

Article

In Situ Formation of Quantum Dots as a Novel Fluorescence Probe for Phosphate Anion Detection

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Abstract

A new fluorescence detection method for PO_4^{3-} was developed through the in situ synthesis of cadmium sulfide quantum dots (CdS QDs). Without PO_4^{3-} , the CdS QDs could not be effectively formed by only the S^{2-} and Cd^{2+} in the solution. As a stabilizer, PO_4^{3-} is an essential component to regulate the in situ synthesis of CdS QDs. The fluorescence intensity following the addition of different concentrations of PO_4^{3-} was monitored for quantification. Under optimum conditions, the fluorescence intensity shows a linear relationship with concentrations ranging from 3.0 to 300 μM , and a detection limit of 2.9 μM . This assay was successfully employed to assess PO_4^{3-} in tap water and wastewater. Compared with traditional methods, which require pre-synthesizing QDs and tethering them with recognition elements to achieve sample detection, the proposed method is simpler and quicker. It takes less than 5 min to complete PO_4^{3-} detection.

Keywords: phosphate anion; in situ synthesis; quantum dots; fluorescence assay; rapid analysis

1. Introduction

Phosphate anion (PO_4^{3-}), as a derivative of phosphorus, is a vital component of organisms and plays a significant role in diverse bioprocesses, such as intermediary metabolism, gene regulation, signal transmission, energy storage and transfer, etc. [1,2]. However, excessive PO_4^{3-} existing in environmental water harms aquatic ecosystems and biological systems [3]. As a common pollutant anion in the environment, PO_4^{3-} can cause water eutrophication which leads to the overgrowth of algae accompanied by red tides, consumes dissolved oxygen required for life, and reduces water quality [4]. In addition, an abnormal level of PO_4^{3-} in the human body disturbs physiological functions [5], such as impairing the absorption of calcium ions, damaging to renal function and causing irreversible harm to the human body [6]. The recommended concentration standard for phosphate anions in drinking water by the World Health Organization (WHO) is 1 mg/L [7]. Therefore, it is urgent to establish an effective method for the determination of PO_4^{3-} in aqueous solutions.

Compared to cations, PO_4^{3-} possesses fewer binding sites and lower reactivity, which poses a major challenge for the detection of PO_4^{3-} in the field of chemosensors [8,9]. To

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date, numerous methods have been established for the detection of phosphate anions, such as fluorimetry [10–12], time-resolved luminescence [13], colorimetry [14], electrochemistry [15], and chemiluminescence [16], etc. Among these, the fluorescence method has gained great attention due to its merits of high sensitivity, rapid detection, simple operation, and in vivo applicability [17,18]. Some sensors have been designed based on electrostatic or hydrogen-bonding interactions to recognize phosphate anions [19]. To avoid the competitive solvation effect generated by the highly polar substrates (for example, water), organic solvents were usually employed as the detection medium [20], which meant these methods could not detect PO_4^{3-} in aqueous solutions. Schubert et al. used a bisterpyridine metallopolymer to achieve selective fluorescence detection of PO_4^{3-} in tap water [21]. However, the complex and time-consuming detection process restricts its wide application. Thus, great efforts have been made to find new fluorescent probes for PO_4^{3-} detection under aqueous or physiological conditions, such as organic dyes [22], metal–organic frameworks (MOFs) [23], gold–carbon nitride dots [24], carbon dots [25] and semiconductor quantum dots [26].

In comparison with traditional organic fluorophores, the associated advantages of quantum dots (QDs) include higher quantum yield, reduced photobleaching, higher molar extinction coefficient, longer lifetime, broad excitation spectra, and narrow emission spectra, making them fascinating fluorescent probes for analysis and detection [27]. Conventionally, semiconductor QDs are pre-synthesized and then used as labels modified with recognition molecules for sensing assays [28,29]. The production and purification processes of QDs before use are time consuming, which may complicate the sensing procedure. The detection principle is usually based on two modes: QDs decorated with recognition elements to determine affinity interactions or used as fluorescence donor/quencher probes for enzyme activity assays [30]. These analytical strategies are often affected by high background signals, which are caused by non-specific adsorption (without the addition of analytes) on the surface of modified QDs or the inadequate quenching of donor pairs. To overcome these drawbacks, novel unconventional routes need to be introduced to synthesize the QDs.

Recently, some biocatalysis methods have been used to synthesize metal nanoparticles (NPs), especially through enzyme-catalyzed reactions [31]. For example, alkaline phosphatase (ALP)-triggered enzyme-catalyzed reaction can reduce silver nitrate to metal silver [32,33]. Tang et al. proposed an enzyme-controllable production system to form copper hexacyanoferrate nanoparticles [34]. Au-based nanomaterials generated by the catalysis of ALP [35] or amino acid oxidase [36] have also been reported. The sensitivity of these assays is constrained by the use of a UV–vis spectrophotometer to determine the output signals generated by the metal NPs. Inspired by this, efforts have been made to produce fluorescent QDs in situ through the enzymatic reactions [37,38]. Usually, different kinds of enzymes are used to regulate the production amounts of capping agents, such as thiolated compounds (L-Cysteine) [39] or sulfur ions [40], which can affect the growth of QDs. Glutathione has been used as a stabilizer by our group to realize the in situ formation of QDs for colorimetric detection of copper ions [41].

Inspired by these reports, we hypothesized that the in situ formation of QDs can greatly reduce background signals and simplify the detection systems. Herein, a novel simple fluorescence assay based on in situ generation of fluorescent CdS QDs was developed for the determination of PO_4^{3-} . To the best of our knowledge, this is the first time PO_4^{3-} has been used as a stabilizer for the rapid synthesis of CdS QDs. Excess Cd was added to facilitate the formation of CdS QDs with high fluorescence intensity, which is consistent with previously reported pre-synthesis methods for QDs [42]. Compared with traditional QDs synthesis methods, the proposed in situ synthesis method is easy to operate and time saving. With the increase in PO_4^{3-} concentration, the fluorescence intensity

of CdS QDs gradually increases. The “turn-on” fluorescent strategy can avoid the false positive signals arising from environmental effects. In addition, this method does not require any costly reagents, and the detection process can be completed within 5 min. The method can be used for detecting PO_4^{3-} in real water samples.

2. Materials and Methods

2.1. Materials and Instruments

Na_3PO_4 was purchased from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China). Na_2S , CaCl_2 , AgNO_3 , KI , MgCl_2 , KCl , CuSO_4 , NaH_2PO_4 , BaCO_3 , Na_2CO_3 , NaHCO_3 , KSCN , and NaClO_4 were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). $\text{Cd}(\text{AC})_2$ was received from Tianjin Dengke Chemical Reagent Co., Ltd. (Tianjin, China). These reagents were analytically pure and were used directly upon receipt. Purified distilled water was used to prepare the solutions (Millipore, Bedford, MA, USA, 18.2 M Ω).

Fluorescence spectra were collected using a Hitachi F-4600 spectrophotometer (Tokyo, Japan) at room temperature. UV–vis absorption spectra were recorded using a Cary-300 ultraviolet spectrophotometer (Agilent, Santa Clara, CA, USA). Transmission electron microscopy (TEM) imaging was performed with a JEM-2100F high-resolution transmission electron microscope (JEOL, Japan) at an accelerating voltage of 200 kV. X-ray photoelectron spectroscopy (XPS) measurements were conducted on a Thermo Kalpha photoelectron spectrometer (Thermo, Czech Republic). The fluorescence quantum yield was measured using an Edinburgh FLS1000 fluorescence spectrometer (Edinburgh Company, Edinburgh, UK). Fluorescence lifetime measurement was conducted using an Edinburgh FS5 fluorescence spectrometer (Edinburgh Company, Edinburgh, UK).

2.2. Optimization of Synthetic Conditions of QDs

The single-variable method was employed to optimize the synthetic conditions, including the concentrations of Na_2S , $\text{Cd}(\text{AC})_2$, as well as the reaction time and buffer pH. Different concentrations of Na_2S (0.012 mM, 0.036 mM, 0.048 mM, 0.060 mM, 0.096 mM, 0.12 mM) were added to the reaction system, while other conditions were kept. The fluorescence spectrum in the range of 290–650 nm was scanned with excitation at 266 nm, and the fluorescence intensity at 470 nm was recorded. As mentioned above, the effects of different concentrations of $\text{Cd}(\text{AC})_2$ (0.25 mM, 0.35 mM, 0.45 mM, 0.55 mM, 0.75 mM, 0.85 mM), reaction time (2, 3, 4, 5, 6 min), and pH (6.0, 7.0, 8.0, 9.0, 10.0) were also investigated.

2.3. Fluorescence Detection of PO_4^{3-} Based on In Situ Formed QDs

Firstly, 5.0 μL of 3.0 mM Na_2S and 11.0 μL of 12.5 mM $\text{Cd}(\text{AC})_2$ were successively added to different concentrations of Na_3PO_4 solution under shaking at room temperature. Then, the final volume of the solution was made up to 250 μL with 20 mM Tris-HCl buffer (pH = 8.0). After reacting for 4 min, the fluorescence spectrum from 290 to 650 nm was scanned with excitation at 266 nm. The fluorescence intensity at 470 nm was recorded for the quantification of PO_4^{3-} . To perform specificity experiments, other ions were used instead of PO_4^{3-} , such as Cl^- , I^- , SO_4^{2-} , and Na^+ , etc.

2.4. Detection of PO_4^{3-} in Real Water Samples

The standard addition method was used to analyze the PO_4^{3-} content in water samples. After being left overnight at room temperature, tap water taken from the laboratory was filtered with a 0.45 μm microporous membrane. Then, different volumes of standard PO_4^{3-} solution were added to the pretreated tap water samples to achieve final

concentrations of 0, 0.10, and 0.30 mM, respectively. Finally, the obtained PO_4^{3-} -spiked water samples were tested according to the detection method for PO_4^{3-} .

3. Results and Discussions

3.1. Determination Principle of the Fluorescence Assay for PO_4^{3-}

The schematic diagram of the fluorescence detection of PO_4^{3-} is shown in Figure 1. In the presence of PO_4^{3-} , S^{2-} and Cd^{2+} , CdS QDs with good dispersion, uniform particle size, and strong fluorescence properties can be produced in situ in an aqueous solution. However, in the absence of PO_4^{3-} as a stabilizer, S^{2-} and Cd^{2+} can only form many non-fluorescent aggregated CdS particles without fixed morphology and size. The concentration of PO_4^{3-} was proportional to the fluorescence intensity. Different fluorescence intensities were obtained by varying the concentration of PO_4^{3-} . As a result, the sensitive “turn-on” fluorescence detection of PO_4^{3-} can be realized.

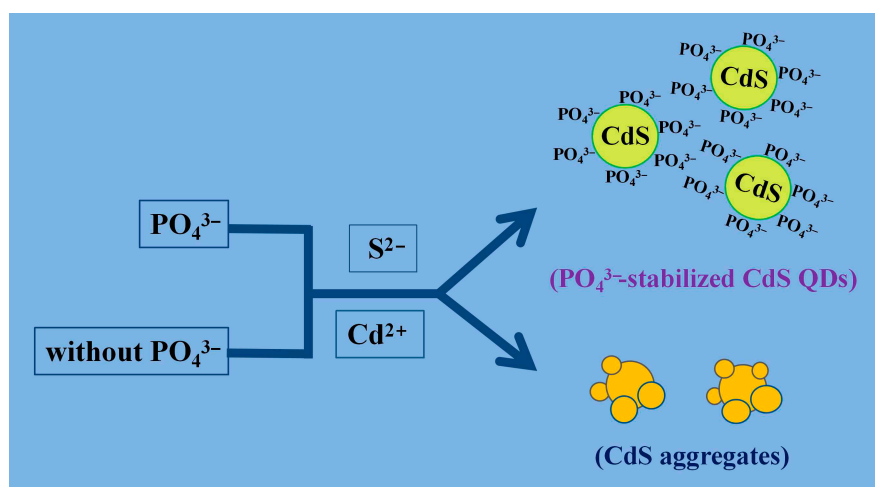


Figure 1. Schematic diagram of the fluorescence assay for phosphate anion detection based on the in situ formation of CdS QDs.

3.2. Characterization of Formed CdS QDs

The UV–vis absorption spectrum, fluorescence excitation spectrum (black line), and emission spectrum (red line) of PO_4^{3-} -stabilized CdS QDs are shown in Figure S1. As can be seen from Figure S1A, the absorption signal in the wavelength range of 470–265 nm was enhanced due to the quantum confinement effect, which confirmed the existence of CdS QDs with a diameter around 4–6 nm [43]. A clear fluorescence peak with a maximum emission signal (F) at the wavelength of 470 nm was observed when excited at 266 nm (Figure S1B), indicating a homogeneous size distribution of the CdS QDs.

The morphology and size of the formed CdS QDs were further evaluated by transmission electron microscopy (TEM, Figure 2). It can be seen from Figure 2A that the synthesized CdS QDs were spherical, with good dispersion and uniform particle size. A particle size histogram has been generated to assess size distribution of CdS QDs. As shown in Figure S2, the median diameter of CdS QDs is approximately 5 nm. Figure 2B shows the HRTEM image of the CdS QDs, where regular lattice fringes can be observed. The measured lattice stripe spacing in the HRTEM image is 0.201 nm, which corresponds to the (220) crystal plane of CdS QDs [44,45].

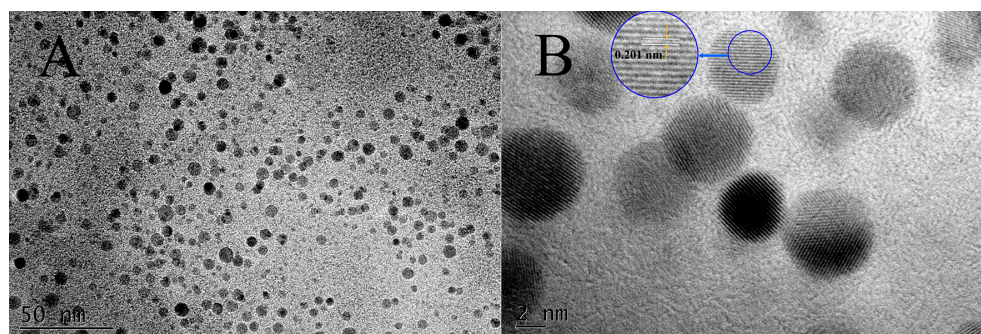


Figure 2. (A) TEM image and (B) high-resolution lattice diagram of CdS QDs.

To investigate the composition of the synthesized QDs, XPS measurements were performed for elemental analysis. As shown in Figure 3, peaks originating from P, O, Cd, and S emerged. The high-resolution spectra of these elements were also recorded (Figure S3). By integrating the XPS peak area of each element, the relative atomic percentages of P, O, Cd, and S in the QDs were determined to be 11.52%, 47.79%, 17.64%, and 3.87%, respectively. The calculated atomic percentage ratio of O to P is close to 4, which is consistent with PO_4^{3-} . The ratio of Cd to S is larger than 1, indicating that there are more Cd atoms than S atoms in a QD, and the prepared QDs have a Cd-enriched surface. This observation is plausible, as a larger amount of Cd precursor was added during the synthesis process. These results indicate that the QDs are mainly composed of PO_4^{3-} and CdS, thus confirming the successful formation of PO_4^{3-} -stabilized CdS QDs.

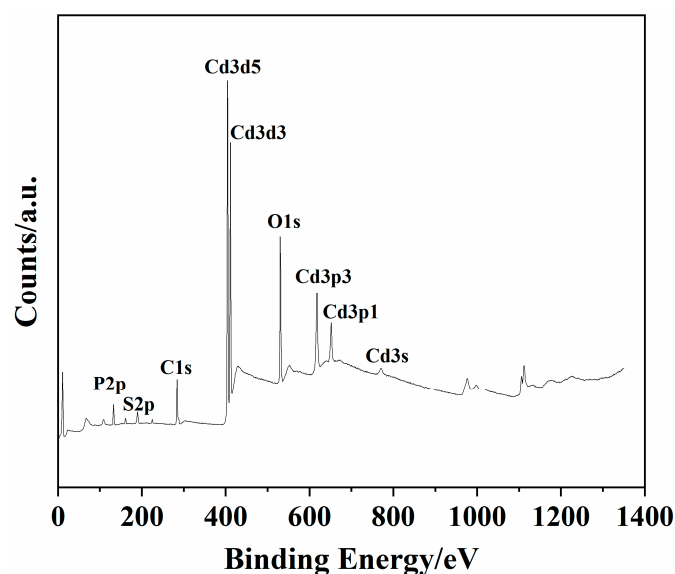


Figure 3. XPS survey of the PO_4^{3-} -stabilized CdS QDs.

3.3. Sensing Mechanism of the Proposed Fluorescence Assay

To confirm the proposed mechanism and feasibility of the fluorescence method, control experiments with one component omitted were performed. The results of these experiments are shown in Figure 4. It can be seen that a significant increase in the fluorescence signal (F) was only observed when all components were present in the detection system (Cd^{2+} , S^{2-} , and PO_4^{3-}) (Curve d). In contrast, if any of the components was absent (Curve a–c), the observed fluorescence signal was very weak. It is worth noting that the fluorescence intensity was further enhanced by increasing PO_4^{3-} concentration (Curve e). These results confirmed that the presence of PO_4^{3-} is indispensable for the formation of fluorescent CdS QDs, and the proposed assay has potential for PO_4^{3-} detection. A TEM

image of the solution containing Cd^{2+} and S^{2-} (without PO_4^{3-} addition) was further acquired. As shown in Figure S4, only bulk aggregated particles are formed in the absence of PO_4^{3-} . Thus, we deduced that PO_4^{3-} acts as stabilizer to facilitate the formation of well-dispersed CdS QDs.

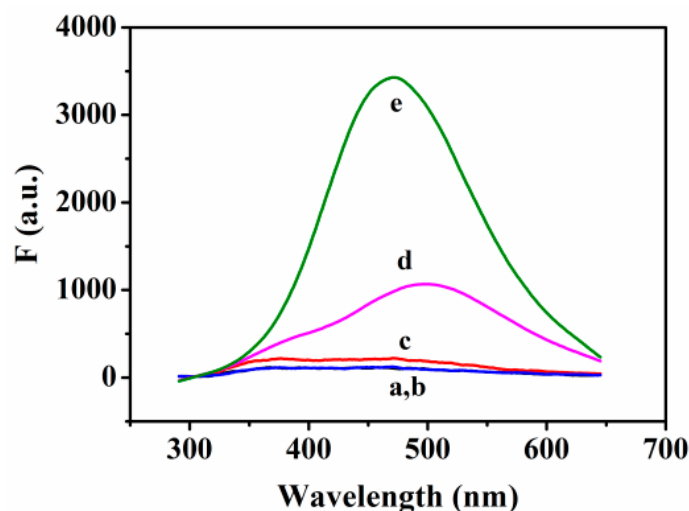


Figure 4. Fluorescence spectra of the system under different experimental conditions (Curve a: $\text{Cd}^{2+} + \text{PO}_4^{3-}$, b: $\text{S}^{2-} + \text{PO}_4^{3-}$, c: $\text{Cd}^{2+} + \text{S}^{2-}$, d: $\text{S}^{2-} + \text{Cd}^{2+} + \text{PO}_4^{3-}$ (0.1 mM)), e: $(\text{S}^{2-} + \text{Cd}^{2+} + \text{PO}_4^{3-})$ (0.25 mM)).

3.4. Optimization of the Experimental Conditions

To obtain optimal response signal, several experimental conditions, including the concentrations of Na_2S , $\text{Cd}(\text{AC})_2$, the reaction time and the pH were optimized. The effect of S^{2-} concentration on the fluorescence signal was investigated. As shown in Figure S5A, the fluorescence intensity increased rapidly when the concentration of Na_2S changed from 0.012 to 0.060 mM, then decreased when the concentration was above 0.060 mM. Thus, a Na_2S concentration of 0.060 mM was selected for the experiments. The effect of Cd^{2+} concentration was also investigated (Figure S5B). The fluorescence intensity reached a maximum value at the concentration of 0.55 mM. Thus, 0.55 mM Cd^{2+} was used in subsequent experiments. The CdS QDs formation time was further investigated. From Figure S5C, it can be seen that the fluorescence intensity tended to stabilize after 3 min. This result indicated that the reaction is very rapid, and the CdS QDs can be formed within 3 min. The effect of pH on the formation of CdS QDs was also investigated. The largest fluorescence intensity was produced at pH 8.0 (Figure S5D).

3.5. Sensitivity of the PO_4^{3-} Detection System

To estimate the detection sensitivity and linear range of the fabricated fluorescence assay, PO_4^{3-} at different concentrations was detected under optimal conditions. It can be seen from Figure 5A that the fluorescence intensity (F) increased significantly with the increase in PO_4^{3-} concentration up to 0.30 mM. It means that the more PO_4^{3-} added, the more CdS QDs were formed. It should be noted that the PO_4^{3-} concentration affects the physical and optical properties of the material. As shown in Figure 5, the increase in PO_4^{3-} concentration leads to a blueshift in the emission peaks (at 470 nm) due to the decrease in the diameter of the resulting CdS QDs, caused by the quantum confinement of the electrons in the quantum dots [46]. The change in the fluorescence intensity caused by different concentrations of PO_4^{3-} was more significant than the wavelength shift value. Therefore, the fluorescence intensity was used as the detection signal in subsequent work. There was a good linear relationship between the fluorescence intensity ratio and PO_4^{3-} concentration in the range of 3.0–300 μM , with a correlation coefficient (R) of 0.995 (Figure 5B).

By calculating the triplicate standard deviation of the blank signal (3σ), the obtained detection limit of PO_4^{3-} was $2.9 \mu\text{M}$. The fluorescence quantum yield and fluorescence lifetime of PO_4^{3-} -stabilized CdS QDs were determined to be 3.12% and 25.37 ns (Figure S6), respectively.

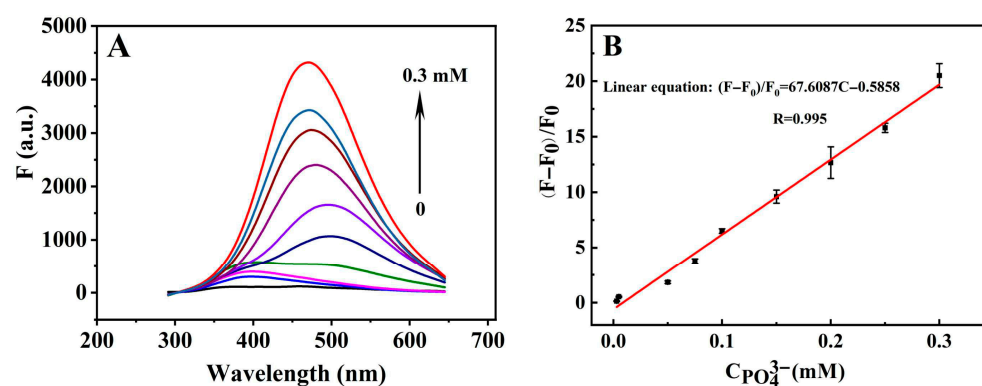


Figure 5. (A) Fluorescence spectra of PO_4^{3-} in the proposed assay with different concentrations (Down to up: 0, 0.003, 0.005, 0.05, 0.075, 0.10, 0.15, 0.20, 0.25, 0.30 mM); (B) The linear relationship between fluorescence signal ratio $(F - F_0)/F_0$ and PO_4^{3-} concentration. (F refers to the fluorescence signal of the CdS QDs at 470 nm, F_0 refers to the fluorescence signal of the blank, and C is the concentration of PO_4^{3-} . The error lines represent the standard deviation of three determinations).

Comparing the results of this study with those of other reported methods (Table S1), the sensitivity of the fabricated assay was higher than or equal to that of some published colorimetric and fluorescence assays [47–55]. In addition, this method has the characteristics of being label-free, easy to operate, rapid and economical. CdS QDs can be synthesized in situ under mild conditions in 5 min and do not require costly reagents such as precious metals, proteases or nucleic acids, etc.

3.6. Selectivity and Recovery Experiment of the Fluorescence Assay

To verify the specificity of the fluorescence assay for PO_4^{3-} , the fluorescence response of 15 types of interfering ions was studied under the same testing conditions. All of the anions were evaluated at concentrations at least 10 times that of PO_4^{3-} . As shown in Figure 6, a significant fluorescence signal (F) recorded at 470 nm can only be induced by PO_4^{3-} , whereas the other ions had little effect on the fluorescence intensity. To investigate the influence of common cations and anions on PO_4^{3-} detection, competition experiments using mixtures of PO_4^{3-} with various ions have been further performed (Figure S7). Under the same concentrations, the fluorescence intensity showed only a slight change in the presence of common ions with the exception of Ag^+ . The fluorescence intensity was significantly decreased in the presence of Ag^+ , which may be attributed to the fluorescence-quenching effect of Ag^+ on CdS QDs. Typically, the concentration of Ag^+ in environmental water samples such as tap water is much lower than that of PO_4^{3-} . When the concentration of Ag^+ decreases to the μM level, its impact on the fluorescence intensity is significantly diminished. These results indicated that other ions in the water will not have potential interference with the detection of PO_4^{3-} and thus the proposed method has good selectivity for PO_4^{3-} .

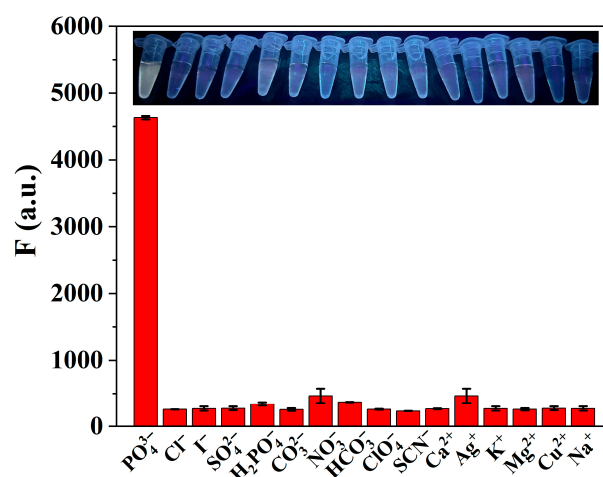


Figure 6. Interference study of the fluorescence assay under the addition of different kinds of ions. (Inset: Photos of the corresponding solution under ultraviolet light. The concentration of PO_4^{3-} was 0.30 mM, and other ions were 5.0 mM).

The practical applicability of the fluorescence assay was verified by determining PO_4^{3-} in tap water and wastewater samples. Different amounts of standard PO_4^{3-} were added to the collected tap water to prepare spiked samples. Each sample was tested three times in parallel ($n = 3$), and the results are listed in Table 1. Recovery rates in the range of 98.0–103.0% were obtained. The relative standard deviation (RSD) was less than 10%. The wastewater collected from a drainage outlet of a rare earth factory in Changting was tested (Figure S8), and the PO_4^{3-} content was determined to be 0.159 mM. The result of our assay was compared with the traditional standard phosphomolybdate blue method to verify its accuracy. The detailed operation of the phosphomolybdate blue method was referred to that of previous work [56,57] and the standard curves for PO_4^{3-} determination are listed in Figure S9. Figure S10 shows the absorption spectrum of the standard method for the detection of PO_4^{3-} in wastewater samples. The content of PO_4^{3-} in wastewater was determined to be 0.166 mM, which is close to the result of the QDs-based assay. These results showed that the proposed assay possesses good accuracy and repeatability for PO_4^{3-} detection in environmental samples.

Table 1. Analysis of PO_4^{3-} in real water samples ($n = 3$).

Samples	Added (mM)	Found (mM)	Recovery (%)	RSD (%)
Tap water	0.00	0.013	-	0.10
	0.10	0.111	98.0	5.70
	0.30	0.322	103.0	9.90

4. Conclusions

In conclusion, a label-free, simple, rapid, and economical fluorescence assay was constructed for the detection of PO_4^{3-} based on in situ synthesized quantum dots, with high sensitivity and specificity. With the increase in PO_4^{3-} concentration, the CdS QDs are rapidly formed in aqueous solutions with an excellent fluorescence enhancement response. The entire detection process can be completed within 5 min. The proposed fluorescence assay can be used to detect PO_4^{3-} in the concentration range from 0.0030 to 0.30 mM with a detection limit of 2.9 μM . In addition, recovery experiments demonstrated the ability of the fluorescence assay to recognize and detect PO_4^{3-} in real water sample analysis. Compared with traditional fluorescence assays in which QDs were pre-synthesized and then tethered with recognition elements to achieve analysis, our assay requires neither complex

chemical modification procedures nor expensive biological agents (such as nucleic acids or proteases). It is worth mentioning that by combining with the enzyme-catalyzed reaction to regulate the production of PO_4^{3-} , the present simple fluorescence assay can find broad application in bioanalytical fields.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/chemosensors14020041/s1>, Figure S1: (A) UV-vis absorption spectrum and (B) fluorescence excitation (black lines) and emission (red lines) spectra of CdS QDs; Figure S2: Histogram of particle size distribution of the synthesized CdS QDs; Figure S3: XPS scan of different elements in the synthesized CdS QDs (A) P2p, (B) O1s, (C) Cd3d, and (D) S2p; Figure S4: TEM image of the solution without addition of PO_4^{3-} ; Figure S5: The influence of (A) Na_2S concentration, (B) $\text{Cd}(\text{AC})_2$ concentration, (C) reaction time and (D) pH on the fluorescence intensity; Figure S6: Luminescence emission decay time of PO_4^{3-} -stabilized CdS QDs; Figure S7: Interference study of the fluorescence assay under the mixed solution of PO_4^{3-} with other ions individually; Figure S8: Fluorescence spectrum of PO_4^{3-} detection in wastewater by the CdS QDs-based method; Figure S9: (A) UV-visible absorption spectra of standard phosphomolybdate blue method for the detection of PO_4^{3-} , (B) linear relationship between different concentrations of PO_4^{3-} against corresponding absorption values; Figure S10: Absorption spectrum of standard phosphomolybdate blue method for the detection of PO_4^{3-} in wastewater sample; Table S1: Comparison of our present work with other methods for PO_4^{3-} detection.

Author Contributions: X.Y.: Methodology, Material preparation, Formal analysis and Investigation, Writing—original draft preparation. Z.L.: Methodology, Investigation, Data collection and analysis. Y.W. (Youjiao Wu): Formal analysis, Investigation, Methodology, Writing—review and editing. X.M.: Resources, Formal analysis, Writing—review and editing. Y.W. (Yiwei Wang): Writing—review and editing, Resources, Supervision, Funding acquisition. S.T.: Methodology, Conceptualization, Formal analysis, Writing—review and editing, Funding acquisition. W.C.: Formal analysis, Supervision, Writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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