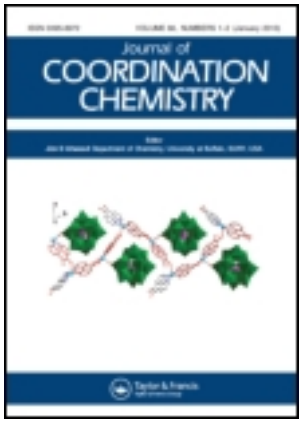

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A coumarin-based fluorescent probe for selective detection of Cu^{2+} in water

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A coumarin-based fluorescent probe for selective detection of Cu^{2+} in water

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Fluorescent Red GK, a commercially available coumarin-based dye, was developed as a "turn-off" fluorescent probe for detection of Cu^{2+} in aqueous solution. It exhibited high selectivity and sensitivity at room temperature. Upon addition of Cu^{2+}, the strong fluorescence of Fluorescent Red GK was severely quenched and its color changed from orange to colorless under illumination with a UV lamp; the color of the solution also changed from pink to colorless. So, it can be used as a specific colorimetric and fluorescent probe for Cu^{2+} with a detection limit as low as 0.0634 μM.

Keywords: Cu^{2+}; Fluorescent Red GK; Fluorescence quenching; Coumarin based; Fluorescent probe

1. Introduction

Copper, the third most abundant transition metal ion in the human body and commonly found as Cu(II) in natural water, plays a pivotal role in environmental, biological, and chemical systems [1–4]. In particular, it is an essential micronutrient that forms part of several proteins involved in a variety of biological processes indispensable to sustain life [5–11]. In addition, excessive uptake or deficiency of copper could do harm to the liver [12–17]. So, the detection of Cu(II) is important and necessary.

The fluorescence method has more advantages due to its operational simplicity, nondestructive methodological sensitivity, high selectivity, rapidity, high sampling frequency and low cost of equipment, and direct visual perception [18].

Recently, fluorophores such as anthracene, coumarin, benzaldehyde hydrazone, and naphthalimide have been reported to be useful as fluorescent ion probes [19–21]. Among them, coumarin and its derivatives have been extensively explored for their unique photophysical properties and high binding ability for various ions [22–26]. In particular, the development of coumarin-based fluorescent probes for Cu^{2+} has gained considerable attention.

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Some examples of selective recognition fluorescent probes based on coumarin for Cu^{2+} have been investigated. Wang *et al.* reported a highly selective fluorescence turn-on chemosensor based on naphthalimide derivatives for detection of copper(II) ions [28]. Kim *et al.* reported a new highly selective, reversible, chromogenic, and fluorogenic chemosensor based on thiazolecoumarin moieties for quantification of copper ions in aqueous DMSO [29]. Amani *et al.* have investigated the recognition ability of a probe by naked-eye colorimetric experiments for Cu^{2+} [30].

We have developed a commercially available fluorescent probe for the detection of Cu^{2+}. In this study, we have developed a commercially available fluorescent probe based on the coumarin dye, Fluorescent Red GK, which can sensitively and selectively detect Cu^{2+} in HEPES aqueous buffer (pH 7.0) and display quenched fluorescence intensities and clear color changes upon recognition.

2. Experimental setup

2.1. *Materials*

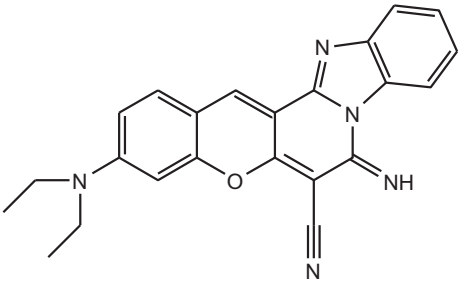
Fluorescent Red GK (scheme 1) was purchased from Beijing City of China. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from Sigma–Aldrich (St. Louis, MO). The solutions were prepared with deionized water. The chemicals used were of analytical reagent grade. All experiments were carried out at room temperature.

2.2. *Instruments*

The UV absorption experiments were carried out on a UV-757CRT spectrophotometer (Shanghai Precision & Scientific Instrument Co., Shanghai, China). Fluorescence measurements were conducted on a Hitachi F-2500 FL spectrofluorimeter (Tokyo, Japan) using an excitation wavelength of 355 nm and emission wavelength of 570 nm. ^{1}H NMR spectra were recorded on a Bruker AVANCE-300 MHz spectrometer.

2.3. *General fluorescence spectra measurement*

By means of the Fluorescent Red GK probe, Cu^{2+} could be detected in aqueous solution. The procedure was as follows: into HEPES aqueous buffer (10 mM/L, pH 7.0) solutions, containing 12.5 μM Fluorescent Red GK (a fluorescence solution), a



Scheme 1. Chemical structure of Fluorescent Red GK.

Cu^{2+} sample was gradually titrated. Meanwhile, changes in the fluorescence intensity were monitored using a fluorescence spectrometer (λ_{ex} = 355 nm, λ_{em} = 570 nm, slit: 15 nm/15 nm).

2.4. *Detection range*

Fluorescence spectra were measured from 425–650 nm with excitation at 355 nm, and the sensitivity for Cu^{2+} was 0.5–50 μM.

3. Results and discussion

3.1. *Fluorescence study*

Figure 1 shows fluorescence emission changes of the probe (λ_{ex} = 355 nm) in pH 7.0 HEPES buffer solution by means of a fluorescence titration experiment. Fluorescent Red GK in 10 mM/L HEPES (pH 7.0) buffer shows a strong fluorescence, whereas a decrease of fluorescence intensity of Fluorescent Red GK (12.5 μM) could be observed with gradual addition of Cu^{2+}. When the concentration of Cu^{2+} added up to 50 μM, the strong fluorescence of Fluorescent Red GK was completely quenched and its color changed significantly from orange to colorless under illumination with a UV lamp.

3.2. *UV-vis Study*

Figure 2 shows change in the UV–vis spectrum when Cu^{2+} was added to the HEPES aqueous buffer (10 mM/L, pH 7.0) solution containing the probe (75 μM). Upon addition of Cu^{2+}, the absorbance at 528 nm gradually decreased. While the concentration of Cu^{2+} added up to 25 μM, the absorbance of the probe was unchanged. Meanwhile, an obvious color

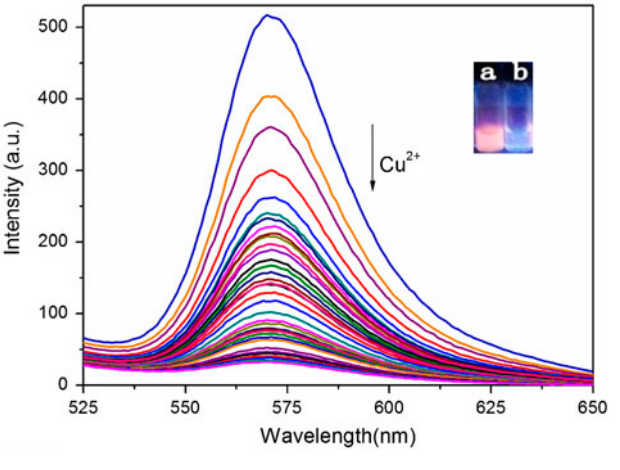


Figure 1. Fluorescence spectra of the probe (12.5 μM) containing HEPES buffer (10 mm, pH 7.0) on gradual addition of Cu^{2+} (0–50 μM). Each spectrum was recorded 2 min after Cu^{2+} addition. Inset: photo a (12.5 μM probe) and photo b (12.5 μM probe with 50 μM Cu^{2+}) were taken under UV 365 nm.

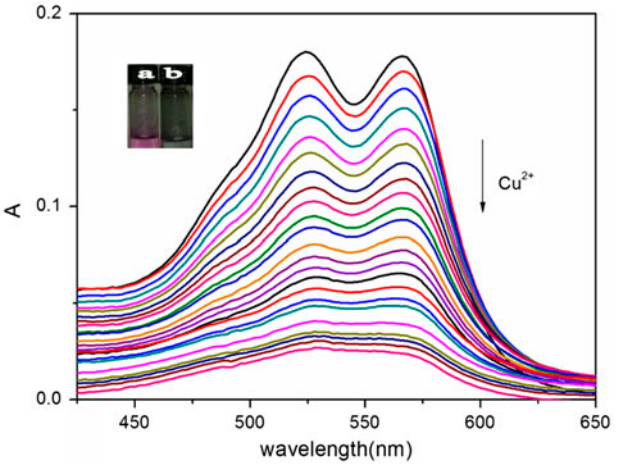


Figure 2. UV-vis spectra of Fluorescent Red GK (75 μM) in 10 mM/L HEPES (pH 7.0) buffer upon addition of gradual addition of Cu^{2+} (0–25 μM). Inset: Color changes of the probe upon addition of Cu^{2+} in 10 mM/L HEPES (pH 7.0) buffer. Photo a (75 μM probe) and photo b (75 μM probe with 25 μM Cu^{2+}).

change from pink to colorless was clearly observed. This strongly suggested that Fluorescent Red GK can serve as a "naked eye" probe for Cu^{2+}.

3.3. *Effect of reaction time*

Time-dependent fluorescence spectra of the system were investigated. As shown in figure 3, the fluorescence signal of the system nearly does not change with increasing reaction time after 2 min. The results revealed that the reaction between Fluorescent Red GK and Cu^{2+} can be completed within 2 min, indicating that the probe has rapid detection ability for Cu^{2+}, so 2 min was selected as the reaction time.

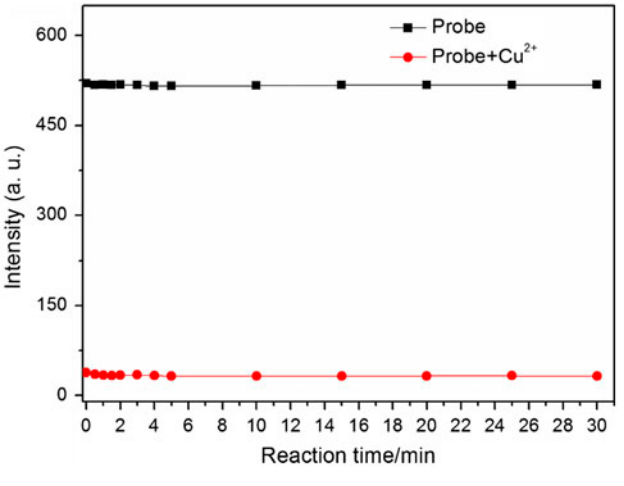


Figure 3. Time-dependent fluorescence change acquired for the probe (12.5 μM) in the absence and presence of Cu^{2+} (50 μM) in 10 mM/L HEPES (pH 7.0) buffer.

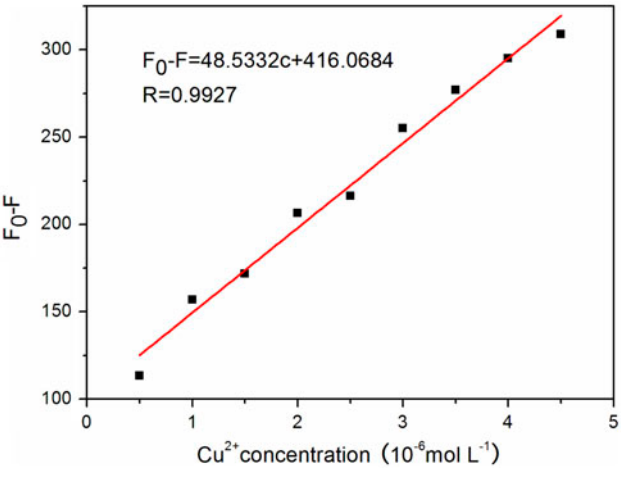


Figure 4. Calibration curve for the Cu^{2+}. Working conditions: pH 7.0; reaction time = 2 min; C_{probe} = 12.5 μM.

3.4. *Work curve*

Under the optimum condition, the calibration curve was constructed (figure 4). The linear range of the method was found to be 0.5–4.5 μM Cu^{2+} with a correlation coefficient of R = 0.9927 (n = 9). In line with IUPAC recommendations, the detection limit was calculated as 0.0634 μM.

3.5. *Metal ion selectivity*

In order to evaluate selectivity of the probe to Cu^{2+}, the fluorescence responses of the probe for various metal cations and its selectivity for Cu^{2+} are shown in figures 5 and 6. When 50 μM Cu^{2+}, Cu^{+}, Na^{+}, K^{+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Fe^{2+}, Ni^{2+}, Pb^{2+}, and Mg^{2+} were

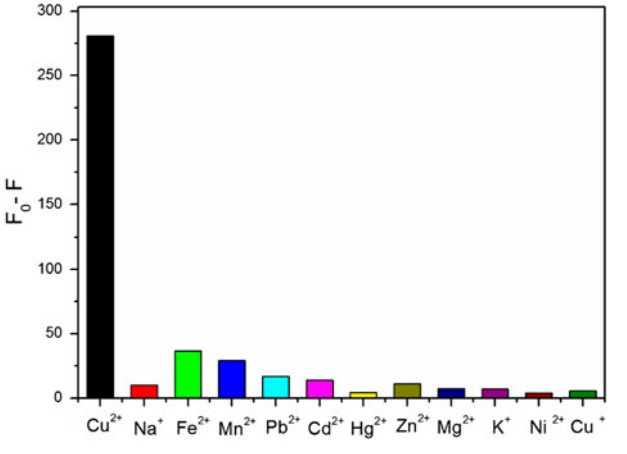


Figure 5. Optical density column graph of probe at 570 nm upon addition of other metal cations.

added to 10 mM/L HEPES (pH 7.0) buffer solution containing the probe (12.5 μM), respectively, it did not cause any apparent color and fluorescence change, only Cu^{2+} generated a large change in the fluorescence spectrum at 570 nm, and the solution color changed to colorless. These results indicated that Fluorescent Red GK showed effective selectivity for Cu^{2+} over other metal ions.

An interference test was performed by measuring the fluorescence of Fluorescent Red GK and Cu^{2+} in the presence of other metal cations (up to 10 equiv) (figure 7). The addition of different metal cations neither influenced the fluorescent intensity nor interfered with the fluorescent decrease by Cu^{2+}. These tests verified the selective nature of this reaction with Cu^{2+}, compared to other metal cations.

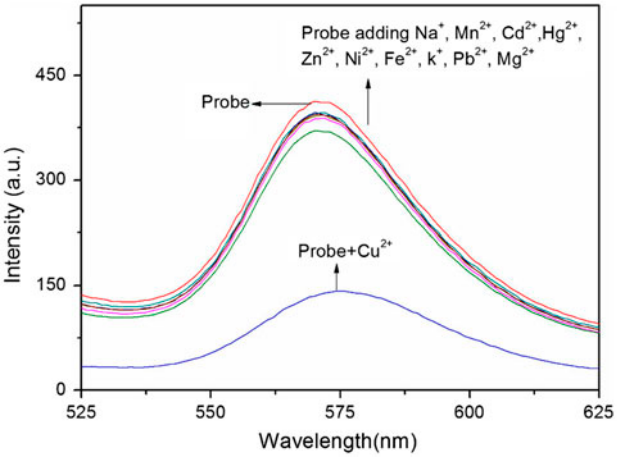


Figure 6. Fluorescence emission spectra of the probe (12.5 μM) in the presence of metal cations such as Cu^{2+}, Cu^{+}, Na^{+}, K^{+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Fe^{2+}, Pb^{2+}, Ni^{2+} and Mg^{2+} (50 μM).

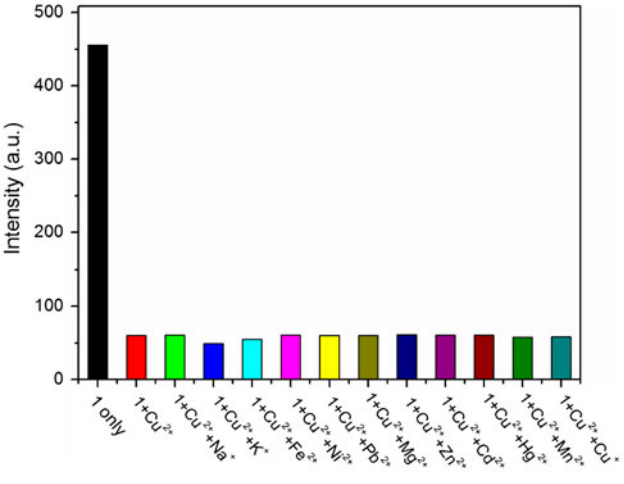


Figure 7. Fluorescence emission spectra of the probe (12.5 μM) after addition of 500 μM of other metal cations to the solution of Fluorescent Red GK + Cu^{2+} (50 μM) in HEPES buffer at pH 7.0.

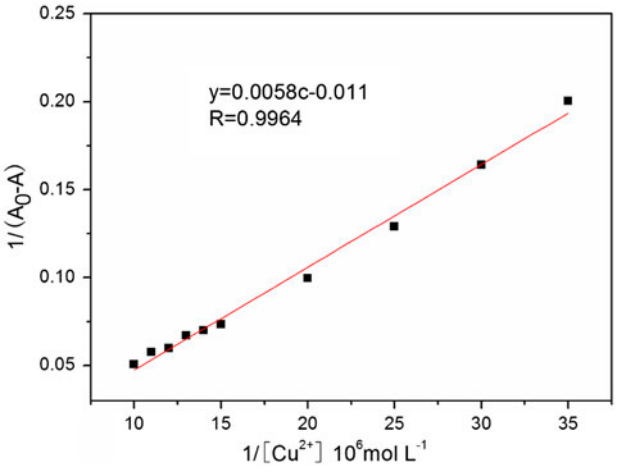


Figure 8. The determination of the stoichiometry between probe and Cu^{2+}.

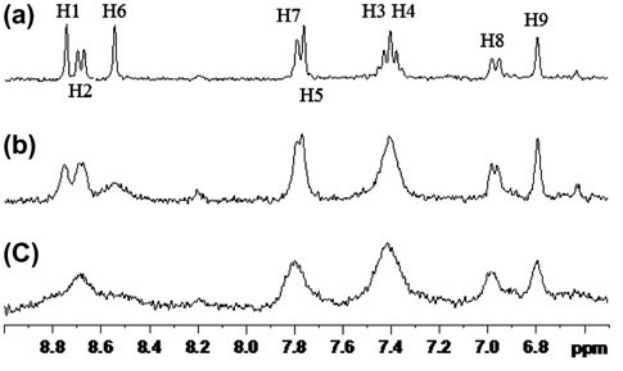
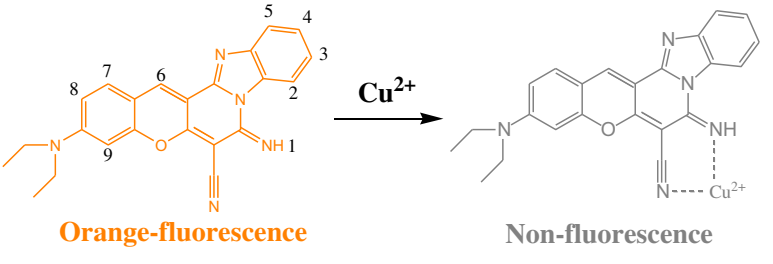


Figure 9. ^{1}H-NMR spectra of (a) Fluorescent Red GK only; (b) Fluorescent Red GK + Cu^{2+} (1:0.5); (c) Fluorescent Red GK + Cu^{2+} (1:1).



Scheme 2. The proposed mechanism for the determination of Cu^{2+}.

Table 1. Performance comparison of various colorimetric and fluorescent probes for Cu^{2+}.

Fluorescence on or off	Linear range, μM	LOD, μM	Testing media	Color change	Response time	Under illumination with a UV lamp	Mechanism	Ref.
On	0.1–0.9	0.035	10 mM Tris-HCl containing 1% DMSO, pH 7.0	–	5 min	From colorless to blue	2-picolinic acid in chemodosimeter **1** acts as an "anchoring group", which brings Cu^{2+} in close proximity to the ester bond	[27]
On	4.0–7.0	0.15	Acetonitrile–water (70:30, v/v), pH 7.0	–	10 s	–	Upon addition of Cu^{2+}, the PET mechanism was quenched	[28]
Off	–	0.04	Aqueous-DMSO (3:1) containing HEPES buffer (10 mM, pH 7.4)	From green to yellow	–	From blue to colorless	ICT	[29]
–	–	–	DMSO	From light pale orange to violet	–	–	On incremental addition of Cu^{2+} to the probe, the peak at 260 nm gradually increased with a red shift and a new band arose at 885 nm.	[30]
On	10–70	23	20 mM HEPES, CH_3CN/HEPES (3:7, v/v) pH = 7.2)	From colorless to pink	1–2 min	From colorless to bright orange	Ring opening of the spirolactam form	[31]
Off	0.1–1.0	0.015	99% water/DMSO (*v*/v) at pH 7.0	–	30 min	From green to blue	Cu(II) promoted hydrolysis of lactone moiety of coumarin yielding a highly fluorescent product	[32]
On	0.5–1.5	16	Ethanol water solution (2:3, v:v, 50 mM HEPES), pH 7.4	–	–	–	Metal ion-induced ring opening of rhodamine spirolactam	[33]
Off	0.008–6	0.003	40 mM HEPES buffer solution at containing 100 mM NaCl and 25 mM KCl, pH 7.4	–	–	–	–	[34]
Off	0–5	10.8	Aqueous solution (containing 5% DMSO	–	1 min	–	PET	[35]
On	2.5–30	1.8	H_2O/CH_3CN (7:3, v/v)	From colorless to jacinth	10 min	From dark to green-yellow	A typical hydrolysis reaction	[36]

(*Continued*)

Table 1. (*Continued*).

Fluorescence on or off	Linear range, μM	LOD, μM	Testing media	Color change	Response time	Under illumination with a UV lamp	Mechanism	Ref.
On	1–14	0.01	50% (v/v) H_2O/CH_3CN buffered by 10 mM HEPES at pH 7.1	From colorless to red	–	–	The color change that occurred as a result of complexation with Cu^{2+} can be attributed to spirolactam ring-opening and hydrolysis of the hydrazide unit to afford C.I. Acid Red 52 (Rhodamine B) in the presence of Cu^{2+}	[37]
On	0.1–1.0	0.045	Ethanol–water solution (6:4, v/v, 50 mM HEPES, pH 7.0)	From colorless to pink	–	–	Metal ion-induced ring opening of rhodamine spirolactam	[38]
Off	–	0.087	THF/water (9:1, v/v) containing HEPES buffer (10 mM, pH 7.4)	–	3 min	From blue to colorless	The capture of Cu^{2+} resulted in the electron or energy transfer from probe to Cu^{2+}	[39]
On	–	–	HEPES buffer (20 mM, pH 7.0) containing 50% (v/v) water/CH_3CN	From colorless to pink	–	–	Metal ion-induced ring opening	[40]
Off	0–5	0.05	HEPES buffer (10 mM, pH 7.0)	–	20 s	From blue to colorless	The paramagnetic effect from spin-orbit coupling of the Cu^{2+} induces fluorescence quenching	[41]
Off	0.5–50	0.0634	HEPES buffer (10 mM, pH 7.0)	From pink to colorless	2 min	From orange to colorless	It may bring Cu^{2+} in close proximity to the –C=N and –C≡N.	This study

3.6. *Proposed mechanism*

According to the linear Benesie Hildebrand expression, the measured intensity $[1/(A - A_0)]$ at 528 nm varied as a function of $1/[Cu^{2+}]$ in a linear relationship ($R = 0.9964$) (figure 8), indicating formation of 1:1 stoichiometry between Cu^{2+} and probe.

To analyze the reaction mechanism of the probe for Cu^{2+}, the product of Fluorescent Red GK–Cu^{2+} was analyzed. Using ^{1}H NMR, we monitored the intermediate formation and compared it with spectra for the probe itself. The ^{1}H-NMR signal of the product of Fluorescent Red GK–Cu^{2+} [figure 9(b) and (c)] was nearly the same as Fluorescent Red GK [figure 9(a)]; we only found a negligible shift of the peak. The ^{1}H of Fluorescent Red GK at δ 8.744 was shifted to 8.752. So, it may bring Cu^{2+} in close proximity to the –C=N and –C≡N groups. The possible reaction mechanism is described in scheme 2.

3.7. *Method performance comparison*

The performance of the proposed probe Fluorescent Red GK was compared with some reported fluorescent probes for Cu^{2+} determination, as shown in table 1. All the fluorescent probes present good selectivity for Cu^{2+} [27–41]. But, some of them need more rigorous testing media [27–33, 36–40] and did not show clear color changes [27, 28, 32–35, 39, 41]. There are still numerous challenges and opportunities remaining for the development of new probes with better performance. Our proposed probe Fluorescent Red GK based on coumarin presents a number of attractive analytical features, such as good selectivity and high sensitivity. The advantages of our proposed method compared with some of the previously published fluorescent probes are as follows: it shows not only clear color changes but also a visual fluorescence change under illumination with a UV 365 nm lamp. In addition, the reaction of our probe with Cu^{2+} could be instantly carried out under room temperature conditions within 2 min.

4. Conclusion

We have developed a colorimetric and fluorescent probe Fluorescent Red GK for Cu^{2+} based on coumarin. Upon addition of Cu^{2+}, the strong fluorescence of Fluorescent Red GK was severely quenched and its color changed from pink to colorless under optimized conditions. Fluorescent Red GK displayed a detection limit as low as 0.0634 μM toward Cu^{2+} and good selectivity over other metal cations. The highly selective quenching of Fluorescent Red GK by Cu^{2+} in aqueous media may be utilized for the diagnosis of various copper-related diseases.

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