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Dual sensing of Hg^{2+} in water by a new naked-eye rhodamine base optical probe

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ABSTRACT

Design and synthesis of a novel fluorophore for Hg^{2+} ions detection, based on a new rhodamine–anthracene derivative is reported. The fluorophore exhibited highly selective and sensitive reversible turn-on fluorescent response toward Hg^{2+} ions, based on the ring-opening mechanism of the rhodamine spirolactam. The noticeable change from colorless to pink upon the addition of Hg^{2+} ions could make it a suitable "naked eye" colorimetric sensor for Hg^{2+} ions. The selective sensing of Hg^{2+} ions in the presence of other metal ions and reversible nature of OFF–ON switching of sensor by Hg^{2+} ions was studied. It provides an easy sensing of Hg^{2+} ions in environmental and biological applications.

Keywords: Optical sensing; Environment; Toxic metals; Rhodamine

1. Introduction

Mercury is one of the most toxic metals in the environment, and absorption of this metal ion in the human body causes the destruction of the human brain and central nervous system, kidneys and endocrine system. Due to the extreme toxicity of mercury to human health, development of the quick, easy, and efficient detection and determination of mercury ion have received an immense interest [1,2]. Traditional quantitative approaches, such as atomic absorption spectrometry and inductively coupled plasma mass spectrometry, have been published for mercury analysis [3–5]. These methods are efficient for Hg^{2+} detection; however, they are time-consuming with sophisticated instrumentation and require highly trained personnel care.

Design and development of colorimetric or fluorescent chemosensors for detection of environmental and biological metal cations are currently of great importance. Thsese chemosensors allow non-destructive determination of metal cations by a simple absorbance and fluorescence enhancement (turn-on) or quenching (turn-off) response.

As well known, the rhodamine with spirolactam structure is non-fluorescent, whereas ring opening of the spirolactam gives rise to a high fluorescence emission. This property provides an ideal model to construct OFF–ON fluorescent switch sensors [6–10]. On the other hand, rhodamine with spirolactam structure has an emission wavelength above 550 nm after ring opening by metal ions. Therefore, it can used as a reporting probe for the analyte, in which the influence of the background radiation (below 500 nm) is removed. Rhodamine derivatives are excellent fluorophores and chromophores. They have attracted considerable interest due to their unique photophysical properties. Among rhodamine derivatives, rhodamine-based spirolactams are considered to offer molecular scaffolds and often are employed in OFF–ON fluorescent or colorimetric chemosensors [11,12]. Thus the design of new rhodamine-based chemosensor is considerable.

Design and synthesis of functional fluorescence and colorimetric dyes as molecular and ion probe for chemosensing applications are undergoing by our research group. We previously reported the fluorescence and colorimetric

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chemosensors, based on functionalized 1,8-naphthalimides and merocyanine derivatives for detection of biologically and environmentally important ions such as fluoride, and pH molecular recognition probes for ions and drugs [13–16].

Herein, we synthesized a new rhodamine B derivative (I) which was utilized as selective colorimetric and fluorescent sensor for mercury ions. Among the various metal ions, the sensor of I exhibits remarkably enhanced absorbance intensity and color change from colorless to pink in acetonitrile (AN). It shows significant OFF–ON fluorescence change. The sensor I was tested on real samples contained Hg^{2+} and was able to successfully detect the Hg^{2+}.

2. Experimental

2.1. Materials and methods

All nitrate salts of Al^{3+}, Fe^{2+}, Cr^{2+}, Pb^{2+}, Co^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+} were purchased from Merck company. Rhodamine B and Anthracene-9-carbaldehyde were purchased from Sigma-Aldrich and used as received. All solvents were of analytical grade and purchased from Merck.

2.2. Instrumentation

Spectrophotometric measurements were recorded using a Shimadzu Multi spec-1501 Photo Diode Array Spectrophotometer. Fluorescence measurements were carried out on a PerkinElmer LS 50B luminescence spectrometer. FTIR spectrums were recorded on a Spectrum One spectrometer using KBr pellets. NMR spectrums were measured with Bruker DRX Avance NMR spectrophotometer on a 300 MHz for proton and 125 MHz for carbon in DMSO.

2.3. Synthesis

2.3.1. Synthesis of (rhodamine-B) lactam–ethylene diamine (1)

The intermediate, compound of 1, was synthesized from rhodamine-B (RB), according to the reported method in literature as follows [17]. Rhodamine-B (2 mmol) was dissolved in 20 mL of ethanol, followed by addition of ethylene diamine (1 mL, 15 mmol). The reaction mixture was refluxed for 24 h. The reaction was cooled to room temperature and the precipitate was collected and washed with ethanol for three times. The crude product was purified by recrystallization from ethanol to give (rhodamine-B) lactam–ethylene diamine (1) in 88.3% yield (Fig. 1): mp 215°C; ^{1}H NMR (300 MHz, DMSO, δ (ppm)): δ 7.75 (d, 1H), 7.47 (m, 2H), 6.99 (m, 1H), 6.37 (d, 6H), 3.27 (m, 10H), 2.24 (m, 2H), 1.07 (t, 12H); 13C NMR (DMSO): δ 168.6, 153.6, 153.3, 148.7, 132.5, 131.3, 128.9, 128.2, 123.8, 122.8, 108.2, 105.6, 97.7, 64.9, 44.4, 43.9, 40.8, 12.7.

2.3.2. Synthesis of chemosensor (I)

A portion of (rhodamine-B) lactam–ethylenediamine (1) (1.0 mmol) and anthracene-9-carbaldehyde (1.0 mmol) was refluxed for 6 h under N_2 atmosphere in ethanol (30 mL) to obtain an intermediate compound of 2. The solid was filtrated and recrystallized from ethanol. Chemosensor of I was obtained by mild reduction of compound 2 with sodium borohydride (Fig. 1). Then it was purified by recrystallization from ethanol as light pink powder (90% yield). ^{1}H NMR (300 MHz, DMSO, δ (ppm)): δ 8.47 (s, 1H), 7.74 (m, 1H), 7.47 (m, 3H), 7.00 (m, 1H), 6.31 (d,6H), 3.50 (s, 2H), 3.31 (d, 8H), 2.98 (t, 3H), 2.18 (t, 2H), 1.45 (m, 3H), 1.04 (m, 12H), 0.86 (m, 4H) $[M]^+$. Elemental analysis: Calc. for $C_{45}H_{46}N_4O_2$: C, 80.12; H, 6.82; N, 8.31. Found: C, 80.19; H, 6.90, N, 8.23%.

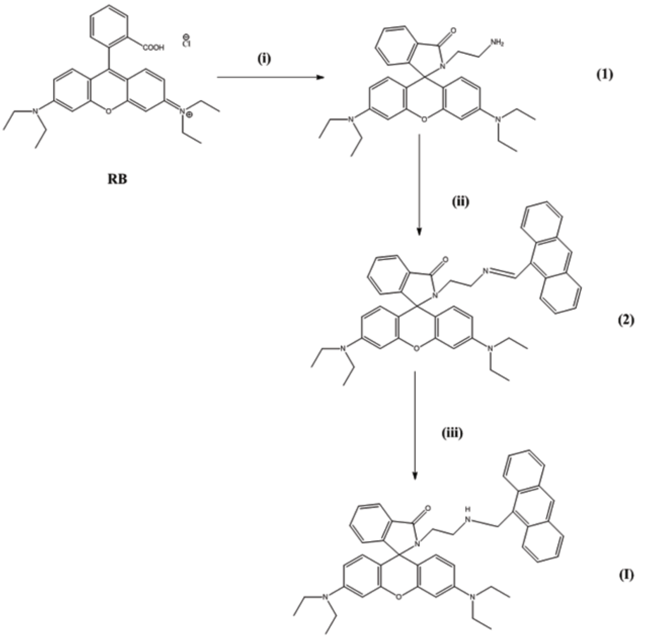

Fig. 1. Synthesis of Compound I. (i) Anthracene-9-carbaldehyde/EtOH, (ii) sodium borohydride.

2.4. UV and fluorescence spectra measurements

Colorimetric sensing of metal ions was carried out using 5 mL of compound (I) (1×10^{-5} M) in acetonitrile and acetonitrile–water in the presence of 10 equiv. of different metal ions (1×10^{-4} M) at room temperature. Spectroscopic changes were recorded using a UV–Vis scanning spectrophotometer. The fluorescence spectra were recorded using PerkinElmer luminescence spectrophotometer at 298 K using a 1 cm path length quartz cuvette with a volume of 4 mL.

3. Results and discussion

3.1. Synthesis

Compound I was synthesized in three-steps, rhodamine-B-ethylenediamine (1) was prepared following the literature procedure [17] from the reaction between rhodamine B and ethylenediamine. Compound (2) was obtained from the reaction of the anthracene-9-carbaldehyde and compound (2). Finally, chemosensor (I) was prepared by reducing the carbon-nitrogen double bond of compound (2), so the nitrogen lone pair electrons become available for better binding to metal ions.

3.2. Colorimetric and selectivity studies

The chemosensor of I displayed remarkable color change via rhodamine lactam ring opening in presence of Hg^{2+}.

The addition of 10 equiv. of various metal ions (Al^{3+}, Fe^{2+}, Cr^{2+}, Pb^{2+}, Co^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}) to a solution of I (1×10^{-5} M) in AN showed a selective color change from colorless toward pink only in case of Hg^{2+} (Fig. 2). This selective color change of I in the case of Hg^{2+} can be used for the "naked-eye" detection of mercury ions.

The UV–Vis absorption spectrum of I in acidic CH_3CN showed a sharp new peak at 550 nm due to ring opening of spirolactam when mercury ions were added (Fig. 3). As previously reported, rhodamine spirolactam ring opening is catalyzed in the acidic condition [18,19]. To study the effect of pH on the response of chromophore, the absorption intensity of chromophore was monitored in the presence and absence of mercury ions in different pH values (Fig. 4). As can be seen, rhodamine spirolactam ring opening spontaneity occurs in the acidic media (pH < 4) due to the strong protonation. However at pH > 4, no significant ring opening occurs and it has a weak absorption intensity. In the presence of mercury ions, absorption of I was an obvious OFF–ON change and absorption increased remarkably in pH range of 2–6, while for pH > 6 the absorption intensity decreased. Thus, chemosensor of I can detect Hg^{2+} ions at pH range of 4–6 because in this region Hg^{2+} ions induce a remarkable fluorescence OFF–ON, whereas in the absence of Hg^{2+} ions I does not show such change. So all measurements were performed in AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7.

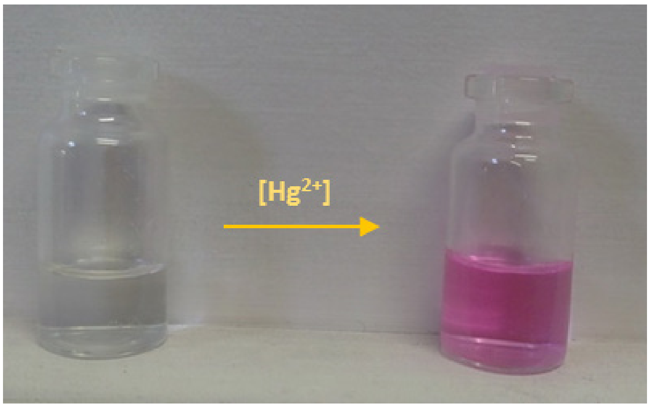

Fig. 2. Photograph of I (100 mM) (left) and in the presence of mercury ions (right) under visible light.

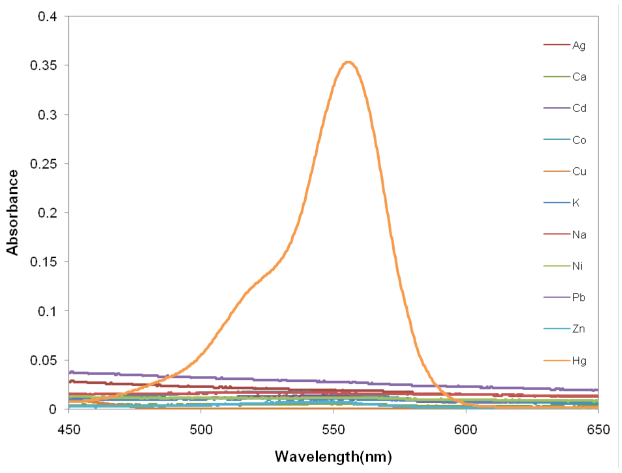

Fig. 3. UV–Vis spectra of I (5×10^{-4} M) in AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7 upon addition of 10 equiv. of various metal ions.

To study the selectivity of I, absorption study was followed by adding 100 equiv. of various metal ions to the solution of I (5×10^{-4} M) in the absence and presence of 10 equiv. of mercury ions. Fig. 5 shows the selective sensing of I for Hg^{2+} over the other metal ions. Adding 100 equiv. of various metal ions to the solution of I did not show a significant peak at 550 nm except for Hg^{2+}. This suggests that other than Hg^{2+}, the remaining metal ions have no effect on I. Furthermore, addition of only 10 equiv. of Hg^{2+} to the solution of I containing 100 equiv. of other metal ions resulted in appearance of a sharp peak at 550 nm, which confirms the sensitivity of the I to Hg^{2+}.

3.3. Fluorescence and selectivity studies

The fluorescence intensity of I (5×10^{-7} M) in AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7 in the presence of

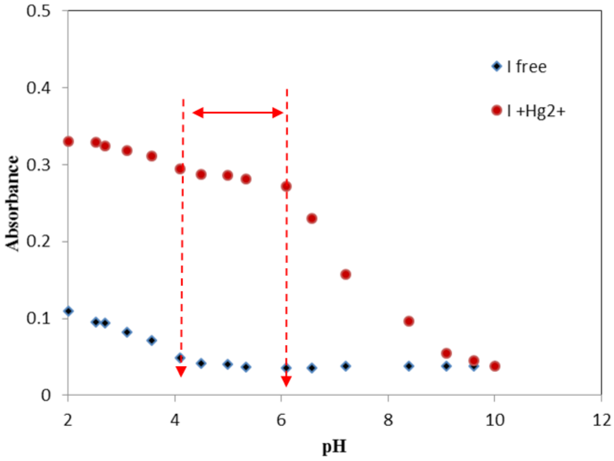

Fig. 4. Effect of pH on the absorption spectra of (I) in the presence (●) and absence (◆) of Hg^{2+} (λ_{max} = 550 nm).

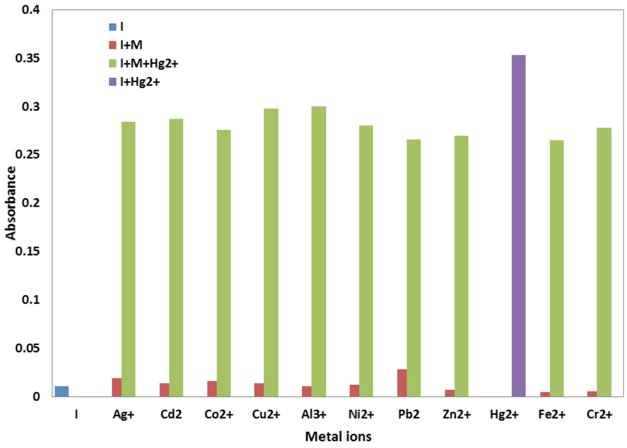

Fig. 5. Absorption response of sensor I solution (AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7) in the presence of 100 equiv. of various metal ions and 10 equiv. of Hg^{2+} at 550 nm.

various metal ions was studied by monitoring the ring opening rhodamine emission due to the excitation at 420 nm. Prepared dye (I) in the presence of mercury ions shows clear emission enhancement.

Fig. 7 shows the mechanism of color change and emission enhancement of I by Hg^{2+}.

The emission spectrum of I (Fig. 8) shows a weak broad peak at 492 nm, suggests that I exists in a ringclosed non-fluorescent spirolactam conformation. Upon adding 10 equiv. of various metal ions, only in the case of Hg^{2+}, a strong peak at 492 nm is observed. This indicates that only Hg^{2+} induces the ring opening of the non-fluorescent spirolactam structure (ring closed) into the fluorescent structure (ring open) of rhodamine. The enhancement factor at 492 nm for I in the presence of Hg^{2+} is 9.6 times more than the emission intensity of I.

Selective sensing of Hg^{2+} over the other metal ions was studied for higher concentrations of metal ions (50 equiv.) to the solution of I (5×10^{-7} M). In the presence of large excess (100 equiv.) of above ions, no significant peak at 492 nm was observed in the emission spectrum, except for Hg^{2+} (Fig. 9). Addition of 10 equiv. of Hg^{2+} to the solution of I containing 100 equiv. of other metal ions also induced the appearance of a peak at 492 nm. This suggests that I performs selective ring opening of spirolactam in the presence of Hg^{2+} even in solutions with large excess of other metal ions.

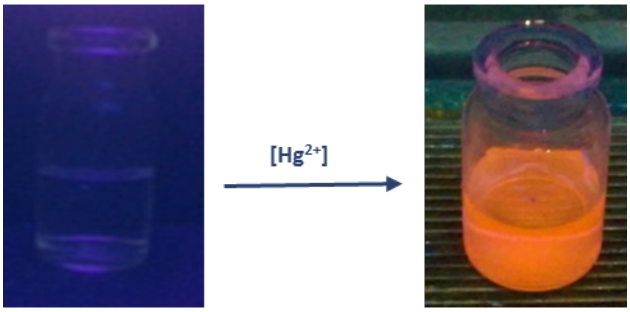

Fig. 6. Photograph of I (100 mM) (left) and in the presence of mercury ions (right) under UV light.

The high sensitivity of I for Hg^{2+} ions was used to generate a calibration curve for quantitative measurement of Hg^{2+} ions in aqueous solution. A fluorescence titration experiment of I (5×10^{-7} M) with Hg^{2+} (5×10^{-7} M) is shown in Fig. 10. By adding Hg^{2+} ions with different concentrations ranging from 0 to 10 μM, the fluorescence intensity of I at 492 nm was recorded to generate a calibration curve (inset (a) of Fig. 10). As shown in Fig. 10, a perfect linearity ($R = 0.99$) was found between fluorescence intensity of I and Hg^{2+} concentration (from 2.1 to 2.6 μM of Hg^{2+}), indicating a linear detection range for Hg^{2+} with a detection limit of 1.8×10^{-7} M. Job's plot is shown in inset (b) of Fig. 10. It clearly confirms a 1:1 interaction between I and mercury ion.

3.4. Reversibility

The reversibility of the chemosensor is an important aspect of it for practical applications. Reversibility of chemosensor was studied by introduction of the EDTA into the system containing I (10 μM) and Hg^{2+} (200 μM). The results showed that the introduction of EDTA (1 equiv. to Hg^{2+}) could immediately restore the absorbance of I. When Hg^{2+} has been added to the system again, the absorbance intensity of I was enhanced. This process was repeated at least three times (Fig. 11). This regeneration indicated that the sensor I could be reused with proper treatment.

3.5. Real sample analysis

Hg^{2+} and other metal ions were added to the synthesized drinking water and tap water obtained from the Tehran city and analyzed by the proposed chemosensor under optimized conditions (Table 1). Results show that I can measure the concentration of Hg^{2+} in water samples with good recovery

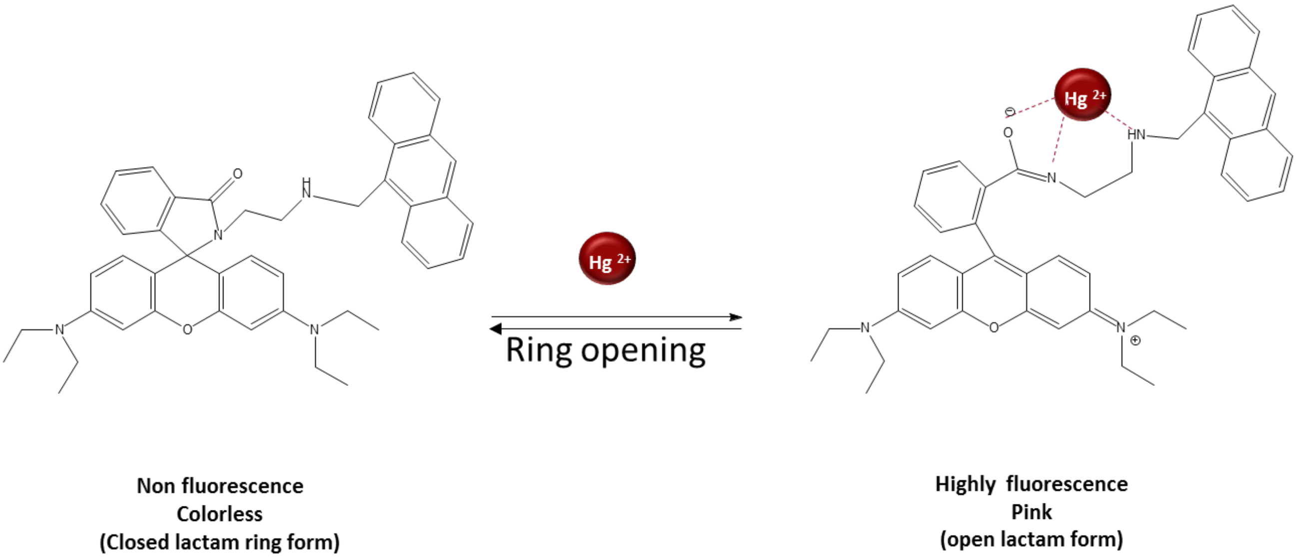

Fig. 7. Mechanism of color change and emission enhancement of I by metal ion.

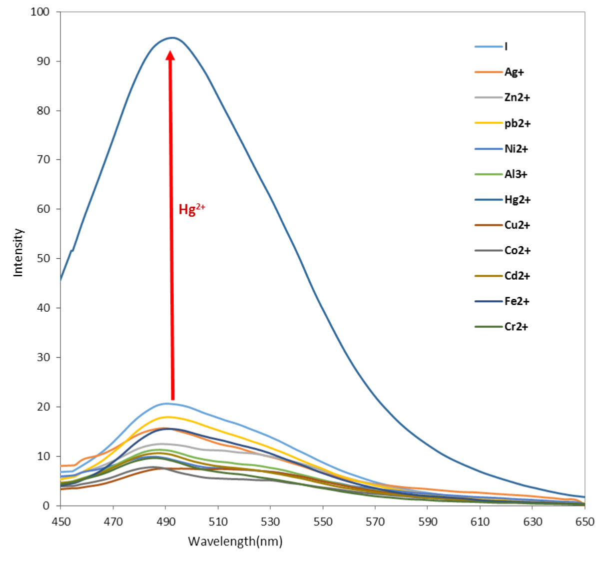

Fig. 8. Emission spectrum of I (5×10^{-7} M) with addition of 10 equiv. of various metal ions in AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7 monitored by exciting at 420 nm.

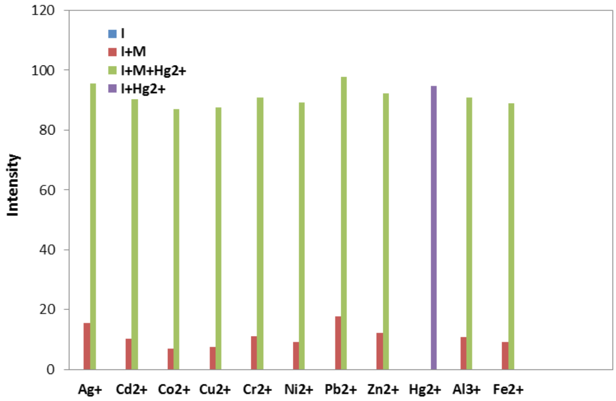

Fig. 9. Emission spectrum of I (5×10^{-7} M) in (AN:H_2O 4;1, acid, acetic/acetate buffer pH = 4.7) in the presence of 100 equiv. of various metal ions and 10 equiv. of Hg^{2+}.

and RSD results, suggesting the potential application of real sample analysis by I.

4. Conclusions

A new rhodamine-based derivative I was synthesized and characterized. The sensor I exhibits reversible and highly selective and sensitive recognition of Hg^{2+} over other metal ions. Upon addition of Hg^{2+}, sensor I shows remarkably enhanced absorbance intensity and color change from colorless to pink in acetonitrile-aqueous buffer solution or pure acetonitrile. It also shows significant OFF–ON fluorescence by color change from colorless to orange. Under the optimized condition of 5×10^{-7} M I in buffer solution (AN:H_2O 1:9, v/v, pH 4.7), the quantification of Hg^{2+} by I showed the detection limit of 1.8×10^{-7} M. The excellent selectivity and sensitivity of I to Hg^{2+} make it a promising sensor for mercury detection in environmental and biological samples.

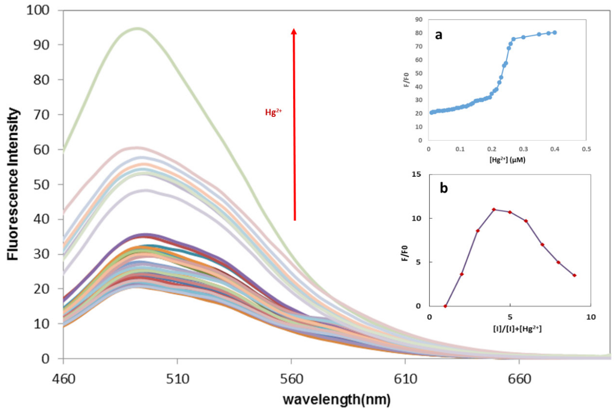

Fig. 10. Variation of the fluorescence spectra of I (5×10^{-7} M) (AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7) vs. Hg^{2+} ions. Inset: (a) emission at 492 nm vs. Hg^{2+} ions added. (b) Job's plot.

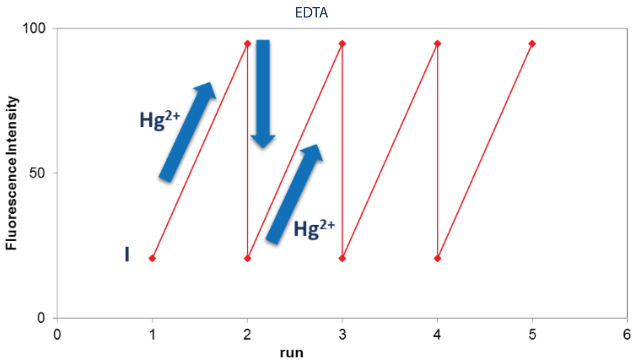

Fig. 11. Reversibility of emission response of I against the addition of alternative equivalents of Hg^{2+} and EDTA.

Table 1
Determination of Hg^{2+} in water samples

Samples	Tap water	Synthesized water
Hg^{2+} added (μM)	0.2	0.2
Hg^{2+} found (μM)	0.25	0.19
Recovery (%)	125	95
RSD[a] (%)		1.6
		1.4

[a] *n* = 3.

[b] Water samples prepared as AN:H_2O 4:1, acid, acetic/acetate buffer pH = 4.7.

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References

[1] Y. Wang, Y. Li, G. Liu, D. Wang, G. Jiang, Y. Cai, Elemental mercury in natural waters: occurrence and determination of particulate Hg(0), Environ. Sci. Technol., 49 (2015) 9742–9749.

[2] K. Leopold, M. Foulkes, P. Worsfold, Methods for the determination and speciation of mercury in natural waters—a review, Anal. Chim. Acta, 663 (2010) 127–138.

[3] L. Ling, Y. Zhao, J. Du, D. Xiao, An optical sensor for mercuric ion based on immobilization of Rhodamine B derivative in PVC membrane, Talanta, 91 (2012) 65–71.

[4] Y. Yu, L.R. Lin, K.B. Yang, X. Zhong, R.B. Huang, L.S. Zheng, p-Dimethylaminobenzaldehyde thiosemicarbazone: a simple novel selective and sensitive fluorescent sensor for mercury(II) in aqueous solution, Talanta, 69 (2006) 103–106.

[5] J. Chamier, J. Leaner, A.M. Crouch, Photoelectrochemical determination of inorganic mercury in aqueous solutions, Anal. Chim. Acta, 661 (2010) 91–96.

[6] F. Yan, D. Kong, Y. Luo, Q. Ye, J. He, X. Guo, L. Chen, Carbon dots serve as an effective probe for the quantitative determination and for intracellular imaging of mercury(II), Microchim. Acta, 183 (2016) 1611–1618.

[7] Q. Ye, F. Yan, Y. Luo, Y. Wang, X. Zhou, L. Chen, Formation of N, S-codoped fluorescent carbon dots from biomass and their application for the selective detection of mercury and iron ion, Spectrochim. Acta A, 173 (2017) 854–862.

[8] N. Horzum, D. Mete, E. Karakuş, M. Üçüncü, M. Emrullahoğlu, M.M. Demir, ChemistrySelect, 1 (2016) 896–900.

[9] J. Zhang, Y. Zhou, W. Hu, L. Zhang, Q. Huang, T. Ma, Highly selective fluorescence enhancement chemosensor for Hg^{2+} based on rhodamine and its application in living cells and aqueous media, Sens. Actuators B, 183 (2013) 290–296.

[10] H. Xu, K. Zhang, Q. Liu, Y. Liu, M. Xie, Visual and fluorescent detection of mercury ions by using a dually emissive ratiometric nanohybrid containing carbon dots and CdTe quantum dots, Microchim. Acta, 184 (2017) 1199–1206.

[11] R. Zhang, F. Yan, Y. Huang, D. Kong, Q. Ye, J. Xu, L. Chen, Rhodamine-based ratiometric fluorescent probes based on excitation energy transfer mechanisms: construction and applications in ratiometric sensing, RSC Adv., 56 (2016) 50732–50760.

[12] J. Kuchlyan, S. Basak, D. Dutta, A.K. Das, D. Mal, N. Sarkar, A new rhodamine derived fluorescent sensor: detection of Hg^{2+} at cellular level, Chem. Phys. Lett., 673 (2017) 84–88.

[13] S. Rouhani, S. Haghgoo, A novel fluorescence nanosensor based on 1,8-naphthalimide-thiophene doped silica nanoparticles, and its application to the determination of methamphetamine, Sens. Actuators B, 209 (2015) 957–965.

[14] S. Rouhani, F. Nahavandifard, Molecular imprinting-based fluorescent optosensor using a polymerizable 1,8-naphthalimide dye as a florescence functional monomer, Sens. Actuators B, 197 (2014) 185–192.

[15] P. Alaei, S. Rouhani, K. Gharanjig, A dual colorimetric and fluorometric anion sensor based on polymerizable 1, 8-naphthalimide dye, Prog. Color Colorants Coat., 6 (2013) 87–96.

[16] S. Rouhani, S. Salimi, K. Haghbeen, Development of optical pH sensors based on derivatives of hydroxyazobenzene, and the extended linear dynamic range using mixture of dyes, Dyes Pigm., 77 (2008) 363–368.

[17] B.N. Ahamed, P. Ghosh, An integrated system of pyrene and rhodamine-6G for selective colorimetric and fluorometric sensing of mercury(II), Inorg. Chim. Acta, 372 (2011) 100–107.

[18] L. Tang, F. Li, M. Liu, R. Nandhakumar, Single sensor for two metal ions: colorimetric recognition of Cu^{2+} and fluorescent recognition of Hg^{2+}, Spectrochim. Acta A, 78 (2011) 1168–1172.

[19] J. Zhang, L. Zhang, Y. Zhou, T. Ma, J. Niu, A highly selective fluorescent probe for the detection of palladium(II) ion in cells and aqueous media, Microchim. Acta, 180 (2013) 211–217.