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Novel hexapodal triazole linked to a cyclophosphazene core rhodamine-based chemosensor for selective determination of Hg²⁺ ions

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Abstract The hexapodal Rhodamine B derivative compound **3** containing a cyclotriphosphazene core was synthesized and characterized by spectroscopic techniques such as FT-IR, ^{1}H, ^{13}C and ^{31}P NMR, HR-MS, MALDI MS and microanalysis. Compound **3** is a naked eye selective sensor with colorimetric and fluorescent properties for Hg^{2+} ions in the presence of other metal ions such as Na^+, K^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Ag^+, Mn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, and Cr^{3+}. The optical sensor properties of compound **3** were investigated using UV–vis and fluorescence spectroscopy. The lowest detection limit of compound **3** was determined as 3.76×10^{-9} M (0.75 ppb) for Hg^{2+} ions. The stoichiometry of compound **3**-Hg^{2+} complex was found to be 1:3 (ligand/metal ion). The reusable test strip was improved by the immobilization of compound **3** into a hydrogel network. The reusability of the sensor and test strip was tested with S^{2-} ion solutions.

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Introduction

Mercury is a toxic species for humans and a large number of living organisms because it can participate in many biological processes thanks to its affinity for thiol groups in the structure of enzymes and proteins [1, 2]. The main sources of mercury contamination are natural and industrial activities such as oceans and volcanic eruptions, gold production, coal plants, the use of fossil fuels, mining, paints, batteries and electronic products [3–6]. Inorganic mercury that is released into the aquatic environment from these sources can enter the food chain due to its conversion to methylmercury by bacteria [7, 8]. Even very low concentrations of mercury threaten human health because it damages vital organs such as heart, kidney, brain and nervous system. High doses of mercury in the human body can cause diseases such as nervous disorders, brain damage, vision and hearing loss [9]. Thus, the highest concentration of mercury in drinking water is set at 2 ppb by the United States Environmental Protection Agency (EPA) [10]. For the determination of mercury conventional methods are used, such as spectrophotometry, atomic absorption spectroscopy (AAS), voltammetry, surface-enhanced Raman scattering (SERS), inductively-coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromotography (HPLC) [11–14]. Recently mercury has been determined with highly sensitivity and selectivity by UV–vis and fluorescence spectroscopy techniques thanks to developed sensors. Some of these sensors are hydroxyquinolines, thiacrown ethers, pyrene, naphthylthiourea and rhodamine derivatives. Among them rhodamine derivatives are among the most common [15–22].

Phosphazene compounds composed by repeating -P (X_2)= N- units form a broad class of inorganic compounds [23]. Both cyclic and linear phosphazene compounds have been the subject of a large number of studies due to their features such as hydrolytic stability, thermal stability, flame retardant properties, biocompatibility, non-toxic degradation products and liquid crystalline behavior [24]. Particularly cyclic phosphazenes are interesting compounds as starting material for the preparation of dendrimeric materials. The main advantage of a cyclotriphosphazene core is that halogen atoms attached to the phosphorus atom are readily substituted with nucleophiles such as alcohols and amines [25–28]. In addition, substituted cyclotriphosphazene core has advantages such as stability under aggressive chemical conditions and optical and electrical inertia [29].

Recently, we reported the synthesis of a hexapodal rhodamine 6G substituted cyclotriphosphazene compound as a highly selective sensor for Fe^{3+} ions [24]. The binding stoichiometry of Host-Fe^{3+} complex was determined as 1:3. Now, in this paper we synthesized a new rhodamine derivative containing triazole linked cyclotriphosphazene core as selective naked eye sensor for Hg^{2+} ions. We also determined the detection limit of compound 3 as 3.76×10^{-9} M (0.75 ppb) for Hg^{2+} ions. Finally, we immobilized the sensor in a hydrogel matrix in order to ensure ease of use in practical application. Thus we have developed a water insoluble reusable test strip as a selective colorimetric sensor for Hg^{2+} ions. We also found that the hydrogel-immobilized system has sensor features even at low concentrations of Hg^{2+} ions.

Experimental

Materials and methods

The reagents and all solvents were purchased from Aldrich chemical company and were used without further purification. THF was dried over sodium-benzophenone system. In the sensor studies were used perchlorate salts of Na^+, K^+, Ca^{2+}, Ba^{2+}, Mg^{2+}, Ag^+, Mn^{2+}, Hg^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, and Cr^{3+} ions as metal ion source and tetrabutylammonium salts of AcO^-, F^-, NO_2^-, HSO_4^-, SCN^-, CN^-, Br^-, Cl^-, I^-, HPO_4^{2-} and NO_3^- anions and potassium salt of PO_4^{3-}, CO_3^{2-} and SO_4^{2-} anions as anion source. 4–(2–Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES) was used as buffer media. All UV–vis absorption spectra were recorded using PG Instruments T80+. The fluorescence spectra were recorded on Shimadzu RF-5301PC spectrofluorophotometer. ^{1}H, ^{13}C, and ^{31}P-NMR spectra were recorded on Bruker Biospin (400 MHz) or Varian Unity INOVA (500 MHz) spectrophotometer by using $CDCl_3$ or DMSO-d_6 as solvent and tetramethylsilane and 85 % H_3PO_4 as interval reference for ^{1}H-NMR and ^{31}P-NMR, respectively.

Mass spectra of the compounds were recorded on Water SYNAT (HRMS ES$^+$) and Bruker Microflex LT MALDI-TOF MS spectrometers. FT-IR was recorded with a Perkin Elmer FT-IR instrument by using an ATR apparatus with 4 cm^{-1} resolution between 4,000 and 650 cm^{-1} and the element combination of compounds were determined by a LECO CHNS-932 elemental analyzer. All UV–vis absorption and fluorescence intensity measurements were repeated three times. The averaged values were given in the figures.

Synthesis of compound 1

The compound 1 was similarly synthesized according to literature procedure [24].

Synthesis of compound 2

Compound 2 was synthesized in two consecutive steps. To a solution of Rhodamine B base (2.21 g, 5.00 mmol) in 1,2-dichloroethane (25 mL) was added $POCl_3$ (1.5 mL) dropwise over 15 min. The reaction mixture was refluxed for 8 h. At the end of this time, the reaction mixture was cooled to room temperature and the solvent was evaporated under reduced pressure to give crude rhodamine B acid chloride. The crude rhodamine B acid chloride and triethylamine (3.5 mL) was dissolved in dry THF (50 mL). A solution of propargyl amine (0.33 g, 6.00 mmol) in dry THF (10 mL) was added dropwise to the above solution over 1 h at 0 °C. The reaction mixture was then stirred at room temperature for 24 h. At the end of this time, the insoluble salts were filtered and the solvent was evaporated under reduced pressure. The dark-red crude product was purified by silica-gel column chromatography with EtOAc: Hexane (2:8) solvent mixture to give the 2.07 g of white crystaline solid (yield 87 %). Mp: 194–195 °C. FTIR-ATR (ν_{max}, cm^{-1}): 1,700 (C=O). ^{1}H NMR (300 MHz, CDCl$_3$, 25 °C): δ 7.94 (m, 1H), 7.45 (m, 1H), 7.12 (m, 1H), 6.48 (d, 2H, J=8.80 Hz), 6.39 (d, 2H, J=2.40 Hz), 6.28 (dd, 2H, J=2.45 Hz), 3.96 (d, 2H, J=2.40 Hz), 3.35 (q, 8 H, J=7.10 Hz), 1.78 (t, 1H, J=2.40 Hz), 1.17 (t, 12H, J=7.20 Hz); ^{13}C NMR (75 MHz, CDCl$_3$, 25 °C): 167.42 (C=O), 153.78, 153.49, 148.87, 132.67, 130.42, 129.12, 128.03, 123.80, 123.03, 108.00, 105.06, 97.79, 78.30 (Ar-C), 76.06 (<u>C</u>≡CH), 64.81 (C≡<u>CH</u>), 44.04 and 28.54 (N-CH$_2$), 12.59 (<u>CH$_3$</u>). HRMS (ES$^+$) m/z: Calcd $C_{31}H_{33}N_3O_2$+(H$^+$), 480.2597. Found: 480.2601. Element analysis Cald. for $C_{31}H_{33}N_3O_2$ C 77.63, H 6.94, N 8.76 %; Found C 77.44, H 6.99, N 8.69 %.

Synthesis of compound 3

N,N,N',N'',N''-Pentamethyldiethylenetriamine (PMDETA, 0.50 mL, 2.40 mmol) was added to a solution of compound 1 (0.2048 g, 0.20 mmol) and the compound 2 (0.69 g, 1.44 mmol) in dry THF (50 ml) and the solution was purged

with argon for 15 min. Afterwards, copper (I) iodide (0.0228 g, 0.12 mmol) was added to the reaction mixture and the mixture was degassed with argon for 15 min. The reaction mixture was stirred at 60 °C for 24 h under argon atmosphere. At the end of this time, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel with CHCl$_3$:methanol (95:5) solvent mixture to give the 0.46 g of a pale pink solid (yield 59 %). Mp: 242 °C. FTIR-ATR (ν_{max}, cm^{-1}): 1,690 (C=O), 1,210 and 1,165 (P=N), 950 (P-O-C). ^{1}H NMR (300 MHz, CDCl$_3$, 25 °C): δ 7.86 (m, 6H), 7.41 (m, 12H), 7.12 (s, 6H, triazol) 7.09 (m, 6H), 6.88 (d, J=8.60 Hz, 12H), 6.75 (d, J=8.60 Hz, 12H), 6.34 (s, 12H), 6.29 (d, J=8.90 Hz, 12H), 6.13 (d, J=8.90 Hz, 12H), 5.32 (s, 12H), 4.43 (s, 12H), 3.28 (q, J=6.90 Hz, 24H), 1.12 (t, J=6.90 Hz, 36H). ^{13}C NMR (75 MHz, CDCl$_3$, 25 °C): 167.89 (C=O), 153.43, 150.18, 148.71, 147.39, 144.74, 132.55, 132.11, 130.83, 128.86, 128.78, 128.08, 123.87, 122.84, 120.98, 107.87, 105.22, 97.87, 65.06, 52.71, 44.35, 35.42, 29.72, 12.63. ^{31}P NMR (202 MHz, CDCl$_3$, 25 °C): 8.19. MALDI-MS m/z: Calcd for C$_{228}$H$_{234}$N$_{39}$O$_{18}$P$_3$ 3901.470 Found: 3901.864. Element analysis Cald. for C$_{228}$H$_{234}$N$_{39}$O$_{18}$P$_3$ C 70.19, H 6.05, N 14.00 %; Found C 70.28, H 5.99, N 13.96 %.

The preparation and utilization as a test strip of sensor-immobilized hydrogel matrix

In the synthesis of hydrogel matrix, 2-Hydroxyethyl methacrylate (HEMA) was used as monomer, N,N'-methylenebisacrylamide (MBA) as cross-linker, N,N,N',N'-tetramethylenediamine (TEMED) as accelerator and ammonium persulfate (APS) as redox initiator. Briefly, to the solution containing HEMA (2 mL) and MBA (0.1 % with respect to the monomer) was added a volume of 50 μL of TEMED and the solution of compound **3** in DMSO (50 mg/250 μL). The mixture was stirred until it became homogeneous. Afterwards, APS as initiator was dissolved in 0.2 mL distilled water (1 mol% with respect to the monomer) and was added to this mixture. The solution was stirred again in order to obtain a homogeneous mixture. Then, the mixture was filled into plastic pipettes with a diameter of 5 mm using an injector. The polymerization reaction was maintained for 12 h at room temperature. At the end of this time, the compound **3** immobilized hydrogels were extracted from the pipettes and were cut to a diameter of 5 mm. Then, the hydrogels were cleaned of unreacted species by storage in distilled water for 24 h. Then, they were used in sensor applications. The swollen hydrogels were put into solutions of Hg^{2+} with a concentration of 0.5, 5 and 50 ppm (50 mL). Thus, the lowest mercury concentration that caused color changes was determined. Reversibility of the sensor-immobilized hydrogel for the detection of Hg^{2+} ions was tested using sodium sulfide (Na$_2$S) solution. To this end, the hydrogels that had changed color after interaction with mercury were put into solutions of sodium sulfide (0.1 M, 50 mL). As a result of this process, the hydrogels returned to their initial color and were cleaned in distilled water for 24 h.

Experimental details for sensor studies

The stock solutions of metal ions and compound **3** in THF:H$_2$O (9:1) were used in all studies and in order to keep a constant concentration of the test solutions, the total volume of the added stock solutions was maintained at less than 100 μl. All spectra were measured at 25 °C in this solvent mixture. UV–vis absorption spectra were recorded using PG Instruments T80+. The change of absorption in λ=562 nm was investigated during sensor studies. Fluorescence measurements were carried out by a Shimadzu RF-5301PC spectrofluorophotometer in THF:H$_2$O (9:1, HEPES buffer, pH=7.0). The effects of transition metal ions on selectively quenching-growing of the emission spectra were investigated in solutions each containing 10 μM of compound **3** and 100 μM metal ions. The change of fluorescence intensities during metal ion titration experiments depending was determined using a series of different concentration Hg^{2+} solutions. The possible competition of other cation types was investigated using separate solutions of compound **3**, each simultaneously containing Hg^{2+} and another cation with excess concentration. Reversibility of the sensor was tested using sodium sulfide (Na$_2$S). Slit width and excitation wavelength was 3 nm and 525 nm in all experiments. Fluorescence quantum yield (QY) is calculated by comparative methods using Rhodamine 6G solution in ethanol as the standard according to described in the literature [24].

Results and discussion

Compound **3**, a selective naked eye fluorescent sensor for Hg^{2+} ions, was synthesized as a result of the "click-reaction" of hexaazide compound **1** with rhodamine-based alkyne compound **2** in the presence of copper (I) catalyst as shown in Scheme 1 with 59 % yield. The structures of compound **2** and compound **3** were characterized by FT-IR, ^{1}H, ^{13}C, ^{31}P NMR, HR-MS, MALDI-TOF MS and elemental analysis (see experimental section and Fig. S1-S7, Supplementary material). In the ^{1}H NMR spectrum of compound **3**, the proton of the triazole ring is observed at δ=7.12 ppm as singlet (6H). The methylene attached to the triazole ring was observed at δ=5.32 and 4.43 ppm as singlet. The signal of all the phosphorus atoms in the phosphazene core of compound **3** was observed at δ=8.19 ppm as singlet in the ^{31}P NMR spectrum. The mass spectrum of compound **3** showed a parent ion peak at
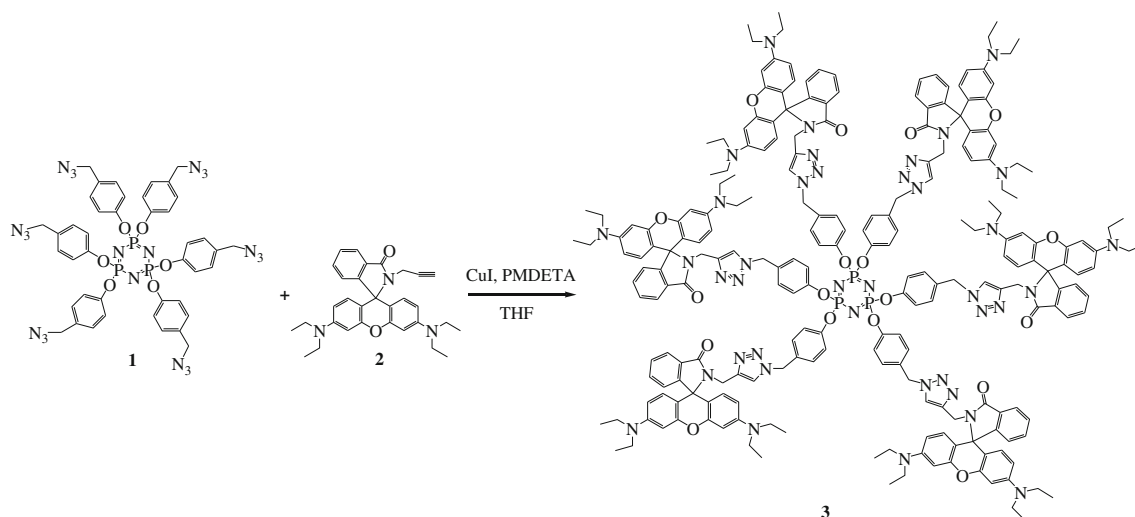
Scheme 1 Synthetic route of compound **3**

3901.864 (M^+). All these spectral analyses confirm the structure of compound **3**.

In order to investigate the sensor properties of compound **3**, various metal ions were added to the solution buffered with HEPES at neutral pH of compound **3** in THF:H$_2$O (9:1). The color and luminescence of rhodamine derivatives change as a result of the opening of the spirocyclic lactam ring in their structure in acidic pH [30]. Therefore, the fluorescence spectra of compound **3** were saved in the absence and presence of Hg^{2+} ions at different pH (2–10). It was determined that the fluorescence intensity of compound **3** in the absence of Hg^{2+} ions was nearly 0 at range of pH=6–10. At the same time, it was observed that the fluorescence intensity of compound **3** in the presence of Hg^{2+} ions remained almost constant in the pH range of 4–7. Therefore, all experiments were carried out at neutral pH=7.0 (Fig. S8, Supplementary material).

The response time of compound **3** was determined by recording the change of absorbance at λ=562 nm according to time after the addition of Hg^{2+} ions to the medium (Fig. S9, Supplementary material). It was found that the system reached equilibrium in 75 min. Therefore, measurements were performed 90 min after the addition of Hg^{2+} ions. In order to investigate photostability of sensor-metal ion complex, fluorescence intensity of the solution of compound **3** was recorded in the presence and absence of Hg^{2+} ions at λ=580 nm for 60 min. Fluorescence intensity value at the end of this time was found to be the same as the initial value. Therefore, it can be stated that compound **3-Hg^{2+}** system has perfect photostability (Fig. S10, Supplementary material).

To observe the naked-eye sensor properties of compound **3** in the presence of metal ions, compound **3** (10 μM) was exposed to different metal ions (100 μM) in THF:H$_2$O (9:1, HEPES buffered, pH=7.0) solution. As indicated in Fig. 1, the color of the solution changed from colorless to pink-purple only in the presence of Hg^{2+} (100 μM). In addition, it was

observed that compound **3** solution had an orange emission in the presence of Hg^{2+} under λ=365 nm UV light.

UV–vis spectra of compound **3** were recorded in the presence of different metal ions (100 μM) in THF:H$_2$O (9:1, HEPES buffered, pH=7.0) solvent mixture. As indicated in Fig. 2 (a) and (b), an intense absorption was observed in the presence of only Hg^{2+} ions at λ=562 nm (related to a delocalized xanthane form of the rhodamine group formed by opening of the spirolactam ring). The reason for this change is that the spirolactam ring opens due to bonding of rhodamine-based molecules with metal ions. This structure is presented in Scheme 2. This change is supported by the fact
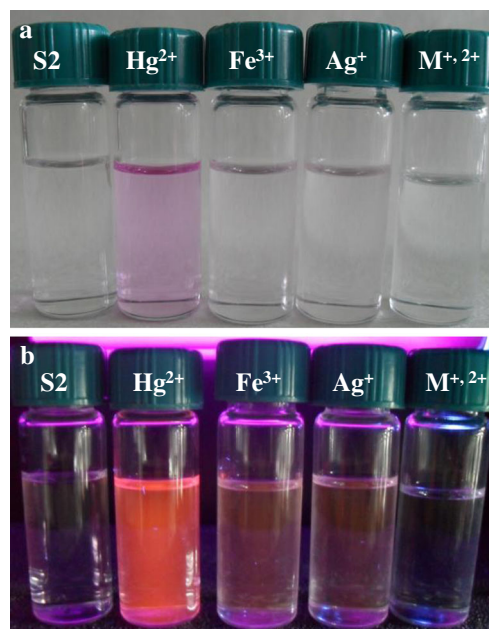

Fig. 1 Solution color of compound **3** in presence of different cations under sunlight **a** and UV light **b**
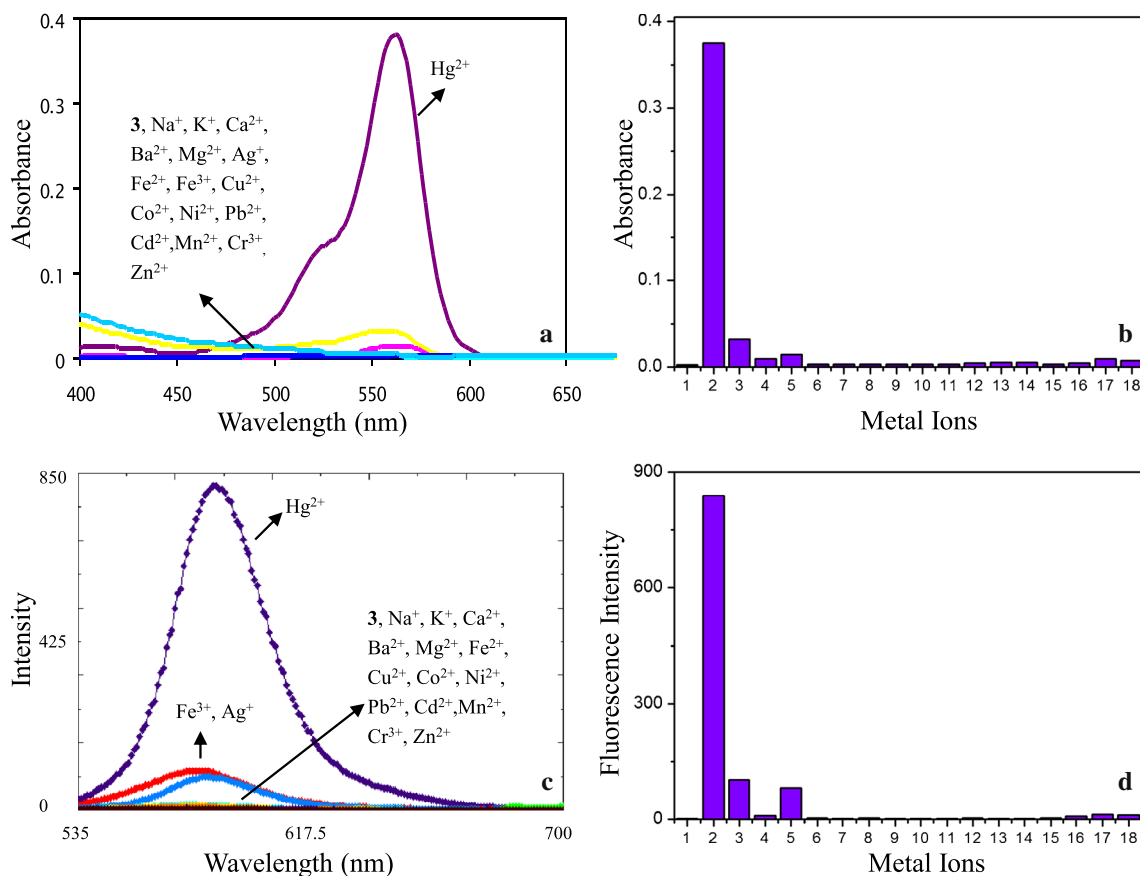
Fig. 2 a Absorption spectra, b Absorption intensity, c Fluorescence spectra, d Fluorescence Intensity of 3 (10 μM) in the presence of different metal ions (100 μM) in THF/H$_2$O (9:1, 0.01 M HEPES buffer, pH=7.0): 1, 3; 2, blank (3+Hg^{2+}); 3, Fe^{3+}; 4, Cu^{2+}; 5, Ag$^+$; 6, Na$^+$; 7, K$^+$; 8, Ca^{2+}; 9, Ba^{2+}; 10, Mg^{2+}; 11, Mn^{2+}; 12, Ni^{2+}; 13, Co^{2+}; 14, Cd^{2+}; 15, Zn^{2+}; 16, Pb^{2+}; 17, Fe^{2+}; 18, Cr^{3+}

that the stretching vibration of compound 3 for C=O stretching observed at 1,690 cm^{-1} shifted to 1,646 cm^{-1} as a shoulder (Fig. S11, Supplementary material). Similar changes in C=O stretching frequency have been reported in the literature after the interaction of the rhodamine ring with metal [24, 31].
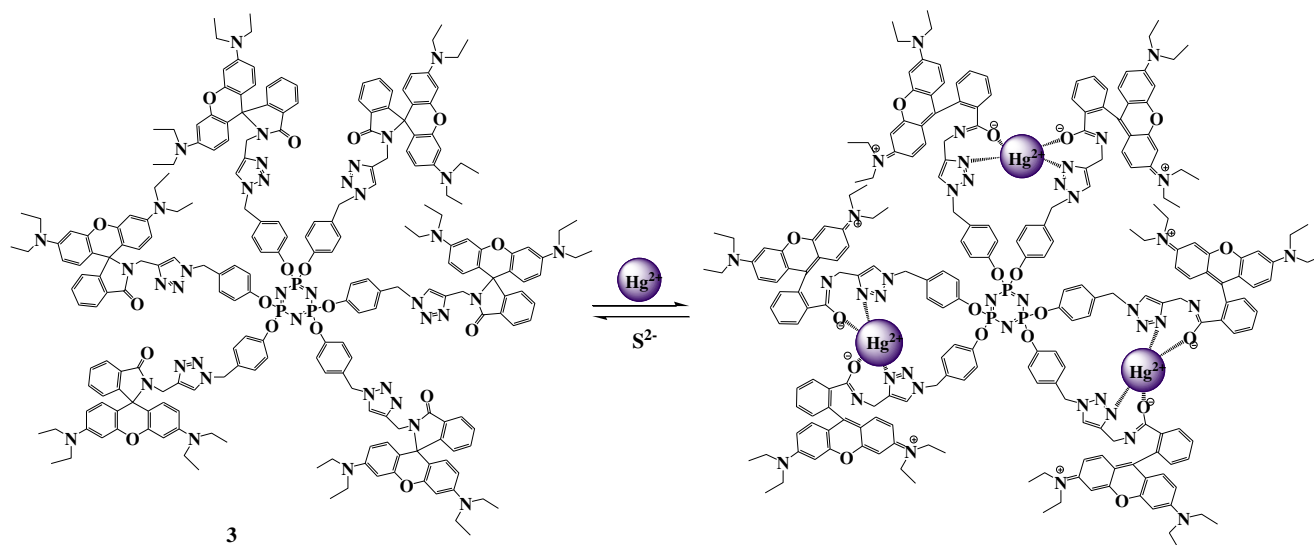
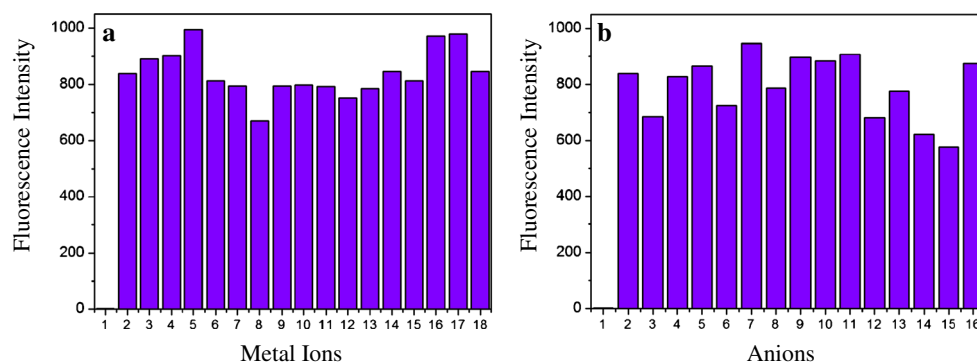Scheme 2 Proposed structure of 3-Hg^{2+} complex

Fig. 3 **a** Fluorescence changes of the solution containing **3** (10 μM) and of Hg^{2+} (100 μM) upon addition of competing metal ions (100 μM) : 1, **3**; 2, blank (**3**+Hg^{2+}); 3, Fe^{3+}; 4, Cu^{2+}; 5, Ag$^+$; 6, Na$^+$; 7, K$^+$; 8, Ca^{2+}; 9, Ba^{2+}; 10, Mg^{2+}; 11, Mn^{2+}; 12, Ni^{2+}; 13, Co^{2+}; 14, Cd^{2+}; 15, Zn^{2+}; 16, Pb^{2+}; 17, Fe^{2+}; 18, Cr^{3+}. (b) Fluorescence changes of the solution containing **3**

(10 μM) and of Hg^{2+} (100 μM) upon addition of competing anions (100 μM) : 1, **3**; 2, blank (**3**+Hg^{2+}); 3, AcO$^-$; 4, F$^-$; 5, NO$_2^-$; 6, HSO$_4^-$, 7, SCN$^-$; 8, CN$^-$; 9, Br$^-$; 10, Cl$^-$; 11, I$^-$; 12, H$_2$PO$_4^-$; 13, NO$_3^-$; 14, PO$_4^{3-}$; 15, CO$_3^{2-}$; 16, SO$_4^{2-}$

In order to investigate the fluorescence response of compound **3** to different metal ions, the fluorescence spectra of compound **3** (10 μM) were recorded in the presence of metal ions (100 μM) in THF:H$_2$O (9:1, HEPES buffered, pH=7.0). For all measurements, excitation wavelength and slit width were 525 and 3 nm. As indicated in Fig. 2 (c) and (d), an intense fluorescence was observed only in the presence of Hg^{2+} ions at λ=580 nm. As shown in UV–vis and fluorescence studies, compound **3** has almost perfect selectivity for Hg^{2+} ions. Quantum yield of compound **3** in the absence of Hg^{2+}

ions was <0.05 %, quantum yield of compound **3**- **Hg^{2+}** solution was found to be 14.6 %.

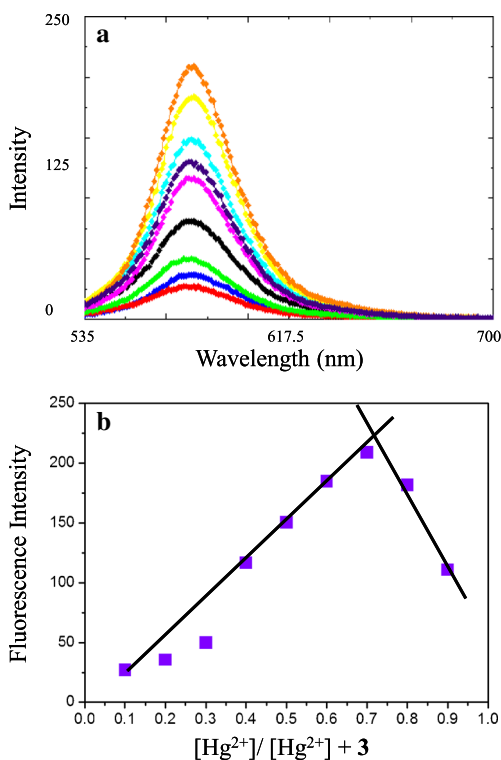The presence of competitive cations was tested by the analysis of the change in fluorescence intensity of compound

Fig. 4 For the determination of binding stoichiometry of **3**-Hg^{2+} complex **a** Fluorescence spectra. **b** Job's plot related to fluorescence intensity
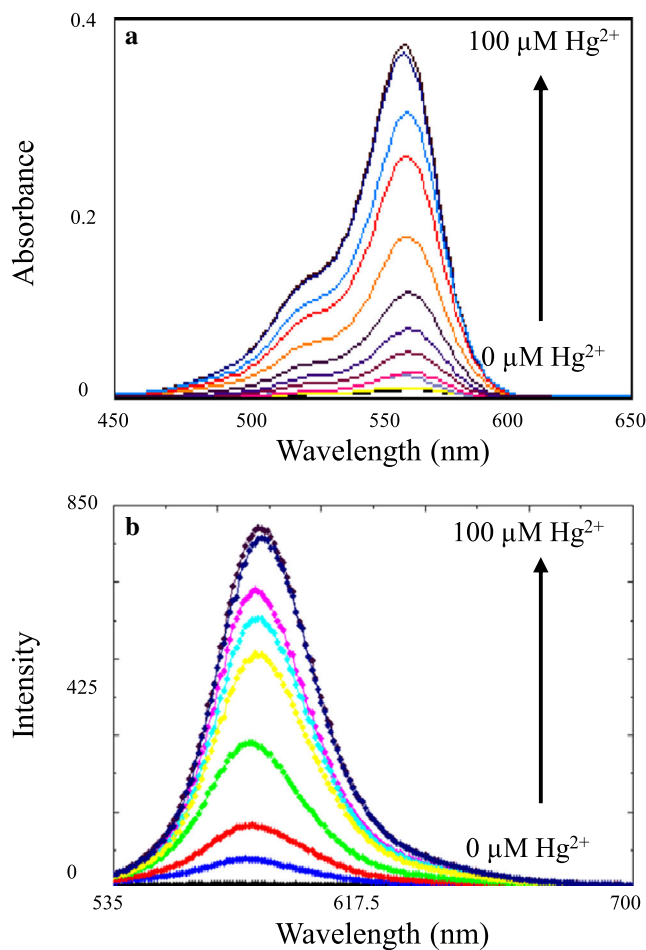

Fig. 5 **a** UV–vis spectra, **b** Fluorescence spectra of **3** in the presence of different concentration of Hg^{2+} ions

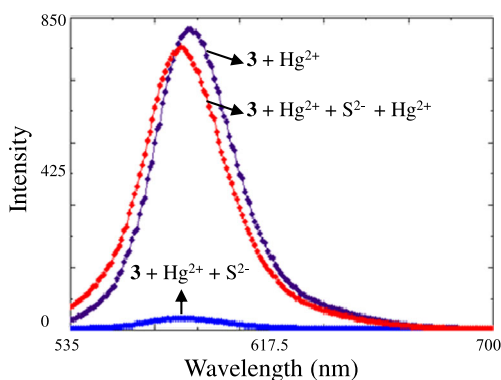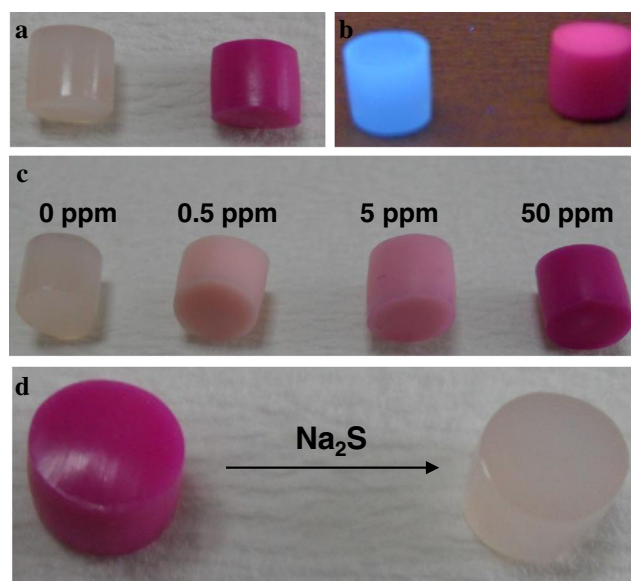**Table 1** Determination of Hg^{2+} in deionized and tap water

Sample	Added amount of Hg^{2+} (ppm)	Recovery (%)
Deionized water	1.0	96.7
	2.5	98.6
Tap water	1.0	94.8
	2.5	95.3

Fig. 7 The Images of sensor immobilized hydrogels in the presence and absence of Hg^{2+} ions **a** under sunlight, **b** under 365 nm UV light, **c** color of hydrogels various concentration of Hg^{2+} ions, **c** reusability of hydrogel based test strip

3-Hg^{2+} complex ion in the presence of different metal ions as competitive cation at λ=580 nm. As indicated in Fig. 3 (a), a negligible effect was observed only in the presence of Ca^{2+} ions. In addition to cation effect, the effect of competitive anions on the sensor properties of compound **3** for Hg^{2+} ions was analyzed. AcO$^-$, F$^-$, NO$_2^-$, HSO$_4^-$, SCN$^-$, CN$^-$, Br$^-$, Cl$^-$, I$^-$, HPO$_4^{2-}$, NO$_3^-$ PO$_4^{3-}$, CO$_3^{2-}$ and SO$_4^{2-}$ anions were added to compound **3**- **Hg^{2+}** complex ion solution. As indicated in Fig. 3 (b), among these anions, only PO$_4^{3-}$ and CO$_3^{2-}$ anions caused a small change in fluorescence intensity. Based on these results, it can be stated that other anions and cations do not have a significant effect on the sensor characteristics of compound **3** for Hg^{2+} ions.

The stoichiometric ratio between compound **3** and Hg^{2+} ions was determined as 1:3 from Job's plot (Fig. 4). As seen in Fig. 4, 0.75 M concentration ratio was obtained from the graph of change in fluorescence intensity to molar concentration ratio of Hg^{2+}. Association constant of the compound **3**- **Hg^{2+}** complex ion was found to be K$_{ass}$= 5.63×10^{13} (Fig. S12, Supplementary material) using the following equation (1) [16];

$$\log \left[F - F_{min} \right] / \left[F_{max} - F_{min} \right] = \log K_{ass} + n\log[M^{n+}] \quad (1)$$

Where, F is the fluorescence intensity of **3** in the presence of various concentrations of Hg^{2+}, F$_{min}$ is the fluorescence intensity of **3** in the absence of Hg^{2+} ions, F$_{max}$ is the fluorescence intensity of **3** in the presence of

excess amount of Hg^{2+} ions and K$_{ass}$ is the association constant of **3**- **Hg^{2+}** complex ion.

The limit of detection (LOD) was determined by metal ion titration for Hg^{2+} ions. To this end, UV–vis and fluorescence spectrums of compound **3** were recorded in the presence of Hg^{2+} ions at various concentrations. As indicated in Fig. 5 (a), while compound **3** did not have any absorption between 450 and 650 nm, an absorbance increase was observed at λ= 562 nm after adding varying amounts of (0–100 μM) Hg^{2+} ions to compound **3** solution (10 μM). A linear relationship was observed in the absorbance increase at 0–35 μM concentration range (A=0.0114 × [Hg^{2+}] μM - 0.0424; R^2=0.9964) (Fig. S13a, Supplementary material). Similarly, fluorescence titration of compound **3** was studied with Hg^{2+} ions. As indicated in Fig. 5 (b), while compound **3** did not have a significant fluorescence within the range of 535–700 nm, a significant increase was observed in fluorescence intensity at 580 nm by adding Hg^{2+} ions (0–100 μM) to compound **3** solution. Similar to UV–vis, a linear response was obtained in fluorescence titration for Hg^{2+} ions in 0–35 μM range (Fig. S13b, Supplementary material). The linear equation can be expressed by I=25.555 × [Hg^{2+}] μM - 98.592 (R^2=0.9959). The detection limit of compound **3** for Hg^{2+} ions from LOD=3δ/k equation [where δ is the standard deviation of the blank solution (compound **3** solution) and k is the slope of calibration plot] of fluorescence intensity line to concentration was found to be 3.76×10^{-9} M [31–33]. This value is lower than the upper limit determined by EPA for potable water [10]. There is a large number of research on rhodamine derivative sensors with 1:1 and 1:2 stoichiometry for Hg^{2+} ions. In addition, Jana et al. reported a rhodamine

Fig. 6 Reversible fluorescence response of **3** for Hg^{2+} ions

derivative sensor with 1:3 stoichiometry. However in that study, no detection limit was reported for Hg^{2+} ions. Furthermore, the detection limit for sensors with 1:2 stoichiometry developed by Bhalla et al. was reported as 5×10^{-9} M [34]. Park et al. [35], Mandal et al. [36] and Wang et al. [37] reported detection limits for the sensors they developed as 2.31×10^{-8}, 1×10^{-9} and 1.74×10^{-6} M respectively. In addition to detection limit, the usability of the sensors in actual water samples is also important. Water samples containing 5 and 10 μM Hg^{2+} were prepared using tap water. Each measurement was performed with three replications. As indicated in Table 1, sensor **3** has high recovery and accuracy in Hg^{2+} water samples.

Reusability of a sensor is also a very important parameter. To this end, whether compound **3** is a reusable sensor for Hg^{2+} ions was tested. This test was performed by adding Na_2S solution to compound **3-Hg^{2+}** complex ion solution [19]. It was observed that the solution lost its pink color after the addition. As indicated in Fig. 6, fluorescence emission at 580 nm was almost lost in the recorded fluorescence spectrum of the mixture. On the other hand, after the addition of Hg^{2+} ions back to the solution, fluorescence emission intensity of the solution was almost the same as the initial value. Thus, it can be stated that compound **3** is a reusable sensor for Hg^{2+} ions.

Ease of use of a sensor in practical application is an important feature. Test strips can be considered a practical way to understand whether an ion is present in a water sample. The presence of an ion can be instantly determined with this method. In recent years, researchers have been focused to develop test strips in design of selective sensors due to their practical use. In some studies, a solution of a synthesized sensor was absorbed by different papers; they were dried and the obtained material was used as a test strip [33, 38]. However, in these studies lack a detailed investigation of reusability of the developed these test strips. In this study, we took the phenomenon of test strip development one step further. We developed a reusable test strip that does not deform even under constant interaction with water. We immobilized compound **3** into a cross-linked hydrogel matrix that does not dissolve in aqueous media but can retain water due to hydrophilic groups [39]. The fact that compound **3** did not dissolve in water and thus remains in the hydrogel network structure were its biggest advantage. The hydrogels that were cleaned with deionized water after synthesis were added to Hg^{2+} solutions with a concentration of 0.5, 5 and 50 ppm (50 mL). As a result of interaction of sensor with mercury ions, the color of the hydrogels changed from white to pink. As indicated in Fig. 7, the lowest concentration that this sensor-immobilized hydrogel system can be used for is 0.05 ppm. Hg^{2+} ions and the gels that changed color were immersed in 0.1 M 50 mL sodium sulfur to analyze reusability of this prepared test strip. It was observed that the color of the

hydrogels changed back to white after this procedure. The hydrogels were then washed with deionized water for 24 h to remove excess sodium sulfur. Thus a reusable test strip that behaves as a calorimetric sensor for Hg^{2+} ions was developed.

Conclusion

In conclusion, a new selective sensor with colorimetric and fluorescent properties for Hg^{2+} ions was synthesized in this study. Its detection limit was determined as 0.75 ppb. The reusable test strip was developed for the detection of Hg^{2+} ions in water samples thanks to immobilization of compound **3** in a hydrogel matrix. In addition, it was determined that compound **3** and hydrogel-immobilized compound **3** were reusable sensors for Hg^{2+} ions.

Electronic supplementary material

The online version of this article contains supplementary material, which is available to authorized users.

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