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Fluorescent difference between two rhodamine-PAHs polystyrene solidphase sensors for Hg(II) detection based on crystal structure and density functional theory calculation



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ABSTRACT

Two novel rhodamine-polystyrene solid-phase fluorescence sensors PS-RB-2 and PS-R6G-2 with pyrene or naphthalene as fluorophore were synthesized for Hg(II) detection. Their structures were characterized by Fourier transform infrared (FTIR) spectra and scanning electron micrographs (SEM). Sensor PS-RB-2 displayed higher selectivity and sensitivity to Hg(II), with a lower detection limit of 0.065 µM. A detection mechanism involving the Hg(II) chelation-induced spirocycle open of rhodamine was proposed and discussed from theoretic level based on crystal structures and density functional theory (DFT) calculations. Sensor PS-RB-2 with recyclable and environment-friendly performance was successfully employed to fluorescent detection of Hg(II) in real water and fish samples, indicating its good potential in practical application. Its solid phase extraction columns were developed for rapid detection of Hg(II) by observing the color change with the naked eyes.

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1. Introduction

Mercury and its ion, which have high toxicity to mammalian cells [1-4], are non-biodegradable persistent contaminant that can be retained in ecosystem for a long time [5-7]. Recently, more and more biofluorescence sensors [8,9], nanoparticles [10] and chemical fluorescent probes [11-14] have been developed for Hg(II) detection because of their simple operation, high sensitivity and selectivity, as well as short detection time. However, they were not appropriate for enrichment, separation and removal of target species or recyclable screening application. Thus, the design and synthesis of the economical and environment-friendly solid-phase fluorescence sensors has become a hot research topic.

Generally, the structure of these sensors is composed of two parts: (*i*) solid-phase carriers, (*ii*) small-molecule fluorescent probe containing recognition group, linker arm and luminescent group. Rhodamine is such a luminophore as an off-on fluorescent probe with high fluorescence quantum efficiency and large absorption coefficients [15–17]. When there is no Hg(II), rhodamine exists in a spiro cycle with nonfluorescence. With the addition of Hg(II), the cycle opens along with apparent color change and strong fluorescence emission. With

Corresponding author. E-mail address: xxh@njtech.edu.cn (X. Xiong). this property, rhodamine is an ideal template for Hg(II) detection [18-20].

In addition, polystyrene microspheres as carriers have been widely used in chromatographic separation, enzyme immobilization and biosensors due to their large surface area, stable property, strong absorption and surface reaction ability [21-23]. In our previous work, a rhodamine-based polystyrene fluorescent sensor I with a low detection limit of 0.439 µM for Hg(II) determination was reported (Scheme 1b) [24]. It exhibited high sensitivity and selectivity, synchronously obtained good reproducibility and stability, and could be used for detecting Hg(II) in real samples. However, just like most rhodamine-based sensors, there is competitive selectivity between Hg(II) and other metal ions such as Cu(II), Pb(II) and Cd(II) [25,26], which affects its application to some extent.

Pyrene and naphthalene as two excellent representatives of polycyclic aromatic hydrocarbons (PAHs) possess strong and stable photochemical properties. As the most effective fluorophores to form folding and π -stacking conformation, they are often used in molecular sensing. In particular, the fluorescence emission of pyrene monomer and excimer can produce a larger stokes shift (> 60 nm), which is better than any other fluorophores [27,28]. Therefore, the ratio-type fluorescent probes can be designed by controlling the conversion of monomer and excimer to realize the recognition of metal ions.

In continuation of our study on novel polystyrene solid-phase fluorescent sensors with lower detection limit, two novel rhodamine-



Scheme 1. Design strategy and synthetic routes of rhodamine-PAHs polystyrene fluorescent sensors.

PAHs sensors PS-RB-2 and PS-R6G-2 with chloromethyl polystyrene microspheres as carriers and different PAHs pyrene or naphthalene as fluorophore were designed and synthesized for Hg(II) detection (Scheme 1a). Their structure-fluorescence relationships were investigated and compared with our previously reported sensor I (Scheme 1b). The detection mechanism was discussed from theoretic level based on crystal structures and DFT calculations. Sensors PS-RB-2 for the determination of Hg(II) in real samples were investigated. Its solid phase extraction columns were also developed for rapid detection of Hg(II) by observing obvious color change.

2. Experimental

Nuclear Magnetic Resonance spectra (NMR) were recorded on a Bruker AV-400 spectrometer in CDCl₃ solvent using TMS as an internal standard. Infrared Ray (IR) spectra were recorded in KBr disk using a Thermo Nicolet 380 FT-IR spectrophotometer. Mass spectra were performed on a Thermo TSQ Quantum Access MAX mass spectrometer. Elemental analyses were performed on a Flash EA-1112 elemental analyzer. Fluorescence spectra were performed on a Shimadzu RF-5301PC spectrofluorometer using 1-cm-path length cuvettes at r.t. The slit widths were set to 5 nm. Unless otherwise noted, all reagents were obtained from Nanjing WANQING chemical Glass ware & Instrument Co., Ltd. The cations Ca(II), Na(I), K(I), Ni(II), Pb(II), Mg(II), Zn (II), Cu(II), Mn(II), Ag(I), Cd(II), Fe(II), Cr(III) and Ba(II) were obtained by using chloride or nitrate.

2.1. Synthesis of compound RB-1

Compound **RB-1** was synthesized according to the literature [29]. To a solution of rhodamine B (0.48 g, 1 mmol) in ethanol (50 mL) was added hydrazine hydrate (5 g, 80%). The mixture was refluxed for 24 h. The solvent was removed under reduced pressure, then water

(100 mL) was added. The mixture was extracted with ethyl acetate (100 mL × 3). The organic layer was combined, dried by Na₂SO₄ and evaporated. The crude product was recrystallized from ethanol to afford pink solid **RB-1** (0.373 g, yield 78%). ¹H NMR (CDCl₃, 400 MHz) δ 7.93 (s, 1H, ArH), 7.45 (m, 2H, ArH), 7.11 (m, 1H, ArH), 6.43 (t, *J* = 2.4 Hz, 4H, ArH), 6.28 (t, *J* = 2.4 Hz, 2H, ArH), 3.61 (s, 2H, NH₂), 3.35 (q, *J* = 7.0 Hz, 8H, CH₂), 1.17 (t, *J* = 7.0 Hz, 12H, CH₃). ESI-MS: Calcd for C₂₈H₃₂N₄O₂ [M + H]⁺: 457.25; found: 457.24. Anal. Calcd. for C₂₈H₃₂N₄O₂: C 73.66, H 7.06, N 12.27; found C 73.45, H 7.03, N 12.32.

2.2. Synthesis of compound R6G-1

Compound **R6G-1** was prepared according to the reported method [30]. Rhodamine 6G (2.4 g, 5 mmol) and NaOH (0.4 g, 10 mmol) were dissolved in ethanol (50 mL) to reflux for 6 h. Then hydrazine hydrate (5 g, 80%) was added and the mixture was refluxed for another 24 h (the color changed from purple to yellow). After cooling to r.t., the precipitation was filtered and dried to give pink solid **R6G-1** (1.8 g, yield 75%). ¹H NMR (CDCl₃, 400 MHz) δ 7.93 (q, $J_1 = 5.4$ Hz, $J_2 = 3.3$ Hz, 1H, ArH), 7.43 (m, 2H, ArH), 7.05 (q, $J_1 = 5.4$ Hz, $J_2 = 3.3$ Hz, 1H, ArH), 6.38 (s, 2H, ArH), 6.25 (s, 2H, ArH), 3.62–3.47 (b, 4H, NH₂, NH), 3.22 (q, J = 7.0 Hz, 4H, CH₂), 1.86 (s, 6H, CH₃), 1.28 (t, J = 7.0 Hz, 6H, CH₃). ESI-MS: Calcd for C₂₆H₂₈N₄O₂: C 72.87, H 6.59, N 13.07; found C 72.63, H 6.62, N 13.12.

2.3. Synthesis of compounds RB-2 and R6G-2

Compound **RB-1** or **R6G-1** (1 mmol) and 1-pyrenecarboxaldehyde or 1-naphthaldehyde (1 mmol) were dissolved in ethanol (100 mL) under N₂ protection, then the mixture was refluxed for 24 h. The crude product was filtered and purified *via* flash column



Fig. 1. Crystal structures of RB-2 and R6G-2.

chromatography on silica gel (AcOEt/petroleum ether, 1: 4v/v) to afford yellow solid **RB-2** (0.48 g, yield 72%) or **R6G-2** (0.43 g, yield 75%).

(*E*)-3',6'-Bis(diethylamino)-2-((pyren-1-ylmethylene)amino) spiro[isoindoline-1,9'-xanthen]-3-one (RB-2). ¹HNMR (CDCl₃, 400 MHz) δ 9.60 (s, 1H, CH), 8.52 (d, J = 8.0 Hz, 1H, ArH), 8.11 (m, 9H, ArH), 7.53 (m, 2H, ArH), 7.20 (d, J = 6.8 Hz, 1H, ArH), 6.64 (d, J = 8.8 Hz, 2H, ArH), 6.55 (d, J = 2.4 Hz, 2H, ArH), 6.28 (q, J = 2.4 Hz, 2H, ArH), 3.32 (q, J = 7.0 Hz, 8H, CH₂), 1.14 (t, 12H, J = 7.0 Hz, CH₃). ESI-MS: Calcd for C₄₅H₄₀N₄O₂: C 80.81, H 6.03, N 8.38; found C 80.55, H 6.06, N 8.43.

(*E*)-3',6'-Bis(ethylamino)-2',7'-dimethyl-2-((naphthalen-1-ylmethylene)amino)spiro[isoindoline-1,9'-xanthen]-3-one (R6G-2). ¹HNMR (CDCl₃, 400 MHz) δ 9.36 (s, 1H, CH), 8.03 (q, *J* = 1.6 Hz, 2H, ArH), 7.88 (d, *J* = 6.8 Hz, 1H, ArH), 7.77 (t, *J* = 1.6 Hz, 2H, ArH), 7.50 (m, 2H, ArH), 7.40 (m, 3H, ArH), 7.17 (t, *J* = 1.6 Hz, 1H, ArH), 6.54 (s, 2H, ArH), 6.26 (s, 2H, ArH), 3.31 (q, *J* = 7.2 Hz, 4H, CH₂), 1.82 (s, 6H, CH₃), 1.25 (t, *J* = 7.2 Hz, 6H, CH₃). ESI-MS: Calcd for C₃₇H₃₄N₄O₂: C 78.42, H 6.05, N 9.89; found C 78.66, H 6.02, N 9.85.

2.4. Preparation of sensors PS-RB-2 and PS-R6G-2

Chloromethyl polystyrene microspheres **PS-CI** (0.2 g) were swollen with dry DMSO (30 mL) overnight, then compound **RB-2** (0.67 g, 1 mmol) or **R6G-2** (0.57 g, 1 mmol) and triethylamine (0.1 g, 1 mmol) were added. The reaction mixture was stirred at 60 °C for 24 h, then filtration. The crude microspheres were purified by Soxhlet extraction with methanol for 24 h and dried under vacuum at 30 °C for 24 h to afford sensor **PS-RB-2** (0.23 g) or **PS-R6G-2** (0.25 g).

2.5. X-ray diffraction crystallography

Suitable crystals of **RB-2** and **R6G-2** were obtained by slow evaporation of methanol solutions at r.t. Crystal data were collected on a Nonius CAD-4 diffractometer with Mo- $K\alpha$ radiation (0.71073 Å) by using an ω - 2θ scan mode at 293 K. The structures were solved by direct methods using SHELXS-97 and refined by full-matrix least-squares procedure on F^2 for all data using SHELXL-97 [31]. All non-H-atoms were refined anisotropically, and the H-atoms were added at the calculated positions and refined using a riding model. The isotropic temp. factors were fixed to 1.2 times (1.5 times for Me) the equivalent isotropic displacement parameters of the C-atom the H-atom is attached to. CCDC-1874778 (**RB-2**) and CCDC-1874772 (**R6G-2**) contain the supplementary crystallographic data for this article. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK).

2.6. Metal ion competition

Sensors **PS-RB-2** or **PS-R6G-2** (2 mg) was swollen with acetonitrile (200 μ L), then 10 μ L (80 μ M) Hg(II) was added. Fluorescence intensity was determined by excitation at 540 nm after adding 10 μ L (80 μ M) Ca(II), Na(I), K(I), Ni(II), Pb(II), Mg(II), Zn(II), Cu(II), Mn(II), Ag(I), Cd (II), Fe(II), Cr(III) and Ba(II) ions, respectively.

2.7. DFT calculation

The DFT calculations were carried out in the ground state (*in vacuo*) with Gaussian 03 [32] software by using B3LYP/6-31G** method. The



Fig. 2. FTIR spectra of PS-Cl, PS-RB-2 and PS-R6G-2.



Fig. 3. SEM of PS-Cl, PS-RB-2 and PS-R6G-2.



Fig. 4. Fluorescence titration spectra of sensors PS-RB-2 (a) and PS-R6G-2 (b) in acetonitrile upon the incremental addition of Hg(II). The excitation wavelength was 540 nm.



Fig. 5. Fluorescence response of PS-RB-2 (a) and PS-R6G-2 (b) to various metal ions in acetonitrile.

geometrical, electronic and energy parameters were extracted from GuassView 3.0 program [32] based on the optimized structures.

2.8. Preparation of real sample solutions

The tap water and environmental lake water were obtained from school and filtered through a 0.22- μ m membrane [33]. The crucian carp samples were purchased from supermarket, weighed into digestion tank and then the concentrated nitric acid (8 mL) was added. The samples were heated at 120 °C for 1 h, cooled and exposed to microwave digestion system until complete digestion. The obtained solution was heated at 120 °C to remove acid. After filtration of the digestion solution, the pH of supernatant value was adjusted to 6–7 using 1 mM NaOH solution [34].

3. Results and discussion

3.1. Synthesis and characterization

Compounds **RB-1** and **R6G-1** were synthesized using rhodamine B or its ester as the parent, *via* the condensation with hydrazine hydrate under refluxing condition, which then afforded **RB-2** and **R6G-2** *via* substitution with 1-pyrenecarboxaldehyde or 1-naphthaldehyde. In order to acquire the optimum yield, different solvents methanol and ethanol were tried. The most satisfactory results were obtained when the mixture was refluxed in ethanol for 24 h. The structures of **RB-2** and **R6G-2** were confirmed by ¹H NMR, mass and single crystal X-ray diffraction analysis. In the ¹H NMR spectra, as a result of deshielding effect of N-atom, the N-adjacent CH₂ appeared as quartet peaks at the low field of



Fig. 6. Detection mechanism of PS-RB-2 and PS-R6G-2 with Hg(II).



Fig. 7. DFT comparison of RB-2 (a) and R6G-2 (b) with Hg(II).

3.32 ppm, whereas the CH₃ in the N-ethyl chain appeared as triplets at the high field of 1.14-1.25 ppm, with coupling constants around 7.0 Hz. All the aromatic protons resonated in the range of 6–9 ppm. The N=CH appeared at the lowest field of 9.36–9.60 ppm.

Compounds **RB-2** and **R6G-2** crystallize in a tetragonal $P4_12_12$ and a monoclinic $P2_1/n$ space group, respectively. Both structures clearly show a unique spirolactam formation. The O(1)-C(18) in **RB-2** (1.223 (7) Å) and O(1)-C(26) in **R6G-2** (1.205(3) Å) represent a typical C=O double bond, and the value is similar to those (1.229 Å) reported for other related derivatives [35]. The N(2)-C(18) in **RB-2** (1.373(7) Å) and N(3)-C(26) in **R6G-2** (1.382(3) Å) are longer than normal N—C amide bond (1.325–1.352 Å) [36]. For **RB-2** (Fig. 1), the pyrene and xanthene rings link to isoindolinone ring, by twisting 26.97° and 89.26°, respectively, whereas they are almost vertical with a dihedral angle of 87.11°. However, the naphthalene and xanthene rings in **R6G-2** are nearly coplanar or vertical with the isoindolinone ring by making a dihedral angle of 9.63° or 89.17°, respectively. Intra- and intermolecular

C-H··· π interactions form three-dimensional network, which reinforce the crystal packing (Table S2). The CIF files of the crystallographic data were used to do the DFT calculations, which could provide a basis for elucidating the difference between their fluorescent properties.

Preparation of sensors **PS-RB-2** and **PS-R6G-2** was carried out in a molar ratio of **PS-CI** to **RB-2** or **R6G-2** 1:1 equiv. in DMSO with triethylamine as acid binding agent. The pure products were obtained by washing with methanol and water for several times, then dried under vacuum at 30 °C for 24 h. By comparison the FTIR spectra (Fig. 2), new peaks generated at 1735 cm⁻¹ and 1716.5 cm⁻¹ were attributed to the C=O stretching vibration. The C—CI stretching vibration [37,38] at 1264 cm⁻¹ was significantly reduced, indicating **RB-2** and **R6G-2** were successfully immobilized to **PS-CI**.

The morphology and structure of **PS-CI**, **PS-RB-2** and **PS-R6G-2** were analyzed by scanning electron microscopy (SEM). As shown in Fig. 3, all the microspheres remained good sphericity and monodispersity, with no significant changes in morphology. However, SEM for **PS-RB-2** and



Fig. 8. Fluorescence intensity change for PS-RB-2 (a) and PS-R6C-2 (b) after alternate treatment by Hg(II) (4 µM) and EDTA (10 µM) solution.

Table 1
Comparison of analytical performance of the three sensors.

Sensor	Carrier	Carrier size (µm)	Detection limit (µM)	Detection range (µM)	Response time (min)	Number of recycles
I	Chloracetyl	200	0.439	1-9	4	≥3
PS-RB-2	Chloromethyl	40	0.065	0.5-4	4	≥3
PS-R6G-2	Chloromethyl	40	0.072	0.5-4	3	≥3

PS-R6G-2 were significantly different from PS-Cl, with longer diameter and rougher surface. The obvious changes in surface morphology indicated that the immobilization was successful.

3.2. Fluorescence properties

To explore the sensing properties, the fluorescence titration of PS-**RB-2** and **PS-R6G-2** with Hg(II) was conducted. The recognition was finished in 4 min with negligible fluorescence changes at pH values of 6.0–9.0. Upon incremental addition of Hg(II), enhanced fluorescence emission was observed at 574 nm (Fig. 4). For PS-R6G-2, Hg(II) expressed a linear concentration range from 0.5 to 4 µM with a correlation coefficient of 0.996. The detection limit was determined to 0.072 μ M based on 3s/k where s was standard deviation and k was the slope of calibration plot [39,40]. However, sensor PS-RB-2 displayed a much higher fluorescence response and a lower detection limit of 0.065 µM in comparison, which was also better than sensor I (Scheme 1b). Moreover, the microspheres surface showed a color change from yellow to rosy red after the addition of Hg(II), which illustrated that sensors could perform fluorescence "off-on" response and visual recognition of Hg(II).

As shown in Fig. 5, weak fluorescence enhancement was observed with the addition of Ca(II), Na(I), K(I), Ni(II), Pb(II), Mg(II), Zn(II), Cu (II), Mn(II), Ag(I), Cd(II), Fe(II), Cr(III) and Ba(II) ions (black bars). However, when Hg(II) ions were added, significant variation was observed (red bars). Compared to PS-R6G-2, PS-RB-2 had better selectivity and fluorescence response to Hg(II) along with obvious color change, from yellow to rosy red, whereas other ions remained yellow. Its antiinterference ability to Cu(II) was also better than sensor I (Scheme 1b). From the selectivity of other metal ions, PS-RB-2 had the similar performance with sensor I.

3.3. Detection mechanism

Detection mechanism of **PS-RB-2** and **PS-R6G-2** with Hg(II) were shown in Fig. 6. At first rhodamine existed in a closed lactam spirocycle with nonfluorescence. With the addition of Hg(II), PS-RB-2 and PS-**R6G-2** could chelate with Hg(II) via N and O atoms, which caused the cycle open along with apparent color change and strong fluorescence emission at 574 nm. To further explain the fluorescent difference from theoretic level, compounds RB-2 and R6G-2 were selected as templates, and the DFT calculations of RB-2 and R6G-2 with Hg(II) were carried out (Fig. 7).

The frontier-orbital energies are closely related to molecular activity in general [41]. *E*_{HOMO} is a rough measure of electron-donating ability, whereas E_{LUMO} acts in reverse [42,43]. The comparison of DFT results for RB-2 and R6G-2 with Hg(II) were shown in Fig. 7, where the positive phase was symbolized with red and the negative phase green. Both of them have delocalized π systems. It is easier for the vertical transition of delocalized π electrons from HOMO to LUMO. It could be seen that the HOMO-LUMO energy gap of neutral RB-2 (0.131 a.u.) was smaller than R6G-2 (0.149 a.u.). The narrow gap implies a higher chemical reactivity because it is energetically favorable to add electrons to a low-lying LUMO or extract electrons from a high-lying HOMO so to form an activated complex in any potential reaction [44]. It indicated that the electronic transfer in **RB-2** was easier and its chelation with Hg(II) might possess a relatively higher reactivity, which correlated well with the fluorescent results. When the chelation took place, the neutral molecule obtained two electrons to form negative ion. In the LUMO of neutral RB-2, electrons were mainly delocalized on the pyrene ring. When electron transitions took place, some electrons entered into the negative HOMO, and then delocalized on the rhodamine ring. Compound R6G-2 had quite similar electron distributions. The vertical electrophilic potential of neutral RB-2 and R6G-2 was 311.0 kj/mol and 410.3 kj/mol, respectively. However, their adiabatic electrophilic potentials (65.5 kj/mol for RB-2 and 148.2 kj/mol for R6G-2) decreased, which meant it was easier to get electrons when the structure changed. In comparison, the narrower energy gap made **RB-2** with a higher reactivity, which led to better fluorescent property.

In addition, EDTA titration was carried out to explore the regeneration ability. EDTA, which had stronger chelation with Hg(II), made fluorescence disappear along with free sensor regain. As shown in Fig. 8, PS-RB-2 and PS-R6G-2 could be reused for more than three times in acetonitrile. However, a decrease in fluorescence intensity was presented with the repeat time increase, which might due to the loss of some rhodamine derivatives on the surface of polystyrene microspheres. In addition, the surface color constantly changed in the process of reuse, indicating the rhodamines immobilized on the microspheres presented an "off-on" state.

To explore the influences of different polystyrene microspheres, the analytical performances of PS-RB-2 and PS-R6G-2 were compared with sensor I (Scheme 1b). Sensor I was employed chloroacetylated polystyrene microspheres (200 µm) as carriers and rhodamine RH as recognition probe. As shown in Table 1, PS-RB-2 and PS-R6G-2 with smaller size had lower detection limit, whereas other properties such as response time, selectivity and recyclability were no degradation. The decrease in size would increase the specific surface area and the loading amount. Moreover, the stability of chloroacetylated microspheres was lower than chloromethyl microspheres due to the greater viability of chloracetyl group. During the synthesis of chloroacetylated microspheres, other side reactions also occurred, which reduced the amount of Cl and then reduced the loading rate. In addition, PS-RB-2 and PS-**R6G-2** could be used at r.t. with no special conditions and the operation was relatively simple. Compared with some Hg(II) chemical sensors [13,14], although they have low detection limit and excellent selectivity, bad reusing property restricted their large scale application. The modification of small-molecule fluorescent probe on solid phase carriers such as polystyrene microspheres can solve this problem. However, the introduction of larger size carriers may reduce their detection performance, such as detection limit. Thus, chloromethyl microspheres with smaller size were selected as carriers, and solid-phase sensors

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Table

Recovery results for spiked Hg(II) in real samples using the developed met	hod.

Sample	Original (µM)	Added (µM)	Detected (µM)	Recovery (%)	Relative error (%)
	0	1.0	0.926	92.60	7.40
Water	0	2.0	2.116	105.80	5.80
	0	3.0	2.811	93.70	6.30
	0.0021	1.0	1.061	106.10	6.10
Lake water	0.0021	2.0	1.969	98.45	1.55
	0.0021	3.0	3.136	104.52	4.52
Crucian carp	0	1.0	0.913	91.30	8.70
	0	2.0	2.082	104.10	4.10
	0	3.0	3.175	105.84	5.84



Fig. 9. The solid phase extraction column prepared by **PS-RB-2** were used to repeatedly detect Hg(II) rapidly (A only **PS-RB-2** in column, B 10 mg L⁻¹ Hg(II) flowed through the column A, C EDTA flowed through the column C several times, D 10 mg L⁻¹ Hg(II) flowed through the column C again).

PS-RB-2 and **PS-R6G-2** with good detection and recovery performance were obtained. Cost saving, environment-friendliness by recycling and high selectivity to Hg(II) should make **PS-RB-2** and **PS-R6G-2** immediately useful for the detection of real samples.

4. Application

Real samples were used to verify the reliability and practicability of this methodology. **PS-RB-2** was used to determine the Hg(II) in tap water, lake water, and crucian carp. All samples were analyzed by standard addition method, which were prepared on the basis of possible metal ions present in the environmental water and fish and analyzed with the proposed method (Table 2). The recoveries for tap water, lake water and crucian carp were 92.60 to 105.80%, 98.45 to 106.10%, and 91.30 to 105.84% respectively, with relative error below 10% in spiked recovery studies. This indicated that the analytical results were within an acceptable range. Therefore, the established method was convenient, fast, environmental friendly and reliable, and could be used for the detection of Hg(II) in actual water and food samples.

More practically, the sensors were recyclable. They could be restored to the previous state by the loss of Hg(II). So simple solid phase extraction columns were developed (Fig. 9), which were filled with **PS-RB-2** to analysis the concentration of Hg(II) in acetonitrile rapidly. The filled layer was light yellow at first, it turned red when 10 mg L⁻¹ Hg(II) flowed slowly. More importantly, it turned to light yellow when EDTA flowed several times. This cyclic process could be repeated at least three times.

5. Conclusion

In summary, a method for the detection of Hg(II) based on rhodamine-PAHs derivatives and polystyrene microspheres was developed. By introducing pyrene or naphthalene fluorophore to the polystyrene microspheres, two novel solid-phase fluorescence sensors **PS-RB-2** and **PS-R6G-2** were synthesized and a lower detection limit (0.065 µM for **PS-RB-2**) was obtained. The sensing mechanism involving the Hg (II) chelation-induced ring-opening of rhodamine spirolactam was proposed and discussed from theoretic level based on crystal structures and DFT calculations. The sensor **PS-RB-2** was successfully applied to monitor Hg(II) in tap water, lake water and crucian carp samples. Its solid phase extraction columns were developed for rapid detection of Hg(II) by observing the color change (from yellow to rosy red). High selectivity and instantaneous response to Hg(II), good anti-interference and

recyclable property make **PS-RB-2** as potential solid-phase fluorescent sensor for further monitoring Hg(II) in environmental samples.

Author contributions section

Yi Li: Conceptualization, Methodology, Formal analysis, Writing -Review & Editing, Visualization, Supervision.

Jinen Xiong: Methodology, Validation, Formal analysis, Investigation, Writing - Original Draft.

Shuang Li: Validation, Investigation, Writing - Original Draft.
Xiangjun Wen: Validation, Investigation, Writing - Original Draft.
Tao Yu: Methodology, Investigation, Supervision, Writing - Review
& Editing.

Yichen Lu: Formal analysis, Supervision, Project administration. Xiong Xiong: Formal analysis, Supervision, Project administration. Yuanjian Liu: Formal analysis, Supervision, Project administration. Xiaohui Xiong: Resources, Writing - Review & Editing, Supervision,

Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

X-ray crystallographic analyses of RB-2 and R6G-2.Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa. 2020.118277.

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