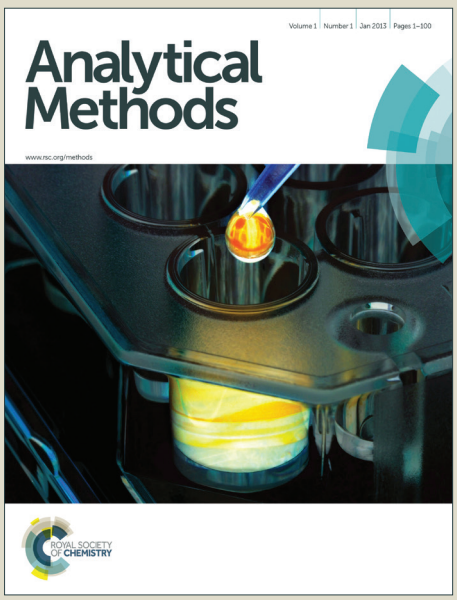
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Journal Name

RSCPublishing

ARTICLE

A PEGylated-Rhodamine based sensor for "turn-on" fluorimetric and colorimetric detection of Hg^{2+} ions in aqueous media

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Subhenjit Hazra,[a] Shruti Balaji,[b] Mainak Banerjee,[a] Anasuya Ganguly,[b] Narendra Nath Ghosh[a] and Amrita Chatterjee[a]*

Abstract: A water soluble turn-on fluorescent probe for the detection of mercury ions in water is developed by appending a water-compatible PEG-unit on to a rhodamine derivative. The probe, **Rh1** responds to Hg^{2+} ions with an intense pink colour and orange fluorescence by Hg^{2+} ion induced opening of the spirolactam ring with high selectivity and sensitivity. The Job's plot reveals that a 1:1 stoichiometry was most favourable for the binding mode of Hg^{2+} and the probe. Preliminary studies revealed that the probe molecule is fairly non-toxic and can successfully penetrate the cell surface of HeLa cells and interact with intercellular Hg^{2+} ions indicating its usefulness for monitoring Hg^{2+} ions in biological samples as well. The probe is highly efficient, cost-effective and shows a low detection limit of 0.14 ppm.

1. Introduction

Mercury is a prevalent pollutant with discrete toxicological profiles and it subsists in a variety of different forms.[1-5] One of the most stable forms, solvated mercuric ion (Hg^{2+}) is a carcinogenic material with high cellular toxicity.[6] Inorganic mercury is a neurotoxin, which causes immune system dysfunction,[7] targets the renal epithelial cells of the kidney causing tubular necrosis and proteinuria.[8,9] Irrespective of the source, Hg^{2+} finally enters freshwater and marine ecosystems, and in the presence of some prokaryotes it changes to methyl mercurials.[10-13] CH_3HgX species, because of their lipid solubility, are readily absorbed by human GI track, cross the blood brain barrier[14] and finally target the central nervous system causing a number of neurological problems including prenatal brain damage, vision and hearing loss, cognitive and motion disorder and even death.[14-18]

Due to its trace amount and the influences of coexisting substances in real samples, the selective sensing of mercury ions are of great significance prior to analysis.[19] Although a large variety of analytical methods, such as atomic absorption and emission spectrometry,[20-22] inductively coupled plasma mass spectroscopy,[23-25] neutron activation analysis,[26] X-ray fluorescence spectrophotometry,[26] have been developed to identify these metal ions, synthetic probes which exhibit signal transduction upon analyte binding have appeared to be more useful and impactful as chemical sensors in both biomedical and environmental research.[27] In particular, fluorescent signalling probes have proven to be more useful and influential tools to monitor *in vitro* and/or *in vivo* biologically applicable species such as metal ions due to their advantages over the other analytical methods in terms of sensitivity, high selectivity, shorter response time, easy sampling, non-destructive and non-invasive properties.[27-44] In this regard, rhodamine-based fluorescent chemosensors have recently witnessed great development for the sensing of mercury ions because of the excellent spectroscopic properties of rhodamine dyes such as high absorption coefficient, high fluorescence quantum yield, absorption and emission at longer wavelength.[28-33,40-44] As well-known, the rhodamine with spirolactam structure is non-fluorescent, whereas, ring-opening of the spirolactam gives rise to a strong fluorescence emission.[29] This property provides an ideal mode to construct OFF–ON molecular switch. However, most of the reported detection protocols of Hg^{2+} ions are based on use of significant amount of organic solvents (e.g., DMF, acetonitrile, ethanol, methanol etc.) to carry out florescence and/or absorbance studies. Use of organic solvent for rhodamine derivatives in spirolactam form is inevitable because the non-ionic aromatic species are barely soluble in water. However, the hard fact is that the organic solvents are toxic in nature and are not appropriate for biological systems. Hence, it is worthy to design a water-soluble chemosensor for detecting

Hg^{2+} in aqueous media. Till date, water soluble probes for selective detection of Hg^{2+} are rare.[42,43] As part of our continued efforts on the development of fluorescent for environmentally and/or biologically toxic analytes,[39,45] we report, herein, the synthesis of a water soluble rhodamine based chemosensor and investigations on its use in Hg^{2+} detection in aqueous media in the absence of organic solvents.

2. Experimental

2.1. Apparatus and reagents

NMR spectra were recorded on Bruker AV300 NMR spectrometer. Mass spectra were obtained from Waters Q-TOF micro mass spectrometer (ESI^+) and Agilent 6400B LC-MS (ESI^+). Fluorescence spectra were taken on a JASCO FP-6300 spectro-fluorometer, the slit width was 5 nm for both excitation and emission. Absorption spectra were recorded on a JASCO V570 UV/Vis/NIR spectrophotometer. For cell imaging Nikon eclipse TS100 microscope was used.

Rhodamine B hydrochloride was purchased from Sigma-Aldrich and used as received. All other chemicals were obtained from different suppliers and were used without further purification. Toluene was dried over sodium wire; acetone was dried over anhydrous K_2CO_3 and kept over molecular sieves; CH_2Cl_2 was dried over anhydrous P_2O_5.

2.2. General procedure

The reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm silica gel plates (60F-254) using UV light (254 or 365 nm) or naked eye for visualization. Probe **Rh1** was dissolved in NaOAc-HOAc buffer solution (using MilliQ, 18 Ω) to make a 2 x 10^{-3} M stock solution, which was diluted to required concentration for measurement. A 2 x 10^{-3} M stock solution of $HgCl_2$ was prepared in NaOAc-HOAc buffer solution (using MilliQ, 18 Ω) and other standard solutions of $HgCl_2$ were prepared by further dilution. For study of the effect of different metal ions and the competitive study of Hg^{2+} with other metal ions, stock solutions (5 mL, 1 mM) were prepared by dissolving respective metal nitrates in buffer. All solutions were subjected to filtration through 0.22 μM syringe filter in order to avoid any interference by any particular matter in fluorescence measurement.

For cell culture and imaging HeLa cells (human cervical cancer cells) maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum were used. The probe was dissolved in water. 5000 cells/well viable HeLa cells were plated in a 96-well plate. After 48 h incubation at 37 ^oC in a humidified 5% CO_2 incubator, 10 μL of the probe in various concentrations were added in duplicates into the culture media present in the wells of the plate to achieve final probe concentrations as 0 μM, 1 μM, 10 μM, 100 μM and 200 μM and incubated for further 7 h, 12 h, 18 h and 24 h. The % cell viability (viable cells in a microscope field expressed as the percentage of total cells in that field) was calculated using the Trypan Blue viability assay at every time point and for every compound concentration.

2.3. Synthesis of probe Rh1

2.3.1. Synthesis of rhodamine B thiohydrazide (2):

Rhodamine-B hydrazide was prepared according to a literature procedure.[40] We followed another literature method to prepare rhodamine-B thiohydrazide with slight modification.[41] In a 50 mL round bottom flask, rhodamine B hydrazide (0.7 g, 0.15 mmol) and Lawesson's Reagent (0.61 g, 0.15 mmol) were dissolved in 25 mL anhydrous toluene, and the reaction mixture was heated at 80 °C for 4 h under N_2 atmosphere. After removal of toluene under vacuum, the residue was purified by flash chromatography using 25% CH_2Cl_2 in petroleum ether as eluent to get pure rhodamine B thiohydrazide (0.15 g, 20% yield). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.16 (12H, t, J = 6.4 Hz, -$NCH_2C\underline{H}_3$), 3.34 (8H, q, J = 6.4 Hz, -$NC\underline{H}_2CH_3$), 6.31 (2H, dd, J = 1.8, 6.6 Hz, Ar-H), 6.46 (2H, d, J = 6.6 Hz, Ar-H), 6.48 (1H, s, Ar-H), 7.09-7.12 (1H, m, Ar-H), 7.16-7.18 (1H, m, Ar-H), 7.23-7.26 (1H, m, Ar-H, overlapped with $CHCl_3$ peak), 7.44-7.46 (1H, m, Ar-H), 7.93-7.95 (1H, m, Ar-H); ESIMS (m/z): 473 (M^+ + 1).

2.3.2. Synthesis of PEG modified 2,4-dihydroxy benzaldehyde (4)

Polyethelene glycol (PEG) modified 2,4-dihydroxy benzaldehyde (**4**) was prepared by a three-step method. First, PEG-400 (6 mL, 16 mmol) and triethylamine (2.3 mL, 16 mmol) were dissolved in dry CH_2Cl_2 (20 mL) and placed in an ice bath. Then, methanesulfonyl chloride (0.62 mL, 8 mmol) was added dropwise to the reaction mixture and the resultant mixture was stirred overnight. The solvent was removed under pressure to get a colorless semi solid consisting of a mixture of desired monomesyl derivative of PEG-400, some dimesyl PEG-400 and unreacted PEG-400. This crude mixture was dissolved in dry acetone and excess amount of NaI (4.8 g, 32 mmol) was added and then the reaction mixture was refluxed for 6 h. Solvent was removed under vacuum to get yellowish thick liquid. The major product from the crude product mixture was isolated by flash column chromatograph using $CHCl_3$ and CH_3OH (95:5 v/v) as eluent and considered as mono-iodo PEG-400 (**3**, 3.2 g, yellow thick liquid, yield: 40%), which was immediately used for further reaction.

Finally, iodide modified PEG-400 (**3**, 3 g, 5.9 mmol) and 2,4-dihydroxy benzaldehyde (980 mg, 7.1 mmol) were dissolved in dry acetone and K_2CO_3 (4 g, 29 mmol)) was added and then the reaction mixture was refluxed for overnight. Solvent was removed under pressure to get yellowish thick liquid. The crude product was purified by flash column chromatograph using $CHCl_3$ and CH_3OH (90:10 v/v) as eluent to afford PEG-400 modified 2,4-dihydroxy benzaldehyde (**4**, 1.6 g) as light yellow viscous liquid in 52% yield. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.92-3.20 (1H, bs, OH), 3.51-3.80 (34-36H, m, -O-C$\underline{H}_2$-C$\underline{H}_2$-O-), 4.08-4.19 (2H, m, -O-CH_2-C$\underline{H}_2$-OH), 6.47-6.72 (2H, m, Ar-H), 7.79 (1H, d, J = 12.3 Hz, Ar-H), 10.32 (1H, s, -C$\underline{H}$O); ^{13}C

NMR ($CDCl_3$, 75 MHz): δ 61.5 (-O-CH_2-CH_2-OH), 70.1 (-O-CH_2-CH_2-O-), 70.4 (-O-CH_2-CH_2-O-), 70.5 (-O-CH_2-CH_2-O-), 72.6 (-O-CH_2-CH_2-O-), 100.0 (Ar-CH), 106.5 (Ar-CH), 125.4 (Ar-C), 129.9 (Ar-CH), 159.0 (Ar-C), 162.0 (Ar-C), 188.3 (Ar-CHO), these are the peaks of major isomer only; ESIMS (*m/z*): 442 (M^+), 486 (M^+), 530 (M^+), these are the major molecular ion peaks obtained from PEGylated aldehyde, **4**.

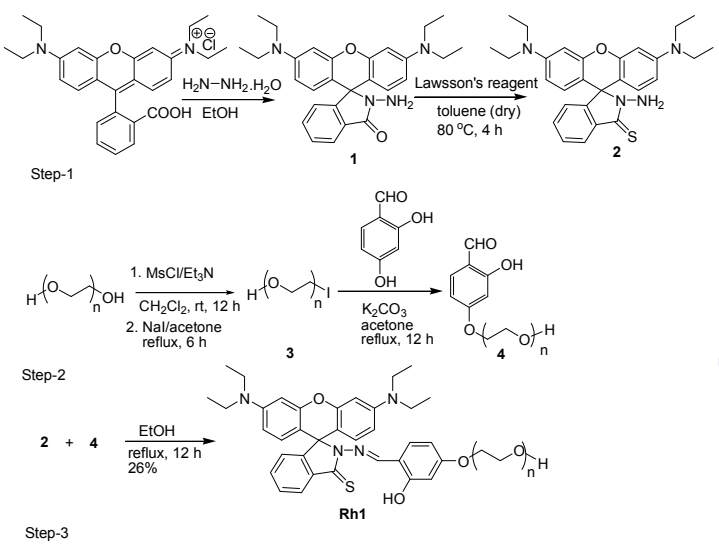
2.3.3. Synthesis of rhodamine derivative, Rh1

To a solution of rhodamine-B thiohydrazide (**2**, 90 mg, 0.19 mmol) in absolute ethanol (30 mL) was added PEG-400 modified 2,4-dihydroxy benzaldehyde (**4**, 100 mg, 0.19 mmol). After the addition, the mixture was refluxed with stirring for 12 h. The solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography using $CHCl_3$ and CH_3OH (90:10 v/v) as eluent to afford the final probe molecule, **Rh1** (48 mg, yield: 26%) as a light orange solid with the recovery of about 50% of starting materials. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.07-120 (12H, m, overlapping peaks, -NCH_2CH_3), 3.24 (8H, bs, -NCH_2CH_3), 3.55-3.76 (34-36H, m, -O-CH_2-CH_2-O-), 4.01-4.08 (2H, m, -O-CH_2-CH_2-OH), 6.16-6.49 (6H, m, Ar-H), 6.64-6.73 (1H, m, Ar-H), 7.00-7.21 (1H, m, Ar-H), 7.25-7.38 (3H, m, Ar-H), 7.71-7.78 (1H, m, Ar-H), 7.84-7.88 (1H, m, Ar-H), 8.70 (1H, bs, Ar-CH=N-N-); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 12.6 (-NCH_2CH_3), 44.2 (-NCH_2CH_3), 61.5 (-O-CH_2-CH_2-OH), 68.0 (-C-, spiro ring carbon), 70.1 (-O-CH_2-CH_2-O-), 70.7 (-O-CH_2-CH_2-O-), 70.8 (-O-CH_2-CH_2-O-), 72.7 (-O-CH_2-CH_2-O-), 97.9 (Ar-CH), 100.0 (Ar-CH), 106.5 (Ar-CH), 107.9 (Ar-CH), 116.7 (Ar-C), 123.1 (Ar-C), 123.8 (Ar-CH), 125.4 (Ar-CH), 127.3 (Ar-CH), 127.6 (Ar-CH), 129.9 (Ar-CH), 133.3 (Ar-C), 138.9 (Ar-CH=N-N-), 148.7 (Ar-C), 152.9 (Ar-C), 158.9 (Ar-C), 160.6 (Ar-C), 161.4 (Ar-C), 199.0 (C=S), these are the peaks of major isomer only; ESIMS (*m/z*): 935 ($M^+ + 1$), 979 ($M^+ + 1$), 1023 ($M^+ + 1$), these are the major molecular ion peaks obtained from **Rh1**.

3. Results and discussion

3.1. Synthesis and spectral characterization of probe Rh1

The water soluble, colourless, non fluorescence probe **Rh1** was synthesized by a three-step procedure (Scheme 1). First, rhodamine-B thiohydrazide **(2)** was prepared adopting a known procedure.[41] This was characterized by ^{1}H NMR and ESI-MS which are in well agreement with the literature data. On the other hand, 2,4-dihydroxy benzaldehyde was modified by selective alkylation of its para-hydroxy group with PEG-iodide (**3**), which was derived from polyethylene glycol-400 (PEG 400). The alkylation occurred selectively on the 4-hydroxy group as the other hydroxyl group is less reactive by forming H-bond with the adjacent aldehyde group of 2,4-dihydroxy benzaldehyde. The PEG modified 2,4-dihydroxy benzaldehyde (**4**) underwent condensation in absolute ethanol under reflux for 12 h to form a stable Schiff's base (**Rh1**), which was used as our probe for the detection of Hg^{2+} ions. As PEG is water soluble it was expected that the PEGylated probe (**Rh1**) will also be water soluble. The probe was indeed found to be readily soluble in water and therefore, the fluorometric studies could be carried out in aqueous media. The formation of the probe **Rh1** was well-supported by ^{1}H NMR, ^{13}C NMR and ESI-MS. ^{1}H NMR showed presence of all expected peaks including the presence of imine proton (at δ 8.70 ppm). In ^{13}C NMR spectrum, the imine carbon and the thione (C=S) peaks were appeared at δ 138.9 and 199.0 ppm, respectively, indicating the formation of **Rh1**. The formation of **Rh1** was further confirmed by taking ESI-MS in which the major peak had appeared at *m/z* 935 (as $M^+ + 1$) for one of the homologues and other peaks at *m/z* 979 and 1023, respectively, maintaining a gap of 44 mass units. All the spectral data firmly established the formation of the desired probe **Rh1**.



Scheme 1. Synthesis of the water soluble probe, **Rh1.**

3.2. Study on pH sensitivity

To study the practical applicability, the effects of pH on the fluorescence response of **Rh1** in the absence and presence of Hg^{2+} ions were evaluated. A solution of high concentration of Hg^{2+} ions might cause precipitation of HgO in strongly alkaline conditions,[43] so, these experiments were carried out at a pH range from 3.0 to 9.5. Fig. 1 shows the fluorescence responses of **Rh1** without and with Hg^{2+} ions as a function of pH. Experimental results revealed that free **Rh1**, at strong acidic conditions (pH <5), shows a noticeable off-on fluorescence

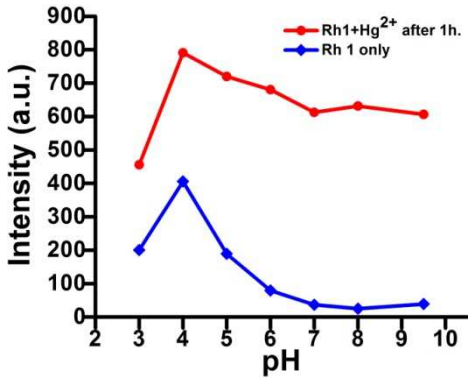


Fig. 1. Fluorescence response of probe **Rh1** (10 μM) after 1 h with and without Hg^{2+} (10 μM, 1 equiv) in different pH buffer (pH 3-9.5) at room temperature (λ_{ex} 525 nm).

with maximum fluorescence response appearing at pH 4 (excited at 525 nm). This can be well-understood considering the possibility of protonation of the spirolactam ring, which leads to the formation of fluorescent ring-open state. However, as the pH increases from mild acidic to neutral to mild basic (pH 5.0-9.5), a very week to negligible fluorescence response could be observed for free **Rh1**, suggesting that the probe molecule prefers to stay in the spirocyclic form. Upon the addition of Hg^{2+} ions, there was an obvious enhancement in fluorescence intensity of **Rh1** at different pH values (pH 3.0-9.5). The pH-controlled emission measurements showed that **Rh1** could react to Hg^{2+} ions within the pH range 5 to 9.5 with minimum change in the fluorescent output which in turn suggests that the probe **Rh1** can perform Hg^{2+} sensing activity in aqueous solution within a wide pH range. Considering the fact that the detection of Hg^{2+} ions *in-vitro* or *in-vivo* would require cellular pH, the media for fluorometric detection of Hg^{2+} ions was set at pH 7.2 for further studies.

3.3. Mechanistic study of Hg^{2+} sensing by Rh1 and spectral features

Rhodamine derivatives in the spirolactam form are generally non-fluorescent and colorless, whereas, when they coordinate with some specific metal ions a strong fluorescence emission and a pink colour are exhibited by spontaneous opening of the spirolactam ring. A rhodamine-based chemosensor can be designed for heavy metal ions (such as Hg^{2+} ion) by the introduction of a proper binding site. Here, the strong affinity of Hg^{2+} towards sulphur has been utilized to design the probe, **Rh1** which contains four ligating sites viz. an S atom, two N atoms from the spirolactam ring and an O atom of the free hydroxyl group of the aromatic aldehyde moiety to strongly anchor Hg^{2+} ions. On addition of Hg^{2+} ions to the aqueous solution (NaOAc-HOAc buffer of pH 7.2) of the probe, an intense pink colour and orange fluorescence was observed. In the emission spectra, an intense emission band appeared at 585 nm which is characteristic of rhodamine B in its ring-open state. Thus, Hg^{2+} ion strongly binds with sulphur and other atoms and triggers off opening of the spirolactam ring, which is associated with a strong fluorescence transduction. It has been observed that the best spectroscopic responses were obtained for the fluorescence and absorbance measurements when Hg^{2+} was allowed to incubate in an aqueous solution of the probe **Rh1** for 1 h at room temperature. The delay in getting complete fluorescence output might be attributed to the fact that a long PEG chain wraps the probe and prevents quick approach of the Hg^{2+} ions towards the binding sites.

For clear understanding of the detection abilities of **Rh1** towards Hg^{2+} ions, a Job's plot analysis was carried out to determine the binding stoichiometry of the **Rh1**-Hg^{2+} complex by maintaining the total probe **Rh1** and Hg^{2+} ion concentration constant (10 μM) and changing the mole fraction of Hg^{2+} from 0 to 1. From the Job's plot shown in Fig. 2 we can observe that the significant enhancement of fluorescence intensity of **Rh1**

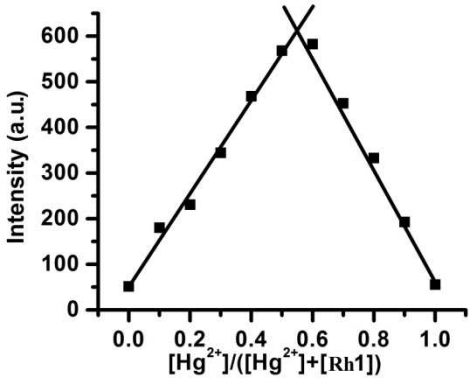


Fig. 2. Job's plot for determining the stoichiometry of probe **Rh1** and Hg^{2+} ions (the total concentration of **Rh1** and Hg^{2+} ions was 10 μM) in buffer (NaOAc-HOAc, pH 7.2) solution at room temperature (excitation at λ_{max} 525 nm).

was resulted due to the complexation with Hg^{2+}. The fluorescence reached the maximum level at a mole fraction of about 0.5 of Hg^{2+} indicating that a 1:1 stoichiometry was most favourable for the binding mode of Hg^{2+} and **Rh1**.

3.4. Spectrofluorometric and spectrophotometric titrations of probe Rh1

The fluorometric titration of probe **Rh1** with Hg^{2+} ions was done by measuring fluorescence emission of the aqueous solution of the probe upon gradual addition of 0-3 equivalent of Hg^{2+} ions. Upon incremental addition of Hg^{2+} ions, a significant enhancement of the intensity of characteristic fluorescence peak of rhodamine B residue at λ_{max} 585 nm was observed (Fig. 3). This reiterates that upon binding with Hg^{2+} the spirolactam ring of the probe goes to its ring-open state. In a similar study, chromogenic response of the probe **Rh1** towards Hg^{2+} was

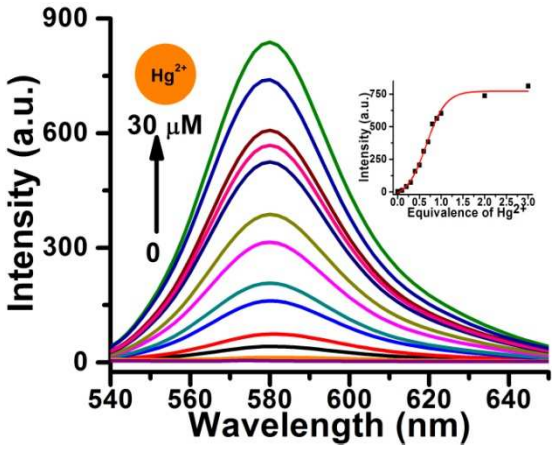


Fig. 3. Fluorescence response of probe **Rh1** (10 μM) after 1 h upon addition of 0–3 equiv of Hg^{2+} in buffer (NaOAc-HOAc, pH 7.2) solution at room temperature (excitation at λ_{max} 525 nm).

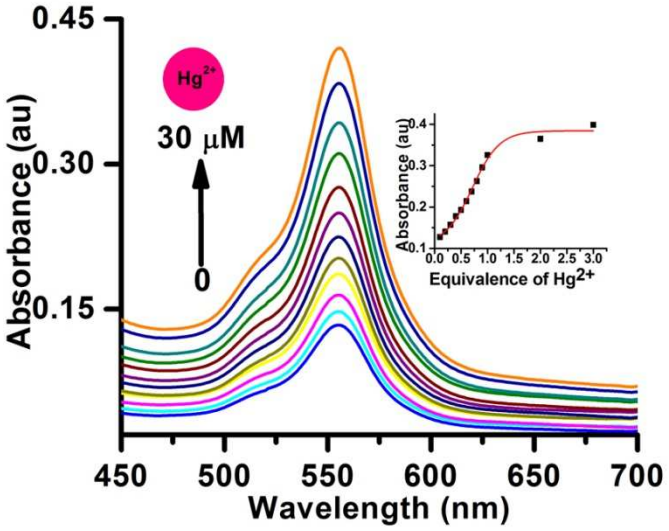


Fig. 4. Absorbance response of probe **Rh 1** (10 μM) after 1 h upon addition of 0–3 equiv of Hg^{2+} in buffer (NaOAc-HOAc, pH = 7.2) solution at room temperature.

examined by measuring absorbance of the aqueous solution of the probe upon gradual addition of 0-3 equivalent of Hg^{2+}. A strong absorbance peak at λ_{max} = 558 nm was developed and intensified with increasing concentration of Hg^{2+} ions (Fig. 4). Both the experiments strongly suggest that the probe is highly effective for the detection of Hg^{2+} ions. Ideally, the probe was expected to show reversible binding ability with the mercury ions. However, to our dismay, the drop in fluorescence intensity was nominal upon addition of excess amount of ligands such as EDTA, Na_2S to **Rh1**-Hg^{2+} complex and incubating it for several hours. Presumably, the long PEG tail makes an amphiphilic barrier around **Rh1** which strongly repels approaching negatively charged ions like S^{2-} by electron rich -O-$\underline{C}H_2$-$\underline{C}H_2$-O- framework. Thus, the probe fails to work as a reversible mercury ion sensor.

3.5. Selectivity of Hg^{2+} over other metal ions

To investigate the specificity of the probe **Rh1**, various ions were examined analogously under the identical condition. For this purpose we have measured the response of probe **Rh1** towards other metal ions such as Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sr^{3+}, Zn^{2+}, Ag^{+} and Cu^{2+}. As depicted in Fig. 5, the interaction of probe **Rh1** with Hg^{2+}

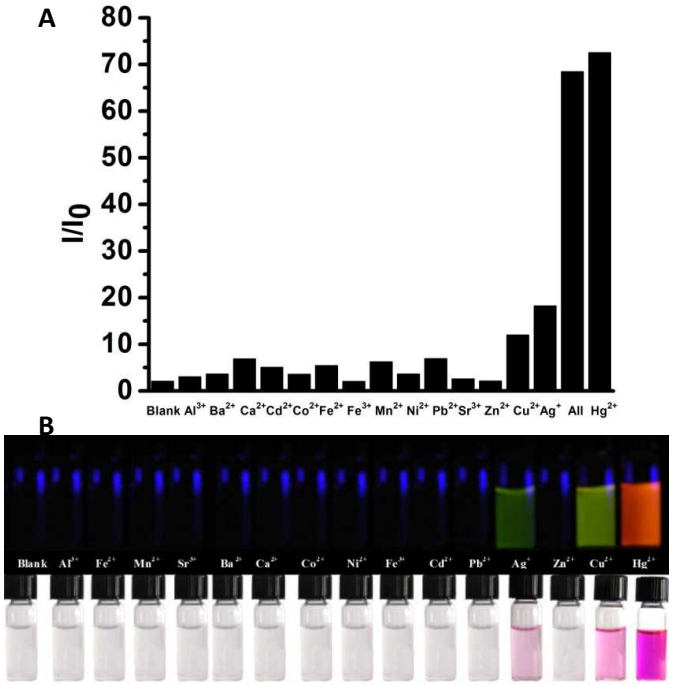


Fig. 5. (A) Bar graph of fluorescence responses of **Rh1** (10 μM) in the presence of 1 equiv of different metal ions after 1 h from the addition of metal ions (λ_{ex} 525 nm, λ_{em} 585 nm). (B) Fluorimetric and colorimetric responses of **Rh1** (10 μM) in the presence of 1 equiv. of different metal ions after 1 h of the addition.

expressed with both strong absorbance and fluorescence responses. On the other hand, probe **Rh1** showed essentially no response toward other ions except Ag^{+} and Cu^{2+}, which show fairly week fluorescence responses. This reflects that weak binding between the ligating sites of the probe and the metal ions like Ag^{+} or Cu^{2+} is possible, which is illustrated by a mere fluorescence output involving the same mechanism as described for Hg^{2+}. However, the binding affinity of Hg^{2+} towards probe **Rh1** is so high that it can easily defeat other competing metal ions in a mixture, which was established by carrying out competition experiments (Fig. 5). As expected, the Hg^{2+}-induced fluorescence output of probe **Rh1** was not much affected by the presence of other metal ions. It divulges that probe **Rh1** preserves excellent specificity for Hg^{2+} in the presence of variety of competing metal ions mentioned above. The specificity is an upshot of the fact that Hg^{2+} being highly thiophillic immediately forms a coordinate bond upon interaction with the probe, which in turn opens up the

spirolactum ring of rhodamine moiety resulting change of fluorescence and color.

3.6. Measurement of detection limit

Probe **Rh1** responds to Hg^{2+} ions linearly below the micro-molar level concentration as shown in Fig. 6 and from that the quantitative detection limit of the probe is approximated to be $7x10^{-7}$ M of $HgCl_2$ or 0.14 ppm.

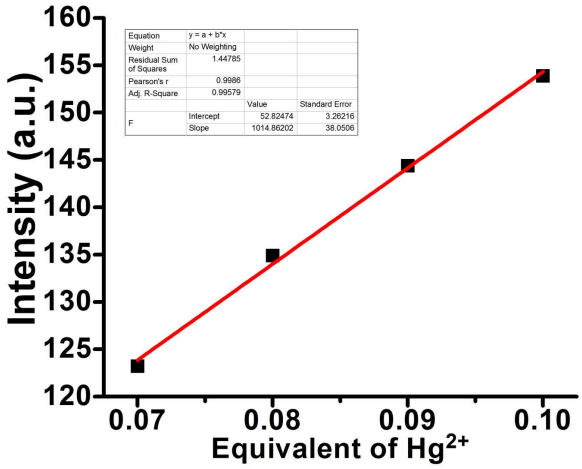


Fig.6. A fluorescence intensity plot of probe **Rh1** against low concentration range of Hg^{2+}. The straight line was obtained from $7x10^{-7}$ M of $HgCl_2$ or 0.14 ppm.

3.7. Fluorescence imaging of living cells

Since the fluorescence intensity of probe **Rh1**, induced by Hg^{2+}, remains unaffected in the presence of other metal ions we thought to explore the practical applicability of probe **Rh1** for monitoring of Hg^{2+} ions in the living cells. For this purpose, first the cell viability of the probe **Rh1** was verified and found no cytotoxic effect on the HeLa cells (Fig. 7). Next, HeLa cells

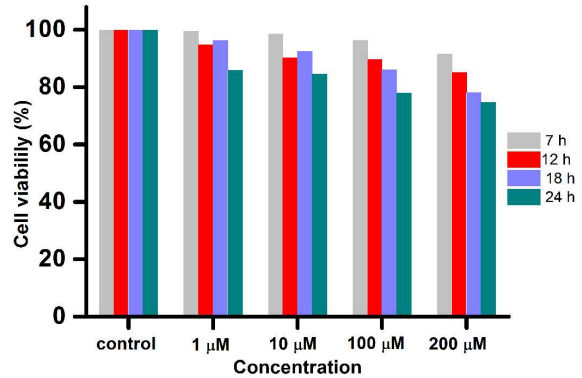


Fig. 7. Cytotoxic assay of the probe, **Rh1** at different concentrations. The percentage of cell survival was monitored after different time intervals. The bar graphs show that near to 80% of total cells are still viable in the presence of 200 μM of **Rh1** in the culture media of HeLa cells. This implies that the probe is fairly nontoxic in nature.

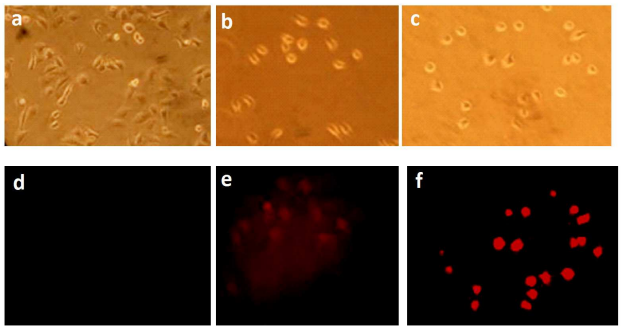


Fig. 8. Detection of Hg^{2+} ions in HeLa cells with probe **Rh1**. Phase contrast (a–c) and fluorescence images (d–e) of HeLa cells after incubating with 0 μM, 0.5 μM and 2 μM of Hg^{2+} ions for 1 h upon a prior 30 min incubation with probe **Rh1** (2 μM), respectively (using a PI filter; Excitation wavelength range: 510-560 nm).

were incubated for 30 min at 37 °C with 2 μM of probe **Rh1** and gave no fluorescence as determined by using a Nikon eclipse TS100 microscope (Fig. 8). After the cells were supplement with different concentration of Hg^{2+} ions 0.5 μM and 2 μM for 1 h, their fluorescence images were recorded and a significant amount of red fluorescence from the intracellular region was observed indicating smooth endocytosis of **Rh1** into the HeLa cells followed by interaction with the intercellular mercury. These preliminary studies revealed that probe **Rh1** is useful for *in vivo* imaging to monitor Hg^{2+} in biological samples as well.

4. Conclusion

In summary, we have appended a water-compatible PEG-unit on to a rhodamine derivative to develop a water soluble fluorescent chemosensor (**Rh1**) for the detection of Hg^{2+} in water as well as in biological systems. The probe goes to its highly fluorescent ring-open state upon complexation with Hg^{2+} ions. **Rh1** features high Hg^{2+} ion selectivity and high sensitivity in aqueous media at neutral pH. The detection limit was found to be 0.14 ppm towards Hg^{2+} ions. Preliminary studies revealed that the probe molecule is fairly non toxic and can successfully penetrate the cell surface of HeLa cells and interact with intercellular Hg^{2+} ions validating its usefulness for monitoring Hg^{2+} ions in biological samples. A numbers of merits like high selectivity and sensitivity, water solubility, no need of organic solvent, use of mild condition, cost effective synthesis make this probe highly considerable for practical use.

Acknowledgements

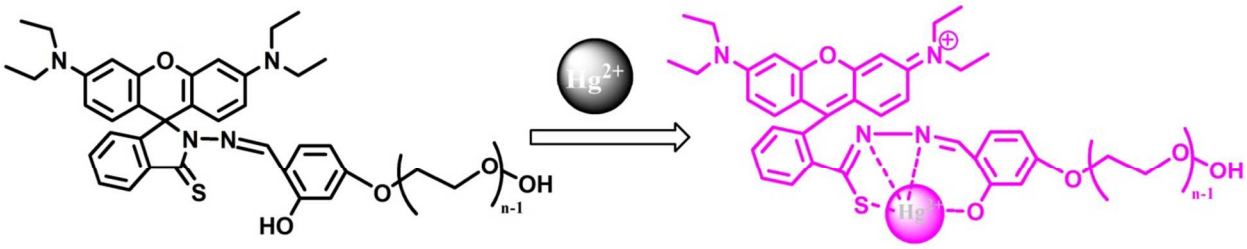
A.C. thanks DST, India (Project No. SR/FT/CS-092/2009) for financial supports.

Notes and references

[a]Department of Chemistry, BITS, Pilani-K. K. Birla Goa Campus, NH 17 B Bypass Road, Zuarinagar, Goa, INDIA.

[b]Department of Biological Sciences, BITS, Pilani-K. K. Birla Goa Campus, NH 17 B Bypass Road, Zuarinagar, Goa, INDIA.

1 L. Magos, *Physiol. Toxicol. Mercury*, 1997, **34**, 321.
2 M. F. Wolfe, S. Schwarzbach, R. A. Sulaiman, *Environ. Toxicol. Chem.,* 1998, **17**, 146.
3 P. B. Tchounwou, W. K. Ayensu, N. Ninashvili, D. Sutton, *Environ. Toxicol.*, 2003, **18**, 149.
4 P. Grandjean, P. Weihe, R. F. White, F. Debes, *Environ. Res.*, 1998, **77**, 165.
5 S. H. Choi, K. Pang, K. Kim, D. G. Churchill, *Inorg. Chem.,* 2007, **46**, 10564.
6 C. R. Baum, *Curr. Opin. Pediatr.*, 1999, **11**, 265.
7 E. K. Silbergeld, I. A. Silva, J. F. Nyland, *Toxicol. Appl. Pharmacol.*, 2005, **207**, S282.
8 R. K. Zalups, S. Ahmad, *J. Am. Soc. Nephrol.,* 2004, **15**, 2023.
9 R. K. Zalups, L. H. Lash, *Toxicol. Appl. Pharmacol.*, 2006, **214**, 88.
10 S. Atilgan, I. Kutuk, T. Ozdemir, *Tetrahedron Lett.*, 2010, **51**, 892.
11 D. W. Boening, *Chemosphere*, 2000, **40**, 1335.
12 H. H. Harris, I. Pickering, G. N. George, Brevia: the Chemical Form of Mercury in Fish Science, Wash. D. C., 2003, vol. 301(5637), p. 1203.
13 S. D. Richardson, T. A. Temes, *Anal. Chem.*, 2005, **77**, 3807.
14 X. Zhang, Y. Xiao, X. Qian, *Angew. Chem., Int. Ed.*, 2008, **47**, 8025.
15 T. Takeuchi, N. Morikawa, H. Matsumoto, Y. *Shiraishi, Acta Neuropathol.,* 1962, **2**, 40.
16 M. Harada, *Crit. Rev. Toxicol.*, 1995, **25**, 1.
17 R. Von Burg, *J. Appl. Toxicol.*, 1995, **15**, 483.
18 T. W. Clarkson, L. Magos, G. J. Myers, N. *Engl. J. Med.*, 2003, **349**, 1731.
19 C. F. Poole, *Trends Anal. Chem.*, 2003, **22**, 362.
20 B. Welz, M. Sperling, Atomic Absorption Spectrometry, Weinheim, Germany, Wiley, 3rd edn, 1999.
21 Y. Gao, Z. Shi, Z. Long, P. Wu, C. Zheng, X. Hou, *Microchem. J.*, 2012, **103**, 1.
22 A. Q. Shah, T. G. Kazi, J. A. Baig, H. I. Afridi, M. B. Arain, *Food Chem.*, 2012, **134**, 2345.
23 J. A. Moreton, H. T. Delves, *J. Anal. At. Spectrom.*, 1998, **13**, 659.
24 J. Djedjibegovic, T. Larssen, A. Skrbo, A. Marjanovic, M. Sober, *Food Chem.*, 2012, **131**, 469.
25 E. Kenduzler, M. Ates, Z. Arslan, M. McHenry, P. B. Tchounwou, *Talanta*, 2012, **93**, 404.
26 Y. Kalyan, A. K. Pandey, P. R. Bhagat, R. Acharya, V. Natarajan, G. R. K. Naidu, A.V. R. Reddy, *J. Hazard. Mat.*, 2009, **166**, 377.
27 P. D. Selid, H. Xu, E. M. Collins, M. S. Face-Collins, J. X. Zhao, *Sensors*, 2009, **9**, 5446.
28 J. Chan, S. C. Dodani, C. J. Chang, *Nat. Chem.*, 2012, **4**, 973.
29 M. J. Culzoni, A. M. de la Pena, A. Machuca, H. C. Goicoechea, R. Babiano, *Anal. Methods*, 2013, **5**, 30 and references cited therein.
30 H. N. Kim, W. X. Ren, J. S. Kim, J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 3210.
31 M. E. Jun, B. Roy, K. H. Ahn, *Chem. Commun.*, 2011, **47,** 7583.
32 M. Beija, C. A. M. Afonso, J. M. G. Martinho, *Chem. Soc. Rev.*, 2009, **38**, 2410.
33 E. M. Nolan, S. J. Lippard, *Chem. Rev.* 2008, **108**, 3443.
34 M. Santra, D. Ryu, A. Chatterjee, S.-K. Ko, I. Shin, K. H. Ahn, *Chem. Commun*., 2009, 2115.
35 X. Wu, J. Chen, J. X. Zhao, *Analyst*, 2013, **138**, 5281.
36 S. Madhu, D. K. Sharma, S. K. Basu, S. Jadhav, A. Chowdhury, M. Ravikanth, *Inorg. Chem.*, 2013, **52**, 11136.
37 B. Biswal, B. Bag, *Org. Biomol. Chem.* 2013, **11**, 4975.
38 T. Mistri, R. Alam, M. Dolai, S. K. Mandal, P. Guha, A. R. Khuda-Bukhsh, M. Ali, *Eur. J. Inorg. Chem.*, 2013, **34**, 5854.
39 V. Kumar, M. Banerjee, A. Chatterjee, *Talanta*, 2012, **99**, 610.
40 H. Wang, Y. Li, S. Xu, Y. Li, C. Zhou, X. Fei, L. Sun, C. Zhang, Y. Li, Q. Yang, X. Xu, *Org. Biomol. Chem.,* 2011, **9**, 850.
41 H. Zheng, Z.-H. Qian, L. Xu, F.-F. Yuan, L.-D. Lan, J.-G. Xu, *Org. Lett.*, 2006, **8**, 859.
42 W. Huang, P. Zhou, W. Yan, C. He, L. Xiong, F. Li, C. Duan, *J. Environ. Monit.*, 2009, **11**, 330.
43 H. H. Wang, L. Xue, C. L. Yu, Y. Y. Qian, H. Jiang, *Dyes Pigm.,* 2011, **91**, 350.
44 X. Li, X. Gao, W. Shi, H. Ma, *Chem. Rev.* 2014, **114**, 590.
45 D. G. Khandare, V. Kumar, A. Chattopadhyay, M. Banerjee, A. Chatterjee, *RSC Adv.*, 2013, **3**, 16981.



A water soluble turn-on fluorescent probe for the detection of mercury ions in water has been developed by appending a water-compatible PEG-unit on to a rhodamine derivative. The probe **Rh1** binds to Hg^{2+} ions with high selectivity and sensitivity in purely aqueous media. Preliminary studies revealed that the probe is fairly non-toxic and can detect intercellular Hg^{2+} ions as well.