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A Novel Fluorescent Sensor Based on Copper Complex for Detection of Glyphosate

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Ultrasensitive, reliable, and prompt prewarning of glyphosate (GLY) residue is essential to ensure environmental and food safety. Hence, a novel fluorescent sensor, *N*'-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)methylene)-2-hydroxy-4-methoxybenzohydrazide (DCB), is proposed. It shows a significant and specific fluorescence quenching response to Cu^{2+} and forms a Cu^{2+} complex (DCS-Cu^{2+}). More importantly, GLY can bind to Cu^{2+} more strongly than DCB, and the DCB-Cu^{2+} complex releases free DCB through competitive coordination. Thus, GLY can restore the fluorescence of DCB which has been extinguished by Cu^{2+}. Through fluorescence enhancement, the DCB-Cu^{2+} complex shows a quick, specific, and sensitive response to GLY, and a good linear relationship between fluorescence intensities and GLY concentrations is found in the range of 0–11 μM with a limit of detection of 0.28 μM (47.34 ng/mL). Furthermore, the suggested sensor has been successfully used for the detection of GLY in actual samples.

1. Introduction

Glyphosate (GLY) is an organophosphorus pesticide (OPP), which is the most common herbicide in agriculture because of its high efficacy, low toxicity, broad spectrum, and affordable price.(1) Unfortunately, owing to the prolonged and uncontrolled usage of GLY, the ensuing residual issues and harmful consequences for humans will be a global problem.(2) Recent studies indicate that GLY may have toxic effects on the genetics, development, and reproduction of organisms.(3,4) In 2015, the International Agency for Research on Cancer identified GLY as a potential carcinogen for humans (group 2A).(5) The US and China have defined the maximum residual level (MRL) for GLY in drinking water as 4.14 μM (0.7 μg/mL), and the European Union has set an MRL for most crops of 0.1 μg/g.(6,7) Therefore, determining GLY in environmental samples is becoming necessary and urgent.

Conventional analytical techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC), ion chromatography (IC), and chromatography-mass

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spectrometry, are being used for the quantitative detection of GLY.[8–12] Even though these techniques can provide sensitive and precise results, they still have some unavoidable drawbacks, such as complicated derivatization steps, drawn-out processes, pricey chemical regents, and the requirement of expensive instruments and professional operators, which restrict their practical applications in GLY detection. Hence, there is a need to develop a simple, quick, and sensitive approach to detecting GLY.

Recently, the fluorescence technique has attracted considerable interest in the analysis of GLY owing to its excellent analytical capabilities, such as simple operation, rapid response, and high sensitivity.[13,14] Qin *et al.* proposed that Cu^{2+}-modulated g-C_3N_4 might be utilized to create an OFF-ON fluorescent platform for detecting GLY.[15] Fu *et al.* designed a sensor for detecting Cu^{2+} and GLY by combining 4-butyl-3-thiosemicarbazide with carbon dots.[16] However, the complex preparation process and unstable properties of these fluorescent materials may limit their practical application. In contrast, small-molecule coumarin derivatives have the advantages of high fluorescence intensity, good solubility and cell permeability, easy synthesis and modification, good fluorescence quantum yield, and excellent photostability.[17] Inspired by the designs of these fluorescence technologies, a novel coumarin fluorescent sensor, *N*'-(7-(diethylamino)-2-oxo-2H-chromen-3-yl)methylene)-2-hydroxy-4-methoxybenzohydrazide (DCB), was designed and synthesized (Fig. 1). DCB showed strong fluorescence which could be quenched by Cu^{2+}. Significantly, the competitive complexation of GLY and DCB with Cu^{2+} caused the quenched fluorescence to increase dramatically with the addition of GLY. Accordingly, the fluorescent sensor based on the DCB-Cu^{2+} complex could be proven effective in detecting GLY.

2. Materials and Methods

2.1 Materials and instruments

The chemicals were purchased from commercial suppliers and used without further purification. Methyl 4-methoxysalicylate and 7-(diethylamino)-2-oxochromene-3-carbaldehyde were purchased from Energy Chemical Co., Ltd. Glyphosate, trichlorfon, phosmet, dimethoate, ethoprophos, fenitrothion, parathion methyl, parathion, and glufosinate ammonium were purchased from Anpel Laboratory Technologies (Shanghai) Co., Ltd.

The ^1H and ^{13}C NMR spectra were obtained with DMSO-d_6 as the solvent on a Bruker AV300 NMR spectrometer. IR spectra were recorded using a Nicolet IN10 spectrometer with a

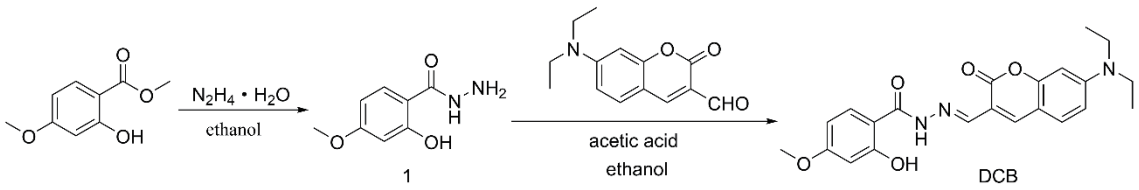

Fig. 1. Synthetic route to compound DCB.

range of 4000–400 cm^{-1}. High resolution mass spectrometry (HRMS) spectra were recorded with the use of a Waters Xevo G2-XS QTOF spectrometer. A Shimadzu UV-2450 UV–visible spectrometer was used to record the absorption spectra. A Hitachi F-7000 fluorescence spectrometer with a xenon lamp as the light source was used to measure the fluorescence emission spectra.

2.2 Synthesis of the compound DCB

2-Hydroxy-4-methoxybenzenecarbohydrazide (1) was synthesized according to a previous study.[18] In a nitrogen atmosphere, compound 1 (0.55 g, 3.0 mmol), 7-(diethylamino)coumarin-3-carbaldehyde (0.74 g, 3.0 mmol), and ethanol (50 mL) were blended. The mixture was refluxed for 6 h with the addition of acetic acid (0.5 mL) as a catalyst. Then, it was cooled to room temperature, filtered, and washed with ethanol to produce an orange solid (1.03 g) with a yield of 83.7%. ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.64 (s, 1H), 11.84 (s, 1H), 8.52 (s, 1H), 8.37 (s, 1H), 7.91 (d, J = 9.4 Hz, 1H), 7.66 (d, J = 8.9 Hz, 1H), 6.77 (dd, J = 8.9, 2.2 Hz, 1H), 6.64–6.44 (m, 3H), 3.80 (s, 3H), 3.48 (q, J = 6.7, 5.7 Hz, 4H), 1.14 (t, J = 6.9 Hz, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.84, 163.24, 161.30, 157.08, 151.89, 143.56, 139.34, 135.31, 131.45, 129.58, 112.75, 110.26, 106.86, 101.77, 96.87, 55.95, 44.74, 12.84; IR (KBr pellet, cm^{-1}) 3292, 2966, 1687, 1595, 1574, 1516, 1422, 1384, 1351, 1262, 1193, 1151, 1074, 1028, 968, 878, 812, 767; HRMS (ESI) calcd for $C_{22}H_{24}N_3O_5$ $[M+H]^+$ 410.1710, found: 410.1712.

2.3 Measurements of absorption and emission spectra

Stock solutions of various metal ions (10 mM, including Zn^{2+}, Cu^{2+}, Ca^{2+}, Cr^{3+}, K^{+}, Al^{3+}, Fe^{3+}, Mg^{2+}, Pb^{2+}, Na^{+}, Sr^{2+}, Ce^{3+}, Ag^{+}, Li^{+}, Cd^{2+}, Hg^{2+}), anions (10 mM, including SO_4^{2-}, F^{-}, SCN^{-}, Br^{-}, $H_2PO_4^{-}$, HPO_4^{2-}, NO_2^{-}, I^{-}, HCO_3^{-}, CO_3^{2-}, Cl^{-}, CH_3COO^{-}, NO_3^{-}), and OPPs (10 mM, including GLY, trichlorfon, phosmet, dichlorvos, malathion, omethoate, dimethoate, ethoprophos, fenitrothion, parathion methyl, parathion, glufosinate ammonium) were prepared. The DCB stock solution (1.0 mM) was prepared in DMF and diluted to a concentration of 10 μM with DMF/HEPES solution (V/V = 8:2, 10 mM HEPES, pH = 7.4). The DCB-Cu^{2+} complex (10 μM) was prepared by combining the DCB solution (2.5 mL, 1.0 mM) with a Cu^{2+} aqueous solution (250 μL, 10 mM), then adjusting the volume to 250 mL with DMF/HEPES. The resulting combination was then incubated for 5 min at 25 °C. Finally, the excitation and emission slit widths on the fluorescence spectrometer were set to 2.5 nm, and fluorescence spectra were measured with λ_{ex} at 460 nm.

2.4 Analysis of GLY in actual samples

To evaluate the actual applicability of the proposed sensor, tap water and Songhua River water were chosen as actual samples to utilize the standard addition method. Water samples were filtered via a 0.22 μm membrane to remove large solids and primary contaminants. Then, these samples were added with various concentrations (2.0, 4.0, 6.0, 8.0, and 10.0 μM) of GLY standard solutions, and DCB-Cu^{2+} was used to analyze the results.

3. Results and Discussion

3.1 Spectral studies of the compound DCB with Cu^{2+}

To evaluate its sensing capabilities, the responses of DCB to various metal ions and anions were studied. As depicted in Fig. 2(a), DCB displayed a strong absorption band at 453 nm, which was caused by an intramolecular charge-transfer transition.[19] Except for Cu^{2+}, it caused a noticeable red shift in the absorbance spectra. When the aforementioned metal ions and anions (50 μM) were added to DCB, the absorbance spectra barely changed. As depicted in Fig. 2(b), except for Cu^{2+}, which remarkably quenched the fluorescence of DCB, other ions had slight effects on its fluorescence. A less substantial response to Zn^{2+} was also detected, but this would be unlikely to influence the intended application of DCB. For the anti-interference studies, Cu^{2+}

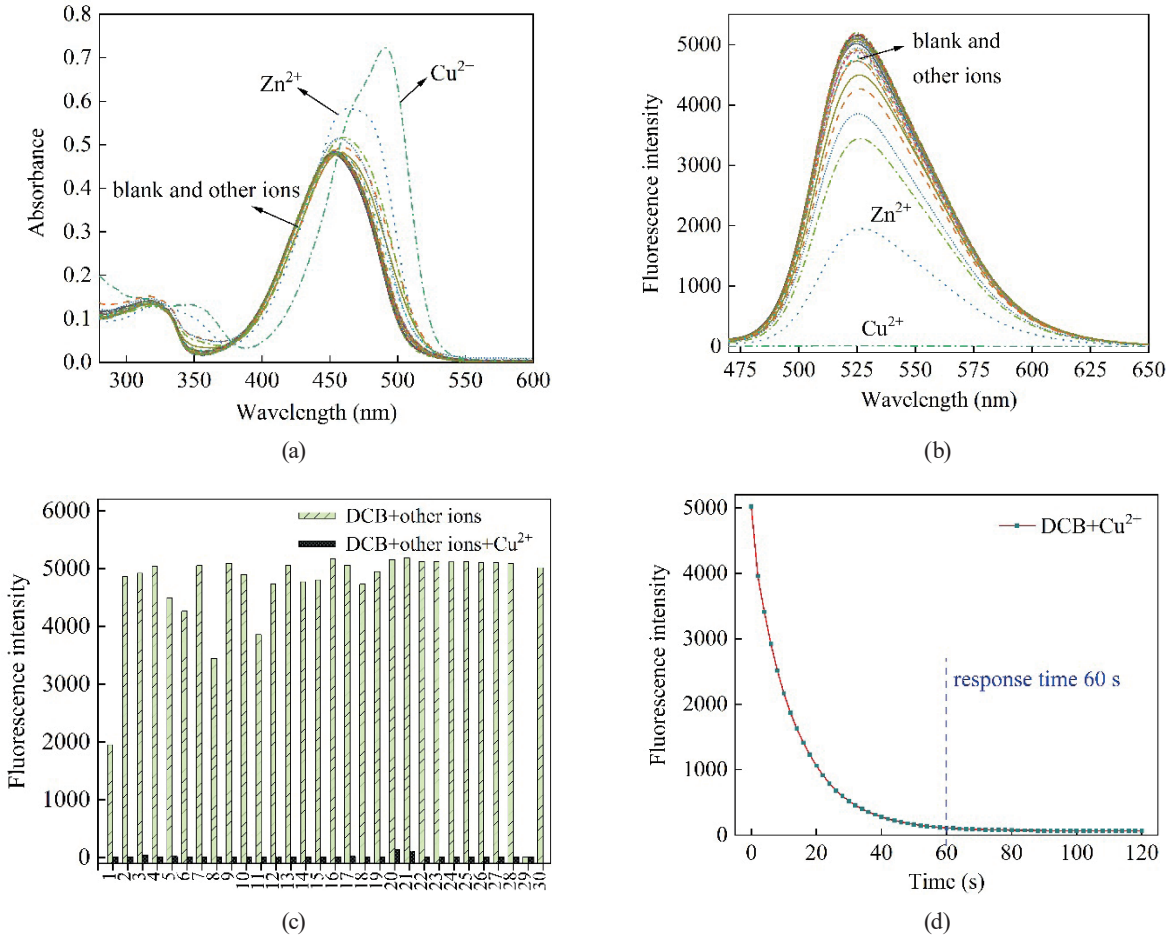

Fig. 2. (Color online) (a) UV-vis and (b) fluorescence spectra of DCB toward various ions. (c) Interference experiments of DCB for fluorescent recognition of Cu^{2+} (1. Zn^{2+}, 2. Ca^{2+}, 3. Cr^{3+}, 4. K^{+}, 5. Al^{3+}, 6. Fe^{3+}, 7. Mg^{2+}, 8. Pb^{2+}, 9. Na^{+}, 10. Sr^{2+}, 11. Ce^{3+}, 12. Ag^{+}, 13. Li^{+}, 14. Cd^{2+}, 15. Hg^{2+}, 16. SO_4^{2-}, 17. F^{-}, 18. SCN^{-}, 19. Br^{-}, 20. $H_2PO_4^{-}$, 21. HPO_4^{2-}, 22. NO_2^{-}, 23. I^{-}, 24. HCO_3^{-}, 25. CO_3^{2-}, 26. Cl^{-}, 27. CH_3COO^{-}, 28. NO_3^{-}, 29. Cu^{2+}, 30. DCB). (d) Time-dependent fluorescence intensity (at 525 nm) changes of DCB to Cu^{2+}.

(50 μM) was added to the DCB solution, which contained interfering metal ions or anions (50 μM). In the presence of these interference ions, Cu^{2+} could completely quench the fluorescence of DCB, demonstrating that coexisting ions had no discernible interference with the detection of Cu^{2+} [Fig. 2(c)]. Excited at 460 nm, DCB displayed strong fluorescence at a wavelength of 525 nm. When Cu^{2+} was added, the fluorescence intensity decreased rapidly and was quenched 60 s later [Fig. 2(d)]. Thus, DCB could be employed as a fluorescence sensor for the selective and rapid recognition of Cu^{2+} with strong resistance to interference.

Fluorescence titration experiments were carried out to determine the sensitivity of DCB for detecting Cu^{2+}. As depicted in Fig. 3(a), with the addition of Cu^{2+} (0–20 μM), the fluorescence intensity of DCB at 525 nm decreased progressively. When the amount of Cu^{2+} reached 15 μM, additional titration had little effect on the emission intensity. This clear fluorescence quenching might be explained by the chelation-enhanced fluorescence quenching effect and the paramagnetic property of Cu^{2+}.[20,21] As depicted in Fig. 3(b), there was an excellent linear relationship between the fluorescence intensity at 525 nm and Cu^{2+} concentration in the range of 0–11 μM, represented by $y = 5076.7167 - 440.2818x$ ($R^2 = 0.9974$). In addition, the association constant was calculated as 7.30×10^4 L/mol using Benesi-Hildebrand equation.[22] According to the reported method, the LOD of Cu^{2+} was 0.32 μM (20.33 ng/mL), which is much lower than the 2.0 μg/mL in drinking water defined by the WHO.[3,14]

3.2 Spectral studies of the complex DCB-Cu^{2+} with GLY

GLY has been shown to exhibit strong chelating properties with Cu^{2+} due to the presence of amine, carboxylate, and phosphonate groups.[3,16] We postulated that GLY might be detected using the complex DCB-Cu^{2+} and investigated the absorbance and fluorescence responses of the complex DCB-Cu^{2+} towards GLY to validate this hypothesis. As depicted in Fig. 4(a), except for GLY, which caused a significant blue shift in absorbance spectra that was consistent with DCB, the complex DCB-Cu^{2+} displayed only minor changes in absorbance spectra with the addition of

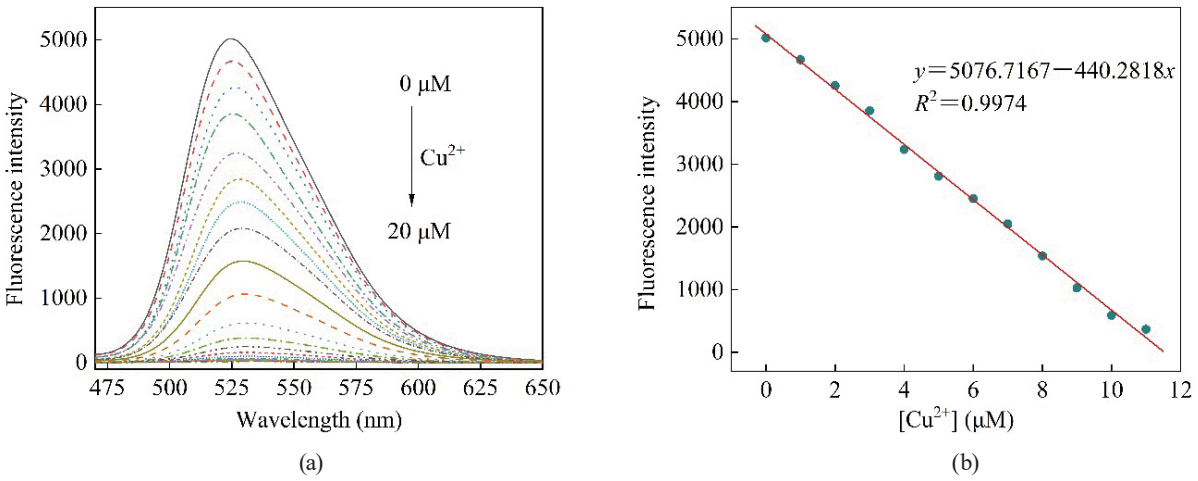

Fig. 3. (Color online) (a) Fluorescence spectra of DCB with varying Cu^{2+} concentrations. (b) Linear relationship between the fluorescence intensity of DCB and Cu^{2+} concentration.

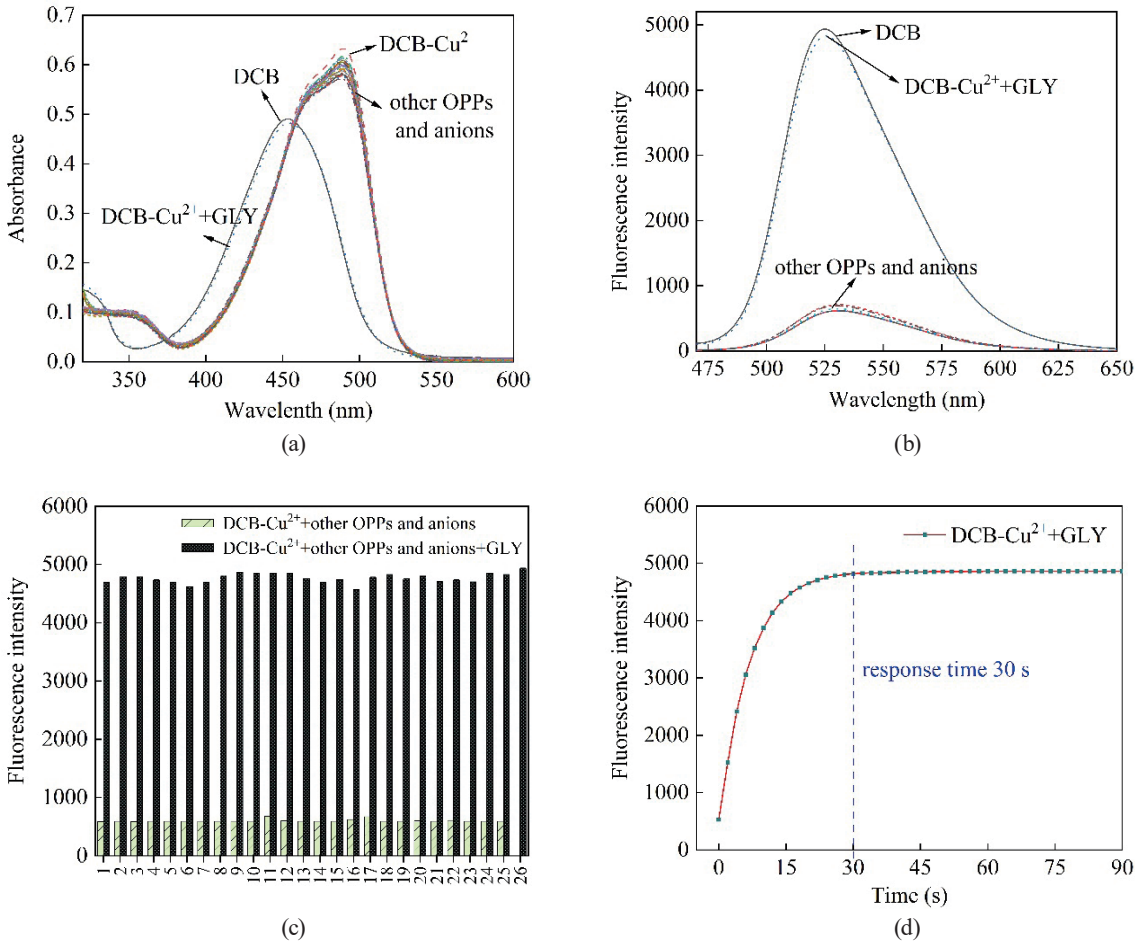

Fig. 4. (Color online) (a) UV-vis and (b) fluorescence spectra of DCB-Cu^{2+} toward various OPPs and anions. (c) Interference experiments of DCB-Cu^{2+} for fluorescent recognition of GLY (1. trichlorfon, 2. phosmet, 3. dichlorvos, 4. malathion, 5. omethoate, 6. dimethoate, 7. ethoprophos, 8. fenitrothion, 9. parathion methyl, 10. parathion, 11. glufosinate ammonium, 12. SO_4^{2-}, 13. F^-, 14. SCN^-, 15. Br^-, 16. $H_2PO_4^-$, 17. HPO_4^{2-}, 18. NO_2^-, 19. I^-, 20. HCO_3^-, 21. CO_3^{2-}, 22. Cl^-, 23. CH_3COO^-, 24. NO_3^-, 25. GLY, 26. DCB). (d) Time-dependent fluorescence intensity (at 525 nm) changes of DCB-Cu^{2+} to GLY.

the aforementioned OPPs and anions (50 μM). As depicted in Fig. 4(b), these OPPs and anions exhibited no discernible changes in fluorescence signal; however, the addition of GLY resulted in considerable fluorescence recovery. Furthermore, with the addition of 11 other OPPs and 13 common anions to the DCB-Cu^{2+} system, the fluorescence intensity changed marginally; in contrast, it increased considerably after the addition of GLY [Fig. 4(c)]. The complex DCB-Cu^{2+} had no interactions with other OPPs or anions, and GLY was detected within 30 s [Fig. 4(d)].

The UV–vis and fluorescence titration experiments of DCB-Cu^{2+} with GLY were next investigated. As depicted in Fig. 5(a), the complex DCB-Cu^{2+} has two absorption bands at 350 and 491 nm. When GLY (0–12 μM) was constantly added, the maximum peak (491 nm) progressively decreased and blue-shifted, and three equivalent points appeared at 332, 373, and 451 nm, indicating that DCB and GLY were capable of forming stable complexes with Cu^{2+}.[23] As depicted in Figs. 5(b) and 5(c), with the addition of GLY, the fluorescence intensity of DCB-

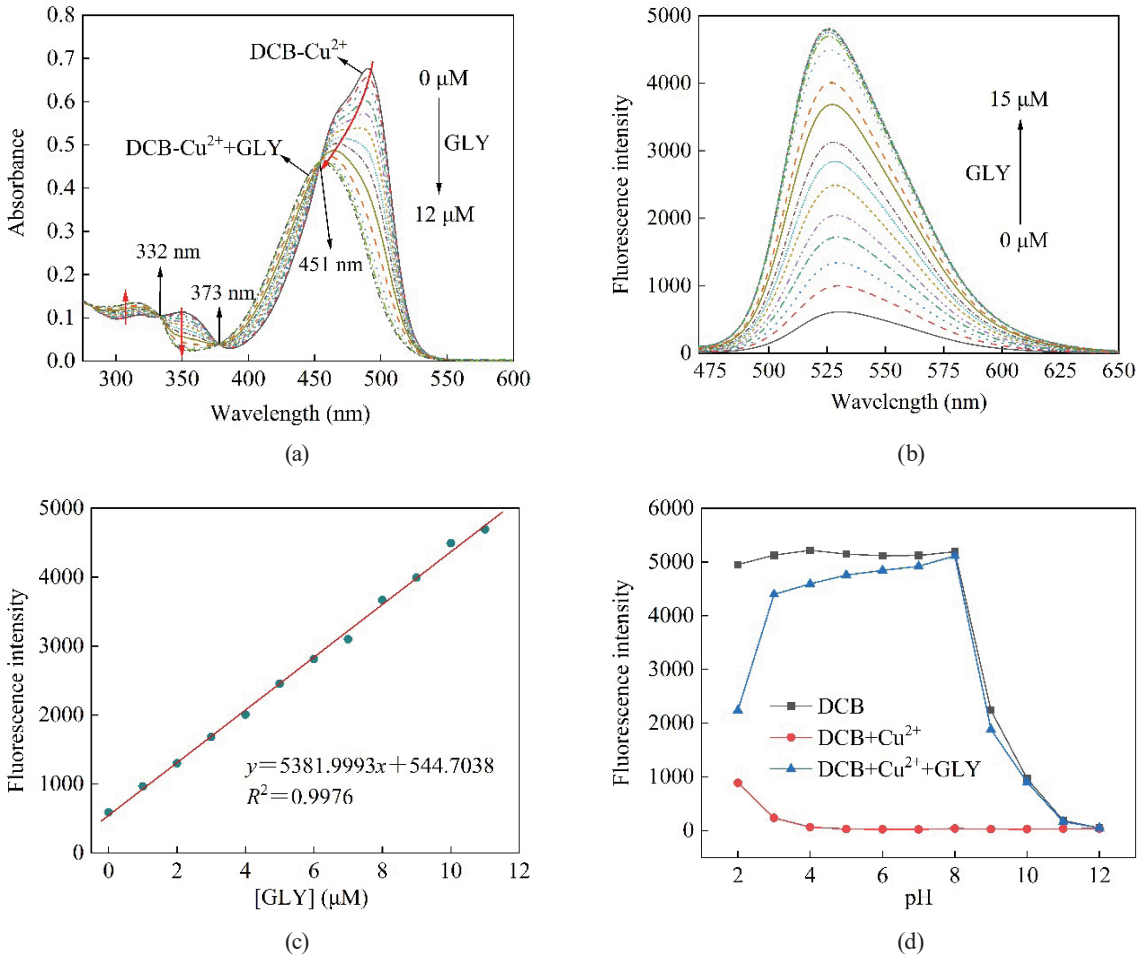

Fig. 5. (Color online) (a) UV-vis and (b) fluorescence spectra of DCB-Cu^{2+} with varying GLY concentrations. (c) Linear relationship between the fluorescence intensity of DCB-Cu^{2+} and GLY concentration. (d) Fluorescence intensities (at 525 nm) of DCB, DCB + Cu^{2+}, and DCB + Cu^{2+} + GLY at different pH values.

Cu^{2+} at 525 nm increased steadily; the relationship between fluorescence intensity and GLY concentration was well defined in the range of 0 to 11 μM, the fitting equation for which is y = 381.9993x + 544.7038 (R^2 = 0.9976), with an LOD of 0.28 μM (47.34 ng/mL). Furthermore, the complex DCB-Cu^{2+} could be used for GLY detection in the pH range of 3–8 [Fig. 5(d)]. All these results showed that the complex DCB-Cu^{2+} could be employed as a specific, sensitive, and instantaneous fluorescent sensor for detecting GLY.

3.3 Mechanism studies

To investigate the detection mechanism further, the stoichiometries of DCB with Cu^{2+} and complex DCB-Cu^{2+} with GLY were determined using Job's plot. As depicted in Figs. 6(a) and 6(b), the stoichiometric ratios of DCB with Cu^{2+} and DCB-Cu^{2+} with GLY were both 1:1. Because DCB was taken out of the complex DCB-Cu^{2+} by chelating Cu^{2+} with GLY, it was presumed that GLY was complexed with Cu^{2+} in a 1:1 ratio.

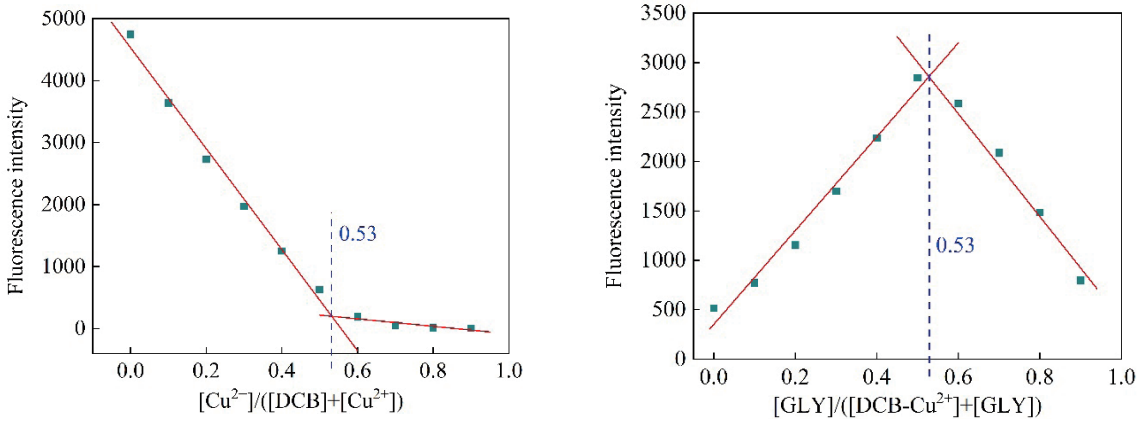

Fig. 6. (Color online) Job's plot of (a) DCB with Cu^{2+} and (b) DCB-Cu^{2+} with GLY.

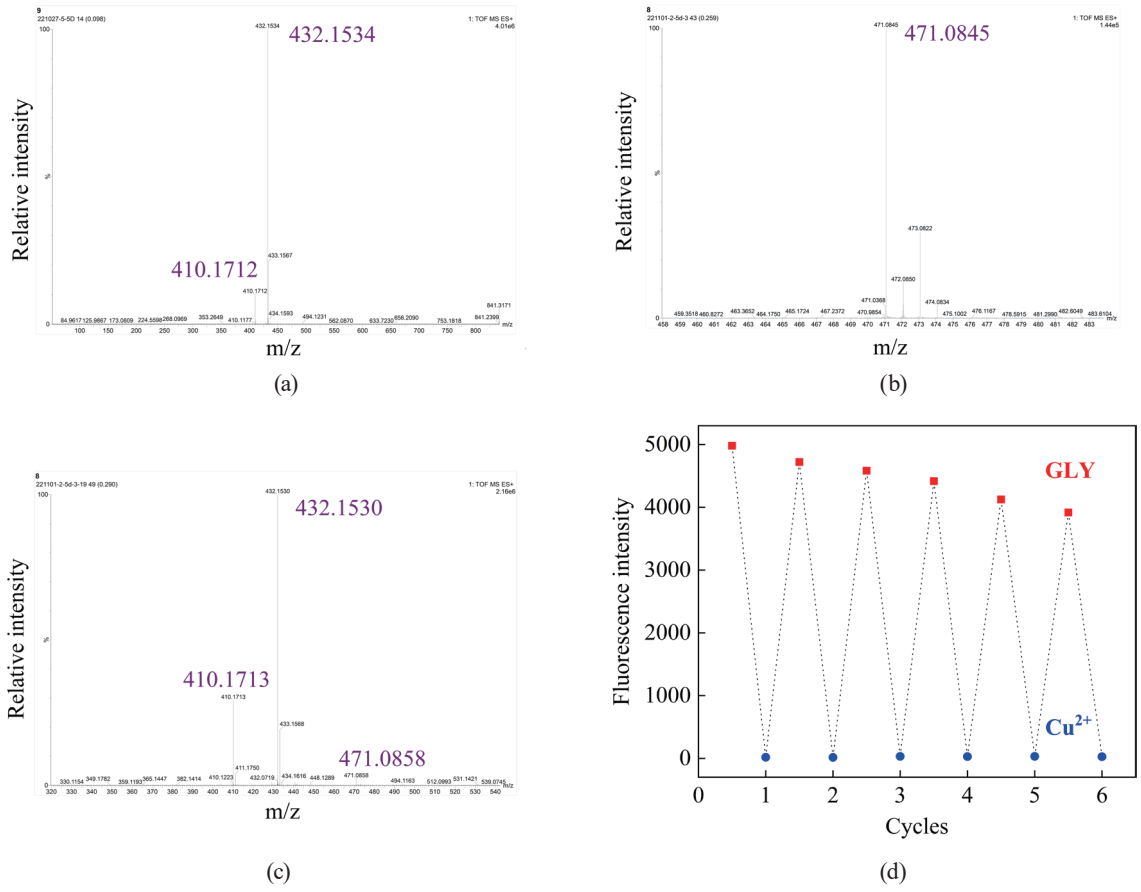

Fig. 7. (Color online) HRMS spectra: (a) DCB, (b) DCB-Cu^{2+}, (c) DCB-Cu^{2+} + GLY. (d) Fluorescence intensity (525 nm) change of DCB upon the alternating addition of Cu^{2+} and GLY.

HRMS was also used to investigate the binding mode. As depicted in Figs. 7(a)–7(c), two dominant peaks were observed using DCB at m/z = 410.1712 and 432.1534, which were attributed to $[DCB+H^+]^+$ (calcd: 410.1710) and $[DCB+Na^+]^+$ (calcd: 432.1530), respectively. After adding

Cu^{2+}, a new peak at m/z = 471.0845 was observed, which was attributed to the formation of $[DCB\text{-}2H^{+}+Cu^{2+}+H^{+}]^{+}$ (calcd: 471.0850). Following further GLY addition, the peak at m/z = 471.0858 almost disappeared, and two strong peaks were generated at 410.1713 and 432.1630. The results supported the proposed mechanism of ligand substitution and provided additional proof that DCB and Cu^{2+} formed a 1:1 stoichiometric complex. Furthermore, the switching of fluorescence intensity at 525 nm could be measured with the addition of Cu^{2+} and GLY continuously and alternately [Fig. 7(d)]. This proved that GLY made a more stable bond with Cu^{2+}, which returned DCB to a free state and restored fluorescence.

The binding mechanism was then confirmed using the IR data of DCB and DCB-Cu^{2+}. As depicted in Fig. 8, comparing the IR spectra, the C=O peak at 1688 cm^{-1} and the C=N peak at 1618 cm^{-1} of DCB were shifted to 1657 cm^{-1} (C=O) and 1599 cm^{-1} (C=N) in the complex. Moreover, during Cu^{2+} complexation, the characteristic frequency of the O−H at 3293 cm^{-1} disappeared. This was attributed to hydroxyl deprotonation and was consistent with the HRMS result of DCB-Cu^{2+}. The research revealed that DCB interacted with Cu^{2+} through the functional groups C=O, C=N, and O−H. On the basis of these results, we proposed a possible responsive mechanism between the complex DCB-Cu^{2+} and GLY, as depicted in Fig. 9.

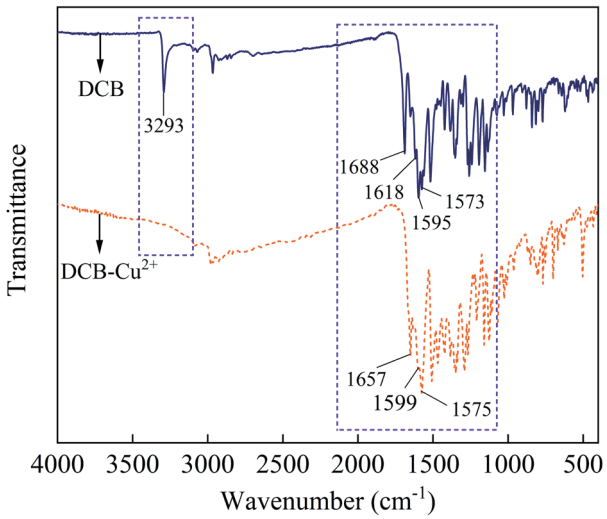

Fig. 8. (Color online) IR spectra of DCB in the absence and presence of Cu^{2+}.

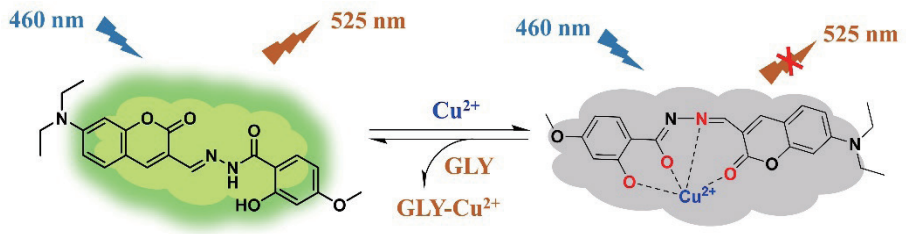

Fig. 9. (Color online) Proposed sensing mechanism of DCB-Cu^{2+} for GLY.

Table 1
Detection of GLY in water samples using the proposed sensor.

Samples	Added (μM)	Found (μM)	Recovery (%)	RSD (%, n = 3)	HPLC Recovery (%)*
Tap water	2.00	1.93	96.60	0.36	96.48
	4.00	3.91	97.82	2.13	97.35
	6.00	6.22	103.68	3.42	96.33
	8.00	7.69	96.08	1.89	97.02
	10.00	10.27	102.71	1.44	95.88
River water	2.00	1.95	97.65	0.42	97.58
	4.00	3.87	96.68	1.21	97.63
	6.00	6.08	101.30	2.88	96.41
	8.00	7.53	94.14	2.37	96.72
	10.00	10.15	101.46	3.67	96.37

*HPLC detections were performed by pre-column derivatization with 9-fluorenylmethylchloroformate.

3.4 Applications in actual samples

To investigate the practical applications of DCB-Cu^{2+}, tap water and Songhua River water were selected for spiked experiments. There was no GLY detected in the samples, which was consistent with the results of the classical approach (HPLC). Then, various concentrations (2.0, 4.0, 6.0, 8.0, and 10.0 μM) of GLY standard solutions were added and monitored using DCB-Cu^{2+} and HPLC. As summarized in Table 1, excellent recoveries of 94.14–103.68% and relative standard deviations (RSD) of less than 3.67% were achieved, which were consistent with the HPLC data. All results suggested that the fluorescent sensor (DCB-Cu^{2+}) had a potential practical application in the environmental monitoring of GLY.

4. Conclusions

In summary, a highly selective and sensitive fluorescence sensor based on a coumarin derivative and a Cu^{2+} complex (DCB-Cu^{2+}) for GLY detection was established. DCB showed a strong chartreuse-emitting fluorescence, and considerable fluorescence quenching when Cu^{2+} was present. As expected, with the addition of GLY, the fluorescence of DCB-Cu^{2+} recovered because GLY had a higher affinity for Cu^{2+} than DCB. Meanwhile, DCB was replaced by GLY to restore its monomeric state. On the basis of this, we developed a fluorescent sensor (DCB-Cu^{2+}) that could detect GLY with high sensitivity and specificity. Furthermore, the sensor was applied to analyze GLY in actual samples with satisfactory results. All these studies showed that the fluorescent sensor (DCB-Cu^{2+}) can have potential applications in environmental monitoring.

Acknowledgments

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