Contents lists available at ScienceDirect

Organic Electronics

journal homepage: http://www.elsevier.com/locate/orgel

Dual-functional chemical sensor for sensitive detection and bioimaging of Zn^{2+} and Pb^{2+} based on a water-soluble polymer

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ARTICLE INFO

Keywords: Dual-functional fluorescent sensor Water-soluble polymer Bioimaging Lead Zinc

ABSTRACT

Free lead and zinc ions are toxic to living cells and environment, we develop a multi-responsive fluorescence probe based on water-soluble double hydrophilic block copolymer. The polymer sensor with Schiff moiety can sensitively, selectively and simultaneously fluorescence probe Zn^{2+} (green) and Pb^{2+} (blue) through the different fluorescence color. The linear concentration ranges for Pd^{2+} and Zn^{2+} were $0.8-10.0 \ \mu$ M and $0-10.0 \ \mu$ M with the detection limits of $0.7 \ \mu$ M and $0.2 \ \mu$ M, respectively. The probe responding to Na^+ , Mn^{2+} , Fe^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Al^{3+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Ag^+ , Ca^{2+} , K^+ , Mg^{2+} , Ba^{2+} , Hg^{2+} , and Pb^{2+} displays almost no significant interference with the assay process. The sensing mechanism of the PSAM probe to Pb^{2+} and Zn^{2+} was investigated by Job's plot experiment, ¹H NMR analysis and density functional theory (DFT), the results show that the nitrogen atom of CH—N and oxygen atom of OH may recognize with ions and the Job's plots for the PSAM complexes demonstrate 1:1 complexation, respectively. To achieve its application, the chemosensor is successfully applied to monitor Pd^{2+} and Zn^{2+} in tap and lake water samples, its ability to monitor free Pd^{2+} and Zn^{2+} ions in living cells or in vivo is also validated by the intracellular imaging.

1. Introduction

Fluorescent sensors for selective identifying metal ions have potential application for the biological imaging and environment because of the facile analysis, high sensitivity, real-time characteristics and especially handy operation [1–8]. Among the heavy metal cations, lead is considered to be one of the most toxic metal ions. Importantly, it is declared one of the 2B carcinogen by W.H.O. Besides, pigments are connected to lead, some nations demand that the contents of lead in pigments need be controlled within 660 ppm in their environment standards. The concentration level of lead that will result in health issue even risk life is different between children and adults. As for adults, concentration level higher than 100 μ g/100 mL in blood is dangerous. Children are more allergic to lead ion, the content will decrease into 72 μ g/mL. Once lead transports into human body, it will remain in the system for period of time, which can lead to anemia, accompanied with headache, dizzies, constipation. Furthermore, residual lead in animal body will cause much health problem such as arteriosclerosis, anemia, muscle paralysis, headache and memory loss, etc [9–19].

Zinc, after iron, copper and aluminum, the fourth most familiar metal to the public, also enjoys very high status in industry, moreover, zinc plays indispensable roles in many pathological and physiological including regulation of metalloenzymes, gene transcription, cell apoptosis, signal transmission [20–24]. Nevertheless, excessive zinc ions in human body are associated with neurodegenerative disorders [25–27]. In this context, it is essential to develop multi-functional sensors for sensitive and selective detection of Pb²⁺ and Zn²⁺.

Accordingly, many types of fluorescent probes for detection of Pb^{2+} and Zn^{2+} ions based on rahodamine [28,29], quinolone [30,31], 1, 8-naphthalimide [32,33], BODIPY [34,35] have been reported. As far as we are concerned, only a few fluorescent probes are developed for simultaneous detection of Pb^{2+} and Zn^{2+} . Joseph et al. [36] reported a

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https://doi.org/10.1016/j.orgel.2020.105711

Received 18 September 2019; Received in revised form 27 February 2020; Accepted 8 March 2020 Available online 14 March 2020 1566-1199/© 2020 Elsevier B.V. All rights reserved.





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triazole-appended alkoxy as the fluorescence sensor for detection of Pb^{2+} and Zn^{2+} in CH_3CN solution based on the different excitation wavelength. Obviously, those small molecule-based fluorescence probes for detections of lead and zinc ions have suffered from many limitations, such as water-insoluble, low sensitivity and poor selectivity. Moreover, considering the bio-imaging applications, small molecule fluorometric sensors expose a few disadvantages such as exuding from the blood vessels and rapid elimination, which will limit the measurement times.

Polymer is a kind of novel sensor, which seems to solve the above problems. Multifunctional fluorescence chemosensors based on amphiphilic copolymer display high selectively and sensitivity towards Al^{3+} in pure aqueous solution [6,37]. A water-soluble conjugated polymer containing rhodamine 6G motieries exhibit high selectivity and good reversibility toward Fe^{3+} in pure aqueous solutions [38]. Herein, multi-functional polymers are promising sensors to monitor of Pb^{2+} and Zn^{2+} in pure aqueous solution in real-time.

We fabricated a multi-responsive double fluorescence sensor (PSAM) using hydrophilic block copolymer towards Pb^{2+} and Zn^{2+} , which contains pendant group of schiff-based moieties (SBAC) as the ion-sensitive receptor. For solving molecular solubility, a hydrophilic group of the acrylamide is introduced into the polymer molecule to achieve water solubility. The PSAM sensor shows good selective response to Pb^{2+} and Zn^{2+} ions in pure aqueous solution with turn-on color. Meanwhile, to further investigate sensor for detection of Pb^{2+} and Zn^{2+} ions in tap and lake water, as well as bio-imaging in living cells.

2. Material and methods

2.1. Materials

All materials were commercially purchased, and utilized as received unless otherwise stated. The chemicals used in the synthesis were acrylamide (Alfa Aesar, 99%), 2, 2'-azobis (2-methylpropionitrile) (AIBN, Merck, 98%), hydrazine monohydrate (Alfa Aesar, 98%), salicylaldehyde (Sinopharm, 98%), triethylamine (Fluka, 99%), acryloyl chloride (Alfa Aesar, 99%), *N*-methyl-2-pyrrolidone (NMP, Sinopharm, 98%), ethanol absolute (VWR, 99.8%), dichloromethane (Sinopharm, 98%), tetrahydrofuran (THF, Alfa Aesar, 98%), and acetone (Sinopharm, 98%). All the metal salts were obtained from Sinopharm Chemical Reagent Co., Ltd. THF and dichloromethane were distilled from fresh sodium and benzophenone before usage.

2.2. Measurements and instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-600 spectrometers, DMSO-d₆ and D₂O were used as the solvent. Molecular weights was measured through gel permeation chromatography (GPC) (Shimdzu, Japan). Absorption spectra were collected on a PerkinElmer Lambda 35 UV/vis spectrometer. Fluorescence spectra were taken on a PE 55 fluorescence spectrometer, the slit width was 4 nm and 4 nm for excitation and emission with 316 nm of the excitation, the emission was acquired from 350 nm to 400 nm, Stock solutions (2.0 mM) of metal cation salts $(Zn^{2+}, Pb^{2+}, Na^+, Ba^{2+}, K^+, Fe^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Cd^{2+}, Al^{3+}, Cr^{3+}, Ni^{2+}, Ag^+, Ca^{2+}, Co^{2+}, Mg^{2+}, Hg^{2+})$ and our polymer sensor were prepared in distilled water. During the test, ensure that the pH of the solution is 7 with Tris-HCl buffer (10.0 mM, pH 7.0). In the selectivity experiments, appropriate amounts of the metal ions stock solutions were placed into 10.0 equiv. of the sensor stock solution. Fluorescence and UV/vis spectroscopy were taken at room temperature. Fourier-transform infrared (FTIR) spectra were performed using KBr discs on a Nicolet 8700 spectrometer in the 4000–400 cm^{-1} region.

2.3. Sample synthesis

2.3.1. Synthesis of 4-aminobenzohydrazide (SH) (Scheme 1(a))

2.0 g (13.2 mmol) of 4-aminobenzoate was dissolved in 20 mL of absolute ethanol, 1.0 mL (19.8 mmol) of hydrazine hydrate was dropwise added under N₂ atmosphere, then the mixture was heated to 78 °C under reflux for 5 h. After the solution was cooled to room temperature, the crude product was obtained by filtering and washing with deionized water, which was then recrystallized in ethanol. Yield (after recrystallization): 1.6 g (80%). FTIR (wavenumber, cm⁻¹): ν_{N-H} , 3430, 3342, 3323, and 3230; ν_{C} =0, 1670. ¹H NMR (600 MHz, 298 K, DMSO-*d*₆): δ (ppm), 9.43 (s, 1H), 7.63 (d, 2H), 5.62 (s, 2H), 4.43 (s, 2H). ¹³C NMR (150 MHz, 298 K, DMSO-*d*₆): δ (ppm), 167.68, 152.53, 129.74, 121.10, and 113.94.

2.3.2. Synthesis of (E)-4-amino-N'-(2-hydroxybenzylidene) benzohydrazide (SB) (Scheme 1(b))

First, 1.6 g (10.5 mmol) of 4-aminobenzohydrazide (SH), 1.3 mL (11.0 mmol) of salicylaldehyde and 30 mL of ethanol were added to a 100 mL three-neck bottle. The mixture solution was stirred at 80 °C for 3 h. After cooling to room temperature, a pale-yellow precipitate was obtained, which was then washed with a small amount of cool ethanol. Yield: 86.0%. FTIR (wavenumber, cm⁻¹): ν_{OHb} NH₃ 3455, 3356, and 3281; ν_{C} =-0, 1664. ¹H NMR (600 MHz, 298 K, DMSO- d_6), δ (ppm), 12.65 (s, 1H), 9.01 (s, 1H), 7.72 (d, 2H), 7.54 (dd, 1H), 5.76 (s, 1H). ¹³C NMR (150 MHz, 298 K, DMSO- d_6), δ (ppm), 163.50, 158.32, 153.50, 147.81, 131.80, 130.62, 130.32, 120.11, 119.67, 119.64, 117.30, and 113.57.

2.3.3. Synthesis of (E)-4-amino-N'-(2-hydroxybenzylidene) benzohydrazide (SBAC) (Scheme 1(c))

0.5 g (1.55 mmol) of compound SB, 0.32 mL (2.32 mmol) of trimethylamine, and 15 mL of NMP were added to a 100 mL three-neck bottle. The mixture was stirred for 10 min at 0~5 °C. 0.197 mL (2.02 mmol) of acryloyl chloride was added to the three-neck bottle with stirring at room temperature for 15 h under N₂ atmosphere. Then the solution was added to deionized water. After the filtration and recrystallization in ethanol, product (SBAC) was obtained. Yield: 0.38 g (70.5%). FTIR (wavenumber, cm⁻¹), $\nu_{OH, NH}$, 3450 (bb), 3320 and 3251; ν_{C} =0, 1660. ¹H NMR (600 MHz, 298 K, DMSO-*d*₆), δ (ppm), 12.01 (s, 1H), 11.32 (s, 1H), 10.11 (s, 1H), 9.01 (s, 1H), 7.97 (d, 2H), 7.90 (d, 2H), 7.57 (d, 1H), 7.34 (t, 1H), 7.02–6.93 (m, 2H), 5.93 (s, 1H). ¹³C NMR (150 MHz, 298 K, DMSO-*d*₆): δ (ppm), 168.10, 163.20, 158.32, 148.90, 143.35, 141.11, 132.30, 130.62, 129.30, 128.20, 120.97, 120.31, 120.25 and 117.30. ESI-MS calcd for C₁₇H₁₅N₃O₃ 309.3270, found 309.3265.

2.3.4. Synthesis of PSAM (Scheme 1(d))

86 mg (0.28 mmol) of compound SBAC, 0.2 g (2.8 mmol) of acrylamide, 1.6 mg (9.7 µmol) of AIBN, and THF (10 mL) was added into a reaction tube equipped with a magnetic stirring bar. After stirring for 2 h at 66 °C under N₂, the reaction mixture was cooled to the room temperature and poured into an excess of cool acetone for the precipitation. The precipitate was redissolved in THF and then added to cool acetone to precipitation again for three times. PSAM was acquired as a paleyellow powder, yield: 70% (0.2 g). GPC results that Mn of 2.7 KDa and M_W/M_n of 1.13 (Fig. S6). The degree of polymerization (DP) of PSAM was analyzed by ¹H NMR (Table S1). Hence, the polymer was named poly(SBAC)₁-co-(AM)₂₀. SBAC content in poly(SBAC)₁-co-(AM)₂₀ block was measured to be ${\sim}4.8\%$ mol by using SBAC as the standard. With the similar procedures, another poly(SBAC)₁-co-(AM)₅₀ were prepared. GPC analysis revealed Mn of 4.6 KDa and M_W/M_n of 1.17 (Fig. S6), respectively. SBAC content in poly(SBAC)₁-co-(AM)₅₀ block was measured to be ~1.9 mol% for poly(SBAC)₁-co-(AM)₅₀ by using SBAC as the standard.



Fig. 1. Fluorescence spectra of $(SBAC)_{1}$ -co- $(AM)_{50}$ with excitation wavelength of Zn²⁺ (316 nm) and Pb²⁺ (327 nm), relative fluorescence intensity (F/F₀) of 0.1 g/L (SBAC)_{1}-co- $(AM)_{50}$ (b) solution after the addition of 1.0 equiv of Zn²⁺, Pb²⁺, Na⁺, K⁺, Ba²⁺, Mn²⁺, Fe²⁺, Fe³⁺, Cu²⁺, Cd²⁺, Al³⁺, Cr³⁺, Co²⁺, Ni²⁺, Ag⁺, Ca²⁺, Mg²⁺, and Hg²⁺. Inset of (b) is the corresponsive images under UV light.



Fig. 2. Fluorescence spectra of 0.1 g/L PSAM with different concentrations of (a) Zn^{2+} , and (b) Pb^{2+} in Tris-HCl buffer solution (pH 7.0). Inset: fluorescence intensity ($\lambda_{em} = 362 \text{ nm}$) vs ion concentration.



Fig. 3. Competitive effects of (a) Zn^{2+} and (b) Pb^{2+} ion with other metal ions in 0.1 g/L of PSAM Tris-HCl buffer solution.



Fig. 4. The time response of 0.1 g/L of PSAM sensing 10 equiv. of Zn^{2+} and Pb^{2+} in 10.0 mM of Tris-HCl solution ($\lambda_{em} = 362$ nm for Zn^{2+} , $\lambda_{em} = 375$ nm for Pb^{2+}).



Fig. 5. Fluorescence intensities of 0.1 g/L PSAM with 10.0 equiv. Zn^{2+}/Pb^{2+} at different pH (1–13), respectively ($\lambda_{ex} = 362$ nm for Zn^{2+} , $\lambda_{ex} = 375$ nm for Pb²⁺).

2.4. Cytotoxicity assays

MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) assay was carried out to evaluate the cytotoxic effect of the sensor in cells. HeLa cells were seeded in 36-well plates and incubated for 24 h to reach ca. 75% confluence before treatment. PSAM (0, 5, 10, 15, 20, and 25 mM, 100 μ L) was add to the 36-well plates, the cells were cultured for 24 h. Then, adding MTT (50 μ L/well, 5 mg/ml) into each well and the cells were cultured for 4 h. After that, adding 200 μ l of DMSO to each well and measuring the absorbance at 316 nm.

2.5. Cell imaging

HeLa cells were cultured by DMEM at 37 $^\circ C$ under 5% CO₂, then stained with PSAM (50 μM) with 25 min and then washed through PBS butter solution. The cells imaging was performed on a fluorescent Inverted microscope (BSF-60).

3. Result and discussion

3.1. Synthesis of poly(SBAC)₁-co-(AM)₂₀ and poly(SBAC)₁-co-(AM)₅₀

We prepared PSAM containing different contents of SBAC moiety through radical polymerization. The structural characterization of the copolymer and the data of GPC and 1H NMR were shown in Figs. S1–5 and Table S2. The percentage content of chromophore in poly(SBAC)₁-*co*-(AM)₂₀ and poly(SBAC)₁-*co*-(AM)₅₀ was determined to be ~4.8 mol% and ~1.9 mmol% by ¹H NMR (Table S1), respectively.

3.2. Fluorescence spectroscopy of PSAM for Pb^{2+} and Zn^{2+}

The fluorescent sensing ability of PSAM was investigated in presence of various metals of 100 μ M in Tris-HCl buffer solution (pH = 7.0) at the room temperature. PSAM of 0.1 g/L without metal ions has a low fluorescence emission peak with excitations of 316 nm (Zn²⁺) and 327 nm (Pb²⁺). After the addition of 1.0 equiv. different metal cation ions such as Na⁺, Ba²⁺, Mn²⁺, Fe³⁺, Cu²⁺, Zn²⁺, Fe²⁺, Cd²⁺, Al³⁺, Cr³⁺, Co²⁺, Ni²⁺, Ag⁺, K⁺, Ca²⁺, Mg²⁺, Hg²⁺, Pb²⁺ to the solutions of PSAM, no or a very weak fluorescence was observed except for Pb²⁺ and Zn²⁺. Because the less solubility of (*SBAC*)₁-*co*-(*AM*)₂₀ in pure water leads to the low fluorescence intensity of the complex (Fig. S9), other metal ions have relatively large interference in detecting Zn²⁺ and Pb²⁺. (*SBAC*)₁-*co*-(*AM*)₅₀ is selected for detailed study. The Zn²⁺ and Pb²⁺ ions cause remarkable fluorescence enhancements at 362 nm and 375 nm compared with (*SBAC*)₁-*co*-(*AM*)₅₀. (Fig. 1a and b). Two different



Fig. 6. Job plot for plot PSAM with (a) Zn²⁺ (b) Pb²⁺ in water solutions. The total concentration on plot PSAM and Zn²⁺/Pd²⁺ is 10 μ M, respectively. (0.1 g/L water solution of PSAMs, [SBAC] = 7.6 μ M; 10.0 mM Tris-HCl, pH 7.0; λ ex = 362 nm for Zn²⁺, λ ex = 375 nm for Pb²⁺, slit widths: λ em. 4.0 nm, λ em. 4.0 nm).



Fig. 7. (a) Partial ¹H NMR spectra of PSAM in D₂O alone and in the presence of Pb²⁺ and Zn²⁺, (b) HOMO and LUMO orbitals of SBAC-Pb²⁺, SBAC-Zn²⁺ and SBAC.

emissions wavelengths indicate that PSAM could be utilized as a dualchannel fluorescent sensor for Zn^{2+} and Pb^{2+} in the completely water solution medium. The difference in fluorescence emission peak for sensing the Zn^{2+} and Pb^{2+} is due to the different energy gaps of two complex, PSAM- Zn^{2+} and PSAM- Pb^{2+} [39].

The emission spectra of PSAM with different concentrations of Zn²⁺ and Pb²⁺ in Tris-HCl buffer solution (pH = 7.0) were illustrated in Fig. 2. As the concentration of Pb²⁺ and Zn²⁺ increased, the fluorescence emission intensity increased until 2.0 equiv. and then there was no further change. The linear concentration ranges for Pd²⁺ and Zn²⁺ were 0.8–10.0 μ M and 0–10.0 μ M with the detection limits of 0.7 μ M and 0.2 μ M, respectively (Fig. S6). Based on Benesi-Hildebrand, the complex constants of PSAM with Pb²⁺ and Zn²⁺ were 4 \times 10⁴ M⁻¹ and 4 \times 10⁵ M⁻¹ respectively. (Fig. S7).

To further evaluate the practical applicability of PSAMs as a selective fluorescence sensor for Zn^{2+} and Pb^{2+} , competitive experiments were carried out in the presence of Zn^{2+}/Pb^{2+} of 0.1 g/L with 1.0 equiv. of

other relevant metal cation ions, such as Zn^{2+} , Pb^{2+} , Na^+ , Ba^{2+} , Fe^{2+} , K^+ , Fe^{3+} , Cu^{2+} , Cd^{2+} , Al^{3+} , Cr^{3+} , Co^{2+} , Mn^{2+} , Ni^{2+} , Ag^+ , Ca^{2+} , Mg^{2+} , Hg^{2+} . As depicted in Fig. 3, Zn^{2+}/Pb^{2+} detection was not disturbed via the presence of detected metal cation ions except Fe^{3+} , Fe^{2+} and Cu^{2+} , due to the strong binding of Cu^{2+} , Fe^{2+} and Fe^{3+} ions with PSAM. PSAM could be as a fluorescence probe for Zn^{2+} and Pb^{2+} in the presence of metal ions. The fluorescent emission peak intensity at 362 nm for Zn^{2+} hited the $F/F_0 = 18$ and at 375 nm of Pb^{2+} was $F/F_0 = 14$. The emission intensity enhancement at 362 nm for Zn^{2+} hits the highest $F/F_0 = 18$ and the emission intensity enhancement at 375 nm of Pb^{2+} was $F/F_0 = 14$. Therefore, we could differentiate the blue and green fluorescent of Zn^{2+} and Pb^{2+} by the different fluorescence color.

Response time for the designed sensor is as important as sensitivity and selectivity. The response time of PSAM (0.1 g/L) for sensing 1.0 equiv of Zn^{2+}/Pb^{2+} in buffer solutions was studied (Fig. 4). The fluorescence analysis displays that the detection between PSAM and Zn^{2+}/Pb^{2+} starts immediately with gently shaking. The fluorescence emission



Scheme 1. Synthesis schematic of the chemical sensor PSAM.



Scheme 2. Proposed binding mode of PSAM with Pb^{2+} and Zn^{2+} .

peak intensity of PSAM in the presence of Zn^{2+}/Pb^{2+} increases in Tris-HCl buffer solution in ${\sim}15$ min, reaches strongest value in 40 min, after that tends to be stable. We can conclude that PSAM and Zn^{2+}/Pb^{2+} complete coordination in 39 min and 30 min, respectively.

The effect of pH on the detection of PSAM was also tested under various pH conditions. From Fig. 5, the fluorescence emission intensity of PSAM no changes in the pH range from 2.0 to 13.0. The pH of the PSAM solution altered by adding of Trils-HCl buffer solution. In the pH range of 7.0–9.0, PSAM displays a high fluorescence response toward Zn^{2+}/Pb^{2+} , demonstrating that the sensor could be used under the biological and environmental condition. For examples, the fluorescence sensor of PSAM could be utilized to be monitor Zn^{2+}/Pb^{2+} in water and living cells, the pH range of which is usually found to be 6.0–7.6 [40].

To elucidate the probing mechanism of PSAM toward Zn^{2+} and Pb^{2+} , titration experiments of PSAM with Zn^{2+} and Pb^{2+} were carried out based on ¹H NMR. The ¹H NMR spectrum of PSAM in D₂O and detailed assignment of each proton was depicted in Fig. 7a. The chemical shifts at 9.87, 7.72, 7.52-6.50, 3.78, 3.65 and 2.30-1.25 ppm are assigned to the proton H_m, H_i, H_e, H_{n+l+k+j+g+f}, H_c, H_d, H_{a+b} of PSAM, respectively. Upon addition of Zn^{2+} , not only chemical shift of H_m at 9.87 ppm disappears, but also that of H_i shifts from 7.72 to 8.14 ppm, indicating that the nitrogen atom of CH=N and oxygen atom of OH may recognize with $Pb^{2+}.$ While upon addition of $Pb^{2+},\,H_m$ peak at 9.87 ppm also disappears, H_i peak alters from 7.72 to 8.31 ppm. For further verifying the probing mechanism of PSAM toward Zn²⁺ and Pb²⁺, energy-optimized structures of monomer SBAC and its complexes are calculated by density functional theory (DFT) at the B3LYP22/LanL2DZ level using the program Gaussian 09 (Fig. 7b). The energy gaps (ΔE) between HOMO and LUMO for SBAC, SBAC-Zn²⁺ and SBAC-Pb²⁺ are 4.12, 3.28 and 3.72 eV respectively. A low ΔE well explains why there are strong spectral absorption and fluorescent emission at long-wavelength for SBAC in the presence of Zn²⁺ and Pb²⁺. Compared with that of SBAC-Pb²⁺, the lower ΔE of SBAC-Zn²⁺ hints the longer fluorescence emission wavelength. According to above results, the probable binding modes of PSAM with Pb^{2+} and Zn^{2+} are proposed in Scheme 2.

The modes between SBAC and the ions, Pb²⁺ and Zn²⁺ were tested by

Table 1 Fluorescent determination results of Zn^{2+} and Pb^{2+} in real samples.

Extract sample	Plus scalar∕µM	Measure value∕µM	Recovery rate/%	Relative standard deviation (RSD)/%
For Zn ²⁺	0.40	0.39	97.50	0.03
Тар	0.50	0.51	100.20	0.05
water	0.60	0.57	95.00	0.02
Lake	0.40	0.43	107.50	0.04
water	0.50	0.49	98.00	0.06
	0.60	0.61	101.17	0.03
For Pb ²⁺	3.00	3.13	104.33	0.02
Тар	4.00	3.75	93.75	0.04
water	5.0	5.27	105.40	0.05
Lake	3.00	2.87	95.67	0.06
water	4.00	4.19	104.48	0.03
	5.00	5.24	104.80	0.04



Fig. 8. Bright and fluorescence images of HeLa cells after incubating with PSAM (50 $\mu mol/L),$ PSAM + $Zn^{2+},$ and PSAM + Pb^{2+} for 0.5 h.

using Job's plot. From Fig. 6, the Job's plots for the PSAM complexes demonstrate 1:1 complexation stoichiometry, respectively.

To evaluate the utility of copolymer fluorescent sensor, PSAM was used as a fluorescent probe to measure Zn^{2+} and Pb^{2+} in bottled water and tap water. No detectable of Zn^{2+} or Pb^{2+} residues was found in the actual samples prior to the addition of Zn^{2+} and Pb^{2+} . Recovery tests were tested from spiked with 85–93% Zn^{2+} or Pb^{2+} . The results were showed in Table 1. As we known, the recovery rate should be limited between 95%~105%, and relative standard deviation (RSD) should be controlled below 5%. The recovery rate of Zn^{2+} ranges from 95.73% to 101.43% and recovery rate of Pb^{2+} ranges from 96.32% to 102.58%. Those results display that the method can provide a potentially efficient detection system for Zn^{2+} or Pb^{2+} in actual samples.



Fig. 9. Fluorescence imaging of Zn^{2+} and Pb^{2+} in zebrafish with PSAM (scale bar = 1000 $\mu M).$



Fig. 10. a. Representative photographs of mice injected with PSAM and with or without Zn^{2+} **b**. H&E-stained major organs (heart, liver, spleen, stomach, kidney and lung) in mice injected with PBS (control) and PSAM after 7 days (scale bar = 50 μ M).

The cytotoxicity of PSAM with the different concentrations of from 0–20 μ M were conducted with living HeLa cells *via* MTT assay (Fig. S8). The result showed that the copolymer is not toxic to cells. Cells were cultured with 50 μ M of PSAM for 25 min at 37 °C under 5% CO₂ for cellular fluorescence imaging. As depicted in Fig. 8, no fluorescence colour was found in the cell image without Zn^{2+}/Pb^{2+} (Fig. 8b). Hereafter, the cells continued to culture 25 min with 50 μ M of Zn²⁺/Pb²⁺ at 37 °C and then were washed three times with PBS. Two fluorescent colors (blue and green) were found from the cell imaging, the cells with Zn²⁺ emit green fluorescence (Fig. 8e and f), while the cells with Pb²⁺ emit blue fluorescence (Fig. 8h and i). We can conclude that the probe of PSAM is a suitable and biocompatible probe for detecting Zn²⁺ and Pb²⁺ ions in living cells.

To demonstrate that the imaging of living animals could be realized for sensing Zn^{2+} and Pb^{2+} by PSAM, the zebrafish was employed to investigate the capacity of PSAM to monitor Zn^{2+} and Pb^{2+} . Zebrafish purchased from Shanghai GeneBio Co., Ltd. Five groups of zebrafishes were co-incubated with 0 μ M (without Zn^{2+} and Pb^{2+}), 5 μ M and 20 μ M of Zn^{2+} , 5 μ M and 20 μ M of Pb^{2+} for 20 min, and then treated with 20 μ M PSAM for further 20 min. As showed in Fig. 9, green and blue fluorescence signal of zebrafish cannot be collected under green or blue channel without metal ions. With the gradual addition of metal ions, a striking enhancement of fluorescence signal in the green or blue channel was collected, confirming that Zn^{2+} and Pb^{2+} can be specifically recognized by PSAM in zebrafish. That implies that PSAM is capable of entering zebrafish and sensing Zn^{2+} and Pb^{2+} efficiently.

Motivated by its remarkable in vitro imaging performance, we further evaluate the feasibility of using PSAM as in vivo imaging. Due to the toxicity of lead ions, we only made zinc ions in animal experiments. All animal experiments are performed in compliance with the Animal Management Rules of the Ministry of Health of the People's Republic of China (Document no. 55. 2001) and the guidelines for the Care and Use of Laboratory Animals of China Pharmaceutical University. As shown in Fig. 10a, when PSAM is injected without Zn²⁺, there is no fluorescence. As the concentration of zinc ions increasing, the fluorescence range increases, the fluorescence image range gradually spreads. It shows that PSAM has good imaging effect and no toxicity in vivo. Non-toxicity of PSAM is further supported by the H&E. To evaluate the systemic toxicity of PSAM, the mice are sacrificed at the 7th day and their major organs (heart, liver, spleen, stomach, kidney and lung) are excised for H&E

staining. Compared with the control group, no pathological changes to the organs are observed (Fig. 10b).

4. Conclusions

In summary, the copolymer of PSAM is synthesized, which can serve as a dual-channel sensor to Pb^{2+} and Zn^{2+} , respectively. The PSAM based on Schiff segment can selectively detect Pb^{2+} (blue) and Zn^{2+} (green) without interference by other metal ions. Real sample analysis demonstrates that PSAM can be applied to a recognition system for Pb^{2+} and Zn^{2+} in natural water samples, showing with good selectivity and sensitivity. Finally, we realize the application of PSAM for monitoring Pb^{2+} and Zn^{2+} ions in vivo and in vitro. This work will provide inspiration for the development of new multi-channel water-soluble chemical sensors.

Declaration of competing interest

The authors declare no competing financial interest.

Acknowledgements

This project supported by the National Natural Science Foundation of China (Grant Nos. 21671037, 21771036), Fujian Provincial Department of Science and Technology (Grant No. 2017J06019).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.orgel.2020.105711.

References

- Y. Li, X.W. Fan, F.Q. Bai, H.X. Zhang, Water-soluble fluorescent probe for multiple ions detection based on different pH moderation, Org. Electron. 70 (2019) 186–192.
- [2] X. Chen, T. Pradhan, F. Wang, J.S. Kim, Fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives, Chem. Rev. 112 (2012) 1910–1956.
- [3] X. Wan, S. Yao, H. Liu, Y. Yao, Selective fluorescence sensing of Hg²⁺ and Zn²⁺ ions through dual independent channels based on the site-specific functionalization of mesoporous silica nanoparticles, J. Mater. Cult. A. 1 (2013) 10505–10512.

G. Wei et al.

- [4] Z. Yan, H. Lei, N. Li, L. Hong, Preparation of 4,4'-bis-(carboxyl phenylazo)dibenzo-18-crown-6 dye and its application on ratiometric colorimetric recognition to Hg²⁺, Spectrochim. Acta A. 79 (2011) 661–665.
- [5] V.K. Gupta, N. Mergu, L.K. Kumawat, A.K. Singh, Selective naked-eye detection of Magnesium (II) ions using a coumarin-derived fluorescent probe, Sensor. Actuator. B Chem. 207 (2017) 216–223.
- [6] J.A. Han, B.D. An, Y. Wang, X. Bao, L.A. Ni, C. Li, L. Wang, X.Q. Xie, A novel type of responsive double hydrophilic block copolymer-based multifunctional fluorescence chemosensor and its application in biological samples, Sensor. Actuator. B Chem. 250 (2017) 436–445.
- [7] J.Y. Zhang, Y.H. Cheng, M. Tebyetekenwa, S. Meng, M.F. Zhu, Y.F. Lu, "Stiff–Soft" binary synergistic aerogels with superflexibility and high thermal insulation performance, Adv. Funct. Mater. 29 (2019) 1806407–1806418.
- [8] Y.H. Cheng, S.J. Liu, F.Y. Song, M. Khorloo, H.K. Zhang, R.T.K. Kwok, J.W.Y. Lam, Z.K. He, B.Z. Tan, Facile emission color tuning and circularly polarized light generation of single luminogen in engineering robust forms, Mater. Horiz. 6 (2019).
- [9] Z.Q. Hu, C.S. Lin, X.M. Wang, L. Wang, L. Ding, C.L. Cui, S.F. Liu, H.Y. Lu, Highly sensitive and selective turn-on fluorescent chemosensor for Pb²⁺ and Hg²⁺ based on a rhodamine-phenylurea conjugate, Chem. Commun. 46 (2010) 3765–3767.
- [10] H. Ju, M.H. Lee, J. Kim, J.S. Kim, J. Kim, Rhodamine-based chemosensing monolayers on glass as a facile fluorescent "turn-on" sensing film for selective detection of Pb²⁺, Talanta 3 (2011) 1359–1363.
- [11] O. Sunnapu, N.G. Kotla, B. Maddiboyina, S. Singaravadivel, G. Sivaraman, A rhodamine based "turn-on" fluorescent probe for Pb(II) and live cell imaging, RSC Adv. 6 (2015) 656–660.
- [12] Z. Yu, W. Zhou, J. Han, Y.C. Li, L.Z. Fan, X.H. Li, Na⁺ induced conformational change of Pb²⁺-stabilized G-quadruplex and its influence on Pb²⁺ detection, Anal. Chem. 88 (2016) 93759380.
- [13] J.Y. Kwon, Y.J. Jang, Y.J. Lee, K.M. Kim, M.S. Seo, W. Nam, J. Yoon, A highly selective fluorescent chemosensor for Pb²⁺, J. Am. Chem. Soc. 127 (2005) 10107–10111.
- [14] X. Zhu, Q. Zheng, G. Wang, N. Fu, Ultrasensitive detection of lead (II) based on the disaggregation of a polyether bridged squaraine fluorescent probe, Sensor. Actuator. B Chem. 237 (2016) 802–809.
- [15] A.K. Jain, V.K. Gupta, L.P. Singh, J.R. Raisoni, A comparative study of Pb²⁺ selective sensors based on derivatized tetrapyrazole and calix[4]arene receptors, Electrochim. Acta 51 (2006) 2547–2553.
- [16] S.K. Srivastava, V.K. Gupta, S. Jain, Determination of lead using a poly(vinyl chloride)-based crown ether membrane, Analyst 120 (1995) 495–498.
- [17] M. Wang, F. Wang, Y. Wang, W. Zhang, et al., Polydiacetylene-based sensor for highly sensitive and selective Pb 2+ detection, Dyes Pigments 120 (2015) 307–313.
- [18] D. Sain, C. Kumari, A. Kumar, H.P. Nayek, S. Dey, Lead ion induced chemodosimeter approach of a tripodal hydroxyl-quinoline based phospho-ester through P-O bond cleavage, Dalton Trans. 45 (2016) 9187–9192.
- [19] B.H. Xu, X.L. Tang, J.A. Zhou, W.M. Chen, H.L. Liu, Z.H. Ju, W.S. Liu, A "turn-on" lanthanide complex chemosensor for recognition of lead(ii) based on the formation of nanoparticles, Dalton Trans. 45 (2016) 18859–18866.
- [20] J.M. Berg, Y. Shi, The galvanization of biology: a growing appreciation for the roles of zinc, Science 271 (1996) 1081–1085.
- [21] S.F. Sousa, P.A. Fernandes, M.J. Ramos, The carboxylate shift in zinc enzymes: a computational study, J. Am. Chem. Soc. 129 (2007) 1378–1385.
- [22] D.D. Mott, M. Benveniste, R.J. Dingledine, pH-dependent inhibition of kainate receptors by zinc, J. Neurosci. Off. J. Soc. Neurosci. 28 (2008) 1659–1671.

- Organic Electronics 82 (2020) 105711
- [23] M. Dhanasekaran, S. Negi, Y. Sugiura, Designer zinc finger Proteins: tools for creating artificial DNA-binding functional proteins, Acc. Chem. Res. 39 (2016) 45–52.
- [24] C.J. Frederickson, J.Y. Koh, A.I. Bush, The neurobiology of zinc in health and disease, Nat. Rev. Neurosci. 6 (2005) 449–462.
- [25] A.I. Bush, W.H. Pettingell, G. Multhaup, M.D. Paradis, J.P. Vonsattel, J.F. Gusella, K. Beyreuther, C.L. Masters, R.E. Tanzi, Rapid induction of alzheimer a beta amyloid formation by zinc, Science 265 (1994) 1464–1467.
- [26] Y.G. Zhang, Z.H. Shi, L.Z. Yang, X.L. Tang, Y.Q. An, Z.H. Ju, W.S. Liu, A facile fluorescent probe based on coumarin-derived Schiff base for Al³⁺ in aqueous media, Inorg. Chem. Commun. 39 (2014) 86–89.
- [27] A.I. Bush, The metallobiology of alzheimer's disease, Trends Neurosci. 26 (2003) 207–214.
- [28] L. He, V.L.L. So, J.H. Xin, A new rhodamine-thiourea/Al³⁺ complex sensor for the fast visual detection of arginine in aqueous media, Sensor. Actuator. B Chem. 192 (2014) 496–502.
- [29] H. Sasaki, K. Hanaoka, Y. Urano, T. Terai, T. Nagano, Design and synthesis of a novel fluorescence probe for Zn²⁺ based on the spirolactam ring-opening process of rhodamine derivatives, Biorg. Med. Chem. 19 (2011) 1072–1078.
- [30] Q. Zhu, L. Li, L. Mu, X. Zeng, C. Redshaw, G. Wei, A ratiometric Al³⁺ ion probe based on the coumarin-quinoline FRET system, J. Photochem. Photobiol. A Chem. 328 (2016) 217–224.
- [31] C.J. Fahrni, T.V. Halloran, Aqueous coordination chemistry of quinoline-based fluorescence probes for the biological Chemistry of Zinc, J. Am. Chem. Soc. 121 (1999) 11448–11458.
- [32] L.Y. Zhao, Q.L. Mi, G.K. Wang, J.H. Chen, J.F. Zhang, Q.H. Zhao, Y. Zhou, 1,8-Naphthalimide-based 'turn-on' fluorescent sensor for the detection of zinc ion in aqueous media and its applications for bioimaging, Tetrahedron Lett. 54 (2013) 3353–3358.
- [33] S.M. Dimov, N.I. Georgiev, A.M. Asiri, V.B. Bojinov, Synthesis and sensor activity of a PET-based 1,8-naphthalimide Probe for Zn²⁺ and pH determination, J. Fluoresc. 24 (2014) 1621–1628.
- [34] A.N. Kursunlu, E. Guler, H.I. Ucan, R.W. Boyle, A novel bodipy-dipyrrin fluorescent probe: synthesis and recognition behaviour towards Fe (II) and Zn (II), Dyes. Pigment. 94 (2012) 496–502.
- [35] A. Ojida, T. Sakamoto, M. Inoue, S. Fujishima, G. Lippens, I. Hamachi, Fluorescent BODIPY-based Zn(II) complex as a molecular probe for selective detection of neurofibrillary tangles in the brains of alzheimer's disease patients, J. Am. Chem. Soc. 131 (2011) 6543–6548.
- [36] S.J. Joseph, S.K. Barik, A. Thakur, R. Ganesamoorthi, S. Ghosh, Triazolyl alkoxy fischer carbene complexes in conjugation with ferrocene/pyrene as sensory units: multifunctional chemosensors for Lead(II), Copper(II), and Zinc(II) Ions, Organometallics 33 (2014) 3096–3107.
- [37] H.N. Kim, Z.Q. Guo, W.H. Zhu, J. Yoon, H. Tian, Recent progress on polymer-based fluorescent and colorimetric chemosensors, Chem. Soc. Rev. 40 (2010) 79–93.
- [38] Y.X. Wu, J.B. Li, L.H. Liang, D.Q. Lu, J. Zhang, G.J. Mao, L.Y. Zhou, X.B. Zhang, W. H. Tan, G.L. Shen, R.Q. Yu, A rhodamine-appended water-soluble conjugated polymer: an efficient ratiometric fluorescence sensing platform for intracellular metal-ion probing, Chem. Commun. 50 (2014) 2040–2042.
- **[39]** S. Samanta, U. Manna, T. Ray, G. Das, An aggregation-induced emission (AIE) active probe for multiple targets: a fluorescent sensor for Zn^{2+} and Al^{3+} & a colorimetric sensor for Cu^{2+} and F, Dalton Trans. 44 (2015) 18902–18910.
- [40] S. Kim, Y.N. Jin, K.Y. Kim, H.K. Jin, J.H. Kim, H.K. Kang, S.W. Nam, S.H. Kim, S. Park, C. Kim, J. Kim, Salicylimine-based fluorescent chemosensor for aluminum ions and application to bioimaging, Inorg. Chem. 51 (2015) 3597–3602.