

# Carbon Dots and Quantum Dots-Based Nanohybrid as a Ratiometric Fluorescent Probe for Fe<sup>3+</sup> and Phytic Acid Sensing

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The selective recognition of metal ions is very important and the development of novel chemosensor is still a challenge. Herein, a single dual-emissive fluorescence probe was developed to detect Fe<sup>3+</sup> and phytic acid (PA). Red quantum dots (CdSe) were embedded into silica nanoparticles for an internal standard emission, whereas blue carbon dots (CDs) were covalently linked onto the surface of silica as a sensing element to achieve a more precise and sensitive detection. The fluorescence of the CDs was effectively quenched by Fe<sup>3+</sup> via a photoinduced electron transfer (PET) process. Fe<sup>3+</sup> could selectively bind to phytic acid, thus leading to the recovered fluorescence of the CDs in the presence of Fe<sup>3+</sup>. Upon addition of PA to the CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex dispersion, the fluorescence of the CDs significantly increased. The ratiometric probe enabled the selective detection of PA within 0.08–1.6 mM and a limit of detection of 1.5 μM. Overall, this method provided high selectivity, stability, and recovery ratio in the detection of Fe<sup>3+</sup> and PA.

## 1. Introduction

Heavy metal pollution, due to industrialization and improper waste disposal, is a world-wide concern in view of the high toxicity of heavy metals and its harm to the environment (Chen et al., 2016). Heavy metal ions in the environment absorbed by human body through the biological enrichment of the food chain will affect human normal physiological activities. As a generally used metal, a small intake of Fe<sup>3+</sup> ions are beneficial to the human body. However, excessive intake of Fe<sup>3+</sup> ions is associated with high toxicity and can be harmful to the liver, lungs, and rectum. Phytic Acid (PA) serves as an energy storage component and regulator of vesicular by binding to various proteins. It has been reported that wheat bran could reduce the early biomarkers for colon cancer risk because of its PA content (Wu et al., 2009). Therefore, PA has been widely used in food, medicine, daily chemical industry, textile industry, plastic industry and polymer industry. However, the excretion of PA into water systems results in the eutrophication of water bodies and many environmental problems. In order to understand the physiological function and provide effective guidance for daily dietary intake, the quantitative analysis of PA is particularly important (Cao et al., 2011).

Probes based on changes in a single emission intensity are likely to be affected by several factors, such as probe concentration, reagent dosage, instrument efficiency, and environmental conditions (Zou et al., 2016). In contrast, ratiometric fluorescence sensing is based on dual-emission as it involves the simultaneous measurement of two fluorescence signals at different wavelengths, which could overcome the above-mentioned unfavourable conditions, thus achieving a more precise and sensitive detection. Ratiometric fluorescent probes for the sensitive detection of pH, biothiols, temperature, and ions have been developed (Yue et al., 2017).

Compared to organic dyes, semiconductor nanocrystalline quantum dots (QDs) show several distinct advantages, such as size-controlled luminescence, as well as greater brightness and stability with respect to

photobleaching. Moreover, the excellent fluorescence stability of QDs can result in improved sensitivity and prolonged lifetimes when they are used as fluorescent probes (Zhang et al., 2011). As a newly emerging class of fluorescent probes, carbon dots (CDs) have also received considerable attention owing to their unique properties, such as high-water solubility, low toxicity, amenability towards surface modification, excellent biocompatibility, and high photostability. Recently, CDs have been applied for the optical sensing of many analytes, including proteins, small molecules, and metal ions (Guo et al., 2015). Specifically, CDs have been widely used to detect various metal ions in aqueous solution due to their high quantum yields and good photostability (Yao et al., 2013). Moreover, CDs are easy to surface modification and have been used as a fluorescent probe for the detection of PA (Gao et al., 2015). By combining CDs with mesoporous silica spheres, a good detection performance of both copper ions and L-cysteine was achieved (Zong et al., 2014). In this study, a fluorescence probe was developed by using CDs as sensing element and CdSe as an internal standard emission. And this hybrid scaffold was further applied for the ratiometric, sensitive, and selective detection of  $\text{Fe}^{3+}$  and PA.

## 2. Materials and methods

Tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilan (APTMS), TritonR X-100, n-hexanol, bovine serum albumin (BSA), ammonia solution ( $\text{NH}_3 \cdot \text{H}_2\text{O}$ ), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and cyclohexane were purchased from Aladdin (Shanghai, China). Lysine and phytic acid (PA) were obtained from Alfa Aesar (Shanghai, China). CdSe/ZnS (QDs) solution was obtained from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). All other reagents were of analytical grade and were commercially obtained. Ultrapure water (resistivity  $>18 \text{ M}\Omega$ ) obtained from a Millipore water purification system was utilised throughout the experiments.

### 2.1 Synthesis of fluorescent CDs

CDs were prepared according to a literature protocol. Briefly, 1.46 g of lysine and 4.2 g of citric acid were dissolved in 40 mL of ultrapure water. Then, the resulting solution was transferred into a poly(tetrafluoroethylene)-lined autoclave (100 mL), which was maintained under autogenous pressure at 200 °C for 5 h. After cooling to room temperature, the resultant CDs were purified by dialysis using a 300 Da cut-off dialysis bag for 48 h to remove unreacted small molecule precursors. The final CDs dispersion solutions were stored at 4 °C for further use.

### 2.2 Preparation of CdSe@SiO<sub>2</sub>

7.7 mL of cyclohexane, 1.77 mL of TritonR X-100, 1.8 mL of n-hexanol, and 400  $\mu\text{L}$  of the QDs solution were mixed under vigorous stirring in a 100 mL flask for 10 min. Subsequently, 200  $\mu\text{L}$  of the ammonia solution and 50  $\mu\text{L}$  of TEOS were added into the flask and the mixture was stirred in the dark for 24 h. Then, 36 mL of isopropyl alcohol was added to break the emulsion. The resultant QDs@SiO<sub>2</sub> nanoparticles were washed with ethanol three times, the QDs@SiO<sub>2</sub> were collected by centrifugation (10,000 rpm, 20 min). Next, 2 mg of the as-synthesised QDs@SiO<sub>2</sub> was dispersed in 20 mL of ethanol under stirring in a 100 mL flask. Then, 100  $\mu\text{L}$  of APTMS was added to the flask, which was maintained at 90 °C for 24 h. After cooling to room temperature, the resultant material was collected by centrifugation (15,000 rpm, 10 min), and redispersed in 4 mL of ultrapure water. The final CdSe@SiO<sub>2</sub> dispersion solutions were stored at 4 °C for future use.

### 2.3 Synthesis of dual emission CdSe@SiO<sub>2</sub>-CDs nanohybrids

6 mL of the CDs solution, 180 mg EDC, and 420 mg NHS were dissolved in 2 mL of ultrapure water and stirred for 30 min in the dark. Then, 1 mL of CdSe@SiO<sub>2</sub> was added into the above mixture and stirred for 12 h at room temperature. Finally, the CdSe@SiO<sub>2</sub>-CDs probes were collected by centrifugation and redispersed in 8 mL of ultrapure water. The final CdSe@SiO<sub>2</sub>-CDs dispersion solutions were stored at 4 °C for future use.

### 2.4 Detection of Fe<sup>3+</sup> ions and PA

Fluorescence detection of  $\text{Fe}^{3+}$  ions: 10  $\mu\text{L}$  of different concentrations of  $\text{Fe}^{3+}$  and 0.7 mL of CdSe@SiO<sub>2</sub>-CDs probes were mixed and incubated at room temperature for 15 min.

Fluorescence detection of PA: 10  $\mu\text{L}$  of  $\text{Fe}^{3+}$  (10 mM) and 0.7 mL of CdSe@SiO<sub>2</sub>-CDs probes were first mixed and incubated at room temperature for 15 min. Then, 10  $\mu\text{L}$  of different concentrations of PA were added to the mixture of the probes. All fluorescent spectra were recorded using an excitation wavelength of 380 nm and excitation/emission slits of 2.5 nm.

## 2.5 Detection of PA in real samples

The real river water samples were collected from local river (Tianjin, China). The samples were filtered through 0.22  $\mu\text{m}$  membrane filters to remove large suspended particles. Because no obvious PA in the collected river water samples was detected by the developed assay, a recovery study was performed for determination of the PA by the standard addition method.

## 3. Results and discussion

### 3.1 Preparation and characterization of CDs and CdSe@SiO<sub>2</sub>

The CDs prepared via the hydrothermal treatment of citric acid and lysine were spherical particles with a diameter of about 10 nm (Figure 1a). In this system, citric acid was used as the carbon source. Previous studies demonstrated that amine-containing molecules were able to passivate CDs with some amines, thus increasing the quantum yield and selectivity in the recognition of target analytes. Therefore, lysine was chosen as the surface passivation reagent for this study. To obtain further information about the CDs, FTIR spectroscopy was employed to characterise the resulting samples (Figure 1c). The peaks at 3,440 and 1,720  $\text{cm}^{-1}$  were indicative of O-H stretching vibrations and C=O stretching vibrations, respectively. The peak at 1,380  $\text{cm}^{-1}$  possibly indicated N-H in-plane bending vibrations. The FTIR spectrum indicated that carboxyl (-COOH), hydroxyl (-OH), and amino (-NH<sub>2</sub>) groups were present on the surface of the CDs particles.

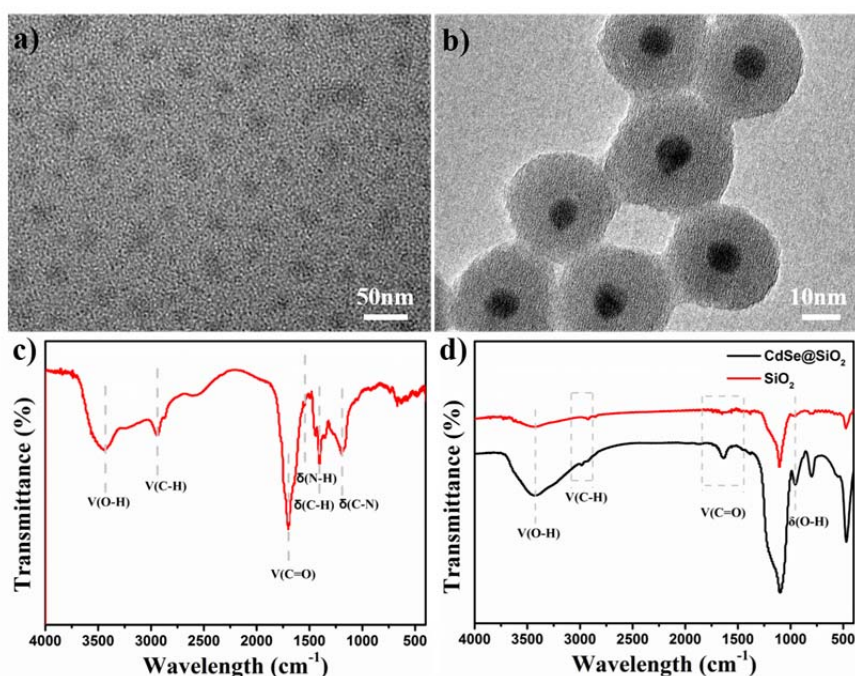


Figure 1: TEM images of the CDs prepared by the hydrothermal treatment of citric acid with lysine (a) and CdSe@SiO<sub>2</sub> (b). FTIR spectra of the CDs (c), and CdSe@SiO<sub>2</sub> and SiO<sub>2</sub> (d).

The as-prepared CdSe@SiO<sub>2</sub> were spherical particles with a diameter of about 35 nm, and CdSe QDs were coated with a silica layer to form a fluorescent core-shell nanostructure (Figure 1b). A silane coupling agent (APTMS) was used to modify the silica surface for further conjugation. The FTIR spectrum of CdSe@SiO<sub>2</sub> exhibited several distinct peaks as compared to that of SiO<sub>2</sub> (Figure 1d). The peaks at 1,675  $\text{cm}^{-1}$  and 950  $\text{cm}^{-1}$  could be attributed to C=O stretching vibrations and O-H out-of-plane bending vibrations, respectively.

The synthesis of the dual-emissive ratiometric fluorescent probe CdSe@SiO<sub>2</sub>-CDs relied on the covalent linking of CDs to the surface of CdSe@SiO<sub>2</sub>-NH<sub>2</sub> by conjugating the surface -COOH with -NH<sub>2</sub> groups through the catalysis of NHS and EDC (Figure 2a). The fluorescence profile of the probe exhibited emission maxima at 633 and 445 nm, which were attributed to CdSe and CDs, respectively, which confirmed that both CDs and QDs were present in the same construct. In addition, the photostability of the probe was confirmed by periodically exposing the aqueous solution of the probe to UV irradiation, followed by acquisition of the emission spectra. No significant change in the relative fluorescence intensity was observed, indicating the photostability of the ratiometric fluorescence probe in the aqueous solution.

### 3.2 Principle behind Fe<sup>3+</sup> ions and PA detection

In CdSe@SiO<sub>2</sub>-CDs, the red fluorescent CdSe core is stabilised within the interior of SiO<sub>2</sub> and it plays a role of the internal standard, whereas the blue fluorescence of CDs at the surface of SiO<sub>2</sub> acts as a sensing unit. The fluorescence of the CDs was significantly quenched in the presence of Fe<sup>3+</sup>, when 10 μL of ferric chloride (10 mM) solution was added to 0.7 mL of CdSe@SiO<sub>2</sub>-CDs probes. The interaction between Fe<sup>3+</sup> and the carboxyl or aromatic hydroxyl groups on the surface of the CDs might be responsible for the formation of CDs/Fe(III) complexes. Previous studies demonstrated that the CDs surface could have a significant impact on the band gap and excited state of the CDs, thus resulting in changes in the steady-state emission and fluorescent lifetimes. Since the outer electronic structure of Fe<sup>3+</sup> is 4s<sup>2</sup>3d<sup>5</sup> and the d orbitals are unfilled, the electrons in the excited state of CDs would be easily transferred into the unfilled 3d orbitals of Fe<sup>3+</sup> upon excitation, leading to the fluorescence quenching of CDs through a photoinduced electron transfer (PET) process (Figure 2b). Because there was little spectral overlap between the absorption spectrum of Fe<sup>3+</sup> and the emission spectrum of CDs, the resonance energy transfer should not be the dominant mechanistic pathway. It is well known that multiphosphates exhibit high affinities towards Fe<sup>3+</sup> ions due to their multidentate nature. Therefore, we inferred that Fe<sup>3+</sup> could selectively bind to PA, and lead to the fluorescence recovery of the CDs, making the CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex a fluorescent light-on probe for the detection of PA. As expected, the fluorescence intensity at 445 nm significantly increased upon addition of PA to the CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex. In a control experiment (i.e., no Fe<sup>3+</sup> ions), no fluorescence intensity change was observed upon addition of PA to the CdSe@SiO<sub>2</sub>-CDs solution. Moreover, we have measured the zeta potential of the CdSe@SiO<sub>2</sub>-CDs at different conditions to test their surface characteristics. The zeta potential of the CdSe@SiO<sub>2</sub>-CDs shifted from -8.93 to 20.42 mV when Fe<sup>3+</sup> were added, but the value decreased to -10.35 mV upon the addition of PA to the CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex solution. This result confirmed that the binding of PA with Fe<sup>3+</sup> released from CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex leads to the recovery of fluorescence intensity.

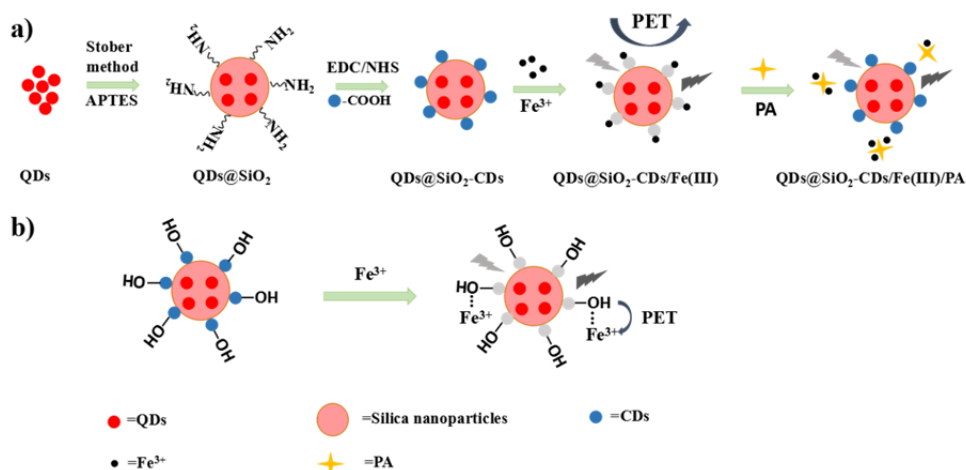


Figure 2: Schematic illustrations of (a) the synthesis of dual-emissive ratiometric fluorescent CdSe@SiO<sub>2</sub>-CDs nanohybrid for the detection of Fe<sup>3+</sup> and phytic acid (PA), and (b) principle behind Fe<sup>3+</sup> detection.

### 3.3 Fluorescence response and selectivity of CdSe@SiO<sub>2</sub>-CDs for Fe<sup>3+</sup> sensing

Upon addition of increasing amounts of Fe<sup>3+</sup>, the fluorescence intensity of the CDs signal was gradually quenched (Figure 3a). The fluorescence intensity ratios of the ratiometric probe, ( $F_0/F_A$ ), was plotted as a function of Fe<sup>3+</sup> concentration, where  $F_0$  and  $F_A$  are the fluorescence intensity of the CdSe@SiO<sub>2</sub>-CDs at 445 and 633 nm, respectively (Figure 3b). The value of  $F_0/F_A$  decreased linearly with increasing Fe<sup>3+</sup> concentrations from 9 to 120 μM ( $R^2=0.995$ ). Within this concentration range, the relative standard deviation (RSD) values were in the range of 1.25 %-5.24 %, which indicated a limit of detection of 0.26 μM. Compared to the reported methods, the developed system exhibited a wider linear range and a lower detection limit for the sensing of Fe<sup>3+</sup>.

To examine the selectivity of the ratiometric fluorescence probe for Fe<sup>3+</sup>, the fluorescence intensity ratios of the ratiometric probe ( $F_0/F_A$ ) were also recorded in the presence of 10 μL of Fe<sup>3+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Ni<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>2+</sup>, Al<sup>3+</sup>, Zn<sup>2+</sup>, Mg<sup>2+</sup>, Cr<sup>3+</sup>, Ba<sup>2+</sup>, Cu<sup>2+</sup> and Pb<sup>2+</sup> (Figure 4a). Notably, 85 % of the fluorescence intensity of the ratiometric fluorescence probe was quenched by Fe<sup>3+</sup> (10 mM), whereas no significant changes in the  $F_0/F_A$

values were noted in the presence of other metal ions at the same concentration, indicating that this probe exhibited excellent selectivity in regard to the sensing of  $\text{Fe}^{3+}$ .

### 3.4. Detection of PA using CdSe@SiO<sub>2</sub>-CDs

The fluorescence intensity of the CDs part of the CdSe@SiO<sub>2</sub>-CDs/Fe(III) system was gradually recovered upon addition of increasing amounts of PA (Figure 5a). The fluorescence recovery factor,  $(F_0 - F_A)/F_A$ , was plotted as a function of PA concentration, where  $F_0$  and  $F_A$  are the fluorescence intensity of the CdSe@SiO<sub>2</sub>-CDs/Fe(III) at 445 and 633 nm, respectively (Figure 5b). The value of  $(F_0 - F_A)/F_A$  increased linearly as a function of PA concentration from 0.08 to 1.6 mM ( $R^2=0.990$ ). Within this concentration range, the RSD values were in the range of 1.95 %-4.78 %, and the limit of detection was estimated to be 1.5  $\mu\text{M}$ . The proposed method is comparable or superior to the literature previous work for PA determination. Furthermore, the fluorescence assay developed in this study is also a low-cost, facile and efficient method.

### 3.5. Analysis of real samples

To further confirm the practicality of the developed nanoprobe, the standard addition method was used in the present assay using river water samples. The dual-emissive ratiometric fluorescent probe was applied to determine the concentration of PA in tap, river, and ultrapure water samples (Table 1). The results of the analysis were further confirmed by the standard addition method and the recoveries of these measurements were 97.8 %, 103.2 %, and 101.8 % for ultrapure water, tap water, and river water, respectively, which indicated that the proposed CdSe@SiO<sub>2</sub>-CDs/Fe(III) probe was suitable for the quantitative determination of PA in real samples.

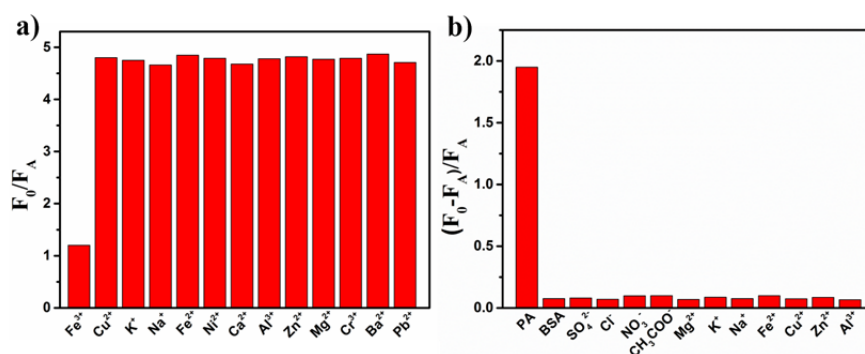


Figure 4: (a)  $F_0/F_A$  of the ratiometric probe upon addition of 10  $\mu\text{L}$  of other metal ions (10 mM concentration). (b)  $(F_0 - F_A)/F_A$  of the ratiometric probe upon addition of 10  $\mu\text{L}$  of other substances (10 mM concentration).

Moreover, to examine the selectivity of the ratiometric fluorescence probe towards PA, the fluorescence intensity ratios of the ratiometric probe  $(F_0 - F_A)/F_A$  were recorded in the presence of 10  $\mu\text{L}$  of PA,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{CH}_3\text{COO}^-$ ,  $\text{Cl}^-$  and BSA (Figure 4b). No obvious change in the fluorescence intensity ratios of the ratiometric probe  $((F_0 - F_A)/F_A)$  was observed in the presence of all substances, except PA.

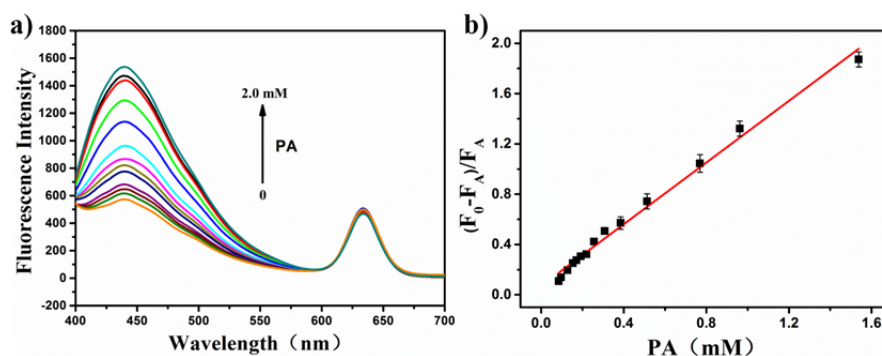


Figure 5: (a) Fluorescent spectra of ratiometric probe in the presence of  $\text{Fe}^{3+}$  upon addition of different concentrations of PA. (b) Plot of the value of  $(F_0 - F_A)/F_A$  as a function of PA concentration.



### 3.5. Analysis of real samples

To further confirm the practicality of the developed nanoprobe, the standard addition method was used in the present assay using river water samples. The dual-emissive ratiometric fluorescent probe was applied to determine the concentration of PA in tap, river, and ultrapure water samples (Table 1). The results of the analysis were further confirmed by the standard addition method and the recoveries of these measurements were 97.8 %, 103.2 %, and 101.8 % for ultrapure water, tap water, and river water, respectively, which indicated that the proposed CdSe@SiO<sub>2</sub>-CDs/Fe(III) probe was suitable for the quantitative determination of PA in real samples.

*Table 1: Recovery and detection of PA in real samples.*

Samples	PA added (mM)	Found (mM)	Recovery
Ultrapure water	0.5	0.489	97.8%
Tap water	0.5	0.516	103.2%
River water	0.5	0.509	101.8%

### 4. Conclusions

Based on the increasingly serious environmental pollution problem, in this work, a single dual-emissive ratiometric fluorescent probe with red and blue emissions was developed, which responds spectroscopically to Fe<sup>3+</sup> and PA. The interaction between Fe<sup>3+</sup> and the chemical groups on the surface of the CDs resulted in significant fluorescence quenching via a photoinduced electron transfer (PET) process. Fe<sup>3+</sup> were found to selectively bind to PA, and the addition of PA to the CdSe@SiO<sub>2</sub>-CDs/Fe(III) complex led to recovery of the fluorescence signal. Based on this fluorescence quenching-recovery phenomenon, a new fluorescence sensing system was designed by using CDs as a sensing unit and CdSe as an internal standard emission to sensitively detect Fe<sup>3+</sup> and PA. This ratiometric fluorescent probe demonstrated high sensitivity and selectivity and has great potential for the efficient detection of Fe<sup>3+</sup> and PA. This method could also provide opportunities for a wide range of applications in real samples.

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