Ratiometric fluorescence and mesoporous structured imprinting nanoparticles for rapid and sensitive detection 2,4,6-trinitrophenol

Ming Li¹, Haijian Liu¹, Xueqin Ren⁎

Department of Environmental Sciences & Engineering, China Agricultural University, Beijing, PR China

1 These two authors contributed equally to this work.

⁎ Correspondence to: Department of Environmental Sciences & Engineering, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, PR China.

E-mail address: renxueqin@cau.edu.cn (X. Ren).

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The present study reports the fabrication of mesoporous-structured ratiometric molecularly imprinted sensors using a combined surface-imprinted and ratiometric fluorescence method. The sensors were subsequently examined in the selective and sensitive determination of 2,4,6-trinitrophenol (TNP). In the preparation of the ratiometric system, the reference dye CdTe quantum dots were embedded in silica core particles via the Stöber method; the functional target sensitive dye AAMBT & SiO₂, which was obtained via polymerization of 2-acrylamide-6-methoxybenzothiazole (AAMBT) with allyltriethoxysilane, was embedded in the mesoporous silica shell. In the surface imprinting process, cetyltrimethylammonium bromide was employed to create mesoporous-structured silica to promote quenching of AAMBT by TNP via resonance energy transfer, thereby enhancing the sensitivity of the sensor. Under optimum conditions, the ratiometric fluorescence molecularly imprinted polymer sensors achieved a detection limit of 43 nM within 3 min. The practical application of the developed sensor in real water samples was successfully demonstrated through analysis of TNP in water samples, achieving satisfactory recoveries of 92–104%. Thus, a convenient and practical method for preparing highly selective and sensitive ratiometric fluorescence sensors is presented herein, providing a prospective method for rapid trace pollutants analysis in complex water samples.

1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic polymeric matrices that possess tailor-made binding sites of specific shape, size, and functional groups conferred by the template used during synthesis (Guo et al., 2015). MIPs have been employed as recognition media to improve the selectivity and efficacy of detection of biological and/or chemical analytes (Da Silva et al., 2014; Hoshino et al., 2008; Lehota et al., 2010; Prasad et al., 2014). MIPs are usually prepared by copolymerization of functional monomers with cross-linkers in the presence of a template molecule. Binding cavities complementary to the template are created after removal of the template molecule, making MIPs highly selective towards the template molecule. Recently, surface imprinting has become a more prevalent technique to prepare MIPs. This technique possesses several advantages such as better site accessibility, lower mass transfer resistance, more effective removal of template, and better defined material shape (Gong et al., 2016; Li et al., 2015a, 2015b; Yang et al., 2016). Surface imprinting can also shorten the time of template recognition and enhance the detection sensitivity. Generally, functional monomers generating signals (i.e., fluorescent or electrical) through interaction with the template molecule are attached to the MIP during polymerization (Moreno-González et al., 2014; Nicholls et al., 2013; Shen et al., 2013). Fluorescent signal is more frequently used for detection, as the rebinding of template molecule to the MIP causes a fluorescence change of the fluorophore moiety close to the binding sites (Cao et al., 2013; Inoue et al., 2013; Li et al., 2015a, 2015b).

Fluorescence-based MIPs (FL-MIPs) are superior for fabricating chemo/biosensors because of their high sensitivity, excellent specificity, and simplicity of detection mechanism (Huang et al., 2013; Orozco et al., 2013; Wagner et al., 2013). The intrinsic merits of FL-MIPs have made them ideal sensors for trace substances detection in a complicated environment. To better visualize the detection, a ratiometric fluorescence technique is introduced to the fabrication process of MIPs. The ratiometric fluorescence technique can improve the detection sensitivity of substances at trace levels and the built-in correction for the environmental effects (Zhu et al., 2014). To fabricate a ratiometric fluorescence-based sensor exciting one single wavelength, two fluorescent dyes are used with one responding to the target and the other one working as a reference (Xu and Lu, 2015a). Organic dyes are...
usually used due to their superior optical properties and chemical functionalization capability (Feng et al., 2015). Another type of dyes used can be quantum dots (QDs) which have special optical properties with broad excitation and narrow emission (Adegoke and Forbes, 2015). Therefore, it is easy to build a ratiometric fluorescence-based sensor by using QDs.

The ratiometric fluorescence-based sensors have been well developed (Yao et al., 2013), while the development of ratiometric FL-MIPs is scarce. Xu and Lu developed a ratiometric FL-MIPs sensor by using CdTe QDs as both the response and reference dyes for the detection of 2,4,6-trinitrotoluene (Xu and Lu, 2015b). In another study, they developed a ratiometric FL-MIPs sensor by using CdTe QDs as the response dye and an organic molecule (hematoporphyrin) as the reference dye for the detection of melanime in milk samples (Xu and Lu, 2015a). A ratiometric FL-MIPs sensor with the combination of a QD and an organic dye is preferred, as QDs can be excited by the excited light of organic dyes owing to the broad excitation spectrum of QDs (Tyrakowski and Snee, 2014; Xu et al., 2013a, 2013b). Moreover, the narrow emission spectrum of QDs can potentially avoid the overlap with the emission spectrum of organic dyes.

In the present study, we developed a novel ratiometric FL-MIPs sensor for visual recognition and detection of 2,4,6-trinitrophenol (TNP), a nitroaromatic explosive Red CdTe QDs and blue-fluorescent organic dye2-acrylamide-6-methoxybenzothiazole (AAMBT) were first synthesized. Then, QDs@SiO2, which represents the core of the MIP and the reference dye, was prepared by embedding QDs into SiO2 via Stöber method. The ratiometric FL-MIPs sensor was then mainly prepared via surface imprinting of QDs@SiO2, with AAMBT as the response dye and TNP as the template. The emission wavelength-dependent photoluminescence quenching behavior of the MIPs by TNP was examined using Stern-Volmer analysis. On the basis of the imprinting recognition and ratiometric fluorescence with color change, the developed sensor could be applied to TNP sensing in water samples with high selectivity and sensitivity.

2. Materials and methods

2.1. Materials

2-amino-6-methoxybenzothiazole (AMB), acryloyl chloride, TNP, melamine (MM), and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma-Aldrich (Shanghai, China). Tetraethylorthosilicate (TEOS), allyltriethoxysilane (ATES), 3-mercaptopropyltrimethoxysilane (MPS), azobisisobutyronitrile (AIBN), (3-aminopropyl)triethoxysilane (APTES), phenol, 2,4-Dinitrophenol (DNP), and 4-Nitrophenol (4-NP) were obtained from Beijing J & K Co., Ltd. (Beijing, China). The remaining reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). all the other solutions of metal ions were all prepared from their chloride salts with doubly distilled water. All reagents were of analytical grade and used without further purification.

2.2. Characterization

Fluorescence measurements were performed with an F-7000 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). Fourier transform infrared (FT-IR) spectroscopic measurements were performed on a Nicolet NEXUS-470 spectrometer (Madison, USA) equipped with a DTGS KBr detector and a KBr beam splitter in the transmission mode. Scanning electron microscope (SEM) was observed by a SU8010 scanning electron microscope (Hitachi, Japan). Transmission electron microscopy (SEM) was observed by a Tecnai G2 F30 transmission electron microscope (Hitachi, Japan) Brunauer-Emmett-Teller (BET) surface areas were determined by nitrogen adsorption/desorption analysis at 77.3 K using a Nova Station A 4200e (Boynton Beach, FL, USA).

2.3. Synthesis of the fluorescent functional monomer

The fluorescent functional monomer was synthesized according to our previous report (Li et al., 2016). Typically, AMB (0.022 mol) was dissolved in a round-bottom flask containing tetrahydrofuran (THF; 40 mL) and water (40 mL). Sodium hydrogen carbonate (0.044 mol) was then added, followed by dropwise addition of acryloyl chloride (0.044 mol) over 15 min into the flask at 0–5 °C. The molar ratio of AMB/acryloyl chloride was set as 1:2. The mixture was stirred for 3 h, and THF was subsequently evaporated. The obtained product was dissolved in ethyl acetate (40 mL), and then stirred for 2 h. After filtering, the ethyl acetate was was washed successively with water (50 mL), saturated sodium bicarbonate solution (50 mL), water (50 mL), and brine (50 mL). Finally, ethyl acetate was removed via rotary evaporation at room temperature. 2-acrylamide-6-methoxybenzothiazole (AAMBT) was obtained and refrigerated before use.

Then, AAMBT (0.01 mmol) and ATES (10 μL) were dissolved in CHC13 (10 mL). ABIN (3 mg) was then added. The molar ratio of AAMBT/ATES was set as 1:4. The mixture was purged with nitrogen for 10 min, and then was stirred at 65 °C for 6 h. After filtering, the solution was washed successively with water (50 mL) and brine (50 mL). Finally, CHC13 was removed via rotary evaporation at 35 °C to give the fluorescent functional monomer AAMBT & SiO2.

2.4. Synthesis of the red-emitting MIP core QDs@SiO2

CdTe QDs were first synthesized via a simple hydrothermal synthesis method. Briefly, thiglycic acid (TGA; 18 μL) was added to a solution (50 mL) containing Cd(NO3)2 (61.69 mg). The pH of the resulting solution was adjusted to 10.5 by addition of 1 M NaOH. Then, a solution (200 mL) containing Na2TeO3 (88 mg) and NaBH4 (80 mg) was added to the above solution. The molar ratio of Na2TeO3/Cd(NO3)2/TGA was set as 1:5:6.5. The resulting mixture was refluxed at 100 °C for 12 h to obtain the TGA-capped red-emitting CdTe QDs solution.

The red-emitting MIP core QDs@SiO2 was prepared by a modified Stöber method. Typically, red QDs solution (10 mL), ultrapure water (20 mL), and ethanol (40 mL) were successively introduced into a 250 mL one-neck flask, and the mixture was stirred for 10 min at room temperature. Then, MPS (40 μL) was introduced, and the resulting solution was stirred for 6 h in the dark. Subsequently, TEOS (1 mL) was added dropwise to the solution, followed by ammonium hydroxide (1 mL), and the mixture was left to react for 12 h. The obtained QDs@SiO2 were centrifuged at 8000 rpm for 10 min and washed with deionized water until the pH of the solution was neutral.

2.5. Synthesis of ratiometric fluorescent MIP

The ratiometric TNP-imprinted materials were prepared on the surface of red QDs@SiO2 by a surface molecular imprinting method. Typically, the obtained AAMBT & SiO2 (2 mg) were dispersed in THF (1 mL). Then, ethanol (5 mL), APTES (20 μL), and TNP (5 mg) were added to the solution string over 15 min to obtain the prepolymers. Then, red QDs@SiO2 (10 mg) were dispersed in water (20 mL) and CTAB solution (0.8 mL, 0.2 M), to which NaOH (0.1 mL, 0.2 M) was added. After stirring for 30 min, the prepolymers and TEOS (200 μL) were added to the QDs@SiO2 solution. The gram ratio of AAMBT & SiO2/QDs@SiO2/TNP was set as 2.5:10. The mixture was stirred for 12 h in the dark, and the obtained materials were centrifuged at 8000 rpm for 8 min. Finally, a mixture of ethanol/acetone (8.2, v/v) was used as the washing solution to remove the CTAB template under ultrasound assistance. Both UV absorption and fluorescence spectroscopy analyses were employed to confirm complete removal of the template. The non-imprinted polymers (NIPs) were prepared in the same way above but without template TNP.
2.6. Detection of TNP

Fluorescence measurements were conducted at the excitation wavelength of 365 nm. The detection process was performed in a NaH₂PO₄/Na₂HPO₄ buffer solution (pH=8). Different concentrations (100–700 nM) of TNP were detected using 5 mL of 200 mg/L ratiometric FL-MIPs. The fluorescence intensity was measured immediately after 3 min of addition of the MIP sensor to the solution of TNP in order to allow stabilization of the fluorescence spectra following reattachment of the template to the FL-MIPs. The fluorescence spectra of the samples were recorded using excitation and emission slit widths of 10 nm, an excitation wavelength of 365 nm, and a photomultiplier tube voltage of 400 V. Control experiments involving NIPs were performed in a similar fashion to those involving MIPs.

2.7. Recovery of TNP in water samples

Water samples examined in the subsequent study included water aliquots from Xiaoping River (Beijing), deionized water, tap water, and mineral water. The river water samples were filtered twice to remove any solid suspensions using qualitative filter membranes with 0.22 µm pores and then centrifuged at 8000 rpm for 15 min. Deionized water and tap water (obtained from our laboratory), and mineral water (purchased from the supermarket) were directly used without any pretreatment. The detection process was performed in a NaH₂PO₄/Na₂HPO₄ buffer solution (pH=8). During the sensing studies, the four water samples were spiked with 100 nM TNP separately, and were detected using 5 mL of 200 mg/L ratiometric FL-MIPs. The fluorescence intensity was measured immediately after 3 min of addition of the MIP sensor to the TNP solution. The relative standard deviation (RSD) was obtained by conducting the experiment in triplicate under the same condition.

3. Results and discussion

3.1. Preparation of the blue-emitting functional monomer

Organic fluorescent monomers have received much attention; however, their application in ratiometric FL-MIPs was seldom reported. In the present study, commercially available benzothiazole derivative AMB was selected as the fluorescent material to prepare the blue fluorescence dye. To enhance the fluorescence intensity of the benzothiazole group, a one-step reaction was performed involving acryloyl chloride as reported in our previous work (Li et al., 2016). Furthermore, in order to be polymerized with TEOS in water surrounding, the organic fluorescent monomer was modified with ATEs in a system without water and oxygen. The solvent was dehydration, and the mixture was purged with nitrogen for 10 min to remove the oxygen. In this case, the double bond between AAMBT and ATEs was polymerized together. The fluorescent monomers AAMBT & SiO₂ were crosslinked with TOES. Hence, the organic fluorescent monomers could be introduced into the MIPs for surface imprinting. Also, the fluorescence spectrum of AAMBT & SiO₂ is studied. As shown in Fig. S1, the excitation and emission peaks are 363 and 427 nm, respectively. As we know, excitation peaks of the synthetic Ds was 365 nm, which was close to the AAMBT & SiO₂. Therefore, AAMBT & SiO₂ and QDs could be used as the dyes excited by one light source in the ratiometric FL-MIPs.

3.2. Preparation and characterization of FL-MIPs

Scheme 1 illustrates the preparation of the FL-MIPs. Firstly, the red-emitting CdTe QDs were embedded in the SiO₂ nanoparticles as the core QDs@SiO₂. In this process, to avoid the fluorescence quenching of QDs by TNP, an appropriate amount of SiO₂ is necessary to hybridize with the QDs. As shown in Fig. S2 (black line), the ratio of the QDs@SiO₂ fluorescence intensity (Iₑ) responding upon 300 nM TNP to the initial fluorescence intensity (I₀) became 1 gradually, indicating that the fluorescence quenching can be avoided under the protection of SiO₂. On the other hand, the thickness of SiO₂ may prohibit the emission spectrum of the QDs (Fig. S2, blue line), so special precaution should be taken to the amount of TEOS used during the enclosing process. Fig. S2 shows the fluorescence intensity of QDs@SiO₂, which was dependent on the amount of TEOS used. A TEOS amount of 1 mL was suitable for polymerizing the QDs@SiO₂ core. Secondly, TNP was pre-polymerized with functional monomer APTES and the fluorescence monomer AAMBT & SiO₂ to form the resonance energy transfer (RET) system via ionic and hydrogen bonding. Finally, the TNP-imprinted silica shell was prepared by a surface imprinting method using TEOS as a cross-linker. During the surface imprinting process, CTAB was used to form the mesoporous structure-imprinted silica shell. Since the fluorescence of AAMBT was quenched and the fluorescence of QDs was preserved, the FL-MIPs displayed red under a UV lamp. After removal of CTAB and TNP, a MIP mesoporous structure and TNP-imprinted shell were obtained on the surface of red QDs@SiO₂. Then, the FL-MIPs displayed blue under a UV lamp due to the recovery of AAMBT fluorescence. The mesoporous structure afforded exposure of more binding sites to the template as well as greater accessibility of the template and fluorescence monomers in the ET system. When MIPs were dispersed in the template solution, the template molecules were rebound to the binding site rapidly. The fluorescence of AAMBT was quenched while the fluorescence of QDs was preserved. Therefore, the TNP template can be visually detected by the FL-MIPs with a color change from blue to red..

FT-IR spectroscopy was used to monitor the progress of the imprinting process during removal and rebinding of TNP to MIPs. As

Scheme 1. Schematic illustrations for the preparation process of FL-MIPs. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)
observed in Fig. 1A, the characteristic peaks at 469 and 798 cm⁻¹ could be ascribed to Si-O stretching vibration, and the wide and strong absorption band at 1100 cm⁻¹ could be attributed to stretching vibrations of Si-O-Si, indicating the presence of SiO₂ matrices in the FL-MIPs. The stretching vibration of N-H observed at 3423 cm⁻¹ could be attributed to stretching vibration of the acylamino group, and the small peaks in the spectra of the MIPs; these peaks were assigned to stretching vibrations of C=O groups in the acrylamide moiety of AAMBT. Furthermore, the presence of these peaks indicated successful embedding of AAMBT into the MIPs. Additionally, as shown in Fig. 1A(a), the new absorption peak observed at 1380 cm⁻¹, which could be assigned to -NO₂ stretching vibration of the acylamino group, and the small peaks in the region of 1450–1390 cm⁻¹, corresponding to the Ar group, proved the successful reattachment of TNP. Thus, these results indicated that the template effectively re-attached to the binding site in the MIP.

The morphology of QDs@SiO₂ and FL-MIP/NIP was characterized by TEM and SEM. Fig. 1B and C revealed that the QDs@SiO₂ core was smooth and uniform, with a diameter of 200 nm and the MIP particles featured a diameter of 260 nm. Furthermore, the MIP particles had a rough surface, indicating that a large number of functional imprinted cavities formed on the surface of MIP. N₂ sorption analysis was conducted to further study the mesoporous structure of the MIPs (Fig. S3). The N₂ sorption isotherm of the obtained TNP-imprinted FL-MIPs displayed a rapid N₂ uptake in the relative pressure range of 0.2–0.4, which is characteristic of mesoporous structures. And the specific BET surface area of the MIPs was 646.0044 m²/g.

The fluorescence stability of the FL-MIPs was subsequently examined by measuring the fluorescence intensity at five-day intervals. The materials were examined under dry and dark conditions. As shown in Fig. S4, the fluorescence intensity remained mostly unchanged at room temperature for at least 100 days, thus demonstrating that the fluorescence of the AAMBT-based MIPs was stable and could be exploited for long periods of time.

3.3. Analytical performance of ratiometric FL-MIPs

The variations (medium, pH, absorption time) in the fluorescence response of the ratiometric FL-MIPs to TNP were studied. Considering the existence environment of waste TNP and the practical application of the FL-MIPs, water was chosen as the dispersion medium. To investigate the adsorption time of the MIPs, dynamic adsorption test was carried out and the results were shown in Fig. S5A. Complete adsorption was achieved in 2 min, so 3 min is chosen as a proper time for the rebinding process. The fluorescence intensity change of the ratiometric FL-MIPs upon different pH values were also studied. As shown in Fig. S5B, pH 8.0 was superior for the adsorption process and the fluorescence intensity ratio was optimum. Because pH could influence both the rebinding of TNP and the emission of the QDs. The FL-MIPs displayed a rapid N₂ uptake in the relative pressure range of 0.2–0.4, which could be attributed to the rebinding of TNP and the emission of the QDs. The FL-MIPs intensity was low at pH values of less than 7. In contrast, with increasing pH, the quenching amount of MIP decreased rapidly because the molecular-imprinted silica layer could ionize at high pH, thus affecting the interaction between the template and the fluorescence sensor. Taking the above factors into account, phosphate buffer solution (0.01 mol/L, pH 8.0) was selected as the optimal binding medium for the subsequent experiments.

The fluorescence spectra of blue-emitting AAMBT & SiO₂, ratiometric FL-MIPs, and red-emitting QDs@SiO₂ solutions were recorded. AAMBT & SiO₂ showed fluorescence peak at 427 nm upon excitation by 365 nm UV light as well (Fig. S6a). The red QDs-embedded silica nanoparticles showed fluorescence peak at 618 nm and exhibited strong red fluorescence upon excitation by 365 nm UV light as well (Fig. S6b). When the blue-emitting AAMBT-embedded silica nanoparticles (Fig. S6a) were coated on the surface of the red QDs-embedded silica nanoparticles, the ratiometric fluorescence response exhibited two well-resolved emission bands upon single-wavelength excitation at 365 nm and displayed blue-pink fluorescence (Fig. S6c). These results indicated that core–shell-structured ratiometric fluorescence probes were successfully prepared.

3.4. Proposed detection mechanism of the FL-MIPs

Generally, MIPs were synthesized via the copolymerization of a functional monomer with a large quantity of cross-linker, in the presence of a template molecule. After the removal of the template, imprinting cavities with the memory of the shape and the functional groups of TNP was formed. For the physical feature, only template or compound with similar shape could enter the cavity; for the chemical feature, functional monomer APTES could attract TNP via ionic interaction. Therefore, when MIPs were dispersed in the template solution, the template molecular TNP could be rebound to the binding site via ionic bond rapidly.

To understand the detection mechanism of TNP by the prepared FL-MIPs, firstly the absorption of TNP and the fluorescence of FL-MIPs were studied, and the results are shown in Fig. 2A. The absorption spectrum of TNP overlapped with the emission spectrum of FL-MIPs. AAMBT and TNP acted as the donor and acceptor respectively, which could induce a resonance energy transfer (RET) process (Zhou et al., 2014). Fig. 2B illustrates the scheme of RET. During UV irradiation, electrons in AAMBT are excited from the HOMO level to the LUMO level. In the absence of TNP, the excited electrons return to the HOMO level, resulting in blue emission. The blue emission of AAMBT was stronger than the red emission of QDs, leading to the observation of blue emission. In the presence of TNP, the energy of AAMBT was transferred to TNP, causing the quenching of...
blue AAMBT fluorescence. A red emission was observed as the red fluorescence of the QDs was preserved. Thus, visual detection of TNP by RET can be achieved.

Additionally, as shown in Fig. 3, the fluorescence intensity of FL-MIPs decreased gradually as re-attachment of TNP proceeded. Moreover, the fluorescence intensity of MIPs (Fig. 3a) was quenched to a greater extent than that of NIPs (Fig. 3b), suggesting the formation of specific recognition sites with predetermined selectivity for TNP in the MIPs. In contrast, NIPs had no imprinting cavities. These results revealed the occurrence of energy transfer from the FL-MIPs to TNP that led to fluorescence quenching.

3.5. Sensitivity and selectivity of the probe

In the absence of TNP, the ratiometric probe emitted two well-resolved emission peaks centered at 430 and 618 nm. Upon addition of TNP, the fluorescence intensity at 430 nm decreased until quenching while the intensity at 618 nm remained unchanged, as shown in Fig. 3. The ratio of the fluorescence intensity was closely related to the TNP concentration range studied i.e., 100–700 nM. Thus, that ratio could be used for quantification of TNP, with a detection limit as low as 43 nM (3 S/N). Owing to the changes in the intensity ratio of the two dyes, the fluorescence color of the ratiometric probe solution changed accordingly, as demonstrated in the inset of Fig. 3. A slight decrease in the emission intensity at 430 nm could result in distinguishable color changes from the original background. Therefore, visual detection of TNP by the naked eye is possible. The dosage response of the mesoporous-structured ratiometric NIPs to TNP was also examined. As observed in Fig. 3B, the fluorescence intensity of NIPs can also be quenched by TNP. However, the degree of quenching was considerably lower, and quenching occurred within a narrower TNP concentration range. Furthermore, fluorescence color changes were not obvious. This phenomenon was attributed to the absence of recognition sites in the NIPs. Hence, TNP cannot penetrate the NIP matrix. Only the fluorescence intensity of AAMBT that is located on the material surface could be quenched, whereas most of QDs did not display spectral changes. The advantages of the ratiometric fluorescence probe were further assessed by comparison with single-fluorescence probes, wherein only AAMBT are embedded. As observed in Fig. S7, color changes displayed by the single-fluorescence probe upon addition of TNP were difficult to observe and the associated response concentration range was narrow. Thus, the comparison study confirmed that the ratiometric fluorescence probe possesses higher sensitivity and reliability than single-fluorescence quenching probes toward visual detection of target molecules. Regarding the mesoporous-imprinted silica, which featured a high surface area, most of the recognition sites were located on the surface of the silica matrix. Hence, the probability of the target molecules entering the recognition sites is higher; accordingly, the extent of fluorescence quenching of AAMBT is greater. Hence, high surface areas and better accessibility to the binding sites are the reason for the enhanced sensitivity. Additionally, to realize highly efficient and robust MIPs, their reusability for repeated operation in real applications is important. Accordingly, the lifetime of the mesoporous-
structured ratiometric fluorescence MIPs was evaluated by monitoring the fluorescence intensity and quenching efficiency for eight successive cycles toward the detection of 300 nM TNP. As observed in Fig. S8, the sensor could maintain its fluorescence intensity and detection sensitivity with no major decreases during those eight cycles.

Subsequently, the selectivity of the mesoporous-structured ratiometric fluorescence MIPs and NIPs was assessed. The fluorescence intensity ratios of the probe were recorded (i.e., $I_{530}/I_{618}$) in the presence of TNP and other template analogs (MM, phenol, 4-NP, DNP). As observed from Fig. 4, 75% of the fluorescence intensity of the MIPs solution was quenched by 500 nM TNP, following with color change under UV light shown in the insert picture. In contrast, the degree of AAMBT fluorescence quenching was lower in the presence of the template analogs, and no obvious color changes was displayed. Unlike the MIP, which had cavities of the same shape as TNP, the analogs did not have cavities, thus resulting in smaller changes in the fluorescence intensity. Therefore, the FL-MIPs exhibited specific recognition toward TNP.

3.6. Practical application of FL-MIPs in real-sample analysis

To evaluate the application of the FL-MIPs, deionized water, tap water, mineral water, and river water were analyzed. The river water samples were filtered twice to remove any solid suspensions using qualitative filter membranes with 0.22 μm pores and then centrifuged at 8000 rpm for 15 min the others were used without any pretreatment.

Because TNP was not detected in the four water samples using the FL-MIPs, a recovery test was performed, wherein the water samples were spiked with TNP at a concentrations 100 nM. As shown in Table 1, the obtained recovery results and the RSD data, which varied from 92% to 104%, agreed with each other. Hence, the results demonstrate that FL-MIPs has promising application in detecting TNP in water samples.

As reported in the literature, many detection methods were employed including photoluminescence, and methods based on the use of copper nanoclusters, organic fluorescence sensors, and MoS2 sensors. These methods use fluorescence or photoluminescence as detecting means. As shown in Table 2, we made a comparison among the previous works and the present work. The limit of detection (LOD) of our method is 43 nM, which is better than other reported methods. The higher sensitivity of the current method is due to the specificity of the MIPs and the sensitivity of the ratiometric fluorescence sensor. Besides, the previous methods have major drawbacks e.g., they require lengthy multi-step procedures and large volumes of hazardous solvents, consequently increasing the level of exposure of workers to these hazardous solvents. Such complex procedures can be simplified using the fluorescent MIPs developed in the present study owing to the high specificity of the imprinted cavities and their sensitivity to fluorescence. As a result, our proposed method is sensitive and selective for practical and rapid determination of TNP in complex environment.

4. Conclusion

Herein, the fabrication and application of a mesoporous-structured ratiometric fluorescence molecularly imprinted sensor in the determination of TNP using QDs as a reference dye and a functional organic dye as a target sensitive dye were presented. The FL-MIPs sensor exhibited excellent selectivity and sensitivity owing to the combined use of the ratiometric fluorescence system and mesoporous silica material. Thus, the developed method proposed herein offers a rapid, convenient, and practical means to prepare highly sensitive and selective ratiometric fluorescence sensors for potential application in trace pollutants analysis of complex samples.
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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2016.09.101.

References
