Efficient Ratiometric Fluorescence Probe Based on Dual-Emission Quantum Dots Hybrid for On-Site Determination of Copper Ions

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Ⓢ Supporting Information

ABSTRACT: Of various chemosensory protocols, the color change observed by the naked eye is considered to be a conceivable and on-site way to indicate the presence of an analyte. We herein designed a ratiometric fluorescence probe by hybridizing dual-emission quantum dots (QDs) and demonstrated its efficiency for on-site visual determination of copper ions. The hybrid probe comprises two sizes of cadmium telluride QDs emitting red and green fluorescence, respectively, in which the red-emitting ones are embedded in silica nanoparticles and the green-emitting ones are covalently linked onto the surface. The fluorescence of the embedded QDs is insensitive to the analyte, whereas the green emissive QDs are functionalized to be selectively quenched by the analyte. Upon exposure to different amounts of copper ions, the variations of the dual emission intensity ratios display continuous color changes from green to red, which can be clearly observed by the naked eye. The limit of detection for copper is estimated to be 1.1 nM, much lower than the allowable level of copper ($\sim$20 μM) in drinking water set by U.S. Environmental Protection Agency. The probe is demonstrated for the determination of copper ions in lake water and mineral water samples, especially for visually monitoring copper residues on herb leaves. This prototype ratiometric probe is simple, fully self-contained, and thus potentially attractive for visual identification without the need for elaborate equipment.
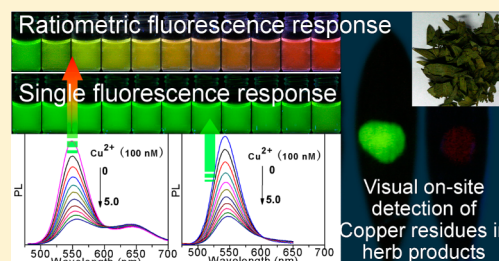
Ratiometric fluorescence technique for constructing chemo/bio sensors has attracted increasing attention in recent years, owing to its advantages in terms of improved sensitivity at trace quantity levels of analyte and built-in correction for environmental effects.[1−8] Those ratiometric sensors employing fluorescent organic dyes are usually susceptible to photobleaching, low fluorescence quantum yield, and exhibit generally broad emission bands and narrow excitation wavelength range.[9−11] Such notorious properties of organic dyes often make it hard to construct efficient ratiometric fluorescence sensors. Fortunately, fluorescent quantum dots have been demonstrated possessing distinct optical properties, including a high absorption coefficient across a broad excitation spectra, narrow emission peak and resistance to photobleaching.[12−14] Most importantly, quantum dots (QDs) with different sizes can be excited by a single wavelength and emit at different bands, affording the convenience of incorporating different-sized QDs and enabling the feasibility to create a multicolor system on the basis of a ratiometric fluorescence probe.[15,16] In this work, we demonstrate a concept to use QDs as the fluorophores for the construction of an effective ratiometric fluorescence probe, showing good sensitivity and selectivity for the visual detection of copper ions (Cu^{2+}) in aqueous solution.

Copper is one of important elements for human health, besides zinc and iron, which ranks third in abundance among the essential transition metals in the human body.[17] It plays pivotal functions in multiple physiological processes dependent on the copper homeostasis in the human body. Excess copper intake can result in kidney disease and disturbs the cellular homeostasis that will cause Wilson, Alzheimer's, and Menkes diseases.[18,19] Accordingly, U.S. Environmental Protection Agency (EPA) has set a limit of 1.3 ppm ($\sim$20 μM) of copper in drinking water.[20] Nevertheless, as a result of its widespread use in industry and agriculture, the potential toxic effects of copper ions to human beings due to its pollution in rivers or seas continue to be a global challenge.[21,22] So practical methods with high sensitivity and selectivity for on-site analysis and rapid determination of copper ions are crucial for human health and environment pollution monitoring.

The fluorescence-based method shows the application potential in revealing trace amounts of analytes because of its sensitivity, simplicity, and cost-effective instrumentation.[23−25] Besides, the changes of fluorescence color observed by the naked eye are considered to be a conceivable way to indicate the presence of an analyte.[26−30] So the development of fluorescence-based sensors for analysis of Cu^{2+} has attracted much attention.[31−43] However, most of these reported fluorescent methods for detecting copper ions are based on the quenching of fluorescence signals.[34−40] A few other
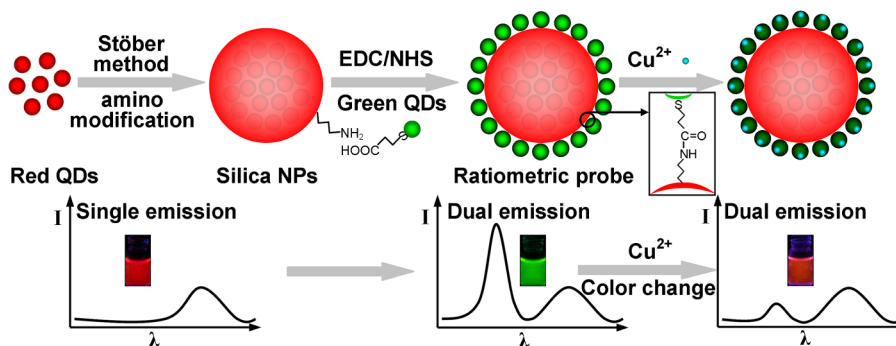
Scheme 1. Schematic Illustration of the Ratiometric Probe Structure and the Visual Detection Principle for Copper Ions

research has been focused on the enhancement of fluorescence, and the sensitivity can be improved by the appearance of a signal on a dark background.[41−43] Both of the two sensors types are based on the change in fluorescence intensity of single lumophore and tend to be interfered by a variety of factors, such as environmental conditions, instrumental efficiency, and the probe concentration. On the contrary, methods based on ratiometric fluorescence could eliminate most of the ambiguities by self-calibration of two or more different emission bands.[5,44−47] Furthermore, if the analyte induces multiple emissions with different colors from a ratiometric sensor, the perceived color changes will be useful not only for the ratiometric sensing but also for rapid visual identification.[26,44,47,48]

Therefore, we proposed a new ratiometric probe for the visual observation of fluorescence color changes induced by analytes without the need of elaborate equipment. We use fluorescent quantum dots to design the ratiometric probe because they not only afford the single-excitation/multiple emissions but also have the feasibility of surface grafting for chemical recognitions. On the other hand, the design of new dual-emission organic fluorophores is limited due to the electronic energy states in organic molecules. As illustrated in Scheme 1, the probe contains two differently sized QDs, and the larger QDs emit red fluorescence and are embedded in the silica nanoparticle. The smaller QDs emit green fluorescence and are covalently attached to the silica nanoparticle surface. The fluorescence of red QDs is stable, and the green QDs are modified with mercaptopropoinic acid to be selectively quenched by Cu^{2+}. In the presence of different amounts of Cu^{2+}, the probe displays continuous color changes from green to red due to the variations of the dual emission intensity ratios, which can be clearly observed by the naked eye. These ratio and color change signatures can be used for both qualitative recognition and quantitative analysis. The probe displays high sensitivity with a detection limit as low as 1.1 nM, which is much lower than the allowable level of copper (∼20 μM) in drinking water set by the EPA. Compared with a single wavelength emissive QD probe, the ratiometeric method exhibits significantly enhanced visual detection sensitivity. Furthermore, the probe can be used for visual identification of Cu^{2+} in real water samples, including lake and mineral water and also has been successfully facilitated for visual on-site detection of Cu^{2+} residues in herb products.

■ EXPERIMENTAL SECTION

Chemicals and Materials. Tetraethylorthosilicate (TEOS), 3-mercaptopropionic acid (MPA), 3-mercaptopropyl-trimethoxysilane (MPS), 3-aminopropyltriethoxysilane (APTS), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were received from Sigma-Aldrich. Sodium borohydride ($NaBH_4$), tellurium powder, cadmium chloride hydrate ($CdCl_2 \cdot 2.5H_2O$), ammonium hydroxide, and absolute ethanol were purchased from Sinopharm Chemical Reagent Company, Ltd. (Shanghai, China). Diarrhea leaf was obtained from a local Chinese medicine shop. Ultrapure water (18.2 MΩ cm) was provided by a Millipore water purification system.

Quantum Dots Embedding in Silica Nanoparticles and Surface Modification. The green and red emissive CdTe QDs were synthesized in the aqueous phase by a previous method.[49] The red emissive QDs (^{650}QDs, λem = 650 nm) embedded silica nanoparticles were prepared by a modification process based on the Stöber method.[50] Typically, 40 mL of ethanol, 15 mL ultrapure water, and 5 mL of red QDs solution were mixed in a 100 mL one-necked flask and stirred for 10 min at room temperature. After packing the flask with aluminum foil, 20 μL of MPS was introduced, and the resultant solution was stirred for 12 h. Then, 0.5 mL of TEOS was added dropwise into the solution, followed by adding 0.5 mL of ammonium hydroxide, and finally the mixture was left to react for another 12 h. To modify the silica surface with amino groups, 100 μL of APTS was added into the above mixture under vigorous stirring. After 12 h of reaction, the products were centrifuged and the precipitate was washed with ethanol and ultrapure water several times. Finally, the resulting nanoparticles were redispersed in 10 mL of ultrapure water for further use.

Synthesis of Dual-Emission Fluorescent Nanoparticles (Ratiometric Probe). To get the dual-emission fluorescent nanoparticles, the green emissive QDs with carboxylic groups were chemically bonded to the amino-modified silica nanoparticles by a condensation reaction. In a typical process, 2 mL of green emissive CdTe QDs (^{550}QDs, λem = 550 nm) solution was mixed with 4 mL H_2O and 2 mL of EDC/NHS (2 mg/mL) under stirring in a 25 mL flask. After 15 min, 1.3 mg of the amino-modified silica nanoparticles was injected into the mixture, and the mixture was kept stirring vigorously for 4 h in the dark. EDC and NHS were used for conjugation of the carboxylic groups of the green emissive QDs and the amino groups of the silica nanoparticle surface. The resultant ^{550}QD-coated silica nanoparticles were collected by centrifugation and washed with ultrapure water three times to remove excess QDs and chemicals. The final product was dispersed in 15 mL of ultrapure water.

Detection of Cu^{2+} Ions. The pH buffering systems were optimized by monitoring the fluorescence stability and reproducibility in different media, including acetate, phosphate, Tris, and HEPES buffers (10 mM). Metal cations can be hydrolyzed at high pH values, and the probes can be corroded at low pH conditions. So the ratiometric probe performs the best in the pH range between 6 and 8.5. The results suggest that HEPES buffer at pH 7.4 is the most suitable media for the detection of the copper ion. Fifty microliters of the as-synthesized ratiometric probes were injected into 2.0 mL of HEPES (pH = 7.4, 10 mM) solution in a spectrophotometer quartz cuvette. Then, 10.3, 10.5, 10.7, 11.0, 11.1, 11.4, 11.7, 11.9, 12.2, and 12.4 μL of copper ions (10^{-5} M) were added into the probe solution one by one, and the final concentrations of copper ions presented are 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 μmol/L. The fluorescence spectra were collected 30 s after each addition because the fluorescence spectra became stable 10 s after the addition of copper ion into the probe solution. The spectra were measured by a fluorescence spectrophotometer (LS-55, PerkinElmer) excited at 365 nm. The color changes were observed under a UV lamp (excitation wavelength at 365 nm).

Selectivity and Interference Experiments. Because Hg^{2+} tends to hydrolyze strongly in water, the solution of Hg^{2+} was prepared in 0.1 M HNO_3 for the experiments. The solutions of other metal ions were prepared in DI water for the experiments. The fluorescent responses of the ratiometric probe to the other metal ions were examined by a similar procedure mentioned above. Fifty microliters of the as-synthesized ratiometric probe solution and a series of selected metal ions (Cd^{2+}, Mg^{2+}, Ni^{2+}, K^+, Ca^{2+}, Co^{2+}, Ba^{2+}, Na^+, Pb^{2+}, and Fe^{3+}) (0.5 μmol/L) were mixed in 2.0 mL of HEPES (pH = 7.4, 10 mM) solution, respectively. Then, the mixtures were added into a spectrophotometer quartz cuvette, and the spectra were measured by a fluorescence spectrophotometer excited at 365 nm. The change of optical color was observed under a UV lamp. For the interference study of the other metal ions, 50 μM of Na^+ and K^+ and 10 μM of Cd^{2+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Ba^{2+}, Pb^{2+}, Ni^{2+}, and Fe^{3+} were mixed with 50 μL of the as-synthesized ratiometric probe in 2.0 mL of HEPES (pH = 7.4, 10 mM) solution, respectively, and the spectra were measured by a fluorescence spectrophotometer. Then, 0.5 μM of Cu^{2+} ions were introduced into the ionic mixture, and the spectra were collected, respectively. The effect of various anions, including nitrate, bromide, acetate, sulfate, sulfite, and chloride, on the fluorescence stability of the ratiometric probe has been examined by addition of corresponding sodium salts.

Detection of Cu^{2+} in Lake Water, Mineral Water, And Seawater Samples. The lake water sample was obtained from a local lake and filtered twice using the filter paper to remove the solid suspensions. The filter paper was selected using ordinary qualitative filter paper, which was produced by Whatman-Xinhua. The bottled mineral water was obtained from the supermarket. Water samples with different concentrations of Cu^{2+} were added to the sensing system, and the resultant fluorescence spectra were measured by the fluorescence spectrophotometer. This probe was used to determine the content of copper in seawater and compared with the result obtained with the ICP method. The seawater was simply filtered before the measurement.

Measurement of Cu^{2+} Residues in Spiked Herb Products. The diarrhea leaf was chosen as the spiked sample to evaluate the probe for the detection of Cu^{2+} residues in herb products. Five milliliters of 0.1 M $CuSO_4$ solution was uniformly sprayed onto the diarrhea leaves, which were then placed in a sealed beaker for 24 h. Then, the above treated diarrhea leaves were dried for 26 h under vacuum, followed by immersing in 30 mL of ultrapure water for 12 h for the extraction of Cu^{2+}. The Cu^{2+} residue solution was thus obtained from the mixture of herb and water after the insoluble draff was removed by a simple filtration. The Cu^{2+} content in the diarrhea leaves was then determined using the ratiometric probe. Typically, 50 μL of the ratiometric probe solution was added to 2 mL of the HEPES buffer (10 mM, pH = 7.4) in a quartz cuvette, and the fluorescence spectra was recorded. Subsequently, 10 μL of the filtrate of diarrhea leaves was injected into the above ratiometric probe solution, and the resultant fluorescence was recorded. The quantity of Cu^{2+} ions in extraction filtrate of diarrhea leaves was determined by a standard correlation curve. For comparison, the quantity of Cu^{2+} of the same sample was also measured by inductively coupled plasma emission spectrometer (ICP) analysis.

On-Site Visual Detection of Cu^{2+} Residues on Herb Leaves. For the demonstration of on-site visual determination of copper ions on herb products, the diarrhea leaves were first treated with copper sulfate, using the similar procedure mentioned above. Ten microliters of the ratiometric probe solution was dripped on the surface of a treated diarrhea leaf, followed by observation under a 365 nm UV lamp. The experiment was repeated three times for consistency. The amount of copper ions on the surface of diarrhea leaves was calibrated by inductively coupled plasma emission spectrometer (ICP) analysis, using the following procedure. First, the total surface area of three pieces of the treated leaves was estimated using digital photographs processed in Matlab software. The three leaves were then immersed in 1 mL of ultrapure water for 12 h for the completed extraction of Cu^{2+}, and the Cu^{2+} content of the solution was determined by ICP analysis. Thus, the quantity of Cu^{2+} on the surface area of diarrhea leaves can be obtained when the superficial area of the three leaves were calculated.

Instrumentation. The fluorescence spectra were recorded by a Pekin-Elmer LS-55 luminescence spectrometer at room temperature. The TEM samples were prepared by dropping a water solution containing the dual-emission QDs hybrid onto a holey carbon film of copper grids and air drying prior to image collection. The sizes and morphologies of the dual-emission QDs hybrid were observed using a JEOL 2010 transmission electron microscope, operating at an accelerating voltage of 200 kV. The atomic emission spectrometry of measurement was performed using a Perkin-Elmer inductively coupled plasma-optic emission spectrometer (ICP-OES), Optima 7300 DV. A GL-9406 portable UV lamp with a 365 nm emission was used for taking photos and no filter was used. All photographs were taken with a canon 350D digital camera without filters.

■ RESULTS AND DISCUSSION

The synthesis procedure and visual principle of the ratiometric probe are shown in Scheme 1. First, the red ^{650}QDs (emission peak centered at 650 nm) are fully wrapped by the silica shell to get the ^{650}QDs-embedded silica nanoparticles. The coated silica shell of the red ^{650}QDs has unique benefits, which not only improves their photo and chemical stabilities but also prevents the direct contact of ^{650}QDs with the external Cu^{2+}, thus providing a reliable reference signal for the ratiometric detection of Cu^{2+}. Because the ^{650}QDs-embedded silica particle

has a strong advantage in capacity for derivatization, the surface of the silica particles is further functionalized with 3-aminopropyltriethoxysilane, in which the end amino groups react with the surface carboxylic groups of the second green [550]QDs. The green [550]QDs are functionalized with mercaptopropionic acid (MPA) and acted as reaction sites for Cu^{2+}.[51] Through a condensation process, the green [550]QDs are chemically bonded on the silica nanoparticle surface to get the ratiometric fluorescence probe with dual emissions at 550 and 650 nm. The obtained hybrid probe can easily be redispersed in water, and yellow-green fluorescence can be observed under a 365 nm UV lamp. Cu^{2+} are considered to be particularly useful to quench the fluorescence of CdTe QDs, under the cooperation of the thiol group on the surface and the cadmium ions in the QDs. So the green fluorescence of [550]QDs can be selectively quenched by Cu^{2+}, whereas the red fluorescence of [650]QD-embedded silica particles remains constant, which result in a noticeable fluorescence color change and thus facilitating the visual detection of Cu^{2+}. Subsequently, the practicability of the novel ratiometric fluorescence probe has been explored for the detection of Cu^{2+} residues in real water samples and herb products.

The architecture and optical properties of the ratiometric probe are studied in detail. Transmission electron microscopy (TEM) images indicate that the as-prepared [650]QDs-embedded silica nanoparticles and the ratiometric probe are well-dispersed with an average size of about 150 nm (Figure S1 of the Supporting Information). As can be seen in the TEM images, the [650]QDs-embedded silica nanoparticle surfaces are very smooth (Figure S1A of the Supporting Information) and the hybrid probe surfaces are comparatively rough (Figure S1B of the Supporting Information), suggesting the successful attachment of the green emissive [550]QDs on the [650]QD-embedded silica nanoparticle surfaces. The fluorescence spectra of green emissive [550]QDs, the ratiometric probe, and red emissive [650]QDs solutions are shown in Figure 1. The [650]QDs-embedded
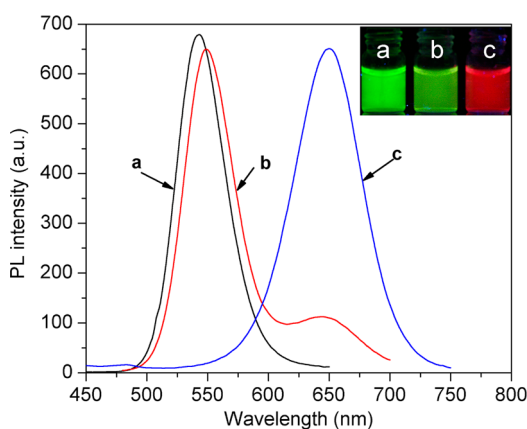

Figure 1. Fluorescence emission spectra of (a) green emissive QDs, (b) the ratiometric probe, and (c) red emissive [650]QD-embedded silica nanoparticles solution. The inset photos show the corresponding fluorescence colors under a 365 nm UV lamp, respectively.

silica nanoparticles show a fluorescence maximum at 650 nm (Figure 1c) and exhibit a strong red fluorescence under a UV lamp ($\lambda = 365$ nm). When the green emissive QDs are coupled to the surface of the [650]QD-embedded silica nanoparticles, the ratiometric fluorescence probe is obtained and also displays very good dispersity (Figure S1B of the Supporting

Information). The fluorescence spectrum of the ratiometric probe (Figure 1b) displays well-resolved dual emission bands under a single wavelength excitation at 365 nm. The corresponding fluorescence color of the ratiometric probe is shown in Figure 1b for a direct comparison with those of the green emissive QDs (Figure 1a) and the red emissive QDs (Figure 1c). Clearly, a significant change of fluorescence color under a UV ($\lambda = 365$ nm) lamp is observed. These results indicate that the green emissive QDs are successfully conjugated onto the surface of the red [650]QD-embedded silica nanoparticles and both are photoluminescent under a single excitation.

The stability of the as prepared ratiometric probe against time is systematically investigated by flashing UV light in an aqueous ratiometric probe solution. After 9 consecutive illuminations at 365 nm (15 min for each time), the relative fluorescence intensity has no apparent change, implying the photostability of the ratiometric fluorescence probe in aqueous solution (Figures S2 of the Supporting Information). The tolerance of the ratiometric probe against various anions (10 mM) is also examined by monitoring the fluorescence intensity upon the addition of the sodium salts. The results suggest that the presence of anions, such as nitrate, acetate, bromide, sulfate, sulfite, and chloride doesn't have much effect on the fluorescence intensity of the probe (Figure S3 of the Supporting Information).

The dose response of the ratiometric probe to Cu^{2+} has been examined. In the absence of Cu^{2+}, the ratiometric probe emits two well-resolved emission peaks centered at 550 and 650 nm under a single wavelength excitation, which can be ascribed to the fluorescence of [550]QDs and [650]QDs, respectively. Upon the addition of Cu^{2+}, the fluorescence intensity at 550 nm of the green MPA-QDs is continuously quenched, whereas the intensity at 650 nm of the embedded red QDs still remains unchanged, as shown in Figure 2A. Owing to the changes in the intensity ratio of the two emission wavelengths, the fluorescence colors of the ratiometric probe solution changed continuously as demonstrated in the inset of Figure 2A. Clearly, even a slight decrease of the emission intensity at 550 nm could result in distinguishable color changes from the original background. Therefore, the visual detection of Cu^{2+} by the naked eye under a UV lamp is feasible. As can be seen from Figure 3, the ratio of the fluorescence intensity is closely related to the amount of Cu^{2+}, ranging from 5×10^{-8} to 5×10^{-7} M, which can be used for the quantification of Cu^{2+} with a correlation coefficient of 0.998, and the detection limit can be as low as 1.1 nM based on the definition of three times the deviation of the blank signal (3σ). Meanwhile, the advantages of the ratiometric fluorescence for visual detection of Cu^{2+} is verified by the comparison with the single fluorescence quenching experiment, in which only a pure green MPA-QD probe is employed for the detection of Cu^{2+} (Figure 2B). Unlike the ratiometric probe, the color changes of the single fluorescence quenching of the green MPA-QDs upon the addition of Cu^{2+} are hard to observe. The comparison clearly shows that the ratiometric fluorescence probe possesses higher sensitivity and reliability than a single fluorescence quenching probe for visual detection (the inset of Figure 2).

To examine the selectivity of the ratiometric fluorescence probe for Cu^{2+}, the fluorescence intensity ratios of the probe were recorded (I_{550}/I_{650}) in the presence of Cu^{2+} and other metal ions, including Cd^{2+}, Mg^{2+}, Ni^{2+}, K^+, Ca^{2+}, Co^{2+}, Ba^{2+}, Na^+, Pb^{2+}, Fe^{3+}, and Hg^{2+}. It can be seen in Figure 4 that the
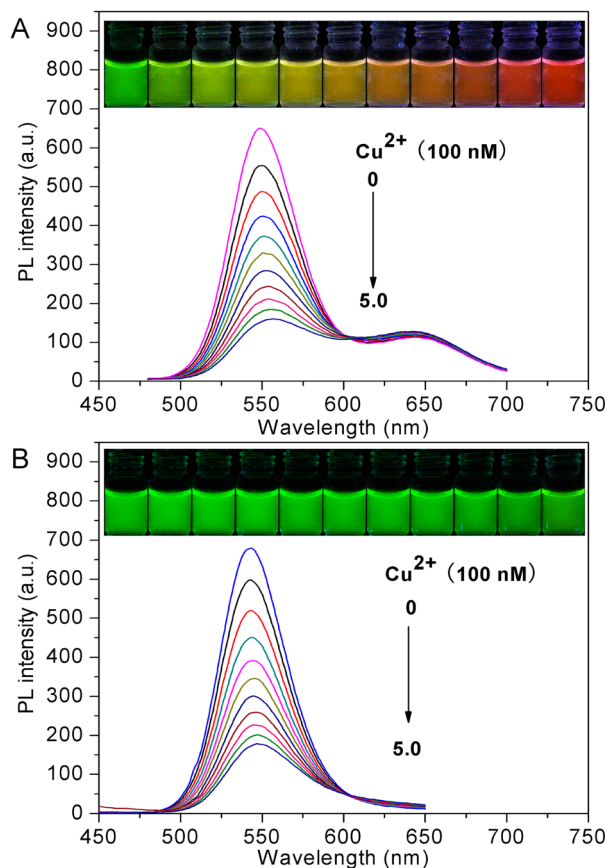
Figure 2. The fluorescence colors and the corresponding fluorescence spectra (λex = 365 nm) of (A) the ratiometric probe and (B) the pure green MPA-QDs upon the exposure to different concentrations of Cu^{2+}. The concentrations of Cu^{2+} from left to right are 0, 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 nM, respectively. The fluorescence photos were taken under a UV lamp (excitation wavelength at 365 nm). The ratiometric fluorescence method shows clearer color changes than the single wavelength fluorescence method.
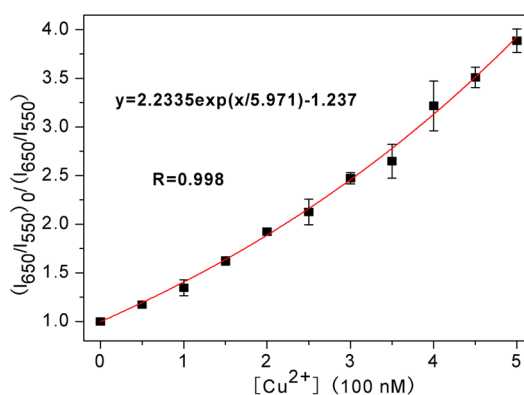

Figure 3. Plot of fluorescence quenching efficiency of the ratiometric probe as a function of the Cu^{2+} concentration. $(I_{550}/I_{650})_0$ and (I_{550}/I_{650}) were the ratio of the fluorescence intensity of the ratiometric probe in the absence and presence of different concentrations of Cu^{2+}, respectively.

fluorescence intensity ratio of the probe (1.2 mg/mL) solution is quenched by about 90% and 50% by Cu^{2+} with the concentration of 250 nM and 500 nM, respectively, which is accompanied by remarkable fluorescence color changes of the
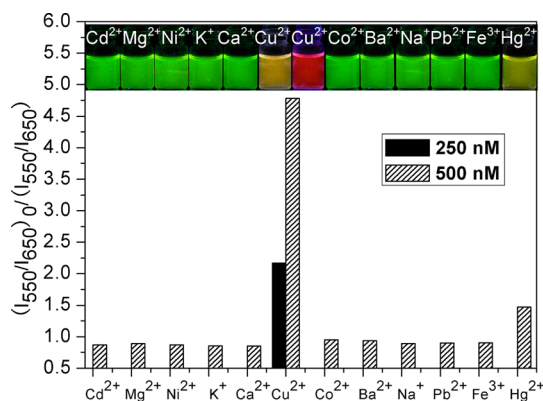

Figure 4. The selectivity of the ratiometric probe to various metal ions in the HEPES buffer (pH = 7.4, 10 mM). The striped bars represent the addition of different ions at 500 nM, and the black bar represents the addition of Cu^{2+} at 250 nM. The inset images show the corresponding fluorescence colors under a UV lamp.

solution under a UV lamp. In contrast, no obvious changes happen in the fluorescence intensity ratio and color after adding other metal ions in the probe solution, as shown in the inset of Figure 4. The results imply the high selectivity of the ratiometric fluorescence probe for visual identification of Cu^{2+} over other metal ions. It is noted that Hg^{2+}, which was prepared in 0.1 M HNO_3, also can slightly quench the green fluorescence of the probe, but its interference can easily be suppressed by a simple sample pretreatment with KI, NaCl, and Rhodamine B (RhB)[52] (Figure S4 of the Supporting Information) for the purpose of detecting Cu^{2+}. The Hg^{2+} ions can form HgI_4^{2+} with I^- and then form a coordination compound with RhB$^+$, which was subsequently removed through filter paper in the NaCl solution. After the simple pretreatment with KI and RhB, the fluorescence responses of the probe to the Cu^{2+} solution and to the pretreated mixture of Cu^{2+} and Hg^{2+} were comparable, as shown in Figure S4 of the Supporting Information. The results suggest that although the simple pretreatment with KI and RhB addition effectively eliminated the interference of Hg^{2+}, the condition needs to be optimized for better performance. The fluorescence intensity ratios of the probe are not influenced by cations at higher concentrations except for the lead ion, as shown in Figure S5 of the Supporting Information. Although lead ion at a comparable concentration shows no interference, it exhibits a negative effect on the determination of copper ion when its concentration is 20 times higher. This issue should be noted for practical applications. For other possible interfering ions, no obvious interferences of the fluorescence signal are observed for copper ion detection, even if the concentrations of the interfering ions are at least 20 times higher than that of Cu^{2+}, as shown in Figure S5 of the Supporting Information. These results indicate that the ratiometric probe is specific toward Cu^{2+} detection.

To further assess its applicability in real water samples, the ratiometric probe was used to detect Cu^{2+} in real water samples spiked with different amounts of Cu^{2+}, including the lake water and mineral water. The real water samples were first treated by filtration to remove solid suspensions. The water samples with different concentrations of Cu^{2+} were then added to the ratiometric probe solution, and the fluorescence spectra were collected. With the addition of Cu^{2+} in the water samples, the fluorescence intensity of the ratiometric probe decreased, resulting in good linearity in a concentration range from 5 ×

10^{-8} to 5×10^{-7} M (Figure 5, Figure S6 of the Supporting Information). The relative standard deviation (RSD) was
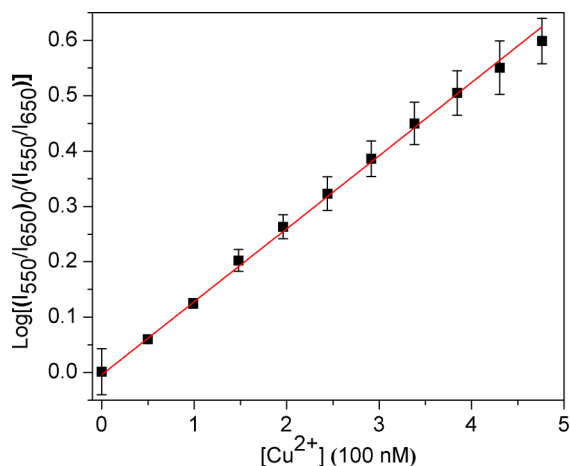

Figure 5. Changes of fluorescence intensity of the ratiometric probe solution upon different concentrations of Cu^{2+} (5×10^{-8} M to 5×10^{-7} M) in real water samples (lake water).

obtained by repeating the experiment 3 times under the same condition. The estimated recoveries of the measurements and the RSD are satisfactory, as shown in Table 1. It can be seen

Table 1. The Detection of Cu^{2+} in Cu^{2+}-Spiked (1) Shushan Lake Water and (2) Mineral Water by the Ratiometric Probe

added Cu^{2+} concentration (ppb)	lake water		mineral water	
	found (ppb)	recovery RSD (%)	found (ppb)	recovery RSD (%)
2.34	2.26	96.6 4.9	2.55	108.9 7.1
5.47	4.95	90.5 1.0	5.87	107.3 1.3
9.41	9.50	101.0 4.8	9.16	97.3 4.0
15.6	16.50	105.7 0.7	15.9	101.9 1.7

that the recoveries of copper ion for real water samples are statistically close to those values added. However, the difference between lake water and mineral water is observed. At low concentration, the recoveries for real lake water are much lower than for mineral water; this result indicates that the humic materials in real lake water exhibit negative interferences for the determination of the copper ion, and the copper ion in the background of mineral water exhibits positive interferences. These results indicate that the reliability of the ratiometric probe for Cu^{2+} determination in real samples can be acceptable. When the probe was applied for the determination of copper ion in seawater background, the standard deviation is relatively high (Figure S6B of the Supporting Information). This could be ascribed to the very complicated components in seawater, including a high concentration of various cations and anions, high salinity, humic, microbes, and much unidentified suspensions. The original content of copper ion in seawater was estimated to be about 105 ppb using this method, and this value is much higher than that obtained using the ICP method. The comparison shows that this is not an all-purpose method performing well in any conditions. However, the principle and idea of the method is actually novel and has been demonstrated to perform well for copper determination in relatively clean water and for the on-site visual detection of the copper ion.

Recently, high level contamination of copper residue in green herb products due to the staining with copper sulfate for good appearance are sometimes reported, so a simple, sensitive, and selective screening method is in demand for rapid monitoring of the quality of herb products on the market. This ratiometric fluorescence probe could play its role in this aspect and its utility for on-site determination of Cu^{2+} residues in herb products was demonstrated. The diarrhea leaves were chosen for the demonstration, and $CuSO_4$ solutions were sprayed onto the diarrhea leaves. These leaves stained with copper sulfate were then kept in a sealed beaker for 24 h and finally dried under vacuum. The appearance of the stained diarrhea leaves looks greener than the untreated ones (Figure S7 of the Supporting Information); however, it is hard to tell for a third party, which one was stained with copper sulfate. This ratiometric probe can visually reveal the secret in this scenario (Figure 6B and Figure S7 of the Supporting Information). The
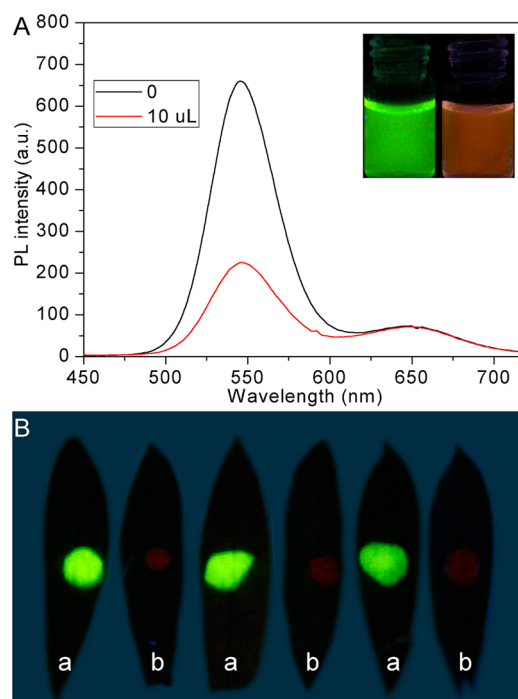

Figure 6. (A) Fluorescence spectra before and after the addition of 10 μL of the filtrate from the diarrhea leaf treated with $CuSO_4$ solution. The inset shows the corresponding fluorescence colors. (B) The fluorescence of the ratiometric probe on the surface of (a) untreated and (b) treated diarrhea leaves. The experiment was repeated three times, and the images were taken under a UV lamp.

ratiometric probe solution was dripped on the surface of the treated leaves; a notable fluorescence color change from yellow-green to red was quickly observed under the UV light excitation (Figure 6B, b), whereas the fluorescence color remained on the surface of the untreated leaves (Figure 6B, a). The experiment was repeated three times for consistency. The spot contained about 0.463 μg/mm^2 of Cu^{2+}, which was estimated on the basis of concentration and superficial area of the leaves. Therefore, the ratiometric probe could meet the sensitivity requirements for rapidly visual on-site detection of Cu^{2+} residues in herb products. The treated herb products were further immersed in ultrapure water (30 mL) for 12 h for complete extraction of Cu^{2+}, and the Cu^{2+} residues solution for determination was then obtained by filtering the herb water mixture. When a small

volume of the target solution was added to the probe solution, a notable fluorescence color change was observed under the UV light excitation (the inset in Figure 6A), which confirmed that this probe could meet the sensitivity requirements for visual detection of Cu^{2+} residues in spiked herbs. The Cu^{2+} residues in the spiked diarrhea leaf were finally estimated to be 2.4 μg/mL based on the fluorescence signals and the standard curve. The value is very close to that calibrated by AES analysis (2.7 μg/mL), confirming the validation of the ratiometric probe in the detection of Cu^{2+} for real samples.

CONCLUSIONS

This work has demonstrated a new concept and utility for visual detection of Cu^{2+} in aqueous solution. The concept takes advantage of the superior fluorescent properties of QDs for visual signal output via ratiometric fluorescence of the dual-emission probe. The presence of Cu^{2+} in solutions can be revealed by the fluorescence color changes of the ratiometric probe. The probe can also be used to quantify Cu^{2+} in solutions on the basis of the measurement of ratiometric fluorescence intensity. This method exhibits significantly enhanced visual detection selectivity and sensitivity compared with single QD-based probes. Moreover, the ratiometric fluorescence probe has been successfully applied in real water samples and herb products, which validate the efficiency of visual on-site determination of Cu^{2+} in complex environments and could be extended to the visual detection of a wide range of metal ions and inorganic molecules.

ASSOCIATED CONTENT

ⓢ Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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