CHEMICAL ENGINEERING TRANSACTIONS

VOL. 81, 2020

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Petar S. Varbanov, Qiuwang Wang, Min Zeng, Panos Seferlis, Ting Ma, Jiří J. Klemeš

**ISBN** 978-88-95608-79-2; **ISSN** 2283-9216

DOI: 10.3303/CET2081231

# Ratiometric Fluorescence Sensor of Copper Ions Based on Dual-Emission Quantum Dots Hybrid

Jingchen Chang[a], Linna Zhang[a], Renliang Huang[b], Wei Qi[a], Zhimin He[a], Rongxin Su[a,*]

[a]State Key Laboratory of Chemical Engineering, Tianjin Key Laboratory of Membrane Science and Desalination Technology, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China
[b]School of Environmental Science and Engineering, Tianjin University, Tianjin 300072, PR China
surx@tju.edu.cn

Detection of copper ions is important for environmental protection and human healthy. In this study, a ratiometric fluorescent probe was developed by using silica nanoparticles capped carbon dots (CDs) as the core and red quantum dots (CdTe) as the shell linking onto the surface of silica. Cu(II) was found to quench the fluorescence of red-emitting CdTe on the shell, while not changing the fluorescence of the core blue-emitting CDs embedding silica nanoparticles. Based on multiple spectroscopic studies, we propose that selective binding of Cu(II) to CdTe of CDs@$SiO_2$-CdTe via a photoinduced electron transfer (PET) process. The as-proposed sensor allows selective determination of Cu(II) in the range from 0.82 μM to 25 μM, with a detection limit of 0.053 μM.

## 1. Introduction

Copper is one of the most important transition metal elements in the human body. A lot of important enzyme-catalyzed reactions require the participation and activation of copper, for example, copper can catalyze the synthesis of hemoglobin (Shou et al., 2015). Based on A large number of clinical research results, excessive intake of copper in human body may cause poisoning, including acute copper poisoning, hepatolenticular degeneration, intrahepatic cholestasis in children and other diseases (Yu et al., 2019). With the rapid development of industry, copper ions are increasingly discharged in both water and soil, that are easily enriched in grains, vegetables, fruits and organisms (Guo et al., 2013). Through the food chain, the copper ions enter the human body and may cause that the content in the body exceeds the limit, causing great harm to people's health (Giergiel et al., 2019). Therefore, it is of great significance for facile and quantitative analysis of copper in environment and organism.

Until now, there are various methods for detecting copper ions, including atomic absorption spectrometry (Adhami et al., 2020), inductively coupled plasma mass spectrometry, inductively coupled plasma atomic emission spectrometry, voltammetry, etc. However, these methods have some disadvantages such as cumbersome operation, expensive instrument and long detection period (Portbury et al., 2016). Compared with the existing elemental analysis methods, fluorescence analysis has the advantages of high sensitivity, simple operation, good selectivity and so on (Samokhvalov, 2020). It has attracted more and more interests in the fields of environmental detection. In recent years, some fluorescent probes for sensing copper ion have been developed (Qiu et al., 2020). A fluorescent probe realised selective detection of copper ions, which modified by tryptophan (Shi et al., 2020). Some scientists successfully prepared fluorescent probes for copper ion detection using CTAB-modified CdSe/ZnS quantum dots (Jin and Han, 2014). And some linked quinoline and coumarin groups to synthesize copper ion fluorescent probes with good interference resistance in the pH range of 5-11 and realised biological imaging of copper ions in living cells (Chen et al., 2013). The above probes based on changes in a single emission intensity. Compared with the traditional single fluorescent probe, ratiometric fluorescence sensing based on dual-emission hybrid probes has many advantages, which can reduce the influence of external environment, substrate concentration and instrument condition, and improves the accuracy of the detection method (Zhang et al., 2018).

Paper Received: 23/06/2020; Revised: 20/07/2020; Accepted: 20/07/2020
Please cite this article as: Chang J., Zhang L., Huang R., Qi W., He Z., Su R., 2020, Ratiometric Fluorescence Sensor of Copper Ions Based on Dual-Emission Quantum Dots Hybrid, Chemical Engineering Transactions, 81, 1381-1386 DOI:10.3303/CET2081231

In this study, we aimed to develop a shell-core dual-emission fluorescence probe using CdTe as sensing element and CDs as an internal standard emission for the ratiometric, sensitive, and selective detection of copper ions (Figure 1). The addition of Cu(II) will quench the shell CdTe fluorescence signal, which shifts the fluorescence of CDs@$SiO_2$-CdTe nanohybrid from red to blue through a photonic electron transfer (PET) process. A smartphone imaging-based sensing platform was developed to obtain the fluorescent images and readout the RGB values, which was used to correlate with the Cu(II) concentration. The ratiometric probe is further used to realize the visual detection of Cu(II) in water samples.

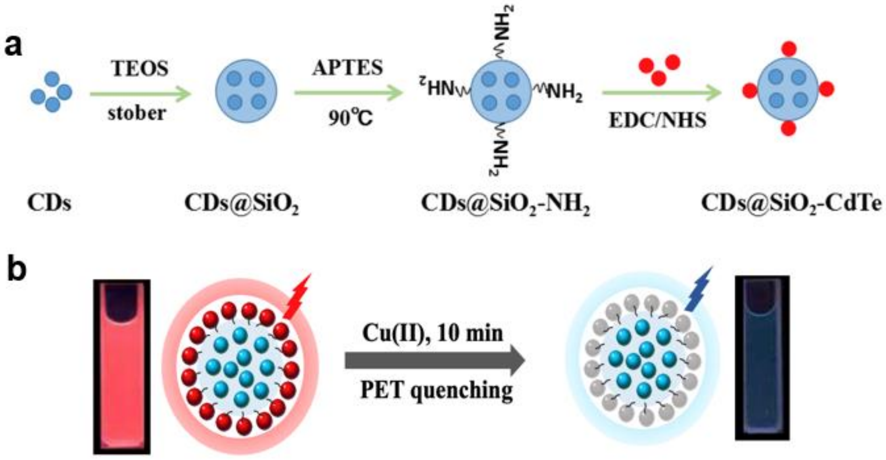

*Figure 1: (a) Ilustrations of the synthesis of the dual-emissive ratiometric fluorescent probe (CDs@SiO2-CdTe). (b) The ratiomatric fluorescence strategy for the detection of Cu(II) by the dual-emission nanohybrid*

## 2. Materials and methods

### 2.1 Materials

Tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilan (APTMS), TritonR X-100, n-hexanol, bovine serum albumin (BSA, 98 %), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and cyclohexane were purchased from Aladdin. CdTe/ZnS (QDs) solution was obtained from Wuhan Jiayuan Quantum Dots Co., Ltd. (Wuhan, China). Nylon Springe Filters (0.22 μm) were purchased from Membrane Solutions Company. Ultrapure water (resistivity >18 MΩ) obtained from a Millipore water purification system was utilised throughout the experiments. All reagents were of analytical grade and were used as received without further purification.

### 2.2 Preparation of the CDs@$SiO_2$

In the experiment, 7.7 mL cyclohexane, 1.42 g triton X–100 and 1.8 mL hexyl alcohol were mixed in a 100 mL round-bottom flask. Then 380 μL water was added, further stirred at room temperature for 10 min to get a stable reverse phase microemulsion system. After that, 50 μL TEOS, 20 μL CDs and 200 μL ammonia were added into the above solution, and then the mixture was kept under stirring for 24 h at room temperature. At the end of the reaction, 36 mL isopropanol was added to the reaction system for demulsifying, and the precipitates were obtained and separated by centrifugation. The CDs@$SiO_2$ were obtained and freeze-dried for future use.

### 2.3 Synthesis of ratiometric fluorescent probe CDs@$SiO_2$-CdTe

CDs@$SiO_2$ amination modification: 4 mg CDs@$SiO_2$ were dissolved into 40 mL ethyl alcohol in a stand-up flask, and then quickly drops 250 μL APTES to the mixture. After the vial was capped, the mixture was heated at 90 °C and stirred vigorously for 24 h. The top of the graham condensing is connected to the drying tube with $CaCl_2$ to prevent water from entering the reaction system. After cooled down to room temperature, the final product was centrifuged at 12,000 rpm for 10 min. 4 mL water was adds to product for future use.

Synthesis of ratiometric fluorescent probe CDs@$SiO_2$-CdTe: 80 mg EDC and 190 mg NHS are respectively dissolved in 1 mL deionized water, and then mixed with CdTe quantum dot solution and stirred for 30 min in the dark. Then, 1 mL of CDs@$SiO_2$ was added into the above mixture and stirred for 12 h at room temperature. Finally, the CDs@$SiO_2$-CdTe probes were collected by centrifugation (8,000 rpm, 30 min), and redispersed in 8 mL of ultrapure water for future use.

### 2.4 Detection of copper ions

Various concentrations of $Cu^{2+}$ (10 μL) were added into the CDs@$SiO_2$-CdTe solution (0.7 mL) in a spectrophotometer quartz cuvette (1 cm×1 cm). Subsequently, the fluorescence spectra were recorded from 400 to 750 nm after each addition, under the excitation at 375 nm, and the slit width of 2.5 nm. The actual water samples were collected from lakes and tap water of Tianjin University in Tianjin. First, the sample was filtered by a water membrane (0.22 μm) to remove the insoluble particles in the solution. And we used standard addition method to evaluate the feasibility of the ratio fluorescence probe in practical system.

## 3. Results and discussion

### 3.1 Characterization of ratiometric fluorescent probe CDs@SiO2–CdTe

As displayed in Figure 2a, the as-prepared CD are spherical particles with an average size of around 4-6 nm. There is a large number of silicoxane on the surface of the as-prepared CD, so we used reverse phase microemulsion method to prepare silica nanoparticles doped with CD. As shown in Figure 2b, the as-prepared CDs@$SiO_2$ nanoparticles were spherical particles with the average size of around 30-40 nm. To further confirm that the CDs was embedded in the sphere of silicon, we compared the FTIR spectra of the blank $SiO_2$ and CDs@$SiO_2$ nanoparticles (Figure 2e). Both nanoparticles showed the characteristic absorption peak of $SiO_2$. Compared with the blank $SiO_2$ nanoparticles, the new bands at around 1, 673 $cm^{-1}$ and 2,985 $cm^{-1}$ in CDs@$SiO_2$ nanoparticles corresponds to the stretching vibration of C=O groups and C-H groups, and the band at around 1,570 $cm^{-1}$ is ascribed to the bending vibration of the N-H group. The bands located in the 1,400-1,470 $cm^{-1}$ range corresponded to the absorption of amino characteristics (Mallela et al., 2018). The FTIR results proved that the CDs have been successfully coated in silica nanoparticle.

A typical TEM image (Figure 2c) shows that the as-prepared CDs@$SiO_2$-CdTe are spherical particles with an average size of around 35-45 nm, and the CdTe nanoparticles can be observed on the surface of $SiO_2$ nanoparticles (Figure 2d). Besides, CDs@$SiO_2$-CdTe hybrid nanoparticles has some different absorption peaks in the FTIR spectrum, including the band at around 1,401 $cm^{-1}$ corresponds to the stretching vibration of C-N groups, proving the existence of amide bonds (Naik et al., 2015). As shown in Figure 2f, the as-prepared CDs@$SiO_2$-CdTe has two fluorescence absorption peaks at 462 nm and 643 nm, representing the fluorescence peaks of CDs and CdTe quantum dots, under the excitation wavelength of 380 nm.

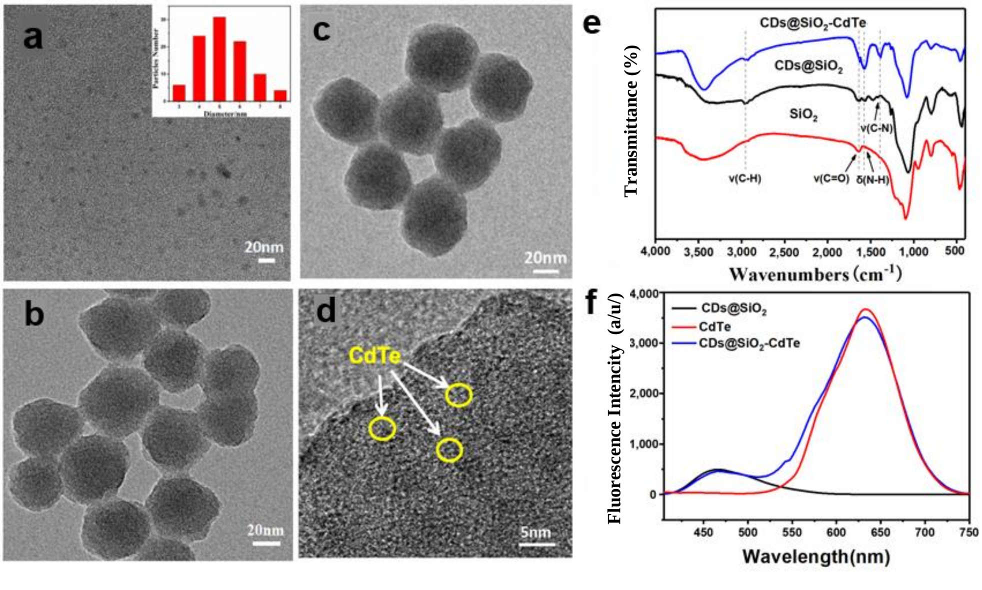

*Figure 2: TEM images of CDs (a), CDs@$SiO_2$ (b), CDs@$SiO_2$-CdTe at low magnification (c) and high magnification (d). The inner picture in Figure 1a shows the size distribution of CDs. (e) FTIR spectra of $SiO_2$, CDs@$SiO_2$ and CDs@$SiO_2$-CdTe. (f) Fluorescent spectra of CDs@$SiO_2$, CdTe and CDs@$SiO_2$-CdTe*

### 3.2 Optimisation of the detection conditions

Figure 3a shows the changes in the fluorescence intensity ratio $F_{643}/F_{462}$ of the ratiometric fluorescent probe CDs @$SiO_2$–CdTe with the reaction time in the presence of a certain concentration of $Cu^{2+}$. The $F_{643}/F_{462}$ value decreased first and then reached a stable value with the increase of time. After 8 min, no significant change was found in the $F_{643}/F_{462}$ value. In order to ensure the complete reaction between the probe and $Cu^{2+}$ in the experiment, we selected 10 min as the reaction time between the $Cu^{2+}$ and the dual-emission fluorescent probe CDs @$SiO_2$-CdTe.

To examine the effect of pH on the detection system, the fluorescence spectra of CDs@$SiO_2$-CdTe were recorded at the pH range of 2.0–12.0. As seen from Figure 3b, the $F_{643}/F_{462}$ value of the dual emission probe CDs@$SiO_2$-CdTe is very small, mainly because the fluorescence intensity of CdTe quantum dots is low when the pH is very low. The lower the pH in aqueous solution, the higher the concentration of $H^+$, the higher the carboxyl protonation degree on the surface of CdTe, which led to the decrease in electrostatic repulsion force, then the aggregation of quantum dots. As a result, the optical performance of CdTe quantum dots will be changed, as well as the decrease or even loss in the fluorescence signal. When the pH was higher than 5, the the $F_{643}/F_{462}$ value is stable at about 7. This ratio fluorescence probe is suitable for the detection system with the pH higher than 5.

The accuracy of the measurement results might be affected by the detection temperature. As shown in Figure 3c, the $F_{643}/F_{462}$ values of the dual emission probe CDs@$SiO_2$-CdTe at different temperature (5 °C , 15 °C , 25 °C and 35 °C) were obtained. The experimental results show that the temperature has little effect on the final test results of the detection system, indicating that the as prepared ratiometric fluorescence probe in this study was not sensitive to temperature. This is good for the real sample dection, since it can be done at various ambient temperatures.

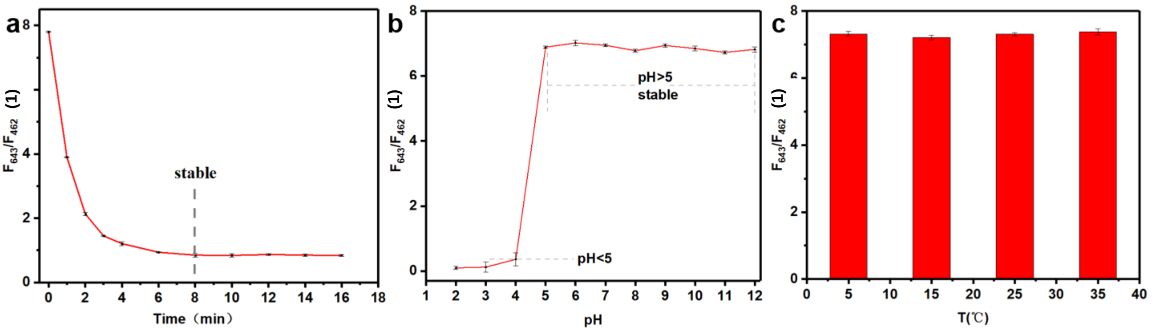

*Figure 3: (a) Time-dependent fluorescence responses (the ratio of the fluorescence signals at 643 nm and 462nm) of the CDs@$SiO_2$-CdTe dispersion upon the addition of 25 μM $Cu^{2+}$. (b) Fluorescence responses of the CDs@$SiO_2$-CdTe dispersion at different pH values. (c) Fluorescence responses of the CDs@$SiO_2$-CdTe at different temperatures*

### 3.3 Ratiometric fluorescent probe CDs@$SiO_2$–CdTe for Cu(II) sensing

In the dual-emission fluorescent probe CDs@$SiO_2$-CdTe, the CDs were applied to give the reference signal, while the shell CdTe QDs were used as a fluorescent probe (Figure 1b and Figure 4). During the dection of Cu(II), the fluorescence intensity of the CDs in the silicon sphere remained unchanged (Figure 4c), while the outer CdTe QDs selectively bound to $Cu^{2+}$, leading to the quenching of the red fluorescence of the outer CdTe quantum points. As shown in Figure 4a, as the concentration of copper ion increases, the fluorescence intensity of CdTe QDs decreased gradually, and at the same time, there was a red-shift in the position of emission peak. The energy of this CdTe-Cu(II) complex is lower than that of a single CdTe quantum dot, so that the emission peak position of CdTe quantum dots will be redshifted. The outer electrons structure of Cu(II) is $3s^2 3p^6 3d^9$, and d orbital contains lone pair electrons. The oxygen atoms of carboxyl groups on the surface of CdTe QDs contain same lone pair electrons, and $Cu^{2+}$ can bind with the carboxyl oxygen atoms of CdTe QDs. The quenching of the CdTe QDs due to photonic electron transfer (PET) process.

As shown in Figure 4a and 4B, the fluorescence intensity of CdTe QDs and the concentration of $Cu^{2+}$ have a good linear relationship ($R^2$=0.996) while the concentration range of copper ions was between 2.0-10 μM, and the detection limit was 0.34 μM. Figure 4c shows that the fluorescence intensity of the ratiometric fluorescent probe at 643 nm corresponding to the signal of CdTe QDs decreased, while that at 462 nm attributed by CDs remained unchanged with the increasing concentration of $Cu^{2+}$. As shown in Figure 4d, the linear range and detection limits of $Cu^{2+}$ were calculated. The fluorescence intensity ratio $F_{643}/F_{462}$ of the ratiometric fluorescent probe CDs @$SiO_2$–CdTe had a linear relationship with $Cu^{2+}$ concentration at the range of 0.82-25 μM ($R^2$=0.992) with the detection limit is 0.053 μM. The ratio fluorescence probe was used to conduct out 5 parallel measurements of a certain concentration of $Cu^{2+}$, and the relative standard deviation range of the results was between 1.63 % and 6.24 %, indicating that the ratio fluorescence probe has good repeatability for quantitative determination of copper ions. Compared with the results of the detection of $Cu^{2+}$ using single CdTe QDs as the fluorescence probe, the detection range of the ratio fluorescent probe CDs@$SiO_2$-CdTe was wider and the detection limit was lower. Meanwhile, the probe itself can further reduce the influence of external environment and changes in instrument conditions on the detection results.

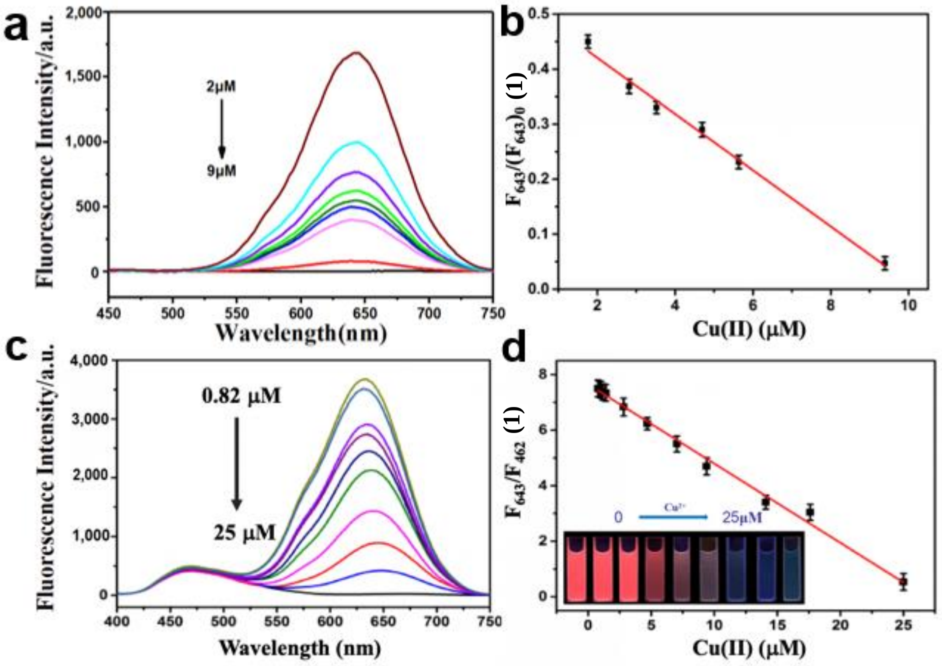

*Figure 4: Fluorescence spectra (a, c) and the corresponding working curve (b, d) of the CdTe probe (upper) and the ratiometric probe (lower) upon the addition of different concentrations of $Cu^{2+}$*

### 3.4 Selectivity analysis

To examine the selectivity of ratiometric fluorescent probe CDs@$SiO_2$-CdTe in $Cu^{2+}$ detection, we selected various common metal ions for interference measurement. First, we added 10 mM interfering substances which has the same volume into the aqueous solution of the ratiometric fluorescent probe, i.e. $K^+$, $Na^+$,$Ba^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Al^{3+}$, $Zn^{2+}$, $Ni^{2+}$, and $Mn^{2+}$, and then determined the ratio of fluorescence signals ($F_{643}/F_{462}$) under the same detection conditions as $Cu^{2+}$ (Figure 5). The experimental results can prove that other substances did not cause the change in the fluorescence intensity ratio of the CDs@$SiO_2$–CdTe, indicating that the ratiometric fluorescent probe can realize the selective detection of $Cu^{2+}$.

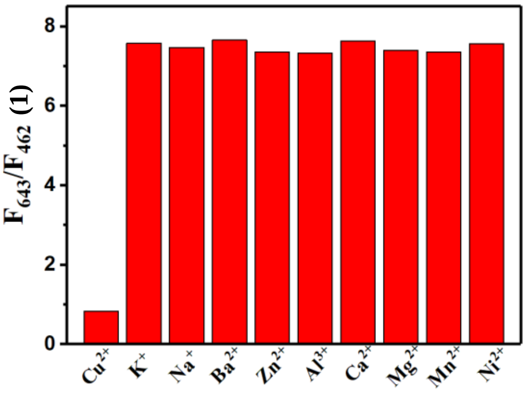

*Figure 5: The $F_{643}/F_{462}$ values of the ratiometric fluorescent probe upon the addition of 10 μL of $K^+$, $Na^+$, $Ba^{2+}$, $Al^{3+}$, $Zn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Ni^{2+}$ and $Cu^{2+}$ (the original concentration of all metal ions is 10 mM)*

## 4. Conclusions

In this work, a dual-emission hybrid probe CDs@$SiO_2$-CdTe with red and blue emissions was developed for fluorescent sensing of Cu(II). The ratiometric fluorescent probe was constructed by reversed-phase microemulsion method and chemical crosslinking method. Cu(II) was found to be able to selectively quench the fluorescence of the CdTe quantum dots on the outer layer of the dual-emission fluorescence probe without affecting the fluorescence of the inner CDs. Besides, it can be used in the environment with pH > 5 and can be done at 0 - 35 °C. This ratiometric fluorescent probe demonstrated high sensitivity and selectivity and has great potential for the efficient detection of Cu(II).

### Acknowledgments

This study was supported by the Natural Science Foundation of China (No. 51473115) and Wuqing S&T Commission (WQKJ201726 and WQKJ201806).

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