

RESEARCH ARTICLE

A ratiometric fluorescent sensor for the detection of phosphate

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Abstract

Over the past few years, ratiometric fluorescent nanoprobe have garnered substantial interest because of their self-calibration characteristics. This research developed a ratiometric fluorescent sensor to detect phosphate. Through encapsulating luminescent materials, gold nanoclusters (AuNCs) and carbon dots (CDs) into a zeolitic imidazolate framework-8 (ZIF-8), the fluorescence signal of AuNCs was enhanced, while that of CDs was suppressed. After phosphate was added, it could decompose ZIF-8, and AuNCs and CDs were released, which weakened the fluorescence signal of the AuNCs while restoring that of the CDs. Thereby, this makes CDs/AuNCs@ZIF-8 a potential fluorescent sensor for phosphate determination. The ratiometric sensor had facile synthesis, good selectivity, and a low detection limit. Therefore, this sensor was an effective tool for the detection of phosphate.

KEYWORDS

carbon dots, CDs/AuNCs@ZIF-8, gold nanoclusters, ratiometric, zeolitic imidazole frameworks

1 | INTRODUCTION

Phosphate is a natural food ingredient and functional additive that is widely used in the processing of seafood, meat products, convenience food, seasonings, and so on.^[1] In addition, its imbalance is linked to hyperparathyroidism, osteomalacia, vitamin D deficiency, and a class of diseases due to the important functions of phosphate in the human body. Therefore, excessive phosphate will cause certain harm to the human body including calcium deficiency and stones, and even aggravate other diseases. Phosphate is a common anion that is of great importance for plant growth and development. However, high concentrations of phosphate can cause environmental pollution, such as seaweed pollution and eutrophication.^[2] Therefore, developing a rapid, simple, and selective sensor could be of great significance.

Carbon dots (CDs) are small fluorescent carbon nanoparticles with sizes less than 10 nm in diameter, and with aggregation-caused quenching (ACQ) characteristics.^[3] Compared with other quantum dots or fluorescent materials, CDs also have other distinct properties such as easier synthesis, good water solubility, high photostability, and good biocompatibility, rendering them attractive for applications

in many fields, including as sensors,^[4,5] optoelectronic devices,^[6] and so on. Gold nanoclusters (AuNCs) have aggregation-induced emission (AIE) properties^[7,8] that have attracted great attention in several fields such as biomedicine and as sensors.^[9] For example, Liu et al. synthesized MoS₂@AuNCs for the detection of alkaline phosphatase.^[10] This sensor took advantage of the AIE properties of AuNCs.

Recently, metal-organic frameworks (MOFs) have been greatly developed in many fields, such as gas adsorption,^[11] heterogeneous catalysis,^[12,13] energy storage,^[14,15] chemical sensing,^[16] biomedical imaging,^[17] and drug delivery.^[18] For instance, Cai et al. packaged AuNCs into ZIF-8 to synthesize AuNCs@ZIF-8 for organophosphorus pesticide detection, so that the exhibited AIE properties and its fluorescence lifetime, quantum yield, and stability were improved.^[19] Recently, fluorescent species loadings in MOFs have been elaborately designed and prepared,^[20,21] which piqued our interest and motivated us to conduct research in this area. In addition, MOF-based nanoprobe can not only prevent the agglomeration of CDs and AuNCs, but also adsorb and enrich analytes to amplify the analytical signal. Therefore, it is possible to construct a ratiometric nanoprobe by encapsulating CDs and AuNCs into MOFs.

In this work, we created a ratiometric fluorescent sensor for phosphate detection. The ratiometric fluorescent sensor was easily synthesized by encapsulating CDs and AuNCs into ZIF-8 by self-assembly (Figure 1). After encapsulation, the fluorescence signal of AuNCs was enhanced because of its AIE properties, while that of CDs was suppressed because of its ACQ characteristics. Upon the addition of phosphate, as phosphate can decompose ZIF-8, AuNCs and CDs were released, which weakened the fluorescence signal of AuNCs, but restored that of CDs. Therefore, CDs/AuNCs@ZIF-8 is a potential ratiometric fluorescent sensor for phosphate determination.

2 | EXPERIMENTAL

2.1 | Chemicals

Hydrochloric acid, $\text{Zn}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2$, Na_2SO_3 , CH_3COONa , NaF , KBr , KI , CaCl_2 , CdCl_2 , MgCl_2 , BaCl_2 , KCl , and NaCl were purchased from Sinopharm Chemical Reagent Co., Ltd, China. Tris(hydroxymethyl)aminomethane (Tris), 2-methylimidazole (2-MIM), $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$, chloroauric acid (HAuCl_4), and glutathione (GSH) were purchased from Aladdin Chemistry Co. Ltd, China. Citric acid (CA) was purchased from J&K Scientific Co. Ltd, China. Urea was purchased from Xilong Chemical Co. Ltd, China. All chemicals in this work were used without further purification.

2.2 | Instruments

Fluorescence emission spectra were recorded on an F-2700 spectrofluorophotometer (Hitachi Co., Ltd, Japan). UV-absorption spectra were recorded on a U-5100 UV-vis spectrometer (Hitachi Co., Ltd, Japan). A quartz cuvette with a path length of 1 cm was used in all experiments. Powder X-ray diffraction (XRD) analysis was performed on a D8 Advance diffractometer (Bruker Co., Germany) using $\text{Cu K}\alpha$ (0.15406 nm) radiation. Scanning electron microscopy (SEM)

experiments were carried out on an Hitachi SU-8020 SEM. All pH values were measured using a PHS-3C pH meter (INESA Scientific Inc., China).

2.3 | Preparation of samples

2.3.1 | Synthesis of CDs

CDs were prepared according to a previous method with minor revisions.^[22] In detail, 15 ml deionized water, urea (3 g), and CA (1.5 g) were mixed, then the mixture was treated at 160°C for 4 h in a Teflon reactor. The product was collected by centrifugation and washed several times with ethanol. The obtained CDs were stored at 4°C for further application.

2.3.2 | Synthesis of AuNCs

AuNCs were prepared according to a previously reported method with minor revision.^[10] Here, 13.6 ml of deionized water, 6 ml of GSH (10 mM), and 0.4 ml of HAuCl_4 (100 mM) were mixed. After 5 min of intensive stirring, the mixture was stirred at 70°C for 24 h until it turned pale yellow. The obtained AuNCs were stored at 4°C for further application.

2.3.3 | Preparation of CDs/AuNCs@ZIF-8

With a few modifications, CDs/AuNCs@ZIF-8 were prepared according to a reported method.^[19] Under vigorous stirring for 3 min, $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in a methanol solution (3.0 ml, 100 mM) was mixed with 850 μl AuNCs and 150 μl CDs. After 2-MIM in methanol solution (3.0 ml, 200 mM) was added, the mixture was stirred for 60 min. CDs/AuNCs@ZIF-8 were collected by centrifugation and washed several times with methanol. The synthesis steps for ZIF-8, CDs@ZIF-8, and AuNCs@ZIF-8 were similar to the above steps.

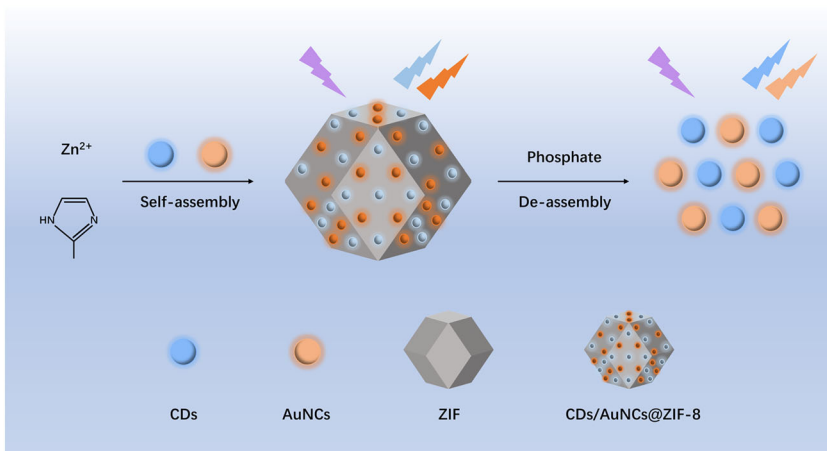


FIGURE 1 Preparation of CDs/AuNCs@ZIF-8 and the mechanism for phosphate detection

2.4 | Ratiometric detection of phosphate

For the ratiometric determination of phosphate, 100 μl of PO_4^{3-} solution at different concentrations, 100 μl of CDs/AuNCs@ZIF-8 (1.5 mg/ml), and 400 μl Tris-HCl buffer (10 mM, pH 8.9) were mixed and then the mixture was incubated for 10 min. The fluorescence emission spectra in the range 400–620 nm were recorded at an excitation wavelength of 330 nm.

3 | RESULTS AND DISCUSSION

3.1 | Characterization of CDs/AuNCs@ZIF-8

SEM images illustrated that both structures for ZIF-8 and CDs/AuNCs@ZIF-8 were regular dodecahedrons (Figure 2a–c). Additionally, compared with ZIF-8, CDs/AuNCs@ZIF-8 had a larger average particle size. The XRD measurements shown in Figure 2d proved the successful synthesis of CDs/AuNCs@ZIF-8.

Figure 3a shows the fluorescence emission spectra of CDs/AuNCs@ZIF-8, CDs, and AuNCs. The emission wavelengths of the CDs and AuNCs were 458 nm and 592 nm, respectively. The sensor had two emission wavelengths at 458 nm and 580 nm after encapsulating CDs and AuNCs into ZIF-8, further confirming the successful synthesis of CDs/AuNCs@ZIF-8. After encapsulation, the fluorescence peak of AuNCs was blue shifted. This phenomenon was consistent with adding methanol to the AuNCs (Figure 3b), which may be due to the increased energy of AuNCs after aggregation. In

addition, the sensor had a similar absorption spectrum characteristic wavelength with a mixed solution of CDs and AuNCs, which also proved that CDs/AuNCs@ZIF-8 was prepared successfully (Figure 3c).

3.2 | Mechanism of sensing

After adding organic solvents, such as acetone, methanol, and dimethylsulfoxide, nanoparticles with AIE properties will aggregate, according to previous reports.^[23,24] As shown in the Figure 3b, after adding methanol, the fluorescence signal of AuNCs increased significantly. In addition, AuNCs aggregated after encapsulation into ZIF-8, and therefore enhanced the fluorescence signal of the AuNCs. CDs demonstrate an ACQ effect, therefore packaging CDs into ZIF-8 would greatly reduce the fluorescence signal of the CDs.

As previous reports have shown, Zn^{2+} has a strong affinity for phosphate, which would decompose ZIF-8.^[25] Therefore, the addition of phosphate would cause the destruction of ZIF-8 and the release of AuNCs and CDs, and therefore reduce the fluorescence signal of AuNCs and enhance that of CDs. The structure of the sensor was altered after the addition of PO_4^{3-} , which indicated that CDs/AuNCs@ZIF-8 was decomposed (Figure 3d).

Fluorescence lifetime is the average residence time of electrons in an excited state after absorbing photons and transiting to an excited state. The internal particles aggregated due to the limiting effect of the MOF, and the vibration and rotation of molecules were inhibited, and therefore the excited state lifetime was increased.^[26,27]

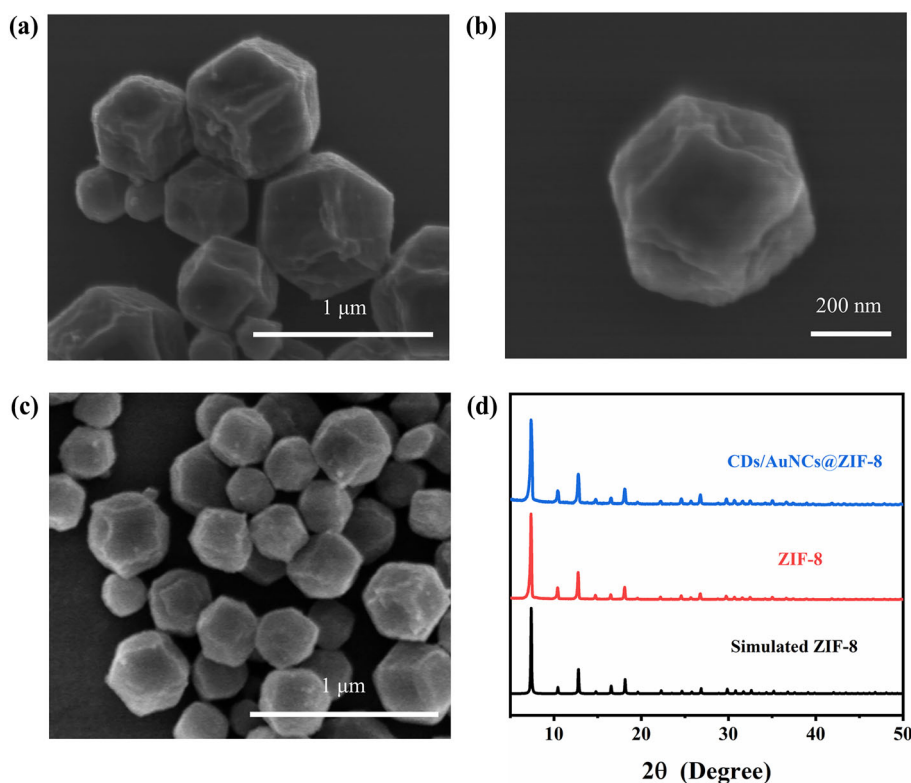


FIGURE 2 (a, b) SEM images of CDs/AuNCs@ZIF-8. (c) SEM image of ZIF-8. (d) XRD patterns of simulated CDs/AuNCs@ZIF-8, ZIF-8, and simulated ZIF-8

FIGURE 3 (a) Fluorescence emission spectra of CDs/AuNCs@ZIF-8, CDs, and AuNCs. (b) Fluorescence emission spectra of AuNCs in the presence or absence of methanol. (c) UV-absorption spectra of CDs/AuNCs@ZIF-8, CDs, AuNCs, and a mixed solution of CDs and AuNCs. (d) SEM image of CDs/AuNCs@ZIF-8 after the addition of PO_4^{3-} . (e) Fluorescence lifetimes of CDs@ZIF-8 and CDs. (f) Fluorescence lifetimes of AuNCs@ZIF-8 and AuNCs

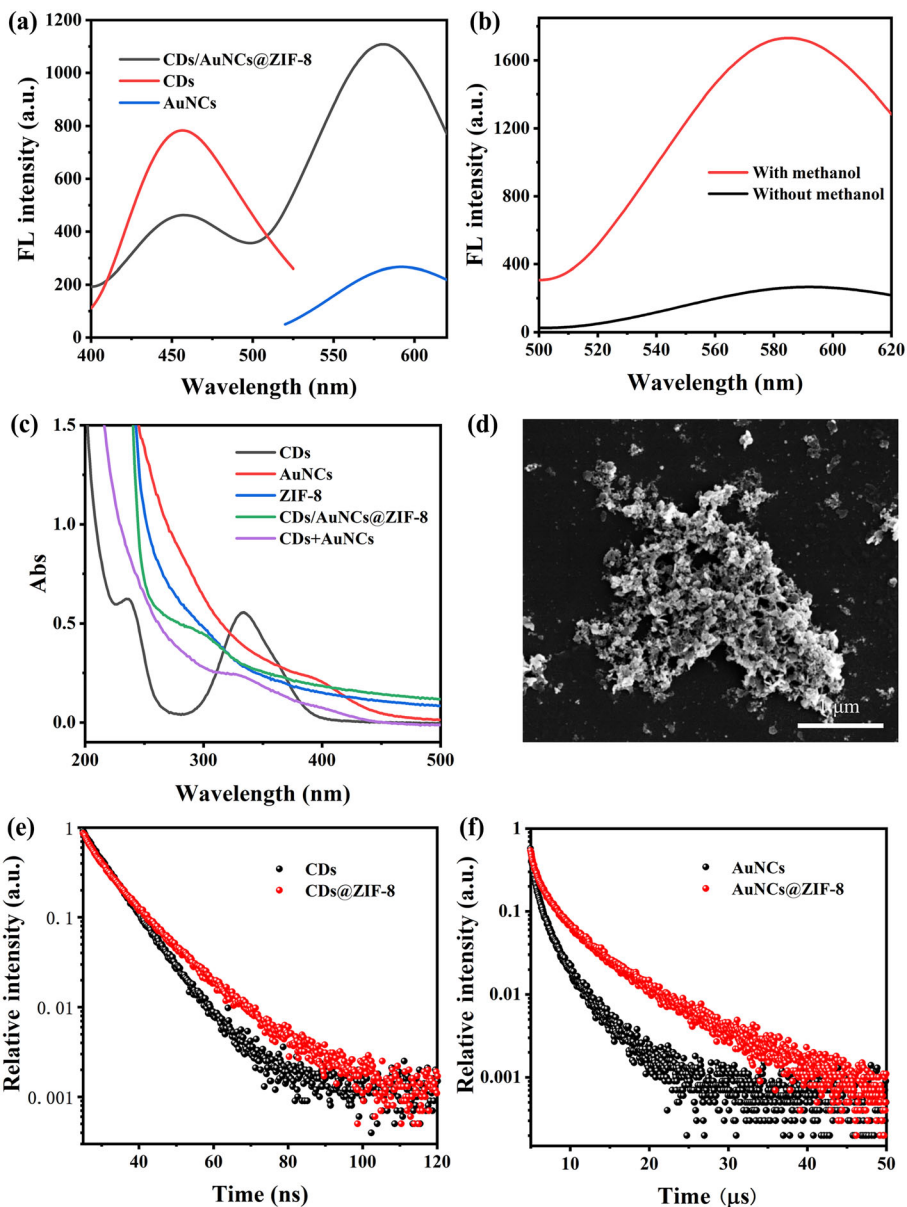
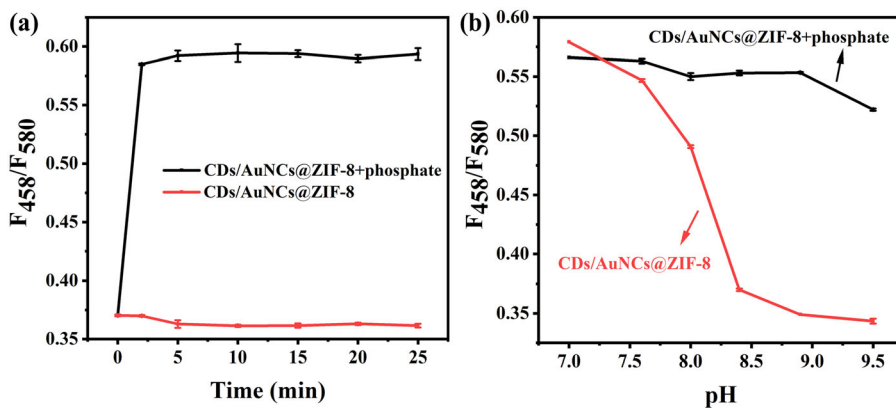


FIGURE 4 (a) Effect of the reaction time on the F_{458}/F_{580} ratio of CDs/AuNCs@ZIF-8 in the presence or absence of phosphate. (b) Effect of pH on F_{458}/F_{580} ratio of CDs/AuNCs@ZIF-8 in the presence or absence of phosphate



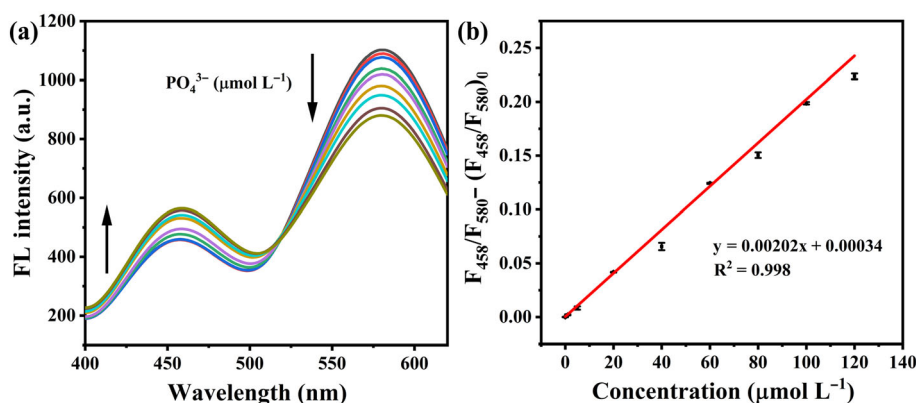


FIGURE 5 (a) Fluorescence emission spectra of the CDs/AuNCs@ZIF-8 after adding phosphate at different concentrations (0, 1, 5, 20, 40, 60, 80, 100, or 120 μM). (b) Linear relationship between $F_{458}/F_{580} - (F_{458}/F_{580})_0$ and phosphate

Materials	Detection range (μM)	Limit of detection (μM)	Reference
Eu-ICP nanospheres	2–100	0.83	[30]
C-dots with Fe^{3+}	10–250	0.8	[5]
UiO-66- NH_2	5–150	1.25	[31]
Tb(H_2O)BTB MOF	40–400	35	[32]
CDs/AuNCs@ZIF-8	1–120	0.27	This work

TABLE 1 Comparison of different fluorescent probes for phosphate determination

Fluorescence lifetimes of AuNCs@ZIF-8 and CDs@ZIF-8 were significantly higher than those of the AuNCs and CDs (Figure 3e,f), which illustrated that AuNCs and CDs aggregated by packaging them into ZIF-8. In addition, a longer fluorescence lifetime will reduce the optimal signal-to-noise ratios.^[28,29] Due to the longer fluorescence life, CDs/AuNCs@ZIF-8 demonstrated better fluorescence characteristics than those of the CDs and AuNCs, therefore this method had a good application potential.

3.3 | Optimization of experimental parameters

The experimental parameters, reaction time, and pH were tested. After 5 min of reaction, the F_{458}/F_{580} ratio remained unchanged, indicating that CDs/AuNCs@ZIF-8 can respond quickly to phosphate (Figure 4a). Therefore, a reaction time of 10 min was adopted. As illustrated in Figure 4b, with an increase in pH, the nanoprobe performed best at pH 8.9, so a buffer solution at pH 8.9 was applied in subsequent experiments.

3.4 | Ratiometric detection of phosphate

With the increase in phosphate concentration, the fluorescence signal was enhanced gradually at 458 nm, while that at 580 nm gradually decreased (Figure 5a). The linear detection range of the sensor was 1–120 μM (Figure 5b). The regression linear equation was $F_{458}/F_{580} - (F_{458}/F_{580})_0 = 0.00202 [\text{phosphate}] + 0.00034$ ($R^2 = 0.9984$). The detection limit (LOD) of CDs/AuNCs@ZIF-8 was 0.27 μM , based on $3\sigma/k$. ($F_{458}/F_{580})_0$ and F_{458}/F_{580} are the ratios of fluorescence signals

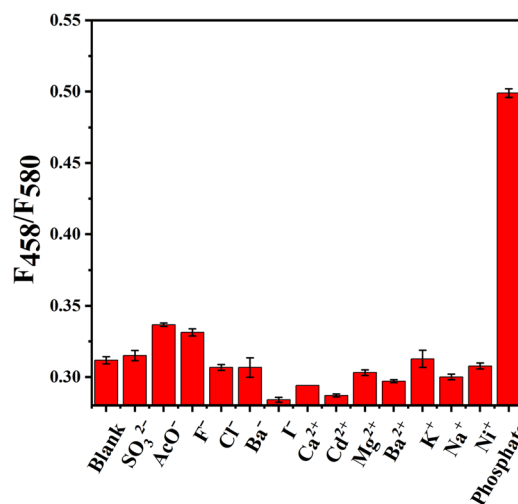


FIGURE 6 F_{458}/F_{580} ratio of CDs/AuNCs@ZIF-8 after adding phosphate (100 μM) and different ions (1000 μM)

at 458 nm and 580 nm in the absence and presence of phosphate, respectively. Table 1 lists the performance comparison of this ratiometric fluorescence sensor with previous methods for phosphate determination.

3.5 | Selectivity of CDs/AuNCs@ZIF-8 to phosphate

In order to determine the specificity of the sensor for phosphate determination, the effect of metal ions (Ca^{2+} , Cd^{2+} , Mg^{2+} , Ba^{2+} , K^+ ,

TABLE 2 Detection of CDs/AuNCs@ZIF-8 to phosphate in tap water and lake water

Samples	Added (μM)	Detected (μM)	Recovery (%)	RSD ($n = 3, \%$)
Tap water	40.0	39.10 ± 1.23	97.8	3.1
	60.0	60.40 ± 2.44	100.7	4.1
Lake water	40.0	40.79 ± 2.19	102.0	5.5
	60.0	58.72 ± 1.55	97.9	2.6

Na^+ , Ni^{2+} , and Fe^{3+}) and anions (SO_3^{2-} , AcO^- , F^- , Cl^- , Br^- , and I^-) was investigated. The concentration of the interfering substance was 10-fold that of phosphate. As displayed in Figure 6, these ions had no significant effect on the F_{458}/F_{580} ratio for CDs/AuNCs@ZIF-8, which proved that the sensor could detect phosphate with a high degree of selectivity.

3.6 | Determination of CDs/AuNCs@ZIF-8 to phosphate in real samples

The feasibility of CDs/AuNCs@ZIF-8 for detecting phosphate in tap water and lake water was investigated. Before spiking, no phosphate was detected in tap water and lake water. As displayed in Table 2, satisfactory recoveries were obtained. The above results showed that this ratiometric fluorescence method was a promising prospect for use in phosphate detection in aqueous samples.

4 | CONCLUSION

This work provided a novel ratiometric fluorescence method to detect phosphate. Compared with the previously reported fluorescent probe for phosphate detection, this probe had the advantages of simple synthesis, good selectivity, and low detection limit. Moreover, the probe had a high practical application value and could be used to analyze phosphate in aqueous samples.

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AUTHOR CONTRIBUTIONS

Jiayi Liu: investigation, conceptualization, data curation, validation, and writing (original draft preparation). **Yibing Liu:** data curation, investigation, validation. **Wei Wang:** data curation, formal analysis, investigation. **Siqi Zhang:** writing (review and editing), data curation. **Li Tang:** writing (review and editing), data curation. **Pinyi Ma:** conceptualization, data curation, writing (review and editing), and software. **Daqian Song:** investigation, resources, writing (review), and software. **Qiang Fei:** project administration, funding acquisition, resources, supervision.

DECLARATION OF INTEREST

The authors declare no competing financial interests.

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