

Synthesis, characterization and ion recognition studies of lower rim 1,3-di{rhodamine} conjugate of calix[4]arene

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Abstract. An amido-linked rhodamine conjugate of calix[4]arene, **L** has been synthesized and characterized. Metal ion recognition properties of **L** have been studied by emission and absorption techniques with 14 different metal ions including the transition ones. Results show that, **L** exhibits ratiometric emission intensity towards Hg^{2+} , Fe^{2+} , Fe^{3+} , Cu^{2+} , Pb^{2+} and Zn^{2+} . Composition of the complex formed in the solution has been found to be 1:2 ($\text{L}:\text{M}^{n+}$), based on the Job's plot. The **L** can also act as a chemosensor for Hg^{2+} through naked eye detection. Fluorescence quenching observed at 485 nm follows an order, $\text{Hg}^{2+} \gg \text{Fe}^{3+} \sim \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Pb}^{2+} > \text{Ca}^{2+}$, while the enhancement observed at 580 nm follows, $\text{Hg}^{2+} \gg \text{Fe}^{2+} \sim \text{Pb}^{2+} > \text{Zn}^{2+}$. Mode of interaction of M^{n+} with **L** is by the ring opening of spirolactam moiety.

Keywords. Rhodamine conjugate of calix[4]arene; ratiometric sensor for Hg^{2+} ; ion recognition; emission and absorption titrations.

1. Introduction

Rhodamine dye is used extensively as a moiety for fluorescent labelling and bio-imaging due to its excellent photophysical properties such as higher absorption and emission wavelengths and high fluorescence quantum yield.¹ Metal ion sensing mechanism of the rhodamine-based receptor is dependent on the changes that occur in the structure between the spirocyclic form and its open one, which in turn changes its emission and absorption spectra. Various rhodamine-based fluorescent metal ion sensors without the calix[4]arene platform have been reported in literature.² These include rhodamine cyclen and thiolactone derivatives³ for Hg^{2+} , and rhodamine hydrazide and binaphthyl conjugates⁴ for Cu^{2+} and bispicolyl amine derivative⁵ for Pb^{2+} . Thus, the calix[4]arene conjugates possessing rhodamine are least explored in literature. Calix[4]arene-based fluorescence resonance energy transfer (FRET) chemosensor containing pyrenyl groups (donor) and a rhodamine group (acceptor) have been reported for Hg^{2+} .⁶ A thia calix[4]arene-based rhodamine receptor has been reported⁷ for selective sensing of Cr^{3+} and Fe^{3+} . It is always of interest to explore ion recognition properties of functionalized calix[4]arenes with rhodamine moiety. Therefore, this

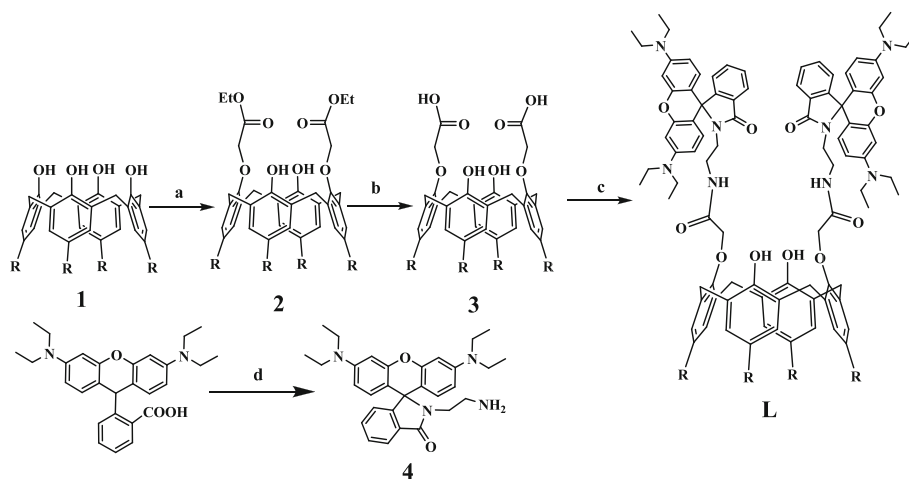
study deals with the synthesis, characterization and ion recognition studies of lower rim 1,3-di(rhodamine) conjugate of calix[4]arene (**L**).

2. Experimental

2.1 Synthesis and characterization of **L**

This has been synthesized in a three-step synthetic procedure as shown in scheme 1. To an ice cold solution of calix[4]arene diacid **3** (0.5 g, 0.65 mmol) in CH_2Cl_2 , Et_3N (450 μL , 3.3 mmol), 1-ethyl-(3-dimethylaminopropyl)-3-carbodiimide hydrochloride salt of EDCI (EDCI.HCl) (0.5 g, 2.6 mmol) and catalytic amount of hydroxybenzotriazole (HOBT) were added and stirred for 30 min. To this solution, rhodamine ethylene amine **4** (0.7 g, 1.4 mmol) was added. Stirring was continued for another 30 min at 0°C. The reaction mixture was allowed to come to room temperature and the stirring continued overnight. Solvent was evaporated under reduced pressure. The solid was re-dissolved in dichloromethane. The reaction mixture was washed with water followed by brine and the organic layer was collected and dried with anhydrous Na_2SO_4 . After filtration, the solvent was removed under reduced pressure. Residue was purified by silica gel column chromatography (petroleum ether/EtOAc) to afford **L**. Yield (0.70 g, 63%). Elemental Anal.

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Scheme 1. Synthesis of **L**: (a) bromoethylacetate/ K_2CO_3 /acetone; (b) NaOH/ C_2H_5OH , reflux; (c) EDCI.HCl/aminoethyl rhodamine (**4**)/DCM/rt/12 h. R = *tert*-butyl; (d) ethylenediamine, ethanol, reflux, 12 h.

$C_{108}H_{128}N_8O_{10}$ (% found) C 76.72, H 7.58, N 6.72; (% requires) C 76.38, H 7.60, N 6.60; fourier transform infrared (FTIR): (KBr, cm^{-1}): 1686, 1634 (ν C=O). 1H nuclear magnetic resonance (NMR) (400 MHz, $CDCl_3$, δ ppm): 8.50 (t, 2H, CONH), 7.75 (m, 1H, lactam-CO-Ar-H), 7.44 (m, 2H, Ar-H), 7.32 (m, 2H, rhod-Ar-H), 6.39(s, 1H, Ar-H), 6.36 (s, 1H, rhod-Ar-H), 6.35 (d, 2H, rhod-Ar-H, $J = 2.47$ Hz), 6.19 (dd, 2H, $J = 2.36$ Hz, $J = 2.46$ Hz), 3.28 (m, 24H, rhod- CH_3), 4.48 (s, 4H, O- CH_2), 4.25 (d, 2H, calix- CH_2), 3.70 (d, 4H, calix- CH_2), 1.23 (s, 18H, calix-*tert*-butyl- CH_3), 1.12 (t, 24H, rhod), 0.97 (s, 18H, calix-*tert*-butyl- CH_3), ^{13}C NMR: ($CDCl_3$, δ ppm): 12.8, 14.3, 22.9, 29.5, 29.9, 31.2, 31.8, 32.1, 31.2, 33.9, 34.1, 38.3, 39.5, 44.5, 65.1, 98.0, 105.7, 108.3, 122.9, 123.9, 125.9, 127.8, 128.9, 130.7, 132.3, 132.6, 142.4, 147.6, 148.8, 149.8, 149.9, 153.3, 154.2, 168.8. ESI MS Calculated for $C_{108}H_{128}N_8O_{10}$:1698.22. ESI MS found: m/z (intensity (%), fragment) 1698.90 (40, $[M]^+$).

2.2 Fluorescence and absorption studies

All the fluorescence titrations were carried out on a Perkin Elmer LS55 at an excitation wavelength of 315 nm in 1 cm quartz cell. Bulk solutions (3×10^{-4} M) of **L** were freshly made before each set of experiments by dissolving the ligand in acetonitrile and then making up with the same solvent. Metal perchlorate salts were made in acetonitrile at 3×10^{-4} M. Fluorescence titrations were carried out after adding appropriate volume of metal salt solution to result in requisite mole ratios of $[M^{n+}]/[L]$, yet maintaining the final $[L]$ as 5 μ M in a total solution volume of 3 mL achieved by diluting

with acetonitrile. Absorption titrations were performed analogously.

3. Results and discussion

Receptor molecule, **L** was synthesized *via* three known steps⁸ starting from *p*-*tert*-butyl calix[4]arene (**1**) as shown in scheme 1. Hydrolysis of diester derivative, **2** under basic condition yields the corresponding di-acid, **3** which upon coupling using EDCI.HCl with aminoethyl rhodamine (**4**) resulted in **L**. In turn, the aminoethyl rhodamine was synthesized from rhodamine B using the literature reported procedure.⁹ All these molecules including **L** were characterized satisfactorily by 1H and ^{13}C NMR, Electrospray ionization – mass spectrometry (ESI-MS), infrared (IR) and elemental analysis. The 1H NMR and mass spectra for **L** are given in figure 1. Cone conformation of **L** is evident from 1H NMR spectroscopy as the bridged $-CH_2$ protons exhibit doublets at 4.25, 3.70 ppm and the *tert*-butyl- CH_3 as singlets at 1.23, 0.97 ppm.

3.1 Fluorescence titration studies

Chemo-sensing characteristics of receptor **L** were studied by fluorescence measurements. Perchlorate salts of 14 metal ions, *viz.*, Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Fe^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Hg^{2+} and Pb^{2+} , were used to evaluate ion binding and recognition characteristics of **L** in acetonitrile. Receptor **L** exhibited absorption bands of rhodamine and calix[4]arene moieties at 315 and 275 nm, respectively, in acetonitrile. When **L** is excited at 315 nm, it emits in the region of

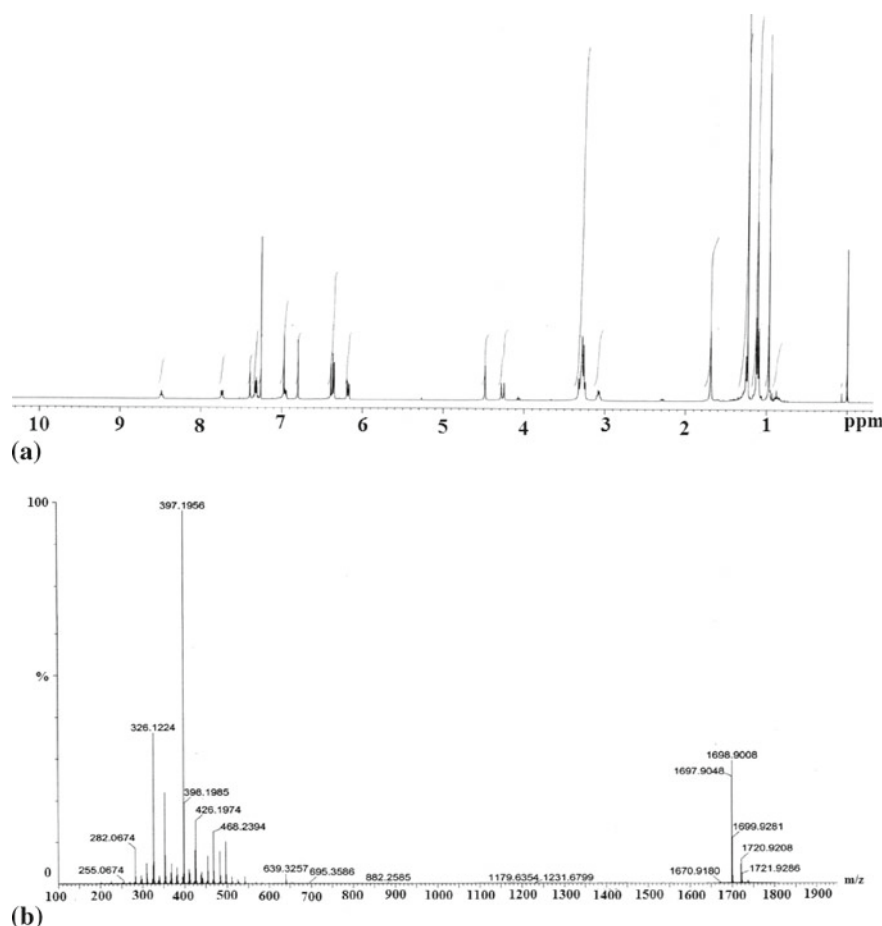


Figure 1. (a) ¹H NMR (in CDCl₃, 400 MHz) and (b) ESI-MS spectra of **L**.

400–600 nm with emission peak maximum at 485 nm, which originates from the rhodamine moiety with spiro-lactam ring. Initially, there is no distinct band at 580 nm, but upon addition of metal ions such as Hg²⁺, the emission intensity of a new band at 580 nm was found to increase ratiometrically (figure 2). Selective enhancement observed at 580 nm is up to 25-fold in case of Hg²⁺. Other metal ions such as Cu²⁺, Zn²⁺, Fe²⁺, Fe³⁺ and Pb²⁺ also show enhancement at 580 nm, but only up to a maximum of 5-fold in acetonitrile. Ratiometric

decrease in emission intensity was observed for metal ions such as Hg²⁺, Cu²⁺, Zn²⁺, Fe³⁺, Fe²⁺ and Pb²⁺. Emission peak observed at 580 nm is due to the ring opening of the spiro-lactam moiety¹⁰ that is attributed to Mⁿ⁺-promoted hydrolysis, which yields highly fluorescent species as given in scheme 2. During metal ion titrations in fluorescence, as ring-opened species grow, concentration of **L** decreases and hence emission intensity of 485 nm band decreases, while that of 580 nm band increases. Emission intensity in case of

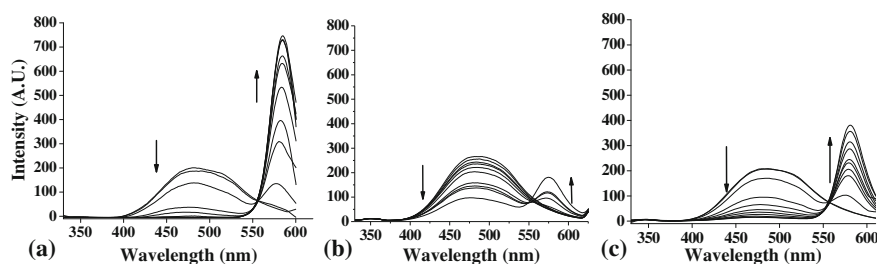
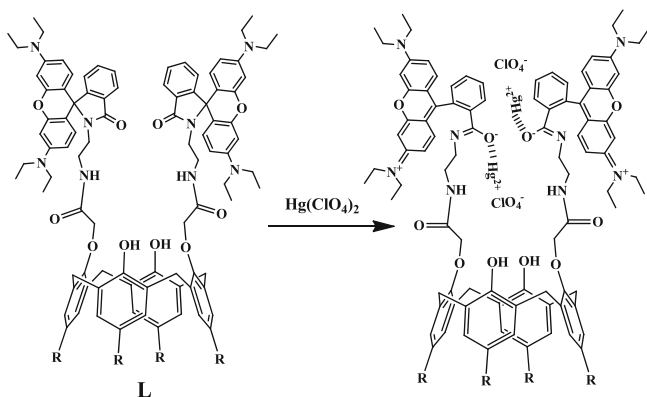


Figure 2. Fluorescence spectral traces observed in the titration of **L** with Mⁿ⁺ to result in [Mⁿ⁺]/[**L**] mol ratio from 0 to 10.0 for (a), (c) and 0 to 2 for (b) at [**L**] = 5 μM in acetonitrile. Arrow indicates the increase or decrease of fluorescence intensity upon increased metal ion addition. (a) Hg²⁺; (b) Cu²⁺; (c) Pb²⁺.



Scheme 2. Proposed ring-opened species formed during upon addition of Hg^{2+} to **L**. $\text{R} = \text{tert-butyl}$.

other metal ions such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Mn^{2+} , Ni^{2+} , Co^{2+} and Cd^{2+} showed no change at 580 nm band. However, it was noticed that metal ions such as Ca^{2+} , Mg^{2+} and Mn^{2+} showed marginal quenching of fluorescence intensity at 485 nm. Based on the data shown in figures 3 and 4, fluorescence quenching of 485 nm follows an order, *viz.*, $\text{Hg}^{2+} \gg \text{Fe}^{3+} \sim \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Pb}^{2+} > \text{Ca}^{2+}$, while an increase is observed with the 580 nm band and this follows an order, *viz.*, $\text{Hg}^{2+} \gg \text{Fe}^{2+} \sim \text{Pb}^{2+} > \text{Zn}^{2+}$.

During titration of **L** with Cu^{2+} and Fe^{3+} up to 2 equivalents intensity of 580 nm band enhances and exhibits stoichiometry. Beyond two equivalents, excess paramagnetic Cu^{2+} or Fe^{3+} quenches emission intensity of the ring-opened **L**. Quantum yield obtained with rhodamine B as a standard is 0.055 for **L** and 0.065 [**L**+ Hg^{2+}] in acetonitrile. Quantum yield for **L** with other metal ions has been measured and the data is, 0.049, 0.057, 0.031 and 0.039, respectively, for [**L**+ Fe^{2+}], [**L**+ Zn^{2+}], [**L**+ Cu^{2+}] and [**L**+ Pb^{2+}]. Minimum detection limit of Hg^{2+} by **L** is 670 ± 5 ppb in acetonitrile and the same for other metal ions is 1.0, 0.84, 0.69, 0.7 and 2.7 ppm, respectively, for Fe^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} and Pb^{2+} . Association constant of **L**

with Hg^{2+} was calculated by Benesi–Hildebrand equation¹¹ and the corresponding association constant (K_a) is $39800 \pm 100 \text{ M}^{-1}$. For other metal ions, *viz.*, Fe^{2+} , Pb^{2+} and Zn^{2+} , association constants are 22580 ± 400 , 21400 ± 250 and $17000 \pm 200 \text{ M}^{-1}$, respectively.

3.2 Absorption studies

Absorption titrations of **L** with Hg^{2+} and other metal ions were carried out at $5 \mu\text{M}$ in acetonitrile. Titration exhibited three isosbestic points at 260, 296 and 334 nm indicating a transition between the unbound **L** and that of the Hg^{2+} -bound one. A new band is formed at 555 nm and absorbance of this band increases significantly as concentration of Hg^{2+} increases (figure 5). The spectra also exhibited an increase in the absorbance in ~ 352 nm band and a decrease in case of ~ 371 and ~ 317 nm bands. Stoichiometry of the complex formed between **L** and Hg^{2+} is 1:2 as derived based on Job's plot. The same stoichiometry is true even for Cu^{2+} and Pb^{2+} (figures 6 and 7). During absorption titration of **L** with Cu^{2+} , absorbance at 555 nm increases up to 2 equivalents and decreases beyond this (figure 6). Comparative histogram shown in figure 8 indicates that Hg^{2+} exhibits maximum changes in the intensity at 555 nm band followed by other metal ions such as Zn^{2+} , Fe^{3+} , Cu^{2+} and Pb^{2+} .

3.3 Visual colour changes

In order to explore the chemo-sensor properties of **L**, colour change experiments were carried out at $10 \mu\text{M}$ of **L** and two equivalents of metal ions in acetonitrile. Distinct visual colour changes were observed for Hg^{2+} immediately after the addition. Appearance of pink colour suggests the ring opening of rhodamine moiety in **L** upon addition of Hg^{2+} . Metal ions such as Cu^{2+} and Pb^{2+} also developed light pinkish colour after 30 min of addition (figure 9). All this suggests that the

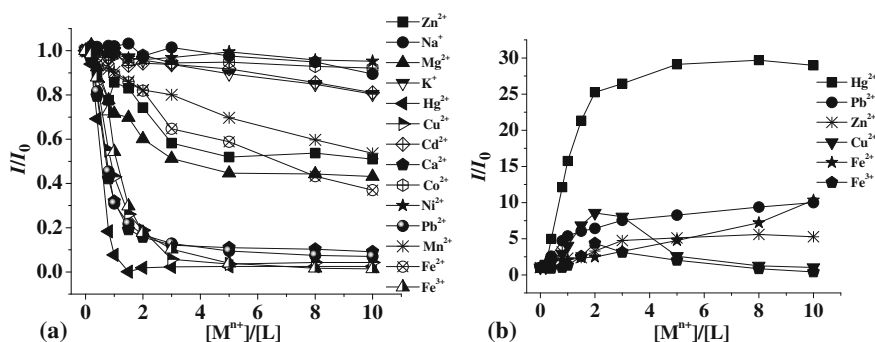


Figure 3. Relative fluorescence intensity plots of (I/I_0) vs. $\text{M}^{n+}/[\text{L}]$ mol ratios at: (a) 485 nm and (b) 580 nm. Concentration of **L**: $5 \mu\text{M}$.

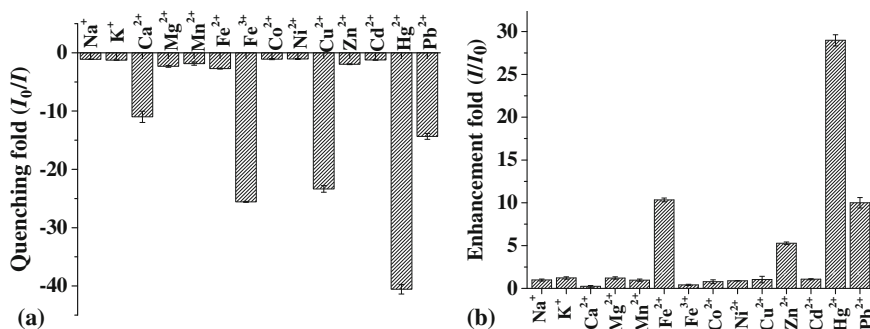


Figure 4. Histograms of highest I_0/I and I/I_0 ratios (a) at 485 nm and (b) at 580 nm indicating quenching and enhancement fold, respectively for the titration of **L** ($5 \mu\text{M}$) by different metal ions at 10 equivalents metal ion addition.

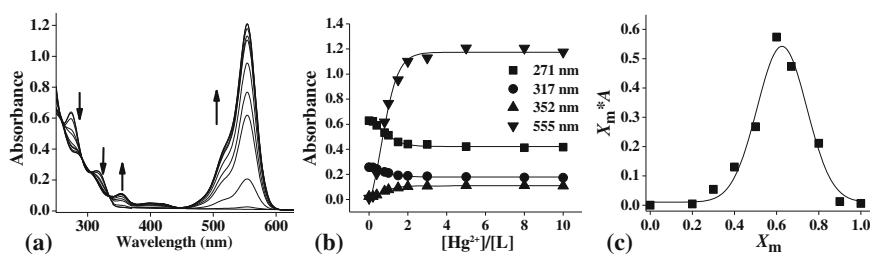


Figure 5. (a) Absorption spectra obtained during the titration of **L** ($5 \mu\text{M}$) with Hg^{2+} up to 10 equivalents. (b) Plot of absorbance vs. $[\text{Hg}^{2+}]/[\text{L}]$ for different bands. (c) Job's plot of X_m versus $A \cdot X_m$, where X_m is mol fraction of the Hg^{2+} added and A is absorbance.

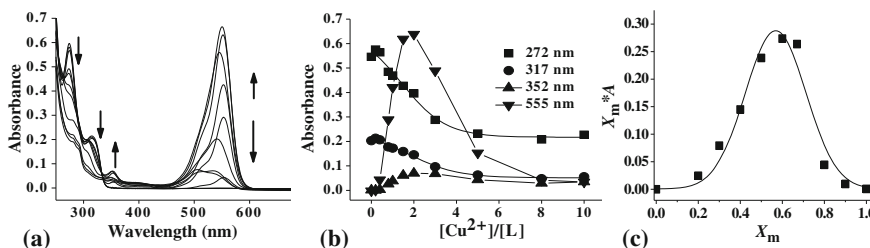


Figure 6. (a) Absorption spectra obtained during the titration of **L** ($5 \mu\text{M}$) with Cu^{2+} up to 10 equivalents. (b) Plot of absorbance vs. $[\text{Cu}^{2+}]/[\text{L}]$ for different bands. (c) Job's plot of X_m vs. $X_m \cdot A$, where X_m is mol fraction of Cu^{2+} added and A is absorbance.

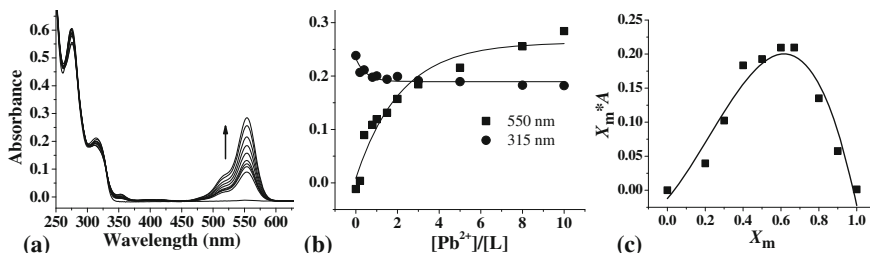


Figure 7. (a) Absorption spectra obtained during titration of **L** ($5 \mu\text{M}$) with Pb^{2+} up to 10 equivalents. (b) Plot of absorbance vs. $[\text{Pb}^{2+}]/[\text{L}]$ for 550 and 315 nm bands. (c) Job's plot of X_m vs. $X_m \cdot A$, where X_m is mol fraction of Pb^{2+} added and A is absorbance.

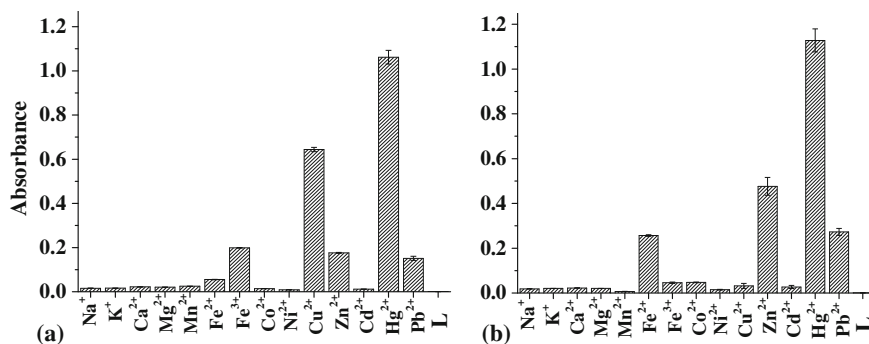


Figure 8. Histograms of absorbance at 555 nm band during titration of **L** at 5 μ M concentration with different metal ions. (a) Absorbance obtained at 2 equivalents of metal ion addition to **L**. (b) Absorbance obtained at 10 equivalents of metal ion addition to **L**.

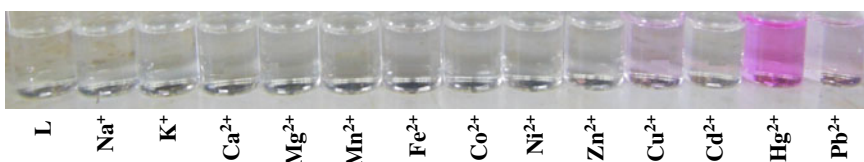


Figure 9. Colour of acetonitrile solutions of **L** (10 μ M) in the presence of different metal ions to give 1:2 mol ratio, after 30 min of their preparation. Until 30 min, no pink colour was developed in case of Cu^{2+} and Pb^{2+} .

reaction is instantaneous with Hg^{2+} , while it is slow with Cu^{2+} and Pb^{2+} . However, all other metal ions did not show any colour change. Thus, visual colour change can be used to sense Hg^{2+} .

4. Conclusion

A new calix[4]arene-based receptor containing rhodamine (**L**) was synthesized and characterized. Chemosensing characteristics of **L** were investigated by fluorescence and absorbance measurements. **L** does not show selective recognition of metal ion during fluorescence and absorption titrations. However, high ratiometric changes occurred in emission intensity with Hg^{2+} at 480 and 585 nm when compared to other metal ions in acetonitrile. Quenching at 485 nm band follows an order, viz., $\text{Hg}^{2+} \gg \text{Fe}^{3+} \sim \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Pb}^{2+} > \text{Ca}^{2+}$, whereas enhancement observed at 580 nm band follows an order, viz., $\text{Hg}^{2+} \gg \text{Fe}^{2+} \sim \text{Pb}^{2+} > \text{Zn}^{2+}$. Mode of interaction of M^{n+} with **L** is by the ring opening of spirolactam moiety as shown in scheme 2 and this has precedence in literature in case of an amide derivative of rhodamine.¹⁰ Composition of the complex has been found to be 1:2 (**L**: M^{n+}) based on Job's plot. Spontaneous pink colour formed in the presence of Hg^{2+} can impart visual chemosensor property to **L** towards this ion over 13 other ions studied.

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