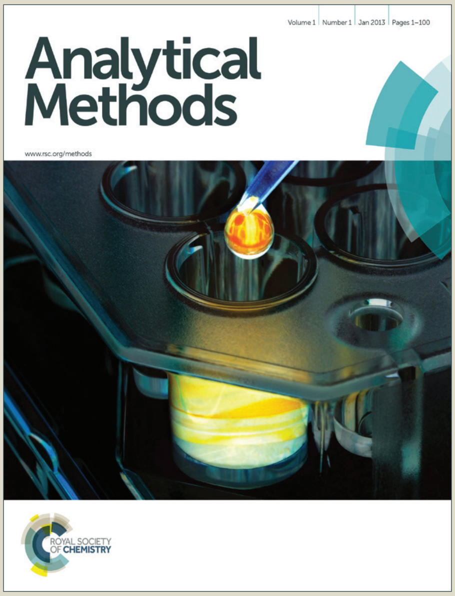
Analytical Methods



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A natural Quercetin-based fluorescent sensor for highly sensitive and selective detection of copper ions

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Abstract

A natural quercetin-based fluorescent sensor for highly sensitive and selective detection of copper ions had been studied. The fluorescent sensor quercetin (Q) after binding to Cu^{2+} ions in pH 7.40 buffered solution generated quenching in fluorescent emission intensity. The binding constant value was obtained 3.56×10^{7}. The sensor Q can be applied to the quantification of Cu^{2+} ion with a linear range of 2.0×10^{-7}-3.0×10^{-6} $mol\cdot L^{-1}$ and the detection limit of 1.0×10^{-7} $mol\cdot L^{-1}$. The sensor showed high selectivity toward Cu^{2+}. As a result the proposed fluorescent sensor was successfully applied for determination of Cu^{2+} in water samples.

Key Words: quercetin, nature, fluorescent sensor, copper ions

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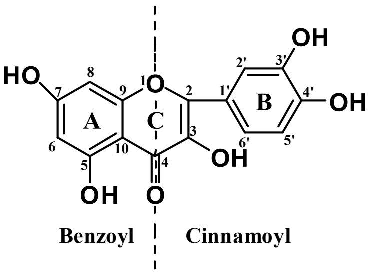
1. Introduction

Copper ions (Cu^{2+}) played a critical role in the area of environmental, biological and chemical systems. However, exposure to a high level of copper can cause gastrointestinal disturbances, liver or kidney damage. Therefore, it was important to develop simple, highly sensitive and selective methods for the detection of Cu^{2+} ion.

Over the past decades, analytical methods were developed to detect Cu^{2+} ion including inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS), electrochemical techniques, fluorescence methods and chromogenic sensors.[1-5] Methods such as ICP-MS and AAS can detect Cu^{2+} ion with high sensitivity and selectivity but with requirements of large instruments. Compared with electrochemical techniques and colorimetric methods, fluorescence methods were much simpler with detection by fluorescence spectroscopy. In recent years, significant emphasis had been placed on the development of new, highly selective fluorescent sensors of copper cation. Thousands of fluorescent sensors for Cu^{2+} ion had been reported,[6-8] however, most of which were obtained by organic chemistry synthesis operation, and few of which was from nature. It was more important to develop natrual products with a drop in oil resources in recent years. While in this study, we found natural quercetin belong to flavonoids could be used as highly sensitive and selective fluorescent sensor for detecting copper ion.

Flavonoid were a ubiquitous group of polyphenolic substances which were present in most plants, concentrating in seeds, fruit skin or peel, bark and flowers. Flavonoids had been shown to have antioxidant, antiinflammatory, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodilator activity. [9] Quercetin (Q) was one of the most common flavonoids present in many foods including apple, tea, onion, nuts, berries, cauliflower and cabbage. Especially, Flos Sophoraes Immaturus (dried flower buds of Sophora japonica L.) contained lots of Q that could be easily extracted with simple process .[10] Due to its strong antioxidant properties, Q had attracted the attention of many researchers, as result of many reports about biological and pharmaceutical properties of Q.[11-14] In recent years, significant emphasis had been placed on the development of fluorescent technique, the fluorescence spectra of Q and its ramification had been studied, it was reported that nucleic acid can enhance the fluorescence intensity of Q. Based on this, a sensitive method for the determination of nucleic acids was proposed .[15-16] However, there were few reports about the research on its ability to act as fluorescent sensors of metal ions. Q was one of the effective metal chelators which possessed three possible chelating sites in competition: the 3-hydroxy-4-carbonyl, the 5-hydroxy-4-carbonyl, and the 3’, 4’-dihydroxyl (catechol) groups[17] (Scheme 1). Complexation of metal cations by Q had already been reported for a large number of metals (Mo(VI), Fe(II), Fe(III), Cu(II), Zn(II), Al(III), Tb(III), Cr(III), Co(II) and many other cations) and in these studies different stoichiometric ratios can be found between different metals and Q.[18-26] Q, like most flavones and flavonols, had strong ultraviolet absorption related to two conjugated systems: between ring B and carbonyl of ring C, between ring A and carbonyl of ring C [24] (Scheme 1). Complexation of metal cations may change its characteristic of spectra. Thus Q had huge potential for detection of metal ions based on the changes of spectra.

Fortunately, fluorescent emission was observed in the buffer solution (pH = 7.40) of Q. Furthermore, we found Q has high selectivity and sensitivity of copper cation related to fluorescence quenching. The concentration of Cu^{2+} can be quantified by the fluorescence titration experiments. From the change of UV absorption spectra, the structure of Q-Cu(Ⅱ) complex and the mechanism of fluorescence quenching had been discussed.



Scheme 1 Structure of the quercetin (3,3’,4’,5,7-pentahydroxyflavone)

2. Experimental

2.1. Reagents

All reagents and solvents were analytical reagent (AR) grade, except MeOH (HPLC), and were used as received unless otherwise indicated. Q was extracted from Flos Sophoraes Immaturus or *Ginkgo Biloba* leaves. Flos Sophoraes Immaturus, were purchased from Bozhou Good health Food Co., Ltd. (Anhui, China). Phosphate Buffer Solution (PBS, pH=7.40) was purchased from Beijing Solarbio Science & Technology Co., Ltd.. All solutions were freshly prepared using ultrapure water before experiments and used immediately.

2.2. Instrumentation

The ^{1}H NMR (600 MHz) spectra were recorded on a Bruker AVANCE Ⅲ 600 spectrometer. Proton chemical shifts were reported in parts per million downfield from tetramethylsilane (TMS). Melting points were determined on WRR visual melting-point apparatus and uncorrected. UV–visible absorption spectra were recorded on a PerkinElmer Lambda 950 spectrophotometer. Fluorescence spectra were recorded on PerkinElmer LS55 spectrophotometer, and excitation wavelength was 390 nm for all measurements.

2.3 Extraction and isolation

Dried Flos Sophoraes Immaturus (20g) were exhaustively extracted with boiling 0.4% sodium tetraborate aqueous solution whose pH was kept between 8~9 by adding calcium hydroxide. After 30min, the extracting solution was filtered at temperature of 60~70℃. The filtrate was acidified to pH 2~3 with hydrochloric acid solution, and the precipitate was obtained, which was recrystallized from water and hydrolyzed in a boiling 2% H_2SO_4 aqueous solution. After 8h, the solution was filtered immediately. The yellow product was washed with water and dried in vacuum desiccators. The product was recrystallized from ethanol to yellow crystals (1.1989 g, yield 6.0%). mp. > 300℃. ESI-MS m/z 301 $[M-H]^-$. ^{1}H NMR (DMSO-d_6 600 MHz): 12.50 (s,1H, 5-OH), 10.78 (s,1H, 7-OH), 9.59 (s,1H, 3-OH), 9.37 (s,1H, 4’-OH), 9.31 (s,1H, 3’-OH), 7.68 (d, J = 1.8 Hz, 1H, 2’-H), 7.54 (d×d, J_1 = 10.8 Hz, J_2=1.8 Hz, 1H, 6’-H), 6.89 (d, J = 8.4 Hz, 1H, 5’-H), 6.41 (d, J = 2.4 Hz, 1H, 8-H), 6.19 (d, J = 1.8 Hz, 1H, 6-H).

2.4 Optimizing experimental conditions

So as to obtain a highly selective and sensitive response for the detection of Cu^{2+} ions, the optimization of the different pH values of PBS was carried out in our experiment. The resulting solutions were investigated by fluorescence spectra with excitation at 390 nm. The buffer solution was composed of CH_3OH-PBS（1:99，V/V), and pH was changed by dilute hydrochloric acid and dilute sodium hydroxide solution.

2.5 Stoichiometric ratio

Job's method [28] (continual variation method) was used to determine the stoichiometric ratio between the Q and the metal ion for their complexation in buffer solution (pH=7.40); the solutions were prepared by mixing of equimolar concentration (1.2×10^{-4} molar) solutions of both components in different ratios varying from 1:9 to 9:1. Then the absorbance was measured at 430 nm. The maximum absorbance at 430 nm (A_2) was recorded, the absorbance of crossover point in Job's plots (A_1) was recorded too. And the stability constant (K_s) between Q and Cu^{2+} was obtained by followed equations:

$$K_s = \frac{1-\alpha}{n^n c^n \alpha^{n+1}} \qquad (2\text{-}1)$$

$$\alpha = \frac{A_1 - A_2}{A_1} \qquad (2\text{-}2)$$

In the equation (2-1), n is the stoichiometric ratio of metal ion and Q , c is the concentration of metal ion.

2.6 Procedures for Sensor selectivity investigation

In order to check fluorescence response of Q to different metal ions, stock solutions (4.0×10^{-3} mol·L^{-1}) of the acetate /nitrate / chlorate salts of different metal ions (Cu^{2+}, Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{2+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}) and the host sensor (1.0×10^{-5} mol·L^{-1}) of Q were prepared in CH_3OH-PBS（1:99，V/V，pH = 7.40）buffer solution, respectively. The test solutions were prepared by placing 2 mL of the sensor stock solutions into a test tube, then adding a small quantity of certain ion stock solutions. In the last test solutions, [Cu^{2+}] = 5×10^{-5} mol·L^{-1}, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} mol·L^{-1}, the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} mol·L^{-1}).

In order to check the influence of other meal ions on fluorescence responses of Q to Cu^{2+}, test solutions were prepared by placing 2 mL of the sensor stock solutions and 5μL of Cu^{2+} stock solutions into a test tube, then adding a small quantity of certain metal ions stock solutions to it. In the last test solutions, [Q]=1×10^{-5} mol·L^{-1}, [Cu^{2+}]=2×10^{-6} mol·L^{-1}, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} mol·L^{-1}, and the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} mol·L^{-1}).

2.7 Detecting Cu^{2+} in water samples

We designed a simple assay for lable-free, sample and fast detection of Cu^{2+} in aqueous solution with high selectivity. Different amounts of Cu^{2+} ions were added to pure water. A little pure water samples were taken and diluted to 1.6×10^{-3} and 2.4×10^{-4} mol·L^{-1} with CH_3OH-PBS（1:99, V/V, pH = 7.40） buffered solution. Then 5 μL of buffered solution containing Cu^{2+} ions were added to the 2 mL host sensor solution containing Q (1.0×10^{-5} mol·L^{-1}). The results were obtained by fluorescence spectrometer.

3. Results and discussion

3.1 The influence of pH on fluorescent intensity of Q

Fig.1 showed fluorescence emission intensity for Q and Q- Cu(Ⅱ) complex with different pH values. It was interesting that its fluorescent intensity of 546.5 nm was increased with pH ranging from 5.00 to 9.00. Fluorescent intensity of Q was increased obviously with pH 5.00~7.80; while, it was increased slowly with pH 7.80~9.00. Under acid condition, the conjugated systems of Q may be broken by oxygen atoms binding with H^+, and there was weak fluorescence signal obtained. With the increase of pH, the hydroxy groups(-OH) of Q became oxygen anion(O^-), which made conjugated system of Q larger, and the fluorescence signal of Q was getting stronger. However, along with the higher degree of alkali, autoxidation of Q occurred more easily.[29] Fluorescent intensity of Q-Cu(Ⅱ) complex was also increased with pH 5.00~7.80, and it was decreased slightly with pH 7.80~9.00. While in the same pH, fluorescent intensity of Q-Cu(Ⅱ) complex was obviously lower than that of Q, which meant fluorescence quenching occurred when Q coordinated with copper ion. Thus Q may act as a fluorescent sensor for detecting the concentration of copper ion with the phenomenon of fluorescence quenching.

In consideration of fluorescence intensity and other factors, we chose pH 7.40 as one of experimental conditions, which was closed to natural environment and living bodies. And under pH 7.40, Q acted as a fluorescent sensor for detecting the concentration of the aqueous copper cations.

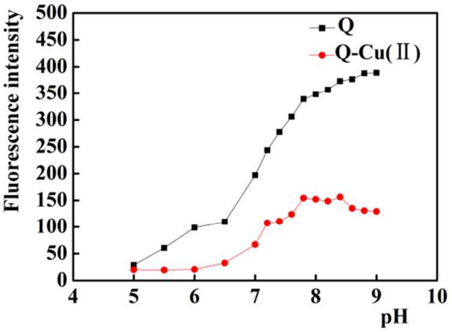
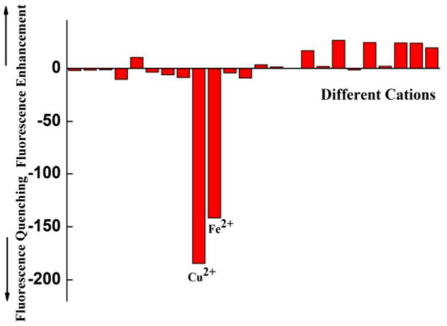


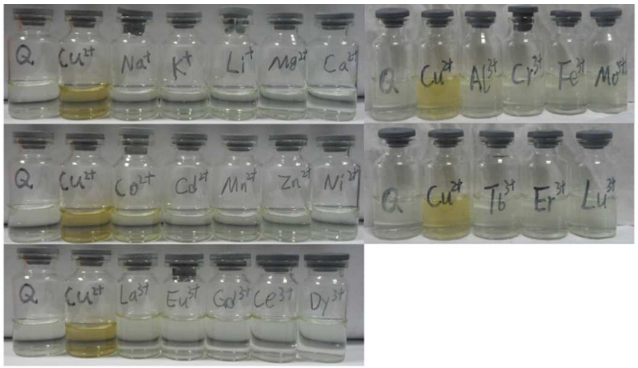
Fig.1 Fluorescent intensity of Q and Q-Cu(Ⅱ) with pH ranging from 5.00 to 9.00.

3.2 Selectivity of the detection system

To check fluorescence response of Q to different metal ions, the effects of other metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{2+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}) on the fluorescence intensity of Q were evaluated. Each type of metal ion was added to the prepared detection system individually, and the efficiency of the fluorescence intensity was recorded. The experimental results shown in Fig.2a and b, which indicated that Cu^{2+} and Fe^{2+} induced obvious fluorescence quenching at their corresponding peaks (546.5 nm). None of the other metal ions generated significant influence on fluorescence signals, even at concentrations as high as 1×10^{-3} mol·L^{-1} of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, and 1×10^{-4} mol·L^{-1} of Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}. However, the Fe^{2+} is not stable and oxidated to Fe^{3+} easily under natural conditions, which cannot affect the sensing process of Cu^{2+}. So, Fe^{2+} is not considered in discussion as follows. Thus, in the presence of no Fe^{2+}, the sensing process happened only in the presence of Cu^{2+} with fluorescence quenching compared to the blank, other metal ions displayed minimal influence, which even could be distinguished by naked eyes (Fig.2b).



(a)



(b)

Fig.2 Fluorescence responses(a) and color changes(b) of Q in CH_3OH-PBS（1:99，V/V，pH = 7.40）buffer solution upon addition of different cations (unlabeled bars are Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}, respectively, λ_{ex}= 390 nm, [Q]= 1×10^{-5} mol·L^{-1}, [Cu^{2+}]=5×10^{-5} mol·L^{-1}, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} mol·L^{-1}, the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} mol·L^{-1}).

Fluorescence responses of Q (1×10^{-5} mol·L^{-1}) to Cu^{2+} (2×10^{-6} mol·L^{-1}) in the presence of certain other metal ion were also measured, and the results indicated that the detection of Cu^{2+} was not influenced by other metal ions. That is, the presence of other metal ions hardly interfered with the fluorescence response of Q-Cu(Ⅱ) system (Fig.3), and Q showed high selectivity on sensing Cu^{2+}.

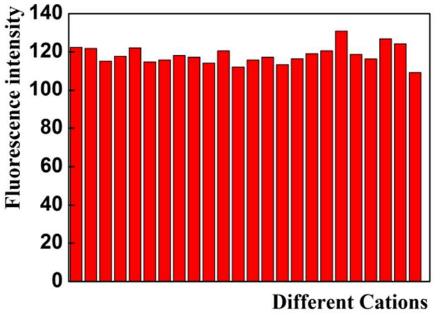


Fig.3 The influence of other metal ions on fluorescent intensity of Q-Cu(Ⅱ) system (unlabeled bars are Q-Cu(Ⅱ) system and other metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}) added to Q-Cu(Ⅱ) system, respectively; λ_{ex}= 390 nm, $[Q]=1 \times 10^{-5}$ mol·L^{-1}, $[Cu^{2+}]=2 \times 10^{-6}$ mol·L^{-1}, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} mol·L^{-1}, the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} mol·L^{-1})

3.3 Sensitivity of the detection system

The detection of Cu^{2+} was performed. The fluorescence intensity (546.5 nm) gradually decreased as the Cu^{2+} concentrations increased (Fig.4). The process of fluorescence quenching was mostly related to static quenching. Static quenching originated from the formation of non-fluorescent Q-Cu(Ⅱ) complex related to paramagnetic effect and heavy atom effect of transition metals.[30]

The fluorescence titration experiments showed that copper ion concentrations were proportional to the decrease in the fluorescence intensity, and gave a good linear change in fluorescence emission intensity in response to the concentration change of Cu^{2+} ranging from 2.0×10^{-7}-3.0×10^{-6} $mol\cdot L^{-1}$. The calibration curve of the relationship between the fluorescence emission intensity and Cu^{2+} concentration was $y = -48.43x + 200.5$ ($R^2 = 0.999$). The detection limit of Cu^{2+} was as low as 1.0×10^{-7} $mol\cdot L^{-1}$.[31] and the standard curve with good linearity can be used to quantify.

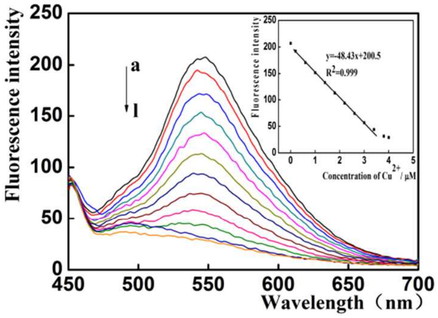


Fig.4 Fluorescence titration of Q and standard curve of fluorescence titration spectra with varying concentrations of Cu^{2+} in CH_3OH-PBS buffer solution（1:99，V/V，pH = 7.40, [Q] = 1×10^{-5} $mol\cdot L^{-1}$, λ_{ex}= 390 nm, from a to l, [Cu^{2+}]= (0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8, 4.0)$\times 10^{-6}$ $mol\cdot L^{-1}$）.

3.4 Proposed mechanism

During detecting Cu^{2+} with Q as fluorescent sensor, Q-Cu(Ⅱ) complex was formed. In order to study the structure of Q-Cu(Ⅱ) complex, Q-Cu(II) complex was characterized by UV-visible and FTIR spectra, and Job’s plots experiments were carried out. UV-visible spectrum of Q and Q-Cu(II) were shown in Fig.5. The UV-visible spectrum of Q in buffer solution showed two major absorption bands at 375 nm (band I) and 262 nm (band II). Band I was considered to be associated with the absorption due to the B-ring cinnamoyl system, and band II with the absorption involving the A-ring benzoyl system.[32] The spectra are related to the $\pi-\pi^*$ transitions within the aromatic rings of the ligand molecule. In comparison with Q absorption spectrum, the band of the Q-Cu(II) complex was shifted to the long-wavelength region，the λ_{max} was shifted to 430 nm - a characteristic of the formation of a complex of the Q. At the same time, and color of the solution was changed into dark yellow during the complexation reaction. Such bathochromic shift can be explained by the extension of the conjugated system with the complexation.

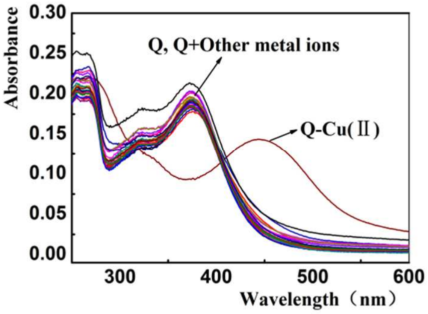


Fig.5 UV/Vis absorption spectra of free Q and Q-Metal ions in CH_3OH-PBS（1:99, V/V, pH = 7.40）buffer solution.

In order to understand the binding stoichiometry of Q-Cu(Ⅱ) complexes, Job's plots experiments were carried out. The intensity of absorbance UV band of Q at 375 nm was decreases and a new characteristic band of complex was observed at 430 nm by the addition of Cu^{2+} (Fig.5). The absorbance plots at 430 nm against the molar fraction of Q (χ) have maximum absorbances at $\chi_L = 0.5$, confirming that the stoichiometric ratio for the complexation of Cu (II) and Q is 1:1 (Fig.6). This result indicated the formation of 1:1 complexes between Q and Cu^{2+}. The stability constant of the complex between Q and Cu^{2+} was calculated to be $K = 3.56\times10^7$, according to equations 2-1 and 2-2, which meant that the Q-Cu(Ⅱ) complex's stability was very high.

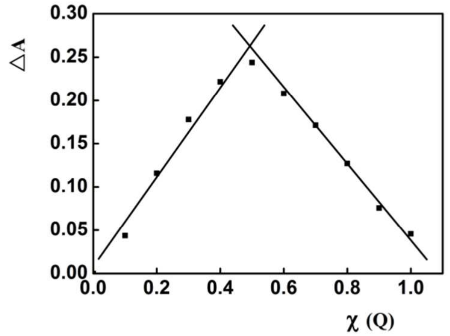


Fig.6 Job's plots of Q-Cu(II) complexes in CH_3OH-PBS（1:99, V/V, pH = 7.40）buffer solution.

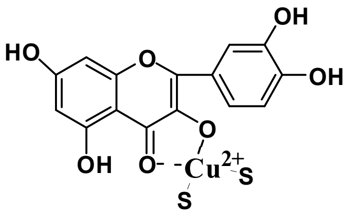
The UV–visible spectra gave significant information about the coordination sites of Q; the interactions of Cu(II) ions with Q at 1:1 quercetin/metal ratio produced bathochromic shift in the absorbance of bands I, which indicated that the 3-hydroxy and 4-carbonyl of ring C coordinated with a copper ion, or hydroxy groups of 3'and 4' positions coordinated with a copper ion.

The main IR data of Q and Q–Cu(II) complex were listed in Table 1. The presence of m (M–O) stretching vibration at 619 cm^{-1} indicated the formation of metal complex. The C=O stretching mode of the free Q occured at 1664 cm^{-1}, which had been shifted to 1626 cm^{-1} by the formation of the complex. This shift suggested the Cu(II) coordination occured through the carbonyl oxygen atom and the 3-OH or 5-OH group of the quercetin.[33]

Table1 Assignment of the IR spectra of the Q and the complex (band position in cm^{-1})

Compound	ν（O-H）	ν（C=O）	ν（C-OH）	ν（C-O-C）	ν（O-Cu）
Q	3405, 3315	1664	1319	1262	
Q-Cu(Ⅱ)	3426	1626	1318	1270	619

These experimental results were consistent with reference report.[22-23] As the 3-hydroxy group had a more acidic proton, therefore the 3-OH and 4-oxo groups were the first sites to be involved in the complexation process. The 3’,4’-dihydroxy groups were the second sites to bind a second metal ion. The 5-OH group was not involved due to lesser proton acidity and the steric hindrance caused by the first complexation. And combining UV and IR spectrum, the most possible coordination site was between 3-hydroxyl and 4-carbonyl of ring C. Scheme 2 is the most possible structure of Q-Cu(Ⅱ) complex.



(S is solvent such as H_2O, CH_3OH *etc.*)

Scheme2 The most possible coordination site of Q-Cu(Ⅱ) complex

3.5 Detecting Cu^{2+} in water samples

After the above studies, we applied this method for detecting Cu^{2+} in water samples with Q as fluorescent sensor. Different amounts of Cu^{2+} ions were added to pure water. A little pure water samples were taken and diluted to 1.6×10^{-3} and 2.4×10^{-4} $mol\cdot L^{-1}$ with CH_3OH-PBS（1:99, V/V, pH = 7.40） buffered solution. Then 5 μL of buffered solution containing Cu^{2+} ions were added to the 2 mL host sensor solution containing Q ($1.0\times10^{-5}mol\cdot L^{-1}$). The results were obtained by fluorescence spectrometer and ICP-MS, respectively.

The results were obtained in Table 2. The recovery was from 100.76% to 102.56% at the Cu^{2+} concentrations from 5.0×10^{-7} $mol\cdot L^{-1}$ to 2.5×10^{-6} $mol\cdot L^{-1}$. Compared with ICP-MS method, the results obtained by fluorescent method were also accurate, which proved that Cu^{2+} can be detected quantitatively using Q as sensor with this method.

Table 2 Determination of Cu^{2+} ion in water samples using the Q-based fluorescent sensor

Sample	ICP-MS ($\times 10^{-6}$ $mol\cdot L^{-1}$)	Found ($\times 10^{-6}$ $mol\cdot L^{-1}$)	RSD (%)	Recovery (%)
Water Sample	0.501	0.514±0.005	1.25	102.56
	1.499	1.512±0.007	0.60	100.87
	2.501	2.520±0.005	0.22	100.76

4. Conclusion

In summary, a novel, simple, rapid, label-free method had been developed to detect Cu^{2+} ions with very high selectivity and sensitivity using natural Quercetin as fluorescent sensor in aqueous media. This gave a linear change in fluorescence emission in intensity in response to the concentration change of Cu^{2+} ranging from 2.0×10^{-7} - 3.0×10^{-6} $mol\cdot L^{-1}$ and the detection limit was 1.0×10^{-7} $mol\cdot L^{-1}$. The method was applied successfully in water samples with stable recovery rate. The detection was based on the formation of the Q-Cu(Ⅱ) complex. The Q-Cu(Ⅱ) complex with the binding stoichiometry of 1:1 under pH 7.40 was very stable ($K_s=3.56\times10^{7}$), and the most possible coordination site was between 3-hydroxyl and

4-carbonyl of ring C in Q. This work opens up new possibilities for Q as chemosensor in quantification, and announces potential applications of Q not only in clinical biochemistry but also in environmental research.

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Caption:

Table1 Assignment of the IR spectra of the Q and the complex (band position in cm^{-1})

Table 2 Determination of Cu^{2+} ion in water samples using the Q-based fluorescent sensor

Scheme 1 Structure of the quercetin (3,3’,4’,5,7-pentahydroxyflavone)

Scheme 2 The most possible coordination site of Q-Cu(Ⅱ) complex

Fig.1 Fluorescent intensity of Q and Q-Cu(Ⅱ) with pH ranging from 5.00 to 9.00.

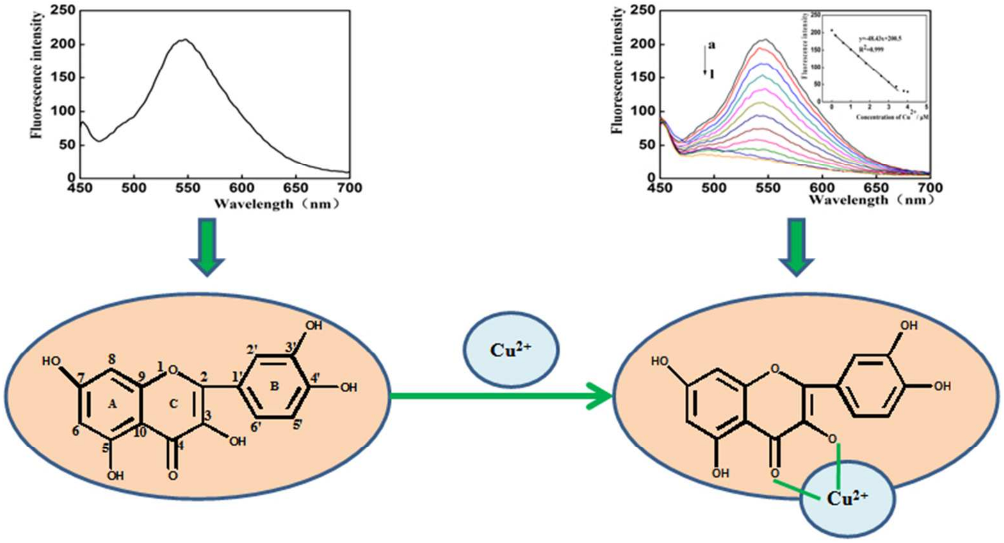
Fig.2 Fluorescence responses(a) and color changes(b) of Q in CH_3OH-PBS（1:99，V/V，pH = 7.40）buffer solution upon addition of different cations (unlabeled bars are Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}, respectively, λ_{ex}= 390 nm, [Q]= 1×10^{-5} $mol \cdot L^{-1}$, $[Cu^{2+}]=5 \times 10^{-5}$ $mol \cdot L^{-1}$, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} $mol \cdot L^{-1}$, the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} $mol \cdot L^{-1}$).

Fig.3 The influence of other metal ions on fluorescent intensity of Q-Cu(Ⅱ) system (unlabeled bars are Q-Cu(Ⅱ) system and other metal ions (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Mn^{2+}, Zn^{2+}, Ni^{2+}, La^{3+}, Eu^{3+}, Gd^{3+}, Ce^{3+}, Dy^{3+}, Al^{3+}, Cr^{3+}, Mo^{5+}, Fe^{3+}, Tb^{3+}, Er^{3+}, Lu^{3+}) added to Q-Cu(Ⅱ) system, respectively; λ_{ex}= 390 nm, $[Q]=1 \times 10^{-5}$ $mol \cdot L^{-1}$, $[Cu^{2+}]=2 \times 10^{-6}$ $mol \cdot L^{-1}$, the concentration of other transition metal cations and rare earth cations was 1×10^{-4} $mol \cdot L^{-1}$, the concentration of alkali metal cations and alkali earth metal cations was 1×10^{-3} $mol \cdot L^{-1}$)

Fig.4 Fluorescence titration of Q and standard curve of fluorescence titration spectra with varying concentrations of Cu^{2+} in CH_3OH-PBS buffer solution（1:99，V/V，pH = 7.40, $[Q] = 1 \times 10^{-5}$ $mol \cdot L^{-1}$, λ_{ex}= 390 nm, from a to l, $[Cu^{2+}]$= (0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8, 4.0)$\times 10^{-5}$ $mol \cdot L^{-1}$）.

Fig.5 UV/Vis absorption spectra of free Q and Qu-Metal ions in CH_3OH-PBS（1:99, V/V, pH = 7.40）buffer solution.

Fig.6 Job’s plots of Q-Cu(II) complexes in CH_3OH-PBS（1:99, V/V, pH = 7.40）buffer solution.



Proposed mechanism
282x160mm (72 x 72 DPI)

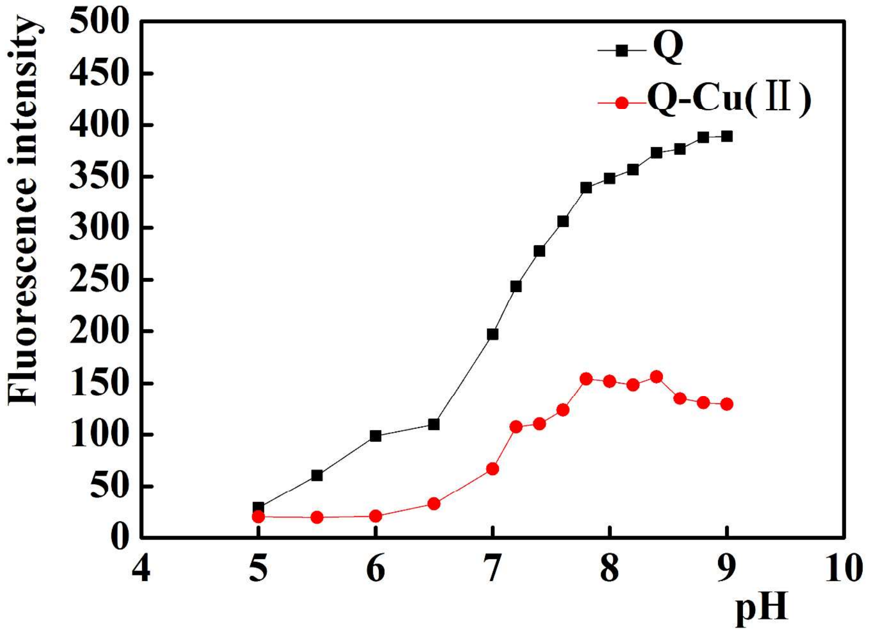


Fig.1 Fluorescent intensity of Q and Q-Cu(Ⅱ) with pH ranging from 5.00 to 9.00.
1173x897mm (72 x 72 DPI)

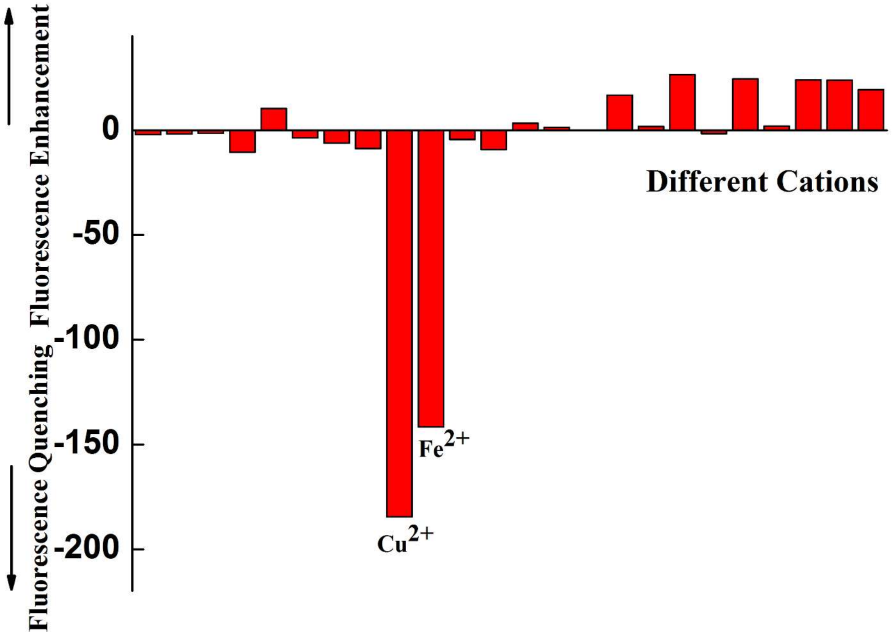


Fig.2a Fluorescence responses of Q in CH3OH-PBS（1:99, V/V, pH = 7.40）buffer solution upon addition of different cations (unlabeled bars are Li+, Na+, K+, Mg2+, Ca2+, Co2+, Cd2+, Mn2+, Zn2+, Ni2+, La3+, Eu3+, Gd3+, Ce3+, Dy3+, Al3+, Cr3+, Mo5+, Fe3+, Tb3+, Er3+, Lu3+, respectively,λλex= 390 nm, [Q]= 1 × 10-5 mol•L-1, [Cu2+]=5 × 10-5 mol•L-1, the concentration of other transition metal cations and rare earth cations was 1 × 10-4 mol•L-1, the concentration of alkali metal cations and alkali earth metal cations was 1 × 10-3 mol•L-1)
1092x812mm (72 x 72 DPI)

Fig.2b Color changes of Q in CH3OH-PBS（1:99, V/V, pH = 7.40）buffer solution upon addition of different cations (λex= 390 nm, [Q]= 1 × 10-5 mol•L-1, [Cu2+]=5 × 10-5 mol•L-1, the concentration of other transition metal cations and rare earth cations was 1 × 10-4 mol•L-1, the concentration of alkali metal cations and alkali earth metal cations was 1 × 10-3 mol•L-1)
605x347mm (96 x 96 DPI)

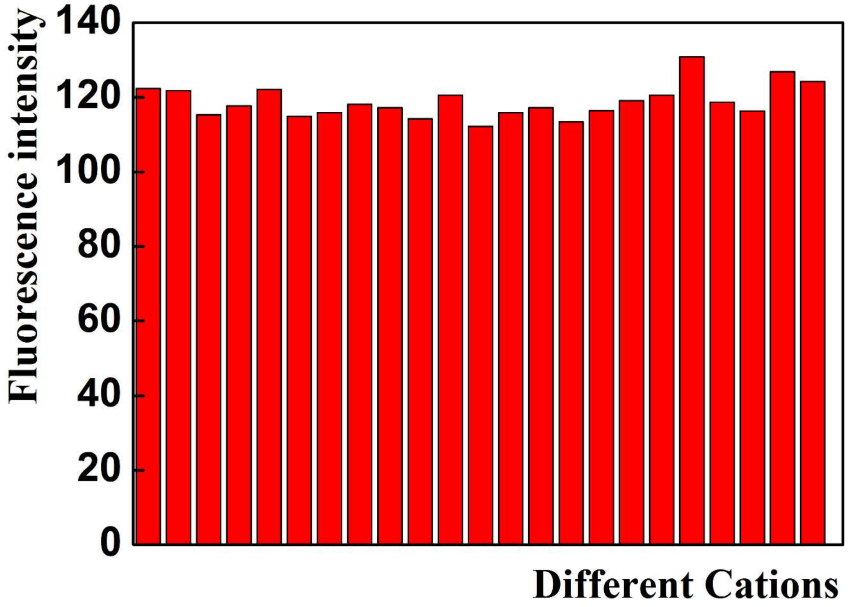


Fig.3 The influence of other metal ions on fluorescent intensity of Q-Cu(Ⅱ) system (unlabeled bars are Q-Cu(Ⅱ) system and other metal ions (Li+, Na+, K+, Mg2+, Ca2+, Co2+, Cd2+, Mn2+, Zn2+, Ni2+, La3+, Eu3+, Gd3+, Ce3+, Dy3+, Al3+, Cr3+, Mo5+, Fe3+, Tb3+, Er3+, Lu3+) added to Q-Cu(Ⅱ) system, respectively; λex= 390 nm, [Q]=1 × 10-5 mol•L-1, [Cu2+]=2 × 10-6 mol•L-1, the concentration of other transition metal cations and rare earth cations was 1 × 10-4 mol•L-1, the concentration of alkali metal cations and alkali earth metal cations was 1 × 10-3 mol•L-1)
1161x900mm (72 x 72 DPI)

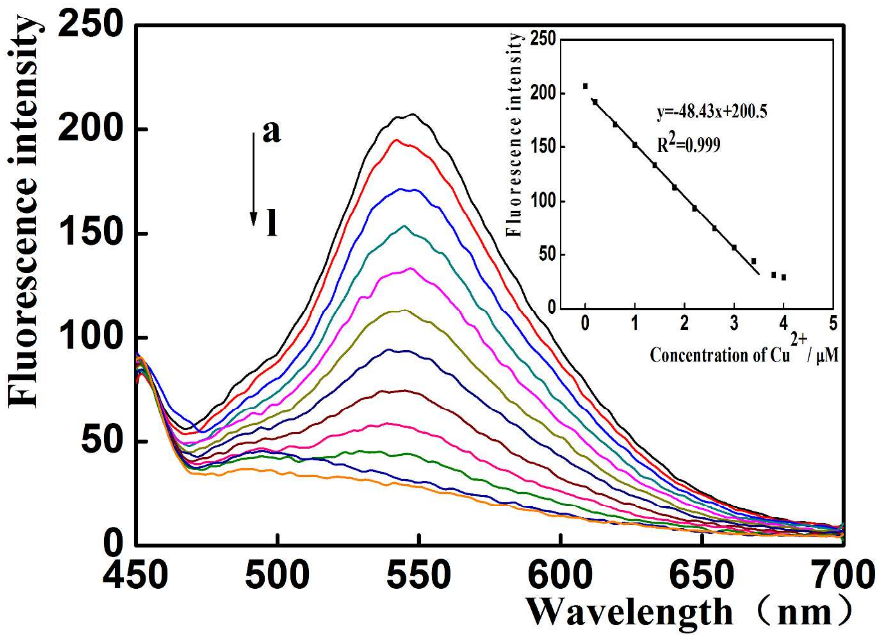


Fig.4 Fluorescence titration of Q and standard curve of fluorescence titration spectra with varying concentrations of Cu2+ in CH3OH-PBS buffer solution（1:99, V/V, pH = 7.40, [Q] = 1 × 10−5 mol•L-1, λex= 390 nm, from a to l, [Cu2+]= (0, 0.2, 0.6, 1.0, 1.4, 1.8, 2.2, 2.6, 3.0, 3.4, 3.8, 4.0)× 10−5 mol•L-1 ）.
1165x909mm (72 x 72 DPI)

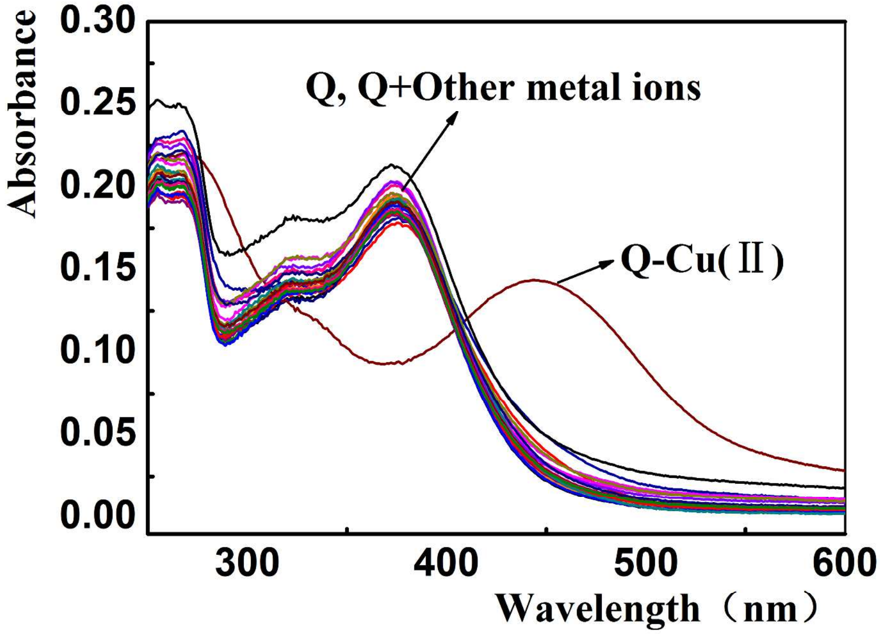


Fig.5 UV/Vis absorption spectra of free Q and Q-Metal ions in CH3OH-PBS（1:99, V/V, pH = 7.40）buffer solution
1182x909mm (72 x 72 DPI)

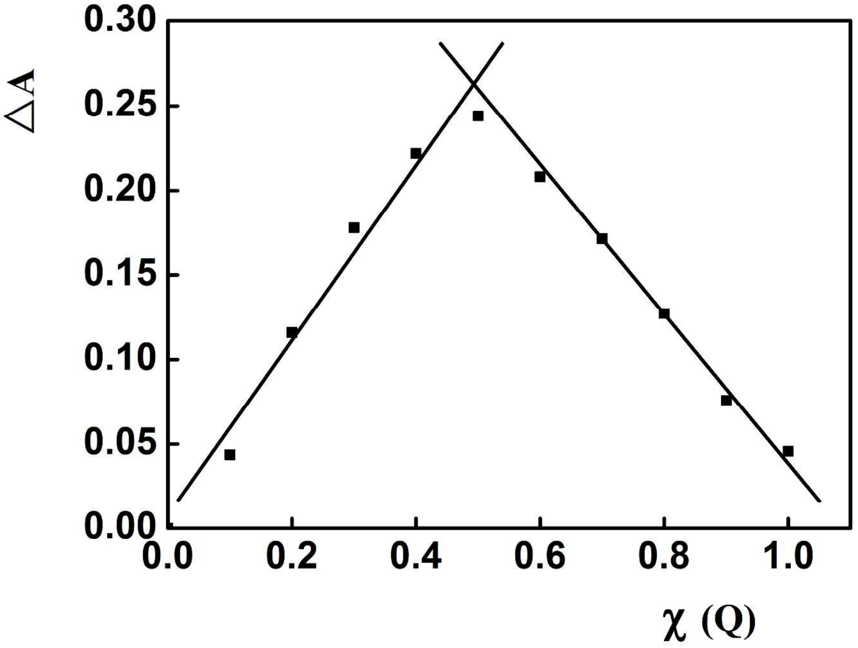


Fig.6 Job’s plots of Q-Cu(II) complexes in CH3OH-PBS（1:99, V/V, pH = 7.40）buffer solution.
1238x957mm (72 x 72 DPI)