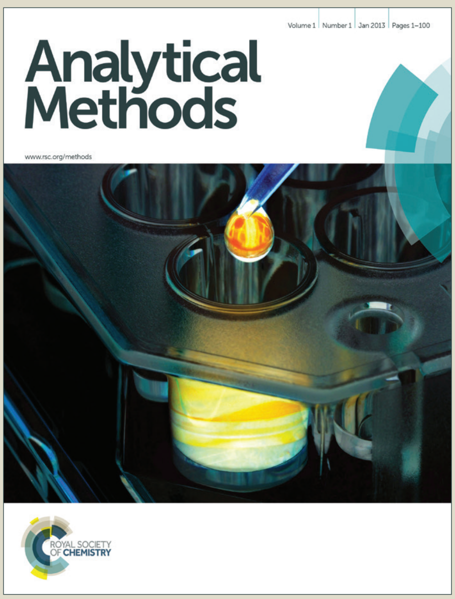
Analytical Methods

Accepted Manuscript



Analytical Methods

Dynamic Article Links ►

Cite this: DOI: 10.1039/x0xx00000x

www.rsc.org/

RESEARCH ARTICLE

Rhodamine-labelled simple architectures for fluorometric and colorimetric sensing of Hg^{2+} and Pb^{2+} ions in semi-aqueous and aqueous environments

Kumaresh Ghosh[*a], Tanmay Sarkar[a], Anupam Majumdar[a], Sushil Kumar Mandal[b] and Anisur Rahman Khuda-Bukhsh[b]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

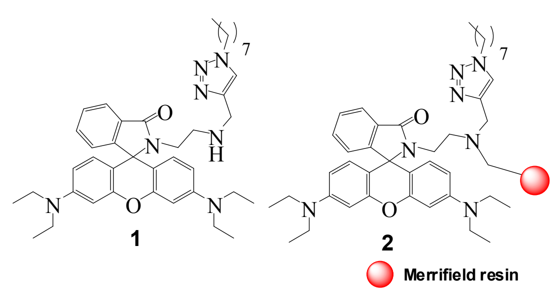
Triazole motif linked rhodamine derivatives **1** and **2** have been accomplished. The chemosensor **1** recognizes both Hg^{2+} and Pb^{2+} ions in CH_3CN/water (4/1, v/v; 10 µM tris HCl buffer, pH 7.0) both colorimetrically and fluorometrically. The chemosensor **1** is cell permeable and detects the intercellular Hg^{2+} and Pb^{2+} ions. The aqueous phase recognition of these ions have also been studied with the resin bound sensor **2** which exhibits more sensitivity and selectivity towards Hg^{2+} over Pb^{2+} ion.

Introduction

Considerable attention on the design of optical chemosensors for selective sensing of heavy metal ions has been focused in recent time as they can cause several diseases in living organisms.[1] Of particular interest is the development of fluoroscent sensor for heavy transition metal ions such as Hg^{2+} and Pb^{2+}, due to their biological and environmental importance.[2] Among the different metal ions, Hg^{2+} ion is considered to be one of the most toxic elements in the environment.[3] Accumulation of mercury even in low concentration in the human body, causes several diseases such as prenatal brain damage, serious cognitive, motion disorders, and Minamata disease.[3] Similarly, Pb^{2+} ion is the most toxic heavy metal ion as like as Hg^{2+}. The accumulation of lead in the body can result in neurological, reproductive, cardiovascular and developmental disorders[4] Therefore, the development of new architectures for the selective determination of Pb^{2+} and Hg^{2+}, is highly desirable.

In this regard, a number of Hg^{2+} and Pb^{2+}-selective chromogenic and fluorogenic sensors are known in the literature.[5,6] Of the different structures, rhodamine labelled compounds are found to be interesting as metal ion binding induced opening of the non fluorescent spirolactam form of rhodamine gives rise to a strong fluorescence emission and color.[7] During the course of our work on the sensing of cations and anions of biological significance,[5z,8] we report in this full account a new rhodamine – based receptor **1** which recognizes both Hg^{2+} and Pb^{2+} ions in semi-aqueous system [CH_3CN/water (4/1, v/v; 10 µM tris HCl buffer; pH 6.8)]. It is mentionable that the chemosensor **1** shows a greater selectivity towards Hg^{2+} ions over Pb^{2+} ions. In an effort to sense such metal ions in water, Merrifield resin bound new structure **2** has been developed. This resin bound structure **2** senses Hg^{2+} ion more effectively than Pb^{2+} ions in water.

The use of solid support in building up new molecular architecture for the recognition of important analytes has been an area of focus in recent times. The development of these systems are easy and they are reusable. In this context, there are some interesting reports on the detection of toxic metal ions using solid supported compounds.[9] Recently, we have reported,[10] for the first time, the sensing of Hg^{2+} ions using Merrifield resin bound rhodamine derivative in aqueous environment. The promising result further tempted us to explore the methodology in wider aspect.



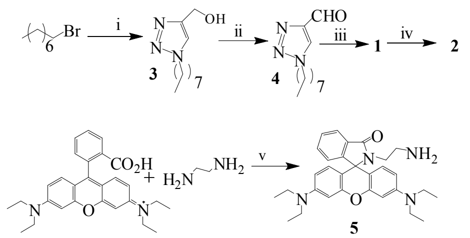
Results and discussion

The synthesis of chemosensors **1** and **2** were achieved according to the Scheme 1. Compound **1** was characterized by ^1H NMR, ^{13}C NMR and mass spectroscopy. Compound **2** was characterized by recording FTIR, fluorescence and SEM images.

[a]*Department of Chemistry, University of Kalyani,* [b]*Department of Zoology, University of Kalyani, Kalyani-741235, India. Email: ghosh_k2003@yahoo.co.in, Fax: +913325828282; Tel: +913325828750.*

†Electronic Supplementary Information (ESI) available: Figures showing absorbance and emission changes of compound **1**, Job plots and other selected curves, characterization spectra See http://dx.doi.org/10.1039/b000000x/

The binding potential of chemosensor **1** in CH_3CN/H_2O (CH_3CN: H_2O = 4:1, v/v, 10 μM tris HCl buffer, pH = 7.0) toward a series of metal ions such as Hg^{2+}, Cu^{2+}, Cd^{2+}, Mg^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+} and Ag^{+} (taken as their perchlorate salts) was established by spectroscopic techniques. Without cations, **1** is



Scheme 1. i. (a) NaN_3, dry CH_3CN, reflux, 6h; (b) propargyl alcohol, ethanol, saturated aqueous solution of $CuSO_4$ and copper turnings, reflux, 10h; ii. PCC, dry CH_2Cl_2, 6h; iii. (a) **5**, CH_3OH, reflux 5 h; (b) $NaBH_4$, CH_3OH, reflux 4h; iv. Merrifield resin, DMF, 60^{0}C, v. EtOH, reflux, 9h.

almost non fluorescent. However, on excitation at 490 nm, a nonstructured emission at 580 nm underwent insignificant change upon interaction with all the metal ions except Hg^{2+} and Pb^{2+} ions

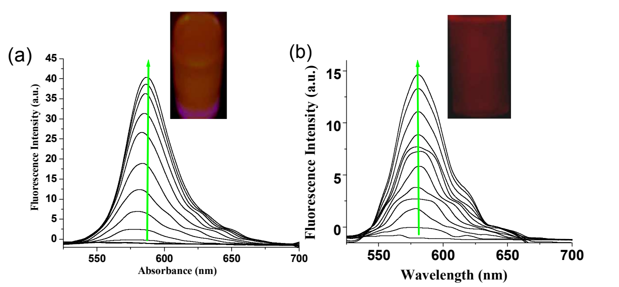


Fig. 1 Fluorescence titration spectra of **1** (c = 2.25 x 10^{-5} M) in CH_3CN/water (4/1, v/v; 10 μM tris HCl, pH = 7.0) upon addition of (a) $Hg(ClO_4)_2$ (c = 4.5 x 10^{-4} M) and (b) $Pb(ClO_4)_2$ (c = 4.5 x 10^{-4} M). Insets represents color change of the receptor solution under illumination of UV light.

(Fig. S1, ESI). On successive addition of Hg^{2+} and Pb^{2+} ions individually to the solution of **1**, a new peak at 580 nm appeared with significant intensity (Fig. 1). Figure 2 shows the change in fluorescence ratio [$(I-I_0)/I_0$] of **1** at 580 nm in the presence of 10 equiv amounts of the different metal ion. Upon interaction with

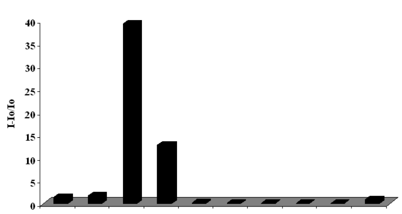


Fig. 2 Fluorescence ratio [$(I-I_0)/I_0$] of **1** (c = 2.25 x 10^{-5} M) at 580 nm upon addition of 10 equiv of metal ions in CH_3CN/water (4/1, v/v; 10 μM tris HCl buffer, pH 7.0).

Hg^{2+} and Pb^{2+} ions, the colorless solution of **1** turned into pink color. In UV-vis spectrum of **1** (c = 2.25 x 10^{-5} M) in CH_3CN/H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 7.0) absorption at 315 nm decreased followed by an appearance of a new absorption at 555 nm upon gradual addition of both Hg^{2+} and Pb^{2+}solutions and resulted in an isosbestic point at 335 nm (Fig. 3). For all of the other ions used for this study, no such change in the absorption spectra of **1** was observed (Fig. S2, ESI). Thus the peaks at 555 nm in UV-vis and 580 nm in emission are the consequence of opening of spiroring in **1** to form metal chelated

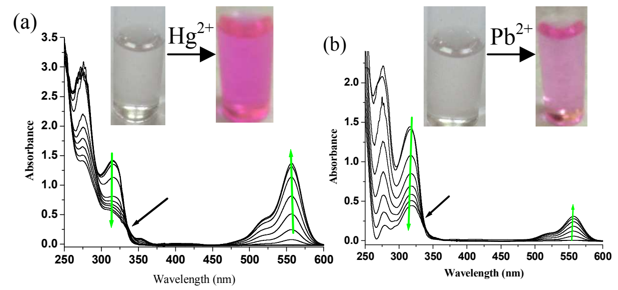


Fig 3 (a) UV-vis titration spectra of **1** (c = 2.25 x 10^{-5} M) in CH_3CN/H_2O (4/1, v/v; 10 μM tris HCl buffer; pH 7.0) upon addition of (a) Hg^{2+}, (b) Pb^{2+}; Insets: Photograph of color change with the addition of Hg^{2+} and Pb^{2+} ions (c = 4.5 x 10^{-4} M).

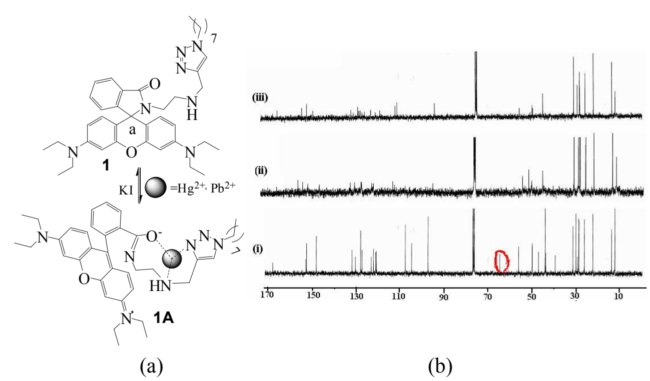


Fig. 4 (a) Suggested metal chelated structure, (b) ^{13}C NMR ($CDCl_3$, 100 MHz) of (i) **1**, (ii) **1**.Hg^{2+} and (iii) **1**. Pb^{2+}-complexes.

equilibrium structure **1A** (Fig. 4a). In the metal chelation, the participation of the triazole ring in the suggested mode in Fig. 4a was understood from the downfield chemical shift of the triazole ring proton by 0.31 – 0.56 ppm (Fig. S3a, ESI). The ring opening in **1** was confirmed by ^{13}C NMR. The disappearance of the signal at 65.1 ppm for the tertiary carbon of the spirolactam ring of **1** (labeled as 'a'; see Fig. 4b) in the presence of Hg^{2+} and Pb^{2+} ions corroborated the opening of the spirolactam ring. The stoichiometries[11] of Hg- and Pb- complexes of **1** were determined to be 1:1 (Fig. S3b, ESI) and the binding constant values[12] were found to be (6.4 ± 0.32) x 10^3 M^{-1} and (2.6 ± 0.47) x 10^3 M^{-1}, respectively. Thus chemosensor **1** binds Hg^{2+} ion ~3 times more strongly than Pb^{2+} ion. To realize the selectivity in the sensing process, the change in emission of **1** was observed in the presence and absence of the other metal ions. In the series, no metal ion except Pb^{2+} interfered in the binding process (Fig. 5). The reversibility in the complexation between **1** and Hg^{2+} was established when the original spectrum for **1** was restored upon the addition of KI (Fig. S4a, ESI). A similar finding was noted with Pb^{2+} (Fig, S4b, ESI). Iodide ion having stronger affinity for Hg^{2+} and Pb^{2+} ions caused demetalation of **1** and regeneration of the spirolactam ring with bleaching of the absorption band at 555 nm and the emission band at 580 nm.

To be acquainted with the sensitivity level of **1** (c = 2.25 x 10^{-5} M) in CH_3CN/water (4/1, v/v; 10 µM tris HCl buffer, pH = 7.0) the change in emission of **1** was recorded with the addition of

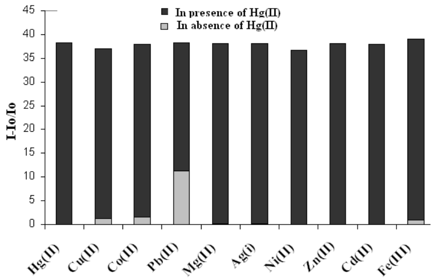


Fig. 5 Fluorescent response of **1** (c = 2.25 x 10^{-5} M) to Hg^{2+} (c = 4.5 x 10^{-4} M) over the selected metal ions (c = 4.5 x 10^{-4} M).

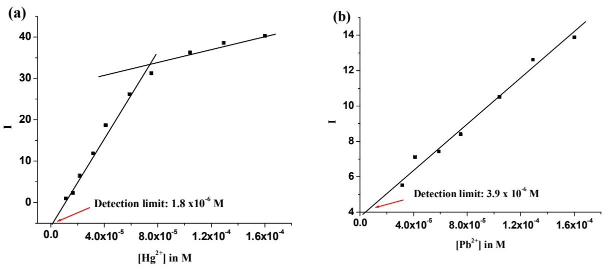


Fig. 6 Plots for the evaluation of detection limits for (a) Hg^{2+} and (b) Pb^{2+} ions from fluorescence of **1**.

Hg^{2+} and Pb^{2+} ions. Hence the detection limits[13] for **1** with Hg^{2+} and Pb^{2+} ions were ascertained to be in the order of 10^{-6} M (Fig. 6). It is to note that the sensor **1** is more sensible to Hg^{2+} ion.

The potential biological application of the receptor was evaluated for *in vitro* detection of Pb^{2+} and Hg^{2+} ions in human cervical cancer (HeLa) cells. The addition of **1** to the cells did not show any cytotoxicity as evident from the morphology of the cells as well as from the MTT assay (Fig. S6, ESI). In this context, Figs 7A and 7F represent the bright field images of the cells after treatment of the cells with **1**. Cells incubated with receptor **1** without Hg^{2+} and Pb^{+2} (Fig. 7B and 7G) and cells incubated with Hg^{2+} and Pb^{+2} without receptor **1** (Fig. 7C and 7H) did not show any fluorescence property. On contrary, cells incubated with the receptor **1** and then with Pb^{+2} ions showed the occurrence of red fluorescence (Fig. 7D). Again cells incubated with the receptor **1** and then with Hg^{2+} ions showed the occurrence of red fluorescence (Fig. 7I). These facts indicate the permeability of the receptor inside the cells and the binding of Hg^{2+}, Pb^{2+} ions with the receptor. The KI adding experiments which could serve as experimental evidence to support the reversibility in structural change, was also applied in human cervical cancer (HeLa) cells. Red coloured cells obtained from the incubation of the receptor followed by treatment with Hg^{2+} and Pb^{2+} became invisible in fluorescence upon addition of KI (100 µM) (Fig. 7E and 7J).

Thus the experimental observations demonstrate that the sensor **1** is able to sense both Hg^{2+} and Pb^{2+} ions in semi aqueous environment with different sensitivities. In vitro detection of these two metal ions is also possible as noted from Fig. 7. However, with a view to sensing these ions in pure water we attached the sensor **1** onto the Merrifield resin to have a new candidate **2**.

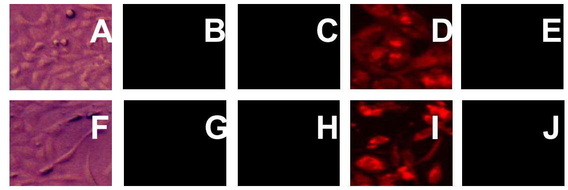


Fig. 7 (A) Phase contrast image of HeLa cells, (B) fluorescence image of HeLa cells, after being incubated with 10 µM chemosensor **1** for 15 min at 37ºC, (C) fluorescence image of HeLa cells in presence of 20 µM extraneous Pb $^{2+}$ ion only, (D) fluorescence image of HeLa cells after being incubated with 10 µM chemosensor **1** for 15 min followed by 15 min incubation with 20 µM extracellular Pb $^{2+}$ ion at 37ºC and (E) disappearance of fluorescence intensity in the HeLa cells treated with the sensor and Pb $^{2+}$ ion after addition of 100 µM KI.(F) phase contrast image of HeLa cells, (G) fluorescence image of HeLa cells after being incubated with 10 µM chemosensor 1 only for 15 min at 37ºC, (H) fluorescence image of HeLa cells in presence of 20 µM extraneous Hg $^{2+}$ ion only, (I) fluorescence image of HeLa cells after being incubated with 10 µM chemosensor 1 for 15 min followed by 15 min incubation with 20 µM extracellular Hg $^{2+}$ion at 37ºC and (J) disappearance of fluorescence intensity in the HeLa cells treated with the sensor and Hg $^{2+}$ ion after addition of 100µM pottasium iodide (KI). For all imaging, the samples were excited at 490 nm.

The sensor bead **2** (Scheme 1) was characterized by SEM, FTIR and fluorescence techniques. In FTIR, the signal at 1684 cm^{-1} for amide in **1** appeared in the bead **2** (Fig. S7, ESI). While in fluorescence marked difference between **1** and **2** was not found (Fig. 8a and 8b), SEM images clearly distinguished the two (Fig. 8c and 8d). Bead **2** was used in the binding studies of metal ions

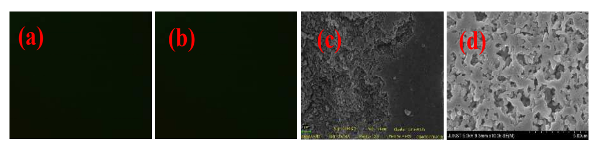


Fig. 8 Fluorescence microscope image of (a) Merrifield resin, (b) receptor **2**; SEM of (c) Merrifield resin; (d) receptor **2.**

in water. In the experiment, 300 µL of aq solutions of Cu^{2+}, Hg^{2+}, Fe^{+3}, Zn^{2+}, Co^{2+}, Cd^{2+}, Ni^{2+}, Ag^{+}, Pb^{2+}, and Mg^{2+} (taken as their perchlorate salts) were individually added to the beads of **2**. After stirring the solution for 4 h, beads were collected and their physical attributes were documented. Instant pinkish red color of beads of **2** was observed in the presence of Hg^{2+} ions (Fig. 9). For Pb^{2+} a light pink color was observed. Besides these, other cations did not bring any color change (Fig. 9) after keeping the beads in contact with the metal salt solutions for 24 h. We presumed that selective adsorption of Hg^{2+} ion on the surface of **2** led to the opening of the spirolactam ring of the receptor site according to the mode shown in Fig. 4a for which color change was noted. In comparison, lower affinities of Pb^{2+} and Fe^{3+} ions brought about similar ring opening reaction at slower rate. Other cations did not participate in this event probably due to their negligible or no adsorption on the surface.

The fluorescence image for beads of **2** in the absence and presence of Hg^{2+} was also collected upon irradiation at 490 nm. As predictable, sensor bead **2** showed fluorescence increase in presence of Hg^{2+} ions (Fig. 10c). In contrast, beads saturated with

Pb^{2+} ions showed relatively weak emission (Fig. 10d) as indicated by light red colour of the beads. This is true for Fe^{3+} ion also.

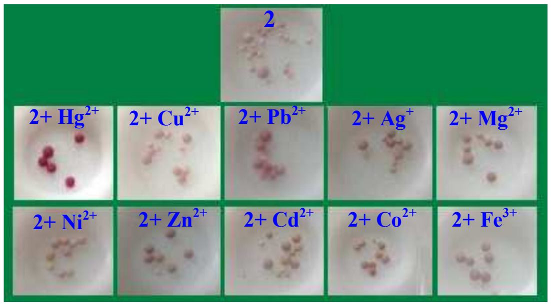


Fig. 9. Color change of bead **2** in the absence and presence of different metal ions in water (in all cases [cation] =4.5 x 10^{-4} M).

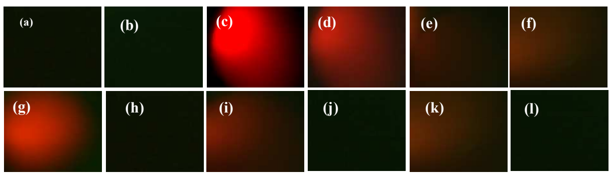


Fig. 10 Fluorescence microscope images of (a) Merrifield resin, (b) bead **2**, and then **2** with300 μL of each metal ion: (c) Hg^{2+}, (d) Pb^{2+}, (e) Cu^{2+}, (f) Zn^{2+}, (g) Fe^{3+}, (h) Ni^{2+}, (i) Co^{2+}, (j) Ag^{+}, (k) Cd^{2+}, (l) Mg^{2+} in water (in all cases [cation] = 4.5 x 10^{-4} M).

The adsorption of Hg^{2+} and Pb^{2+} ions on the surface of bead **2** was established by recording the SEM images, UV-vis, and fluorescence spectra of the bead. SEM images recorded for bead itself and after treatment with Hg^{2+} and Pb^{2+} ions shows marked change in surface morphology (Fig. 11).

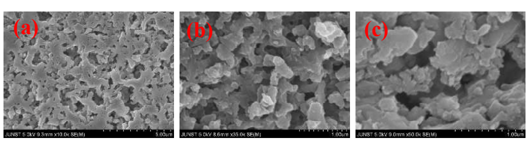


Fig. 11. SEM pictures of (a) **2**; (b) **2** + Hg^{2+}; (c) **2** + Pb^{2+}.

UV-vis and emission spectra of the metal treated beads in the solid state are displayed in Fig. 12. In both absorption (Fig. 12a) as well as emission (Fig. 12b) the change is worthy for Hg^{2+} and Pb^{2+} ions. Appearance of the peaks at ~ 575 nm in emission and 565 nm in UV-vis spectra described the opening of spirolactam ring of the receptor site in **2** like **1**. The color (Fig 9) and peak intensities (Fig. 12) of **2** in the presence of Hg^{2+} ion are much significant relative to the case with Pb^{2+} and importantly, the detection of Hg^{2+} is possible in water. In FTIR spectral comparison, the stretching at 1664 cm^{-1} for lactam amide in **2** was diminished to the greater extent in the presence of Hg^{2+} ion (Fig. S8, ESI). This is in contrast to the case of Pb^{2+} ion where the amide stretching of **2** remained almost unaltered (Fig. S8, ESI).

Like **1**, the sensor bead **2** showed reversible binding of Hg^{2+} ion. The reversibility in the sensing process was established by performing KI adding experiment. Mercury adsorbed pinkish red colored beads were stirred with aq solution of KI for 4 h. As expected, the beads became less fluorescent and also the colour was discharged. Figure 13 reports the change in emission and absorbance in the solid state before and after addition of KI.

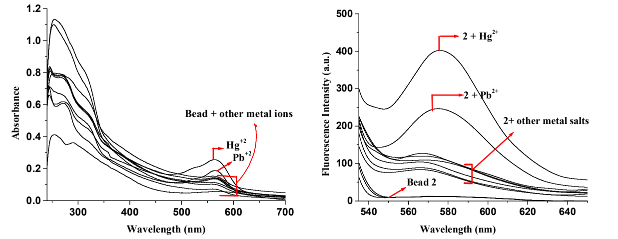


Fig. 12 (a) Absorption and (b) emission spectra for bead **2** (in solid state) after keeping the beads in contact with the aqueous solution of different metal salts.

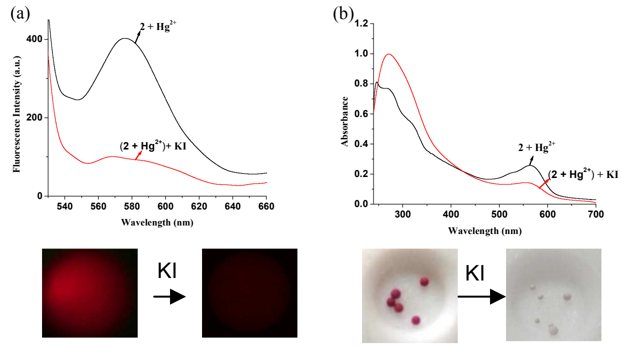


Fig. 13 Change in (a) fluorescence (Inset: Color change under exposure of UV light) and (b) absorbance of **2-** Hg^{2+} complex (Bottom: Colour change in visible light) in the solid state after treatment of aq solution of KI (c = 2.1 x 10^{-3} M).

The color of the beads was regenerated on addition of Hg^{2+} ion. This metalation and demetalation processes remained active for four times (Fig. 14, ESI).

In order to realize the sensing limit of **2** towards Hg^{2+} the beads to **2** were added to aq solutions of $Hg(ClO_4)_2$ of different concentrations. Pinkish red color intensity of the beads was found to be different with variation in concentration of Hg^{2+} as shown in Figure 14i. On decreasing concentration of Hg^{2+} ions, color of the beads became lighten. This was understood more clearly from the fluorescence image of the beads (Fig. 14ii).

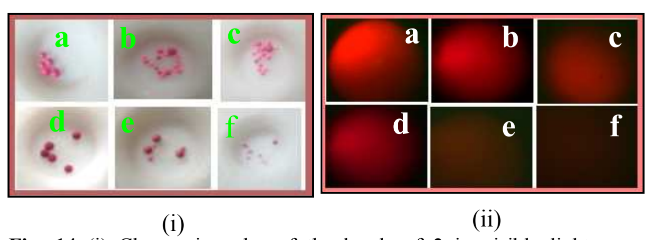


Fig. 14 (i) Change in color of the beads of **2** in visible light upon treatment with aq solution of $Hg(ClO_4)_2$ of different concentrations: a. 2.25 x 10^{-2} M, b. 4.5 x 10^{-3} M, c. 8.69 x 10^{-4} M, d. 4.5 x 10^{-5} M, e. 4.15 x 10^{-6} M, f. 4.15 x 10^{-7} M; (ii) Color change of the same under UV exposure.

The characteristic features of Fig. 14 reveals that the beads of **2** are able to report the presence of Hg^{2+} in water at ease upto 10^{-6} M concentration range. This is in contrast to our recent report where the sensing is possible upto 10^{-4} M.[10] The selectivity of bead **2** towards Hg^{2+} over Pb^{2+} and other cations was understood from the change in color of the bead in the presence of other metal ions examined (Fig. 15).

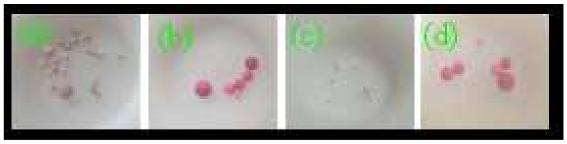


Fig. 15 (a) Bead **2**; (b) **2** + Hg^{2+} (300 µL); (c) **2** + all metal salts (300 µL each metal salt) except Hg^{2+}; (d) **2** + all metal salts (300 µL of each metal salt) + Hg^{2+} (300 µL); (concentration of all metal salts: 5.2 x 10^{-4} M).

Conclusions

We have shown that triazole motif coupled rhodamine derivative **1** is a good candidate for sensing of both Hg^{2+} and Pb^{2+} ions in aq. CH_3CN solvent. The performance of **1** was found to be better for Hg^{2+} than Pb^{2+} ion. The present example is in contrary to the reported triazole labelled rhodamine receptor which senses Pt^{2+} ion.[14] The chemosensor **1** is cell permeable and can detect the intercellular Hg^{2+} and Pb^{2+} ions by exhibiting fluorescence image of cells. In this context, only few rhodamine – based receptors are reported to sense Hg^{2+} and Pb^{2+} from different spectra channel.[15] The present simple system with moderate sensitivity is a new addendum in this horizon. In addition, the Merrifield resin bound version **2** of the chemosensor **1** is found to be useful in detecting selectively Hg^{2+} ion over the Pb^{2+} ion in water both colorimetrically and fluorometrically. The beads are easy –to – prepare and reusable.

Experimental

(1-octyl-1H-1,2,3-triazol-4-yl)methanol (3):
To a stirred solution of octyl bromide (0.5 g, 2.59 mmol) in CH_3CN, NaN_3 (0.25 g, 3.86 mmol) was added. The mixed solution was refluxed for 6 h. After completion of reaction, monitored by TLC, solvent was removed under vacuum; water was added and extracted with CH_2Cl_2 (30 mL x 3). The organic layer was separated and dried over Na_2SO_4 and the solvent was removed in a rotary evaporator to afford blackish gummy compound 1-azidooctane (0.3 g, 75%). Without characterisation, to a refluxing solution of octylazide (0.25 g, 1.61 mmol) in ethanol, propergyl alcohol (0.09 g, 1.61 mmol) was added followed by saturated aqueous solution of $CuSO_4$ and copper turnings. The mixed solution was refluxed for 10h. After completion of reaction, solvent was removed under vacuum; water was added and extracted with CH_2Cl_2 (30 mL x 3). The organic layer was separated and dried over Na_2SO_4 and the solvent was removed in a rotary evaporator. Finally, the crude product was purified by column chromatography using 50% petroleum ether in ethyl acetate to afford yellow gummy 1-octyl-1H-1,2,3-triazol-4-yl)methanol **3** (0.22 g, 64%), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (s, 1H), 4.79 (brs, 2H), 4.33 (t, 2H, J = 8 Hz), 3.21 (brs, 1H), 1.91 – 1.89 (m, 2H), 1.31 – 1.26 (m, 10H), 0.87 (t, 3H, J = 8 Hz); FT IR (ν in cm^{-1}, KBr) 3325, 2955, 2922, 1645, 1548.

1-octyl-1H-1,2,3-triazole-4-carbaldehyde (4):
To a stirred solution of 1-octyl-1H-1,2,3-triazol-4-yl)methanol **3** (0.2 g, 0.946 mmol) in dry CH_2Cl_2, PCC (0.265 g, 1.04 mmol) was added and stirring was continued for 6h. After completion of reaction, monitored by TLC, solvent was removed under vacuum; water was added and extracted with CH_2Cl_2 (30 mL x 3). The organic layer was separated and dried over Na_2SO_4 and the solvent was removed in a rotary evaporator. Finally, the crude product was purified by column chromatography using 40% petroleum ether in ethyl acetate to afford yellow gummy 1-octyl-1H-1,2,3-triazole-4-carbaldehyde **4** (0.15 g, 74%), ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.1 (s, 1H), 8.08 (s, 1H), 4.42 (t, 2H, J = 8 Hz), 1.94 (m, 2H), 1.33 – 1.25 (m, 10H), 0.87 (t, 3H, J = 8 Hz); FT IR (ν in cm^{-1}, KBr) 3385, 2956, 2924, 17031, 1701, 1533.

Compound (1):
To a stirred solution of aldehyde **4** (0.1 g, 0.48 mmol) in dry CH_3OH (20 mL), the amine **5** (0.23 g, 0.48 mmol) which was obtained according to the reported procedure,[16] was added. The mixed solution was refluxed for 5 h. After completion of reaction MeOH was evaporated off. The crude Schiff base (0.25 g, 0.37mmol), obtained by this way was redissolved in dry CH_3OH (30 mL) and $NaBH_4$ (0.02 g, 0.48 mmol) was added portion wise at 0 ^{0}C under nitrogen atmosphere. The reaction mixture was refluxed for 4 h. The solvent was removed under vacuum; water was added and extracted with CH_2Cl_2 (30 mL x 3). The organic layer was separated and dried over Na_2SO_4 and the solvent was removed in a rotary evaporator. Finally, the crude product was purified by column chromatography using 2% methanol in $CHCl_3$ as eluent to give brownish gummy **1** (0.19 g, 77%), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (s, 1H), 7.63 (s, 1H), 7.45 – 7.43 (m, 2H), 7.08 (t, 1H, J = 4 Hz), 6.41 (d, 2H, J = 8 Hz), 6.37 (s, 2H), 6.27 (d, 2H, J = 8 Hz), 4.28 (t, 2H, J = 8 Hz), 3.85 (brs, 1H), 3.36 – 3.30 (m, 12H), 2.54 (brt, 2H), 2.04 (m, 2H), 1.28 (m, 10H), 1.16 (m, 12H), 0.86 (t, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.7, 153.6, 153.2, 148.8, 132.4, 130.9, 128.6, 128.0, 123.8, 122.7, 121.7, 121.4, 108.1, 105.2, 97.7, 65.1, 56.4, 50.3, 50.2, 47.3, 44.3, 31.6, 30.2, 29.0, 28.9, 26.4, 22.5, 14.0, 12.5; FT IR (ν in cm^{-1}, KBr) 3400, 2927, 1684, 1634, 1615, 1515. ESI-HRMS m/z: $(M+H)^+$ Calcd 678.4417; Found: 678.4554.

Compound (2):
Compound **1** was immobilized on Merrifield resin by refluxing a solution of **1** in DMF with Merrifield resin overnight. Filtration followed by thorough washing with DMF to remove the excess amount of **1** gave Merrifield resin bound sensor bead **2**. The beads were characterized by recording FTIR, fluorescence image and also SEM.

Cell culture:

HeLa cells, obtained from a human cervical cancer cell line, were procured from National Center for Cell Science, Pune, India, and used throughout the study. Cells were cultured in DMEM (Gibco BRL) supplemented with 10% FBS (Gibco BRL), and a 1% antibiotic mixture containing PSN (Gibco BRL) at 37ºC in a humidified incubator with 5% CO_2 and cells were grown to 80-90% confluence, harvested with 0.025% trypsin (Gibco BRL) and 0.52 mM EDTA (Gibco BRL) in phosphate-buffered saline

(PBS), plated at the desired cell concentration and allowed to re-equilibrate for 24 h before any treatment.

Cell imaging study:

Cells were rinsed with PBS and incubated with DMEM containing chemosensor making the final concentration up to 10 μM in DMEM [the stock solution (1 mM) was prepared by dissolving chemosensor **1**to the mixed solvent (acetonitrile: water = 1:9 (v/v)] for 15 min at 37ºC. After incubation, bright field and fluorescence images of HeLa cells were taken by a fluorescence microscope (Model: LEICA DMLS) with an objective lens of 20X magnification; fluorescence images of HeLa cells after being incubated with 20 μM extraneous Pb^{2+} ion only were also taken. Fluorescence images of HeLa cells incubated with 10 μM chemosensor **1** for 15 min followed by addition of 20 μM Pb^{2+} ion were taken and consequently fluorescence images were taken after further addition of KI (100μM).

Similarly another set of experiments were carried out with Hg^{2+}ion instead of Pb^{2+}ion and phase contrast and fluorescence images were taken in the same manner as mentioned above.

Cell cytotoxicity assay:[17]

To test the cytotoxicity of chemosensor **1**, 3-(4,5-dimethylthiazol-2-yl)-2,S-diphenyltetrazolium bromide (MTT) assay was performed as per the procedure described earlier.[15] After treatment with chemosensor **1** at different doses of 1, 10, 20, 50 and 100 μM, respectively, for 6 h, 10 μl of MTT solution (10mg/ml PBS) was added to each well of a 96-well culture plate and again incubated continuously at 37ºC for a period of 3 h. All media were removed from wells and 100 μl of acidic isopropyl alcohol was added into each well. The intracellular formazan crystals (blue-violet) formed were solubilized with 0.04 N acidic isopropyl alcohol and absorbance of the solution was measured at 595 nm wavelength with a microplate reader (Model: THERMO MULTI SCAN EX). The cell viability was expressed as the optical density ratio of the treatment to control. Values were expressed as mean ± standard errors of three independent experiments. The cell cytotoxicity was calculated as % cell cytotoxicity = 100% - % cell viability.

Acknowledgements

T.S. thanks CSIR, New Delhi, India for a fellowship.

References

1. (a) T. Chen, W. Zhu, Y. Xu, S. Zhang, X. Zhang and X. Qian, *Dalton Trans.,* 2010, **39**, 1316; (b) A. J. Bryan, A. P. de Silva, S. A. de Silva, R. A. D. D. Rupasinghe and K. R. A. S. Sandanayake, *Biosensors*, 1989, **4**, 169; (c) E. M. Nolan and S. J. Lippard, *Acc. Chem. Res.*, 2009, **42**, 193; (d) A. P.de Silva, D. B. Fox, A. J. Huxley, M. Moody and T. S. *Coord. Chem. Rev.*, 2000, **205**, 41; (d) D. T.Quang and J. S.Kim, *Chem. Rev*., 2007, **107**, 3780.
2. R. N. Reeve, *Introduction to Environmental Analysis*, 1st ed., John Wiley and Sons Ltd, 2002.
3. (a) G. E Mckeown-Eyssen, J.Ruedy and A. Neims, *Am. J. Epidemol.,* 1983, **118**, 470; (b) P. W. Davidson, G. J. Myers, C. Cox, C. F.Shamlaye, D. O. Marsh, M. A.Tanner, M.Berlin, J. Sloane-Reeves, E.Cernichiari, O.Choisy, A.Choi and T. W.Clarkson, *Neurotoxicology.*, 1995, **16**, 677.
4. (a) N. Rifai, G. Cohen, M. Wolf, L. Cohen, C. Faser, J. Savory and L. Depalma, *Ther. Drug Monit.* 1993, **15**, 71 and references cited therein; (b) A. J. S. Liu-Fu, Lead Poisoning, A Century of Discovery and Rediscovery, in *Human Lead Exposure*, ed. H. L. Needleman, Lewis Publishing, Boca Raton, FL, 1992.
5. Sensors for Hg^{2+} ion: (a) M.Yuan, Y. Li, J. Li, C. Li, X. Liu, J. Lv, J. Xu, H. Liu, S. Wang and D. Zhu, *Org. Lett*., 2007, **9,** 2313; (b) J. Hu, M. Zhang, L. B.Yu and Y. Ju, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4342; (c) E. M. Nolan and S. J. Lippard, *Chem. Rev.*, 2008, **108**, 3443; (d) H. N. Kim, M. H. Lee, H. J. Kim, J. S. Kim and J. Yoon, *Chem. Soc. Rev.*, 2008**, 37**, 1465; (e) J. Wang, X. Qian and J. Cui, *J. Org. Chem.*, 2006, **71**, 4308; (f) A. Cabellero, A. Espinosa, A. Tarraga and P. Molina, *J. Org. Chem.*, 2008, **73**, 5489; (g) M. G. Choi, D. H. Ryu, J. L. Jeon, S. Cha, J. Cho, H. H. Joo, K. S. Hong, C. Lee, S. Ahn and S. –K.Chang, *Org. Lett.*, 2008, **10**, 3717; (h) J. Wang and B. Liu, *Chem. Commun.*, 2008, **39**, 4759; (i) M. H. Lee, J. –S. Wu, J. W.Lee, J. H. Jung and J. S. Kim, *Org. Lett*., 2007, **9**, 2501; (j) J. H. Soh, K. M. K. Swamy, S. K. Kim, S. Kim, S. H. Lee and J. Yoon, *Tetrahedron Lett*., 2007, **48**, 5966; (k) J. S. Kim, L S. Y.ee, J.Yoon and J.Vicens, *Chem. Commun.* 2009, **32***,* 4791; (l) M. Suresh, A. Ghosh and A. Das, *Chem. Commun.* 2008, **33**, 3906; (m) M. Suresh, A. Shrivastav, S. Mishra, E. Suresh and A. Das, *Org. Lett.*, 2008, **10**, 3013; (n) M. Suresh, S. Mishra, S. K. Mishra, E. Suresh, A. K. Mandal, A. Shrivastav and A. Das, *Org. Lett.*, 2009, **11**, 2740; (o) H. N. Kim, W. X. Ren, J. S. Kim and J.Yoon, *Chem. Soc. Rev.*, 2012 , **41**, 3210; (p) M. Suresh, A. K. Mandal, S. Saha,; E. Suresh, A. Manodoli, R. Di Liddo, P. P. Pamigotto and A. Das, *Org. Lett.*, 2010, **12**, 5406; (q) C. Guo and J. Irudayaraj, *Anal. Chem.*, 2011, **83**, 2883; (r) A. Mitra, A. K. Mittal and C. P. Rao, *Chem. Commun,*. 2011, **47**, 2565; (s) Y.Wu, H.Jing, Z. Dong, Q. Zhao, H. Wu and F. Li, *Inorg. Chem.* 2011, **50**, 7412; (t) S. Saha, P. Mahato, G. U. Reddy, E. Suresh, A. Chakrabarty, M. Baidya, S. K.Ghosh and A. Das, *Inorg. Chem.* 2012, **51**, 336; (u) S. Saha, M. U. Chhatbar, P. Mahato, L. Praveen, A. K. Siddhanta and A. Das, *Chem. Commun.*, 2012, **48**, 1659. (v) S. K. Kim, K. M. K.Swamy, S. –Y.Chung, H. N. Kim, M. J. Kim, Y. Jeong and J. Yoon, *Tetrahedron Lett*., 2010, **51**, 3286; (w) H. Yang, Z. Zhou, K. Huang, M. Yu, F.Li, T.Yi and C. Huang, *Org. Lett.,* 2007, **9**, 4729; (x) J. Huang, Y. Xu and X. Qian, *J. Org. Chem.*, 2009, **74**, 2167; (y) M. Alfonso, A. Tarraga and P. Molina, *Org. Lett.*, 2011, **13**, 6432; (z) K. Ghosh, T. Sarkar and A. Samadder, *Org. Biomol. Chem.*, 2012, **10**, 3236.
6. Sensors for Pb^{2+} ions: (a) C. –T. Chen and W. –P. Huang, *J. Am. Chem. Soc.,* 2002, **124**, 6246; (b) S. Deo and H. A. Godwin, *J. Am. Chem. Soc.,* 2000, **122**, 174; (c) R. S. Addleman, J. Bennett, S. H. Tweedy, S. Elshani and C. M. Wai, *Talanta*, 1998, **46**, 573; (d) Y. Shen and B. P. Sullivan, *J. Chem. Educ.,*1997, **74**, 685; (e) A. Beeby, D. Parker and J. A. G. Williams, *J. Chem. Soc., Perkin Trans. 2* 1996, 1565; (f) W. –S. Xia, R. H. Schmehl, C. –J. Li, J. T. Mague, C. P. Luo, and D. M. Guldi, D. M. *J. Phys. Chem. B* 2002, **106**, 833-843; (g) J. Wang, X. Qian and J. Cui, *J. Org. Chem*., 2006, **71**, 4308; (h) H. Zhen, Z.-H. Qian, L. Xu, F.-F. Yuan, L.-D. Lan and J.-G. Xu, *Org. Lett*., 2006, **8**, 859; (i) X.-Q. Zhan, Z.-H. Qian, H. Zheng, B.-Y. Su, Z. Lan and J.-G. Xu, *Chem. Commun*., 2008, 1859; (j) W. Shei and H. Ma, *Chem. Commun*., 2008, 1856; (k) T. Hayashita, D. Qing, M. Minagawa, J. C. Lee, C. H. Ku and N. Teramae, *Chem. Commun.,* 2003, 2160;
7. (a) X. Chen, T. Pradhan, F.Wang, J. S. Kim, J.Yoon, *Chem. Rev.*, 2012, **112**, 1910 and references cited therein; (b) J. Du, M. Hu, J. Fan, X. Peng, *Chem. Soc. Rev.*, 2012, **41**, 4511.
8. (a) K. Ghosh, T. Sarkar, A. Sommader and A. –R. Khuda-Bukhsh, *New J. Chem.*, 2012, **36**, 2121; (b) K. Ghosh and T. Sarkar, *Supramol. Chem.,* 2012, **24**, 748; (c) K. Ghosh and D. Tarafdar, *Supramolecular. Chem.,* 2013, **25**, 127; (d) K. Ghosh, A. R. Sarkar, A. Sommader, A. –R. Khuda-Bukhsh, *Org. Lett.*, 2012, **14**, 4314; (e) K. Ghosh and A. R. Sarkar, *Org. Biomol. Chem.* 2011, **9**, 6551; (f) K. Ghosh, D. Kar and P. Ray Chowdhury, *Tetrahedron Lett.,* 2011, **52**, 5098; (g) K. Ghosh and T. Sarkar, *Supramolecular Chem.,* 2011, **23**, 435; (h) K**.** Ghosh and I. Saha, *New J. Chem.,* 2011, **35**, 1397; (i) K. Ghosh, A. R. Sarkar and A. P. Chattopadhyay, *Euro. J. Org. Chem.,* 2012, 1311.
9. (a) M. H. Lee, S. J. Lee, J. H. Jung, H. Lim, J. S. Kim, *Tetrahedron*., 2007, **63**, 12087; (b) C. Wang, S.Tao, W. Wei, C. Meng, F. Liu, M. Han, *J. Mater. Chem.*, 2010, **20**, 4635; (c) M. Zhu, C. Zhou, Y. Zhao,

Y. Li, H. Liu, Y. Li, *Macromol. Rapid Commun.*, 2009, **30**, 1339; (d) G. Ye, F. Bai, G. Chen, J. Wei, J. Wang and J. Chen, *J. Mater. Chem.,* 2012, **22**, 20878.
10. K. Ghosh, T. Sarkar and A. Majumdar *Asian J. Org. Chem.*, 2013, **2**, 157.
11. P. Job, *Ann. Chim.*, 1928, **9**, 113 – 203.
12. B. Valeur, J. Pouget, J. Bourson, M. Kaschke, N. P. Enesting, *J. Phys. Chem.* 1992, **96**, 6.
13. A. Caballero, R. Martinez, V. Lloveras, I. Ratera, J. Vidal-Gancedo, K. Wurst, A. Tarraga, P. Molina, J. Vaciana, *J. Am. Chem. Soc.*,2005, **127**, 15666.
14. H. Kim, S. Lee and J. Tae, *Org. Lett.*, 2010**, 12**, 5342.
15. (a) J. Y. Kwon, Y. J. jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.,* 2005, **127**, 10107; (b) Z. –Q. Hu, C. –S. Lin, X. –M. Wang, L. Ding, C. –L. Cui, S. –F. Liu and H. Y. Lu. *Chem. Commun.,* 2010, **46**, 3765.
16. X. Zhang, Y. shiraishi and T. Hirai, *Org. Lett*., 2007, **9,** 5039.
17. T. Mossman, *J Immunol Methods,* 1983, **65**, 55.