 3Hz, ^2J = 6Hz), 7.06-7.10 (dd, 1H, ^1J = 3Hz, ^2J = 9Hz), 6.86 (d, 1H, J = 9Hz), 6.39-6.44 (m, 4H), 6.23-6.27(dd, 2H, ^1J = 3Hz, ^2J = 8.7Hz), 6.06-6.10 (dd, 1H, ^1J = 2.4Hz, ^2J = 11.1Hz), 6.01 (d, 1H, J = 2.4Hz), 3.27 (m, 16H), 2.17 (m, 18H).

iii) Synthesis of 3',6'-bis(diethylamino)-2-(2-(2hydroxybenzylamino)ethyl) spiro[isoindoline-1,9'-xanthen]-3-one (3):

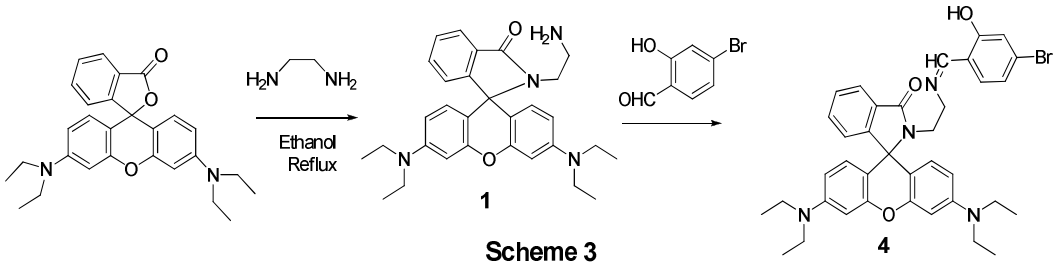


Scheme 2

N-(Rhodamine B) lactam-ethylenediamine (200mg, 0.38mmol) was dissolved in 5 ml THF, followed by the addition of salicylaldehyde (0.04 gm, 0.32 mmol). The reaction

mixture was stirred for 5h. The solvent was evaporated and the product was filtered and solid was washed with ether. The product **3** was obtained with 80.1% yield; m.pt : 90-100 ˚C; ^{1}H NMR (300MHz, $CDCl_3$) : δ 13.25 (bs, 1H, OH), 7.79-7.82 (dd, 1H, ^{1}J = 2.4Hz, ^{2}J = 3.9Hz), 7.73 (s, 1H), 7.51-7.55 (dd, 1H, ^{1}J = 2.4Hz, ^{2}J = 5.4Hz), 7.40-7.44 (dd, 2H, ^{1}J = 3Hz, ^{2}J = 4.8Hz), 7.33-7.36 (dd, 2H, ^{1}J = 2.4Hz, ^{2}J = 5.4Hz), 7.25 (d, 1H, J = 2.4Hz), 7.04-7.08 (dd, 1H, ^{1}J = 3.0Hz, ^{2}J = 4.5Hz), 6.80 (d, 1H, J = 6.7Hz), 6.41-6.45 (dd, 3H, ^{1}J = 3Hz, ^{2}J = 5.4Hz), 6.25-6.30 (dd, 2H, ^{1}J = 2.4Hz, ^{2}J = 6Hz), 3.32-3.47 (m, 12H), 1.14-1.20 (m, 12H).

iv) Synthesis of 2-(2-(4-bromo-2-hydroxybenzylideneamino)ethyl-3',6'-bis (diethylamino)spiro[isoindoline-1,9'-xanthen]-3-one (4):



Scheme 3

N-(Rhodamine B) lactam-ethylenediamine (260.5 mg, 0.5 mmol) was dissolved in 5ml THF, followed by the addition of 4-bromo-2-hydroxybenzaldehyde (100 mg, 0.5 mmol). The reaction mixture was stirred at 60˚C for 24 h. The solvent was evaporated and the product was filtered and solid was washed with ether. The crude product was obtained which was further recrystallized from CH_3CN to obtain pure compound **4** in 65.2% yield; m.pt : 90-100 ˚C ; ^{1}H NMR (300MHz, $CDCl_3$) : δ 13.35 (bs, 1H, OH), 7.90-7.93 (dd, 1H, ^{1}J = 3.0Hz, ^{2}J = 3.9Hz), 7.87 (s, 1H), 7.42-7.45 (dd, 2H, ^{1}J = 4.5Hz, ^{2}J = 5.4Hz), 7.30-7.34 (dd, 2H, ^{1}J = 2.4Hz, ^{2}J = 4.8Hz), 7.20 (d, 1H, J = 2.1Hz), 7.07-7.09 (dd, 1H, ^{1}J = 3.0Hz, ^{2}J = 5.4Hz), 6.84 (d, 1H, J = 8.7Hz), 6.37-6.41 (dd, 3H, ^{1}J = 1.2Hz, ^{2}J = 9Hz), 6.20-6.24 (dd, 2H, ^{1}J = 9Hz, ^{2}J = 2.7Hz), 3.29-3.45 (m, 12H), 1.18-1.23 (m, 12H).

Conclusions

1. The chemosensors **2-4** based on Schiff bases of N-(Rhodamine B)lactam-ethylenediamine and substituted aldehydes has been synthesized and characterised through NMR spectroscopy.

2. The chemosensor **2** shows the selective behaviours towards Fe^{2+} ions and can be used as naked eye sensor for Fe^{2+}.

3. The chemosensors **3-4** shows the differential behaviour towards Cu^{2+} and Fe^{2+}. These two shows visible color change from colorless to brown with Fe^{2+} and pink with Cu^{2+} ions.

4. The chemosensors **3-4** can be used to estimate the Cu^{2+} and Fe^{2+} ions simultaneously with two different visible color changes.

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