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Ultrasensitive Cu^{2+} sensing by near-infrared-emitting CdSeTe alloyed quantum dots

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ABSTRACT

The near-infrared (NIR)-emitting CdSeTe alloyed quantum dots (AQdots) that capped with L-cysteine were applied for ultrasensitive Cu^{2+} sensing. The sensing approach was based on the fluorescence of the AQdots selectively quenched in the presence of Cu^{2+}. Experimental results showed a low interference response towards other metal ions. The possible quenching mechanism was discussed on the basis of the binding between L-cysteine and the metal ions. In addition, biomolecules have low effect on the fluorescence due to the minimized interferences in NIR region. The response of the NIR optical sensor was linearly proportional to the concentration of Cu^{2+} ranging from 2×10^{-8} to 2×10^{-6} mol L^{-1}. Furthermore, it has been successfully applied to the detection of Cu^{2+} in vegetable samples.

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1. Introduction

In recent years, contamination by large amount of heavy metals poses a serious threat to human health and the environment [1,2]. Copper is one of the heavy metals and essential element for many living organisms, it becomes toxic at high concentrations [3]. Therefore, the design and development of selective as well as sensitive method for Cu^{2+} detection in environmental and biological samples is of great importance for analytical chemists. Many methods for the detection of Cu^{2+} have been developed such as atomic absorption spectroscopy [4,5], inductively coupled plasma mass spectroscopy [6,7], X-ray fluorescence [8]. These methods offer good limits of detection (LODs) and wide working concentration ranges. However, these methods may require the use of sophisticated and relatively costly apparatus, and are unsuitable for on-line or in-field monitoring [9].

Chemical sensors can overcome these disadvantages and become increasingly important for monitoring metal ion. Some sensors have been developed for the determination of Cu^{2+}. A variety of organic fluorophore-based sensors have been proposed to selectively respond to Cu^{2+} due to their relatively high sensitivity [10–13]. However, organic fluorophore often suffer from the disadvantages like narrow excitation bands, broad emission spectra and fast photobleaching. Recently developed quantum dots (Qdots) have substantial advantages over organic dyes, including excellent fluorescence properties, high photochemical stability and excellent resistant to chemical degradation [14–16]. Therefore, the Qdots-based sensors have great potential to overcome the problems encountered with existing organic fluorophore-based sensors.

Therefore, the synthesis and application of these Qdots are of great interest in the development of novel sensitive sensors. So far only a few studies on Qdots system for Cu^{2+} detection have been reported. Chen and Rosenzweig showed the first example of thioglycerol-coated CdS Qdots sensor for Cu^{2+} [17]. Later, the optical detection of Cu^{2+} with peptide-coated CdS Qdots [18], $Cd_{10}S_{16}$ molecular cluster [19], MPA-capped CdSe [20] or CdTe [21], ZnS Qdots [22] and CdSe/CdS Qdots [23] was also proposed. However, most of the existing Qdots-based sensors were emitting at wavelengths below 550 nm. In addition, selectivity, stability and limited applicability to real sample were still among the limitations for the Qdots-based sensors proposed [24]. Therefore, the stable Qdots with near-infrared (NIR)-emission between 650 and 900 nm are of particular interest for the detection because many molecules' autofluorescence and absorbance can be reduced to the minima in this region [16,25,26]. Up to now, the sensors based on NIR-emitting Qdots for metal ions detection are very rare.

In this study, the NIR-emitting CdSeTe AQdots were prepared in aqueous solution with L-cysteine as stabilizer, and then it was demonstrated as a selective fluorescent Cu^{2+} probe with low detection limit. Moreover, the proposed sensing system has been applied for the determination of Cu^{2+} in vegetable samples and the recovery test was satisfactory.

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2. Experimental

2.1. Apparatus

The fluorescence spectra of the Qdots samples were obtained with an Edinburgh FLS920P fluorescence spectrometer (Edinburgh Instruments Ltd., UK). The optical absorption spectra were measured using the Shimadzu 3600 UV–vis spectrometer (Shimadzu, Japan). The TEM images were acquired using a JEOL JEM-2100 (JEOL, Japan) transmission electron microscope operating at an acceleration voltage of 200 kV. The elemental analysis of Qdots was performed on VG PQExCell ICP-MS system.

2.2. Reagents

Selenium powder (Se, 99.5%, 200 mesh), tellurium powder (Te, 99.8%, 200 mesh) and mercaptopropionic acid (MPA, 99%) were purchased from Acros Organics (NJ, USA). L-Cysteine (98%) was purchased from Sigma–Aldrich. Cadmium chloride was purchased from Tingxin Chemical Reagent (Shanghai, China). Sodium borohydride was purchased from Tianjin Chemical Research Institute (Tianjin, China). The stock standard solutions (10 mmol L^{-1}) of Cu^{2+} were prepared by dissolving appropriate amounts of $Cu(NO_3)_2{\cdot}3H_2O$ in high purity water. Working solutions were prepared by appropriate dilutions of their stock solutions. All other reagents were of analytical grade and used without further purification. The ultrapure water with 18.2 MΩ cm^{-1} (Millipore Simplicity, USA) was used throughout the experiments.

2.3. Preparation of the AQdots

The L-cysteine-capped CdSeTe AQdots with NIR-emission were prepared through the incorporation of selenium ions into CdTe nanocrystals as reported in our previous method [27]. L-Cysteine as a sulfur amino acid was selected as capping agent for its good compatibility and the protection of Qdots-induced toxicity [28]. In the synthesis, all reactions were carried out in oxygen-free water under nitrogen. In brief, $CdCl_2$ (0.25 mmol) and L-cysteine (0.6 mmol) were dissolved in water (200 mL) and adjusted to pH 11.5 with 1 mol L^{-1} NaOH. The resulting transparent solution was deaerated by bubbling of nitrogen for 20 min and was heated to 95 °C. Under vigorous stirring, the freshly prepared 0.125 mmol of NaHTe and 0.04 mmol of NaHSe aqueous solutions were injected to the above solution. Afterward, the reaction mixture was further refluxed at 95 °C under a nitrogen atmosphere. The emission wavelength of the AQdots could be tuned by changing the refluxing time. Part of the refluxing solution was taken out at regular intervals for characterization. After 3 h, the CdSeTe AQdots with emission wavelength of 685 nm (CdSeTe685) was obtained.

2.4. Selectivity measurements

The following inorganic salts were used for the cation selectivity experiment: nickel acetate tetrahydrate, zinc acetate, barium chloride, calcium chloride, iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate, sodium chloride, potassium chloride, lanthanum nitrate hexahydrate, aluminum nitrate, cobaltous sulfate, magnesium chloride, copper nitrate and lead nitrate. A 10 mmol L^{-1} salt stock solution was prepared with ultrapure water. Subsequently, the salt solutions were prepared by serial dilution with Tris–HCl solution (pH 7.4).

2.5. Procedure for spectrofluorometric detection of Cu^{2+}

Copper nitrate was used for the Cu^{2+} sensitivity studies. Various concentrations of Cu^{2+} were prepared using serial dilution of the copper nitrate stock solution to test the sensitivity limits of the AQdots. A known concentration of Cu^{2+} solution was added into the AQdots nanoparticle solution and mixed thoroughly for 10 min. The fluorescence intensity of the solution was recorded at 685 nm with the excitation wavelength of 300 nm. Both slit widths of excitation and emission were 2 nm.

2.6. Physical characterization of the fluorescence quenching

An amount of 5 μL of 1 mmol L^{-1} Cu^{2+} or Al^{3+} was added to 1 mL of 160 nmol L^{-1} CdSeTe685 AQdots solution. After incubation for 20 min, the suspended solutions of the nanoparticles were deposited onto copper grids with carbon support by slowly evaporating the solvent in air at room temperature. The high-resolution transmission electron microscopy (HR-TEM) images of Cu^{2+} and Al^{3+}-treated CdSeTe685 AQdots were acquired using a JEOL JEM-2100 (JEOL, Japan) transmission electron microscope.

2.7. Sample preparation

The vegetable samples of bean, cucumber and tomato were purchased from local market. And the accurately weighted samples were ground and then digested with 20 mL concentrated nitric acid on a gas burner until the solution became wet salt. Then, it was dissolved with 2 mol L^{-1} HNO_3 and heated until it was clear. Finally, the solution was diluted with water.

3. Results and discussion

3.1. Spectral characteristics of the AQdots

The ultrafiltration-purified L-cysteine-capped CdSeTe AQdots were well dispersed in PBS or Tris–HCl solution (10 mmol L^{-1}, pH 7.4). The absorption spectra and fluorescence spectra of the AQdots were obtained and shown in Fig. 1. The maximal fluorescence wavelength was 685 nm. It can be seen that the full width at half-maximum (FWHM) fluorescence intensity is about 60 nm and symmetric, showing that the CdSeTe AQdots were nearly of monodisperse and homogeneous. In addition, the fluorescence intensity of these AQdots was pH-dependent [29] as shown in Fig. 1B. The low fluorescence intensity in acidic medium is the result of dissociation of the nanoparticles due to protonation of the surface-binding thiolates [30]. With the increase of pH, the deprotonation of the thiol group in the L-cysteine molecule occurs. This deprotonation may strengthen the covalent bond between Cd and L-cysteine molecule, which leads to the fluorescence intensity increases with pH increasing. However, the fluorescence intensity was declined with the further increase of pH value.

3.2. Selective fluorescence quenching of the AQdots by Cu^{2+}

The experiment of selective fluorescence quenching was performed with the CdSeTe AQdots (160 nmol L^{-1}, λ_{max} = 685 nm). The purified AQdots solutions contained no free L-cysteine. The AQdots were dispersed in Tris–HCl buffer solution (10 mmol L^{-1}, pH 7.4) for its pH-dependent fluorescence intensity and then added to the same amount of metal cation solutions (1 μmol L^{-1}). The fluorescence intensity of these samples was measured and the results were shown in Fig. 2. It was found that the fluorescence intensity of AQdots has more sensitivity to Cu^{2+} than to Pb^{2+}, Fe^{2+} and no response to other cations such as K^+, Na^+, Mg^{2+}, Al^{3+}, Ca^{2+} and Zn^{2+}. In contrast, they demonstrated complete fluorescence quenching in the presence of Cu^{2+}. The quenching effect of Cu^{2+} on fluorescence emission of CdSeTe AQdots was found to be concentration dependent. Therefore, this selective response towards Cu^{2+} can be

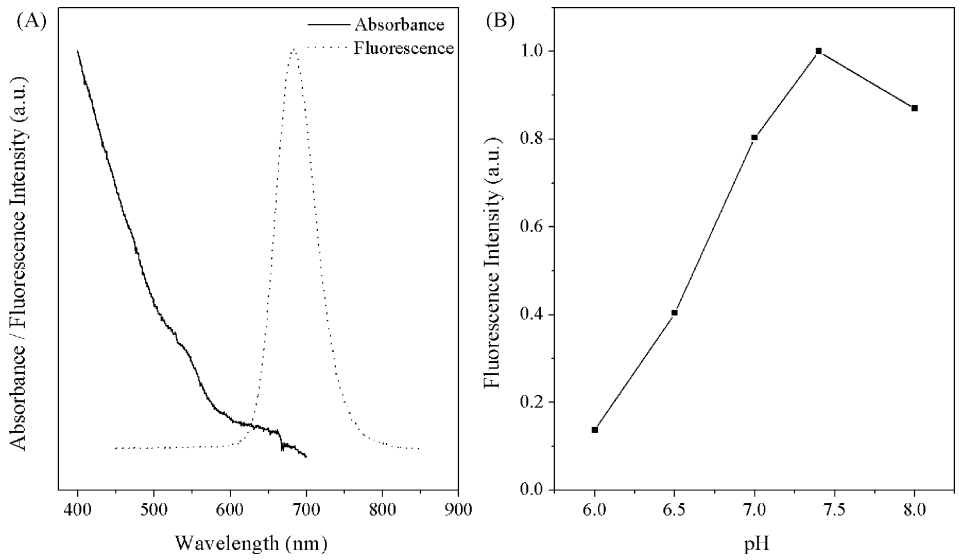

Fig. 1. (A) Absorption and fluorescence spectra of the L-cysteine-capped CdSeTe AQdots. (B) Fluorescence intensity of the CdSeTe AQdots (λ_{max} = 685 nm) in PBS buffer (10 mmol L^{-1}) at different pH values.

used for the development of a sensitive and selective sensor for Cu^{2+}.

3.3. Mechanism of the quenching effect by Cu^{2+}

L-Cysteine as a sulfur amino acid was an ideal capping agent for the CdSeTe AQdots synthesis. The cysteine capping layer was very important to improve water solubility and stability of the AQdots. Therefore, the metal–sulfide bond strength, which was characterized by their respective K_{sp} value, may be an important parameter for the fluorescence quenching effect. And the selective fluorescence quenching could be rationalized by the competitive cysteine binding between the AQdots and the metal ions present in the solution. The K_{sp} value of Cu–S (6.3×10^{-36}) is much lower than that of Cd–S (8.0×10^{-27}), Pb–S (8.0×10^{-28}), Zn–S (2.9×10^{-25}) and Fe(II)–S (6.3×10^{-18}) [31]. Therefore, we proposed that the cysteine capping layer was preferentially displaced from the surface of the CdSeTe AQdots upon the binding of Cu^{2+}. The displacement of cysteine capping consequently created imperfections on the AQdots surface, resulted in fluorescence quenching. In order to verify this hypothesis, the quenching effect of different metal ions on the fluorescence intensity of the MPA-capped CdTe Qdots (λ_{max} = 650 nm) was also investigated (Fig. 2). The MPA-capped CdTe Qdots were synthesized according to the reported methods [32]. It can be found that the quenching effect of the MPA-capped CdTe Qdots displays similar results with the L-cysteine-capped CdSeTe AQdots.

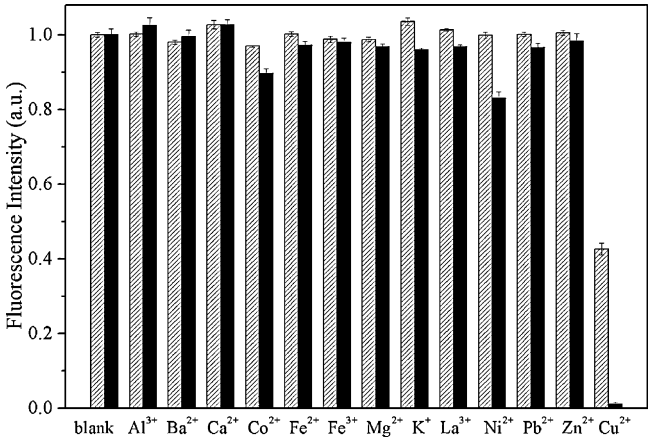

Fig. 2. The quenching effect of different ions on the fluorescence intensity of the MPA-capped CdTe Qdots (λ_{max} = 650 nm) (▨) and the L-cysteine-capped CdSeTe AQdots (λ_{max} = 685 nm) (■) in 10 mmol L^{-1} Tris–HCl buffer at pH 7.4. The excitation wavelength was 300 nm.

In addition, the fluorescence quenching experiments were conducted in the presence of free cysteine. The reduced fluorescence quenching in the presence of Cu^{2+} was observed in the CdSeTe AQdots solutions containing free cysteine. And similar reduced fluorescence quenching of the MPA-capped CdTe Qdots was observed in the presence of Cu^{2+} when the free MPA increased in the CdTe Qdots solution. This also suggested that the thiol capping agent of the Qdots was related to the fluorescence quenching effect. And the aggregation of the AQdots was also observed at high Cu^{2+} concentrations, leading to precipitation in the presence of 10 μmol L^{-1} Cu^{2+}. At high Cu^{2+} concentrations, loss of more cysteine would eventually cause the AQdots to aggregate and precipitate. The control experiment showed no aggregation of the AQdots in the same concentration of Al^{3+}. The low magnification TEM image (Fig. 3B and C) of CdSeTe confirmed the aggregation of the AQdots.

Based on the investigation, we concluded that the competitive binding of the cysteine with Cu^{2+} was the primary mechanism for the fluorescence quenching. The fluorescence intensity of CdSeTe AQdots was highly sensitive to their surface protection. Even the removal of a limited amount of surface-bound cysteine would lead to dramatic reduction in fluorescence intensity. Therefore, the lower detection limit and higher sensitivity were achieved with the lower concentration of cysteine-capped CdSeTe AQdots.

3.4. The detection of Cu^{2+}

The detection of Cu^{2+} is of particular interest due to copper importance in environment and living organisms. The CdSeTe AQdots displayed great fluorescence quenching at low concentration of Cu^{2+} compared to other metal ions. The fluorescence quenching was best described by the Stern–Volmer equation,

$$\frac{F^0}{F} = 1 + K_{SV}[Q],$$

where F and F^0 are the fluorescence intensity of the CdSeTe AQdots in the presence and absence of Cu^{2+} respectively, [Q] is the con-

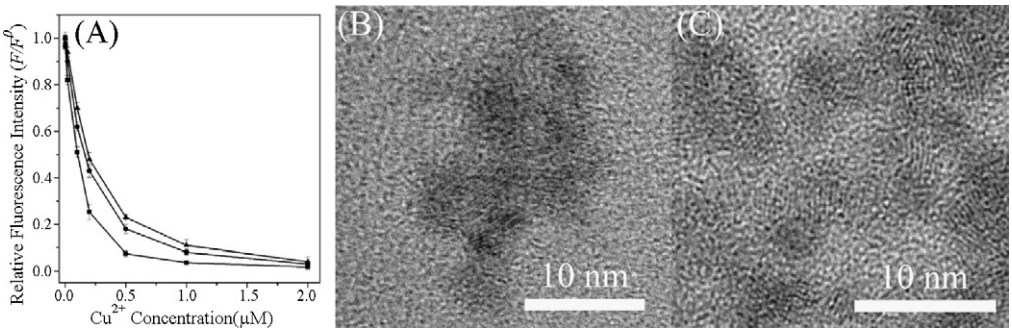

Fig. 3. (A) Fluorescence quenching by Cu^{2+} for 80 nmol L^{-1} of CdSeTe AQdots (λ_{max} = 685 nm) in the absence of free L-cysteine (■) and presence of 40 μmol L^{-1} (●) and 60 μmol L^{-1} (▲) of free L-cysteine, respectively. The excitation wavelength was 300 nm. (B) The TEM image of the CdSeTe AQdots (λ_{max} = 685 nm) in the presence of 5 μmol L^{-1} Cu^{2+}. (C) The TEM image of CdSeTe AQdots (λ_{max} = 685 nm) in the presence of 5 μmol L^{-1} Al^{3+}.

centration of the quencher (i.e., Cu^{2+}) and K_{SV} is the Stern–Volmer constant.

The influence of the concentration of L-cysteine-capped CdSeTe AQdots on fluorescence quenching by Cu^{2+} was studied. The strong fluorescence quenching was observed at low CdSeTe AQdots concentration in the presence of the same Cu^{2+} concentration as shown in Fig. 4A. Fig. 4B illustrated the high reproducibility of the CdSeTe AQdots response to Cu^{2+}. The linear relationship of the Stern–Volmer plot of F^0/F to Cu^{2+} concentration (Fig. 4C) was obtained. The linear relationship between $1/K_{SV}$ and CdSeTe AQdots concentration as shown in Fig. 4D, which suggested that the detection limit can be reduced with the decrease of AQdots concentration. Therefore, the CdSeTe AQdots concentration of 80 nmol L^{-1} was recommended. The calibration plot of F^0/F versus [Q] shows a good linear relationship (R^2 = 0.9976) for Cu^{2+} concentration in the range of 2×10^{-8} to 2×10^{-6} mol L^{-1} (Fig. 4C). The limit of detection was evaluated using $3\sigma/S$, and is found to be 7.1×10^{-9} mol L^{-1}, where σ is the standard deviation of the blank signal, and S is the slope of the linear calibration plot.

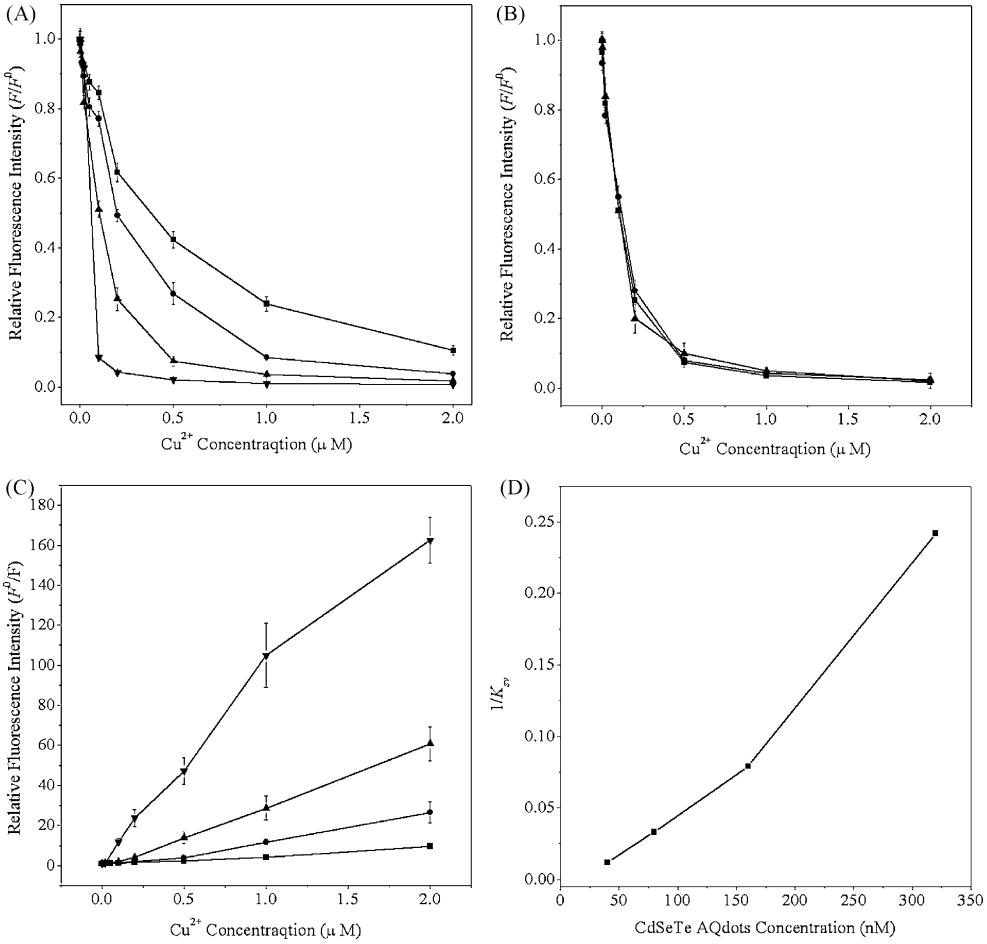

Fig. 4. (A) The quenching effect of Cu^{2+} concentration on the fluorescence intensity of 40 nmol L^{-1} (▼), 80 nmol L^{-1} (▲), 160 nmol L^{-1} (●) and 320 nmol L^{-1} (■) CdSeTe AQdots. (B) The reproducible determination of the fluorescence quenching effect of the 80 nmol L^{-1} CdSeTe AQdots. (C) Stern–Volmer plots of the different CdSeTe AQdots concentration. (D) Linear correlation of $1/K_{SV}$ values of the CdSeTe AQdots. The L-cysteine-capped CdSeTe AQdots (λ_{max} = 685 nm) was used. The excitation wavelength was 300 nm.

Table 1
Detection of Cu^{2+} in real samples.

Samples	Cu^{2+} concentration (mg/kg)				
	Proposed method	ICP-MS method	Added	Total	Recovery (%)
Bean	3.51	3.10	5	8.87	107.2
Cucumber	2.62	2.94	5	7.86	104.8
Tomato	1.96	1.65	5	7.44	109.6

3.5. Sample analysis

The proposed method has been applied to the analysis of vegetable samples including bean, cucumber and tomato. A control experiment was carried out using inductively coupled plasma-optical emission spectroscopy (ICP-OES) method. Table 1 gives the analysis results of Cu^{2+} in these vegetable samples. With standard addition method, the recoveries were found to be 104.8–109.6%. The analysis results agreed with those obtained with ICP-MS methods.

4. Conclusions

The near-infrared (NIR)-emitting L-cysteine-capped CdSeTe alloyed quantum dots (AQdots) have been demonstrated selective fluorescence quenching in the presence of Cu^{2+}. The selective quenching effect could be rationalized by the competitive cysteine capping layer binding between the CdSeTe AQdots and the metal ions present in the solution. Based on this finding, we proposed a novel NIR fluorescence sensor for sensitive Cu^{2+} detection in aqueous medium. The limit of detection (LOD) of this method is higher than that of the previous reported methods. Under the optimum conditions, the detection range of the proposed method was from 2×10^{-8} to 2×10^{-6} mol L^{-1} with the LOD of 7.1×10^{-9} mol L^{-1}. Furthermore, the practical utility of the CdSeTe AQdots sensor has been demonstrated by the determination of trace Cu^{2+} in vegetable samples, obtaining satisfactory results with the reference method. Our preliminary studies have demonstrated the potential of this method for practical applications.

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