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Highly Selective and Sensitive Detection of Hg^{2+} Based on Förster Resonance Energy Transfer between CdSe Quantum Dots and g- C_3N_4 Nanosheets

Shan Wang^{*} , Ruiqing Liu and Chenchen Li

Abstract

In the presence of Hg^{2+} , a fluorescence resonance energy transfer (FRET) system was constructed between CdSe quantum dots (QDs) (donor) and g- C_3N_4 (receptors). Nanocomposites of g- C_3N_4 supported by CdSe QDs (CdSe QDs/g- C_3N_4 nanosheets) were fabricated through an electrostatic interaction route in an aqueous solution. The nanocomposites were characterized by X-ray photoelectron spectroscopy, X-ray diffraction, Fourier-transform infrared spectroscopy, and transmission electron microscopy. Results showed that the g- C_3N_4 nanosheets were decorated randomly by CdSe QDs, with average diameter of approximately 7 nm. The feasibility of the FRET system as a sensor was demonstrated by Hg (II) detection in water. At pH 7, a linear relationship was observed between the fluorescence intensity and the concentration of Hg (II) (0–32 nmol/L), with a detection limit of 5.3 nmol/L. The new detection method was proven to be sensitive for detecting Hg^{2+} in water solutions. Moreover, the method showed high selectivity for Hg^{2+} over several metal ions, including Na^+ , Mg^{2+} , Ca^{2+} , Pb^{2+} , Cr^{3+} , Cd^{2+} , Zn^{2+} , and Cu^{2+} . The CdSe QDs/g- C_3N_4 nanosheet conjugate exhibited desirable long-term stability and reversibility as a novel FRET sensor. The novel FRET-based fluorescence detection provided an attractive assay platform for quantifying Hg^{2+} in complex water solutions.

Keywords: FRET, CdSe QDs, g- C_3N_4 nanosheet, Sensor

Background

The main cause of mercury poisoning in humans was polluted natural waters [1]. Hg^{2+} ion metabolism by aquatic microbes produces methyl mercury, which was a potent neurotoxin associated with cognitive and motion disorders [2]. Therefore, mercury detection methods that are rapid, cost-effective, facile, and applicable to complex environments are necessary. Particularly, nanomaterials with unique optical properties can be employed to develop optical sensors with high sensitivity and selectivity [3]. Semiconductor quantum dots (QDs), fluorescent metal nanoclusters (NCs), noble metal nanoparticles (NPs), and carbon nanodots (CDs) were commonly used in the design of Hg^{2+} optical sensors because of their distinct properties, such as easy synthesis, high stability,

functionalization, and biocompatibility. Many fluorescent sensors for Hg^{2+} had been reported [4–8]. For example, Huang et al. [9] developed a time-gated Förster resonance energy transfer (FRET) sensor for Hg^{2+} detection. Moreover, different FRET systems had been developed for the detection for Hg^{2+} [10–12]. Notably, FRET systems could be similarly built using nanoparticles, such as QDs, as well as organic and inorganic NPs [13–15]. Among the nanoparticles, g- C_3N_4 nanosheets had attracted widespread interest [16, 17]. Although g- C_3N_4 nanosheets have been applied as sensors, a FRET detection system with g- C_3N_4 nanosheets and CdSe QDs for metal ions has not been reported. FRET-based fluorescence sensing systems offer multiple advantages [18].

In the present study, a new FRET-based fluorescence sensor was developed to detect mercury ions in aqueous media by using g- C_3N_4 nanosheets and

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CdSe QDs particles as vehicles. The proposed mechanism was illustrated in Fig. 1.

Methods

Materials

Mercury (II) chloride (HgCl_2) was purchased from Tong Ren Chemical Research Institute (Guizhou, China). Urea and CdSe QDs were purchased from Aladdin Reagent Company (Shanghai, China). Other reagents and chemicals were of analytical reagent grade and used without further purification. All solutions were prepared using purified water from a Milli-Q gradient water purification system (Millipore Inc., USA; nominal resistivity 18.2 M Ω cm).

Characterization

An X-ray diffractometer (Rigaku D/max-2400) was used to obtain diffraction patterns. Ultraviolet–visible (UV–vis) spectra were recorded on a UV–vis 800 spectrophotometer at room temperature. Fourier-transform infrared (FTIR) spectra were recorded on a Nicolet-nexus670 spectrometer using KBr. Fluorescence measurements were performed at room temperature with an RF-5301PC fluorescence spectrometer. X-ray photoelectron spectroscopy (XPS) measurements were performed using a multifunctional spectrometer (Thermo Scientific).

Construction of FRET Sensor between the g- C_3N_4 Nanosheets and CdSe QD Particles

In a typical procedure, g- C_3N_4 (125 mg, which was synthesized according to our previous report [19]) was dispersed in 250 mL of water (1:1) and ultrasonicated for 5 h at ambient temperature. Then, CdSe QDs (1.838 g, 0.0216 mol) were dissolved in the solution by sonication for 2 h. Given that the amine

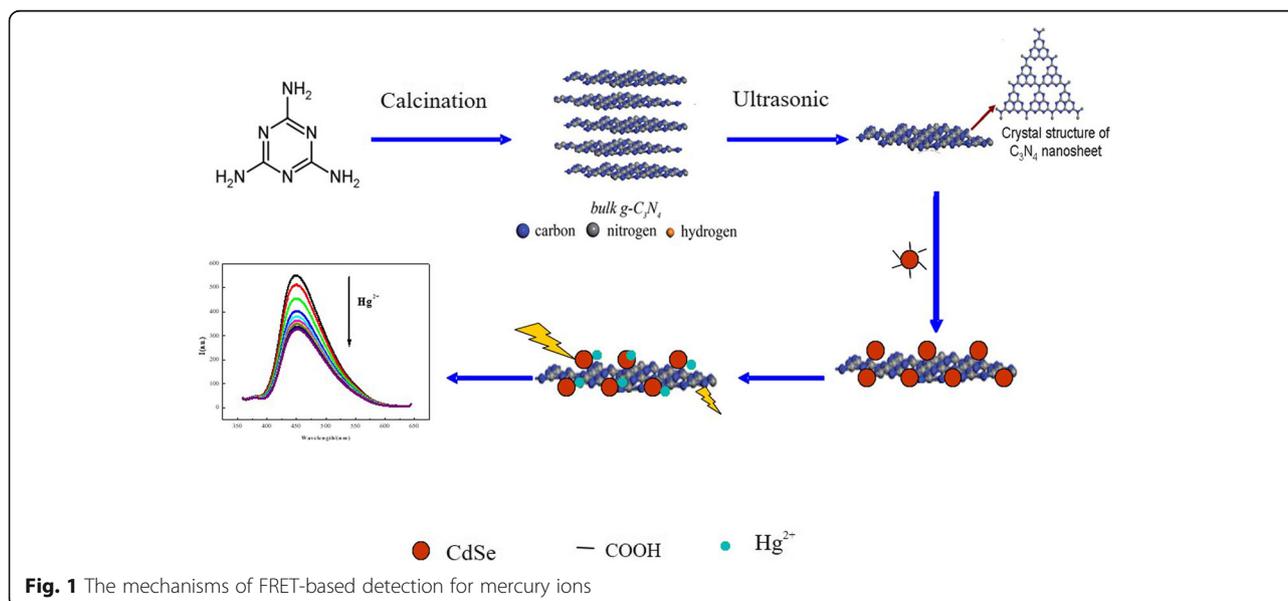
group on the g- C_3N_4 nanosheets and CdSe QDs had a carboxyl group, g- C_3N_4 nanosheets and CdSe QDs nanoparticles would be combined by electrostatic interaction. All solutions were prepared in Milli-Q gradient water (pH = 7). The CdSe QDs/g- C_3N_4 nanosheet conjugate emission spectra were recorded. All samples were excited at 334 nm, which was near the minimal acceptor absorption.

Fluorescence Detection of Hg^{2+}

Hg^{2+} was quenched at room temperature in water. During a typical operation, 10 μL of the CdSe QDs/g- C_3N_4 nanosheet conjugates was added to 3 mL of ultrapure water, and then the calculated amount of Hg^{2+} was added. The emission spectra of the CdSe QDs/g- C_3N_4 nanosheet conjugates were recorded 2 mins later at room temperature.

Interference and Competition Analyses

The response of the FRET nanoprobe to other metal ions (Na^+ , Mg^{2+} , Ca^{2+} , Pb^{2+} , Cr^{3+} , Cd^{2+} , Zn^{2+} , and Cu^{2+}) was studied through fluorescence spectroscopy. Studies were carried out using the CdSe QDs/g- C_3N_4 nanosheet conjugates emitting at 450 nm. The conjugate solution was placed in a 1-cm optical path quartz fluorescence cuvette. Fluorescence intensity was measured at emission wavelength of 450 nm under excitation wavelength of 334 nm in the presence of each possible interference (32 nM). Competition assays were also performed for all the possible interferences previously analyzed. For competition experiments, 32 nM Hg^{2+} aqueous solutions were prepared.



Results and Discussion

Characterization

The structure and morphology of $g\text{-C}_3\text{N}_4$ nanosheets were characterized by TEM, XPS, and XRD. The TEM image in Fig. 2a showed that the $g\text{-C}_3\text{N}_4$ nanosheet possessed a graphene-like morphology that mainly consists of a few layers [19]. Figure 2a showed the XRD patterns of the $g\text{-C}_3\text{N}_4$ nanosheets. The strong XRD peak centered at 27.4° corresponded to the typical graphitic interlayer stacking (002) peak of $g\text{-C}_3\text{N}_4$. The small peak at 13.1° corresponded to the periodic in-plane structural packing feature within the sheets [20, 21]. XPS measurement was used to analyze the valence states of $g\text{-C}_3\text{N}_4$ nanosheets. The XPS spectrum in Fig. 2c showed the C–C bonded to N at 284.8 and 288.0 eV, and the N 1s spectrum was at 397.04 eV. In Fig. 2d, the peak at 811 cm^{-1} was attributed to the vibration of the triazine ring. The peaks around 1000 cm^{-1} represented the stretching modes of CN heterocycles, and the peak at 1800 cm^{-1} corresponded to C–NH–C. The peaks at $300\text{--}3600\text{ cm}^{-1}$ corresponded to N–H and O–H stretching vibrations [22].

UV-vis and Fluorescence Properties of CdSe QDs/ $g\text{-C}_3\text{N}_4$ Nanosheets

Fluorescence and UV-vis absorption spectra were obtained to evaluate the optical properties of CdSe QDs/ $g\text{-C}_3\text{N}_4$ nanosheets. As shown in Fig. 3a, a large

peak at approximately 334 nm was observed in the UV-vis absorption spectrum. Moreover, the fluorescence emission and excitation peaks were observed at 452 and 334 nm in the synchronous fluorescence spectroscopy in Fig. 3b and were associated with the emission fluorescence and ultraviolet light excitation of nanosheets. The emission peaks showed a shift compared with the pure $g\text{-C}_3\text{N}_4$ nanosheets at 14–16 nm (emission and excitation peaks were observed at 438 and 310 nm as presented in Fig. 3c), which could be ascribed to the FRET. The influence of excitation wavelengths on fluorescence intensities was also confirmed.

Effect of pH to the Fluorescence of the CdSe QDs/ $g\text{-C}_3\text{N}_4$ Nanosheet Conjugates

Figure 4 showed the fluorescence of the CdSe QDs/ $g\text{-C}_3\text{N}_4$ nanosheet conjugates at different pH values. The pH value increased from 3 to 7 with the fluorescence intensity. However, the fluorescence intensity gradually decreased when the pH value varied increased from 7 to 10, which could be attributed to the effect of pH on the change in the surface charge owing to protonation–deprotonation due to the existence of amino groups in the structure of $g\text{-C}_3\text{N}_4$ nanosheets. In this study, the CdSe QDs/ $g\text{-C}_3\text{N}_4$ nanosheet conjugates were conducted for the detection of Hg^{2+} ions, and the pH value of 7 was selected as the optimum pH value. The fluorescence emissions were

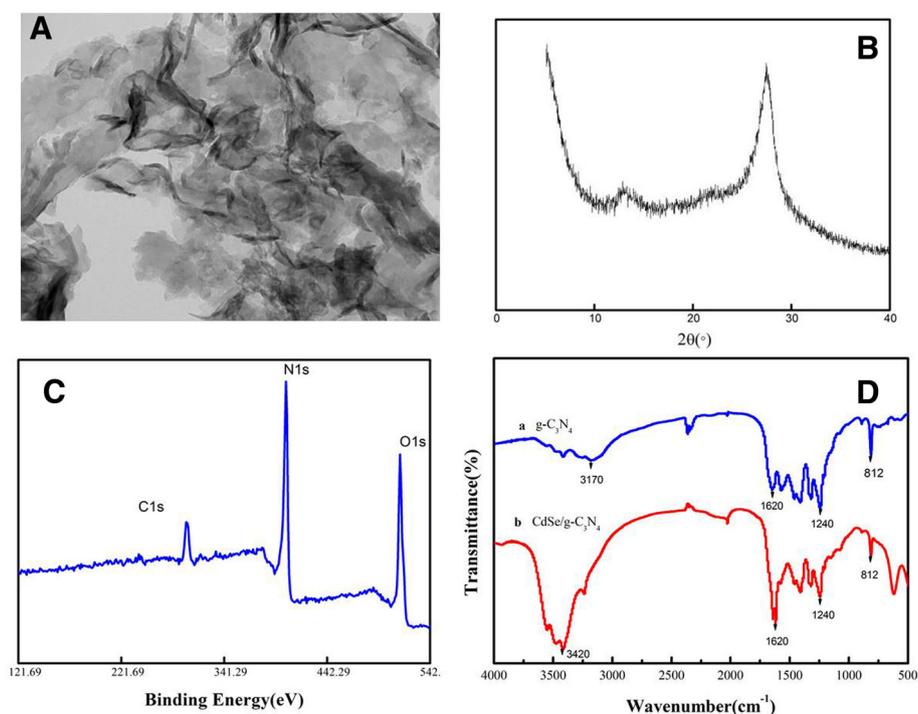
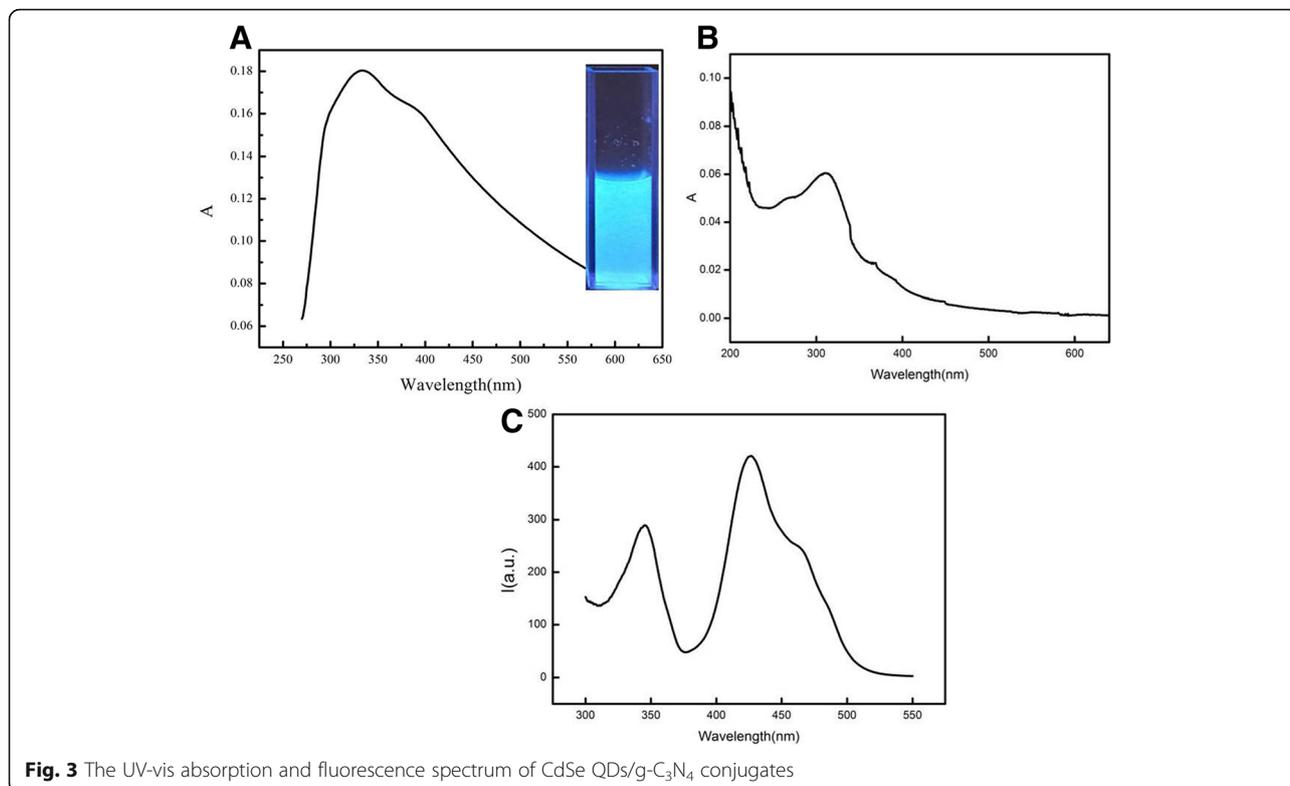
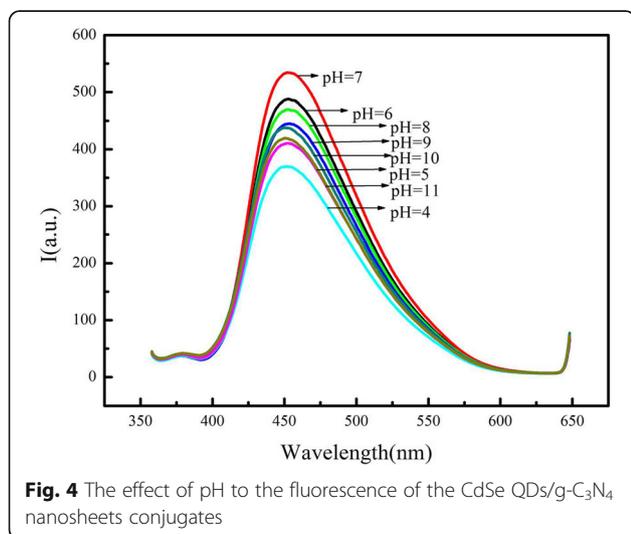


Fig. 2 Characterization of the as-prepared $g\text{-C}_3\text{N}_4$ nanosheet. **a** TEM image. **b** XRD image. **c** XPS spectrum. **d** FTIR spectrum



measured at pH 7 containing different concentrations of NaCl to obtain the stability of the CdSe QDs/g-C₃N₄ nanosheet conjugates under high ionic strength circumstances. Only a slight change was observed under high ionic strength in the fluorescence intensities of the CdSe QDs/g-C₃N₄ nanosheet conjugates. The result showed that high ionic strength had minimal effects to the fluorescence intensities of the conjugates.

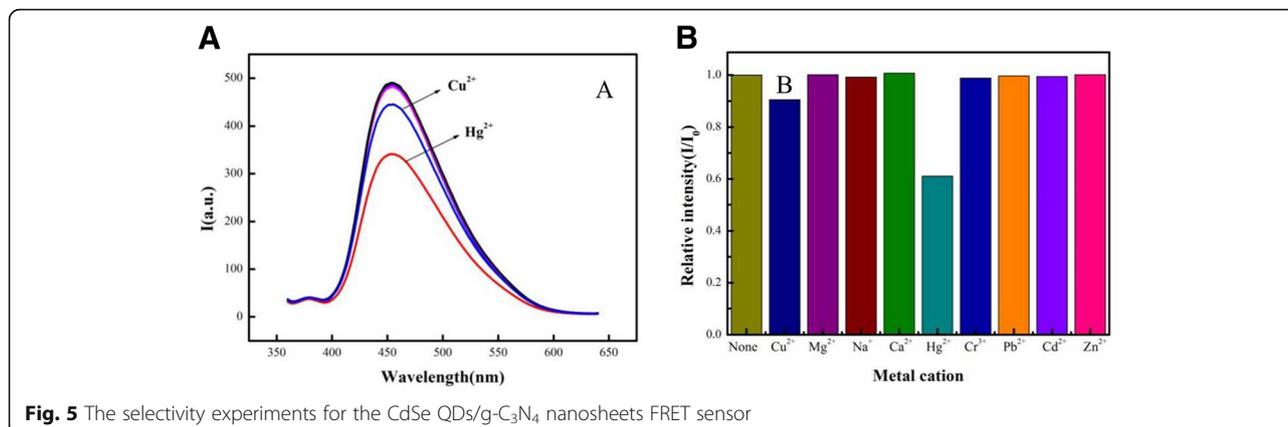


Selectivity of CdSe QDs/g-C₃N₄ Nanosheet FRET System in Detecting Mercury Ion

Selectivity is an important parameter of a new sensing system. The selectivity of the CdSe QDs/g-C₃N₄ nanosheet FRET sensor was evaluated using various metal ions (e.g., Cu²⁺, Mg²⁺, Na⁺, Ca²⁺, Hg²⁺, Cr³⁺, Pb²⁺, Cd²⁺, and Zn²⁺); the results were shown in Fig. 5a. Compared with the blank sample without ions, the fluorescence ratio of Hg²⁺ increased obviously, while the fluorescence intensity of other metal ions changed slightly or remains the same. These results indicated that the FRET sensor showed more selectivity than the others (Fig. 5b). Thus, the CdSe QDs/g-C₃N₄ showed high selectivity toward Hg²⁺. This phenomenon was distinct in comparison with pure g-C₃N₄ nanosheet, which was selective for Cu²⁺ and Hg²⁺ [23, 24].

Feasibility of the FRET Fluorescence Process in Detecting Hg²⁺

To study the practicability of the FRET sensor, the CdSe QDs/g-C₃N₄ nanosheet fluorescence detection of Hg²⁺ was performed. The presence of Hg²⁺ resulted in decreased fluorescence intensity as shown in Fig. 6, which illustrated that Hg²⁺ could effectively quench the FRET sensor. In order to study the sensitivity, the response of the sensor to different Hg²⁺ concentrations was further evaluated by fluorescence spectroscopy, and the results were shown in Fig. 6a. The fluorescence intensity of g-C₃N₄ nanosheets



gradually decreased with increasing of Hg²⁺ concentrations. Figure 6b explained that the I/I_0 was dependent on the concentration of Hg²⁺, where I_0 and I were the fluorescence intensity in the absence and presence, respectively, of Hg²⁺. Moreover, the relation of I/I_0 between concentrations of Hg²⁺ was linear, and the equation of linear regression was $I = -9.6 \times 10^7 + 550.5(R^2 = 0.9882)$, as shown in the inset of Fig. 6b. Compared with recently reported luminescence methods, the proposed method had lower detection limit and higher sensitivity [25, 26]. The g-C₃N₄ nanosheets and CdSe QDs displayed no obvious quenching response to other metal ions apart from Hg²⁺, which suggested a relatively high selectivity for this method.

The other coexisting cations that affect the detection of mercury ion were detected as well. The response of the CdSe QDs/g-C₃N₄ nanosheet-based sensing system toward Hg²⁺ ion in the presence of alkali, alkaline earth, and other transition metal ions was shown in Table 1. The coexistence of most of the metal ions did not interfere with the binding of Hg²⁺, which indicated that the interference of these coexisting ions on the Hg²⁺ sensor was negligible.

In addition, long-term stability is a superior property of sensors. The absorbance and the fluorescence during the

continuous investigation every 3 days within 2 weeks indicated that the activity of CdSe QDs/g-C₃N₄ nanosheets remained above 92% of the initial efficiency though they were stored at ambient environment. The results indicated that the CdSe QDs/g-C₃N₄ nanosheets as FRET sensors had good long-term stability.

Compared with previous reports concerning fluorescence assays for Hg²⁺ (results are listed in Table 2), the CdSe QDs/g-C₃N₄ nanosheet fluorescence probe based on FRET with the concentration of Hg (II) in the range of 0–32 nmol/L at pH = 7 exhibited a limit of detection at 5.3 nmol/L. Thus, our method obtained a superior detection limit and linear range.

Application of the FRET Sensor

The CdSe QDs/g-C₃N₄ nanosheets as a FRET sensor successfully provided a good platform for detecting Hg²⁺ in real samples because of their sensitivity and selectivity. Well, lake, and tap waters were selected as real samples for analysis in which the recovery of Hg²⁺ were in the range of 95.4–101.6% (Table 3). The relative standard deviation (RSD) of Hg²⁺ was in the range of 0.64–1.72%. The result stated clearly that the designed method can be efficiently

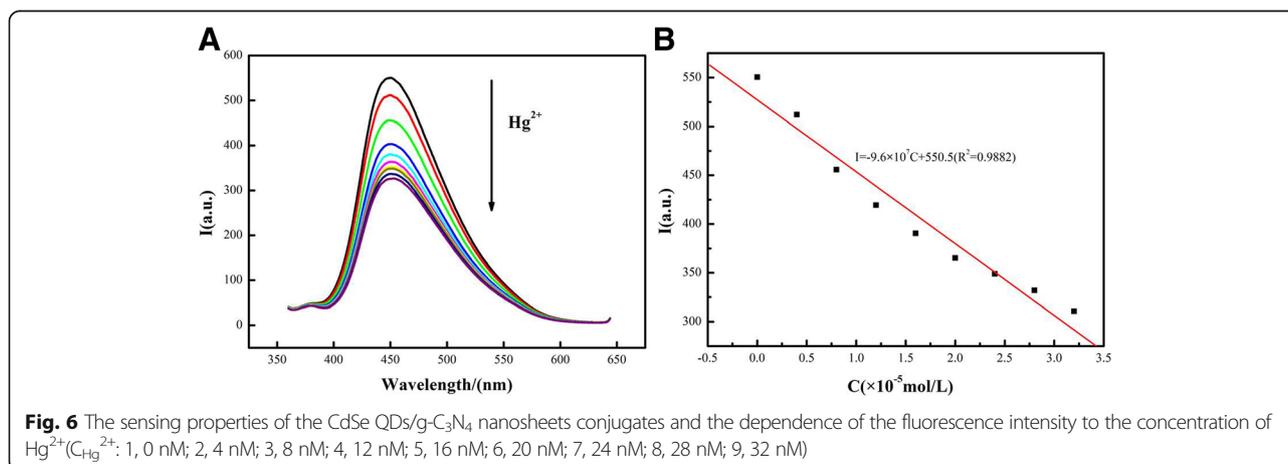


Table 1 Effect of interfering species on the fluorescence intensity of FRET sensor solution under optimum conditions

Coexisting Substance	Concentration/nmol/L	$\Delta F/F_0$ (%)
Ca ²⁺	1.2	-2.6
K ⁺	0.2	+3.9
Li ⁺	0.5	+3.5
Mg ²⁺	1.0	+3.2
Al ³⁺	2.0	-1.8
Pb ²⁺	3.0	+3.4
Mn ²⁺	0.5	-0.8
Ba ²⁺	2.5	-3.1
Cr ³⁺	10	+2.6
Fe ³⁺	8.0	+4.7
Fe ²⁺	5.0	-2.8

$\Delta F = F - F_0$, where F and F₀ are the fluorescence intensity in the presence and absence of interfering species

used to detect Hg²⁺ in practical applications. The acceptable values of RSD and relative error confirmed the high sensitivity, high precision, and high reliability of the proposed FRET sensor for Hg²⁺ determination in practical applications.

Conclusions

A FRET-based system was developed for detecting Hg²⁺ within g-C₃N₄ nanosheets/CdSe QDs. The detection limit for Hg²⁺ ion was 5.3 nM, with a linear

Table 2 The comparison of the previously reported and the present fluorescence method for mercury ions

Fluorescence sensor	Detection limit (nM)	Solvent (medium)	Sensing mode	Reference
Silica particles	100	H ₂ O	Ratiometric	[27]
CdTe QDs	1.5	H ₂ O	Fluorescence turn off	[28]
Tetraphenylborate anion	100	Membrane	Fluorescence turn off	[29]
Rhodamine derivative	20	H ₂ O	Fluorescence turn on	[30]
CdS/ZnS QDs	40	H ₂ O	Fluorescence turn off	[31]
Gold nanorods	500	H ₂ O	Absorbance turn off	[32]
Mesoporous silica	1000	CH ₃ CN	Fluorescence turn on	[33]
Mn:CdS/ZnS QDs	0.18	H ₂ O	Fluorescence turn on	[34]
GO/ Au NPs	25	H ₂ O	PET	[35]
CdTe QDs/rhodamine B	20.3	H ₂ O	FRET	[36]
CdTe QDs/g-C ₃ N ₄ nanosheets	5.3	H ₂ O	FRET	This work

Table 3 The results of the determination for mercury ions in practical samples

Samples	Found (nM)	Added (nM)	Found (nM, n = 3)	Recovery (%)
Well water	Not found	5	4.93	98.6
		10	9.54	95.4
		15	15.21	101.4
Tap water	Not found	5	5.06	101.2
		10	10.16	101.6
		15	15.18	101.2
Lake water	Not found	5	5.02	100.4
		10	10.13	101.3
		15	15.29	101.9

response ranging from 0 to 32 nM. The applicability of this sensor was demonstrated by measuring the content of Hg²⁺ in real samples. Given the long-term stability, low cost, and facile preparation of the CdSe QDs/g-C₃N₄ nanosheet conjugates, the fluorescence assay could be used as an environmental protection sensor. This strategy would provide an alternative approach for constructing FRET-based sensors for Hg²⁺ in aqueous media, including environmental and biological samples.

Highlights

1. Fluorescence resonance energy transfer (FRET) system was constructed between CdTe quantum dots (QDs) (donor) and g-C₃N₄ (acceptor) in the presence of Hg²⁺ for the first time.
2. The nanocomposites of g-C₃N₄ supported by CdSe QDs (CdSe QDs /g-C₃N₄) were fabricated through a simple electrostatic interaction route in an aqueous solution.
3. The feasibility of the FRET system as a sensor was demonstrated for detecting Hg (II) in water solution. At pH 7, a linear relationship was observed between the quenched fluorescence intensity of the concentration of Hg (II) in the range of 0–32 nmol/L. The detection limit was 5.3 nmol/L.
4. The novel FRET-based fluorescence detection may provide an attractive assay platform for quantifying Hg²⁺ in complex water solutions.

Abbreviations

FRET: Förster resonance energy transfer; FTIR: Fourier-transform infrared; UV-vis: Ultraviolet-visible; XPS: X-ray photoelectron spectroscopy; XRD: X-ray diffractometer

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Availability of Data and Materials

The data sets supporting the results of this article are included within the article and its additional files.

Authors' Contributions

SW designed the experiment, performed the experiment, and wrote the whole paper. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing financial interests.

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