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Short communication

Determination of curcumin by its quenching effect on the fluorescence of Eu^{3+}–tryptophan complex

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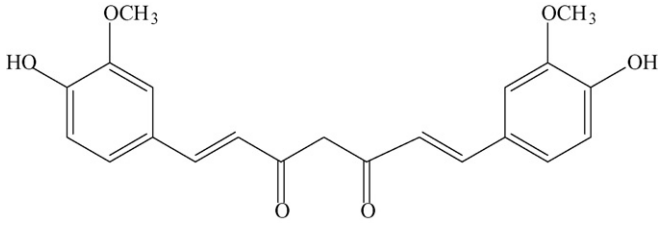
Abstract

Curcumin ($C_{21}H_{20}O_6$, CU) is a natural antioxidant, which is considered to be a very useful compound in health matters, and is employed in the treatment of cardiovascular and arthritic illnesses. It is found that the fluorescence intensity of Eu^{3+}–tryptophan (Trp) can be greatly quenched by curcumin in the buffer of pH 7.7. Under optimum conditions, the quenched intensity of fluorescence is in proportion to the concentration of curcumin in the range of 1.0×10^{-8} to 1.2×10^{-4} mol L^{-1}. The detection limit (S/N = 3) is 9.0×10^{-10} mol L^{-1}. The synthetic and actual samples are satisfactorily determined. In addition, the interaction mechanism is also investigated.

Keywords: Fluorescence quenching; Curcumin; Eu^{3+}–CU–Trp

1. Introduction

Curcumin [1.7-bis (4-hydroxy-3methoxyphenyl)-1, 6-heptadiene-3, 5-dione], the main constituent of the rhizomes of the plant curcumin longa, is a common ingredient used in spices, cosmetics and traditional Chinese medicine. The structure of curcumin is as follows:



It has been reported that curcumin has many pharmacological functions, such as antioxygenation, antibiosis and antitumor. Therefore, the investigation for the determination of curcumin is important for clinical medicine and pharmacology [1–4]. Methods such as thin-layer chromatography [5–7], high performance liquid chromatography [8–11], spectrofluorimetry [12,13] and UV–vis spectrophotometry [14–16] have been employed to determine curcumin in a variety of matrices such as curcuma longa, food stuffs and biological materials. Curcumin has extensive absorption around 420 nm and can emit the fluorescence around 530 nm in organic solvent, but they decrease strongly in aqueous solution. This fact makes the determination of trace amount of curcumin in aqueous solution be difficult.

In this paper, we found that the fluorescence intensity of Eu^{3+}–tryptophan (Trp) system can be considerably quenched by curcumin. Based on this phenomenon, the novel method for determining of curcumin concentration is developed. The formation condition and the effect factors of the Eu^{3+}–Trp–CU system are studied here. The quenching mechanism of the system is discussed.

2. Experiment

2.1. Chemicals

Stock standard solution of Eu(III) (1.00×10^{-2} mol L^{-1}) was prepared by dissolving 0.3520 g Eu_2O_3 (99.99%, Yuelong Chemical Co., Shanghai, China) in 1:1 hydrochloric acid, then evaporating the solution to be almost dry and diluting to 100 mL with water.

A stock solution of curcumin (CU) (1.00×10^{-3} mol L^{-1}) was prepared by dissolving CU in ethanol, and then diluted to 1.00×10^{-4} mol L^{-1} with ethanol as the working solution.

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The Trp solution (1.00×10^{-4} g mL^{-1}) was prepared by dissolving 0.01 g Trp (Bio Basic Inc., Canada) in 100 mL volumetric flask with water. This solution needed to be stored at 0–4 °C.

A series of Tris–HCl buffer solutions (0.10 mol L^{-1}) was used for the pH adjustment.

All the chemicals used were of analytical reagents grade and doubly deionized water was used throughout.

2.2. Apparatus

Normal fluorescence measurements were recorded with a F-2500 spectrofluorimeter (Hitachi, Japan). All pH measurements were made with a pHS-2F digital acidity meter (Leici, Shanghai, China).

All absorption spectra were recorded with an UV-2401 spectrophotometer (Shimadzu, Japan).

2.3. Procedure

To a 10 mL test tube, working solutions were added in the following order: 1.0 mL of 0.1 M Tris–HCl (pH 7.7), 0.5 mL of 1.0×10^{-4} g mL^{-1} Trp, 0.9 mL of 1.0×10^{-3} M Eu^{3+} and appropriate amount of curcumin solutions. The mixture was diluted to 10 mL with doubly distilled water, and allowed to stand for 15 min. The fluorescence intensity was measured at $\lambda_{ex}/\lambda_{em}$ = 280 nm/350 nm in a 1 cm quartz cell, the excitation and emission slits were both 10 nm with a scan speed of 1500 nm min^{-1}. The quenched fluorescence intensity of Eu^{3+}–Trp by curcumin was represented as ΔI_f (%) = $(I_0 - I_f)/I_0 \times 100\%$. Here, I_f and I_0 were the intensities of the systems with and without curcumin, respectively.

2.4. Sample treatment

Extraction of curcumin from sample was done according to the previously reported procedures [12]: the samples of dried (100 °C for 24 h) curcumin spices (curry was acquired in the commercial food establishment) were carefully ground in an agate mortar to obtain a fine intimately mixed powder. Then, a few of the powders were dissolved in appropriate ethanol. Undissolved particles were removed by centrifugation. The clear centrifugation and combined ethanol washings were transferred into a 50 mL volumetric flask and diluted to the mark with ethanol. An appropriate volume of this solution was pipetted into a 10 mL flask and its curcumin content was determined by standard addition method according to the procedure described above.

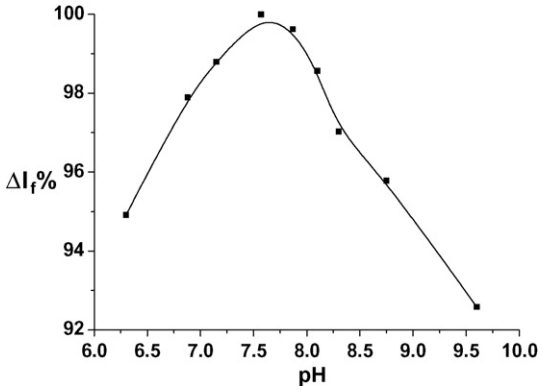

Fig. 2. Effect of pH on the intensity of fluorescence; conditions: Trp, 5.0×10^{-6} g L^{-1}; Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M.

3. Result and discussion

3.1. Fluorescence spectra

Excitation and emission spectra of Eu^{3+}–Trp–CU: (1) Trp, (2) Trp–Eu^{3+}, (3) Trp–CU and (4) Trp–Eu^{3+}–CU systems are shown in Fig. 1. From this figure, it can be seen that after the excitation of 280 nm, the strong of characteristic fluorescence of Trp at the emission peak of 350 nm can be quenched by the addition of Eu^{3+} or CU. This indicates an interaction of Trp with Eu^{3+} or CU. Moreover, when Eu^{3+} and CU are added into the system together, the fluorescence intensity of Eu^{3+}–Trp–CU system was extremely quenched than Trp–CU or Trp–Eu^{3+} system. It is the result of the interactions of Eu^{3+}, Trp and CU.

3.2. Effect of pH and the choice of buffer solution

The effect of pH on the quenched fluorescence intensity ΔI_f (%) of the system is shown in Fig. 2. The experimental

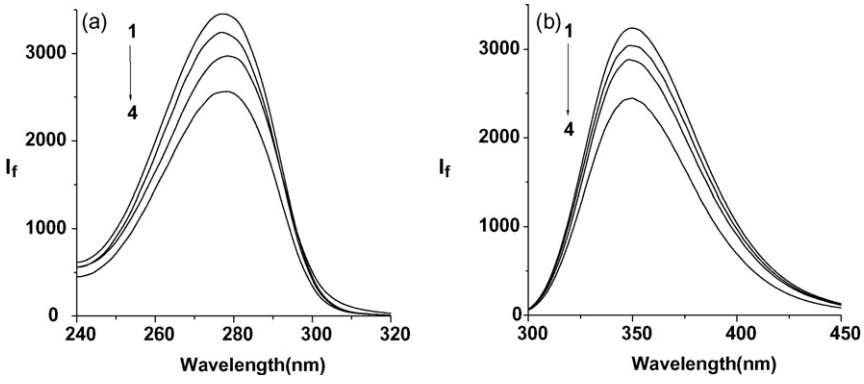

Fig. 1. Excitation spectra (a) λ_{em} = 350 nm and emission spectra (b) λ_{ex} = 280 nm. (1) Trp, (2) Trp–Eu^{3+}, (3) Trp–CU and (4) Trp–Eu^{3+}–CU; conditions: Trp, 5.0×10^{-6} g L^{-1}; Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

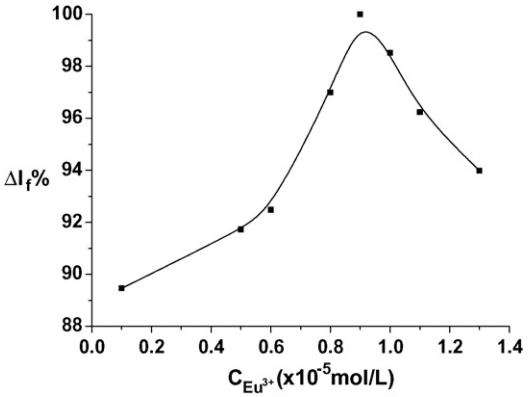

Fig. 3. Effect of Eu^{3+} concentration on the intensity of fluorescence; conditions: Trp, 5.0×10^{-6} g L^{-1}; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

results indicate that the ΔI_f (%) reaches a maximum around pH 7.7.

The effect of different buffers on the ΔI_f (%) of this system is also tested at the same pH (pH 7.7). The ΔI_f (%) for KH_2PO_4–NaOH, trihydroxymethylaminomethane (Tris)–HCl, citric acid–Na_2HPO_4, Na_2HPO_4–NaH_2PO_4 and hexamethylenetetramine (HMTA)–HCl are 83.8, 100, 93.8, 90.7, 96.1, respectively. So Tris–HCl is the most suitable buffer.

It is believed that the complexes of Eu^{3+} always have been formed at the neutral or weak basically medium and the molecular solutions such as HMTA and Tris are contributed to the fluorescence intensity.

3.3. Effect of Eu^{3+} concentration

The effect of the concentration of Eu^{3+} is tested as shown in Fig. 3, and the ΔI_f (%) of the system reaches a maximum when Eu^{3+} concentration is 9.0×10^{-5} M. Therefore, 9.0×10^{-5} M of Eu^{3+} is chosen in the further research.

3.4. Effect of Trp concentration

The effect of Trp concentration on the fluorescence intensity of Eu^{3+}–Trp–CU system is studied. From Fig. 4, it is found that the quenched fluorescence intensity of Eu^{3+}–Trp–CU system reaches a maximum when the concentration of Trp is 5.0×10^{-6} g mL^{-1}. So 5.0×10^{-6} g mL^{-1} of Trp is chosen in the research.

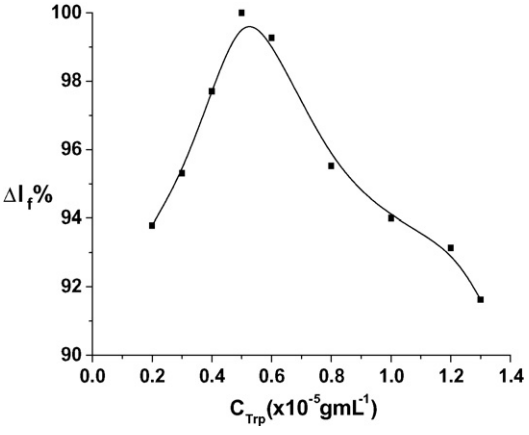

Fig. 4. Effect of Trp concentration on the intensity of fluorescence; conditions: Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

Table 1
Interference from foreign substances

Foreign substances	Concentration (10^{-8} mol L^{-1})	ΔI_f (%)
L-Pro	8500	−4.47
L-α-Ala	6000	−4.75
L-Leu	100	−3.53
L-Arg	1500	−4.92
L-Glu	10	−4.94
Gly	7000	−4.30
K^+, Cl^-	15000	−3.67
Mn^{2+}, SO_4^{2-}	3500	−4.45
NH_4^+, Cl^-	75000	−3.50
Zn^{2+}, Cl^-	5000	−4.44
Ca^{2+}, Cl^-	36000	−3.86
Na^+, Cl^-	14500	−4.19
Na^+, SO_4^{2-}	2500	−3.59
Fe^{3+}, SO_4^{2-}	1500	−4.95
Fe^{2+}, SO_4^{2-}	1500	−3.05
K^+, NO_3^-	30000	−3.99
Mg^{2+}, SO_4^{2-}	500	−3.23
Glucose	5000	−3.50
Sucrose	2500	−3.58
D-Fructose	8500	−4.99
DNA[a]	10	−4.6
RNA[a]	170	−4.94
EDTA	200	−4.87

[a] 10^{-8} g mL^{-1}.

3.5. Signal stability

Under the optimum condition, the effect of time on the fluorescence intensity is studied. The result shows that the ΔI_f (%) reaches a maximum after 30 min and remains stable for over 2 h.

3.6. Interfering substances

The interferences of various ions including common anions, cations, mino acids and glucide are tested according to the standard procedure. From Table 1, it is found that these foreign substances have not or little effects on the determination of CU under the permission of ±5% errors.

4. Analytical applications

4.1. The calibration graph and detection limits

Under the optimum conditions defined, the calibration graphs for curcumin is obtained and shown in Table 2. It can be seen that there is a linear relationship between the ΔI_f (%) and the concentration of curcumin in the range of 1.0×10^{-8} to 1.2×10^{-4} mol L^{-1}. The detection limit (S/N = 3) is 9.0×10^{-10} mol L^{-1}.

Table 2
Analytical parameters of this method

Linear range (mol L^{-1})	Linear regression equation	Correlation coefficient	Limit of detection (mol L^{-1})
1.0×10^{-8} to 1.2×10^{-4}	$Y = 4.411 + 4.008 \times 10^{6}x$	0.9997	9.0×10^{-10}

Table 3
The results of samples determination

Spectrophotometric method (mg g^{-1}) [16]	Nitrile fluorimetric method (mg g^{-1}) [12]	This method (mg g^{-1})	Average (mg g^{-1})	R.S.D. (%)
1.29	1.28	1.26, 1.27, 1.29, 1.28, 1.27	1.27	1.14

4.2. Determination of actual sample

The standard addition method was used for determination of CU in actual sample. The results are shown in Table 3.

The curry samples are treated according sample treatment mentioned above and determined using the proposed method. As can be seen, the results obtained by this method agree with those of both β-CD spectrophotometric and nitrile fluorimetric methods.

The above results indicate that the accuracy and precision of the method are satisfactory.

5. Interaction mechanism of the system

5.1. Formation of Eu^{3+}–Trp–CU complex

Whereas resonance light scattering (RLS) technique is available to provide some insight into the process responsible for the formation of the complex, as shown in Fig. 5. It can be seen that the intensity of RLS is greatly enhanced when Eu^{3+} or CU is added into Trp system; and it is greatly enhanced if Eu^{3+} and CU are added into Trp simultaneously. It is concluded that there exists a large congeries in Eu^{3+}–Trp–CU system.

The earlier publication [17] shows that at Trp can bind with Eu^{3+} and form Eu^{3+}–Trp complex. Furthermore, because of the unsaturated binding number of Eu^{3+} in Eu^{3+}–Trp system, so the abundant Eu^{3+} can bind with CU of the structure β-diketone.

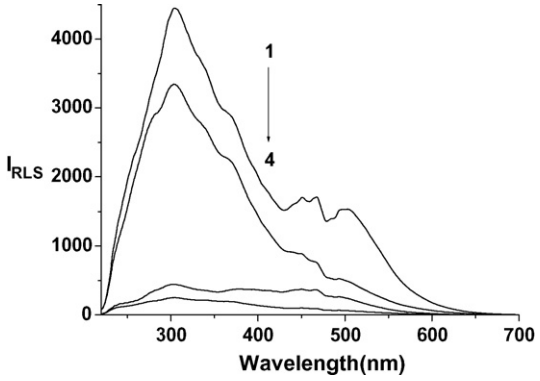

Fig. 5. The RLS spectra of the system: (1) Trp–CU–Eu^{3+}, (2) Trp–Eu^{3+}, (3) Trp–CU and (4) Trp; conditions: Trp, 5.0×10^{-6} g L^{-1}; Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

5.2. The distance of Eu^{3+}–Trp–CU

Fig. 1 shows that under the excitation of 280 nm, the emission peak is at 350 nm for Trp. When Eu^{3+} or CU is added into Trp, the fluorescences of Trp decrease, furthermore there is an overlap between the absorption spectrum of Eu^{3+} or CU and the emission spectra of Trp as shown in Fig. 6. The above facts show that there is intermolecular energy transfer from Trp to Eu^{3+} or CU under the excitation of 280 nm. Moreover, when Eu^{3+} and CU are added into Trp, the fluorescence of Trp decreased greatly. In order to know more details of this system, the energy transfer efficiency (E_a) and the interaction distance between donor and acceptor can be evaluated using Förster theory [18,19],

$$E_a = \frac{A_a}{A_d}\left(\frac{I_{ad}}{I_a} - 1\right) \quad (1)$$

$$E_a = 1 - \frac{I_{da}}{I_d} \quad (2)$$

$$R_0^6 = 8.8 \times 10^{-25} \cdot k^2 \cdot n^{-4} \cdot \phi_d \cdot J \quad (3)$$

$$E_a = \frac{R_0^6}{R_0^6 + r^6} \quad (4)$$

Table 4
The efficiency of energy transfer (E_a) and the critical transfer radius (R_0)

System	Donor	Acceptor	E_a	J (10^{-14} cm^6 M^{-1})	R_0 (nm)	r (nm)
Trp–CU	Trp	CU	0.11	1.649	2.13	3.00
Trp–Eu	Trp	Eu	0.062	2.882	1.38	2.17
Eu–CU–Trp	Trp	CU	0.22	2.013	1.90	3.90
	Trp	Eu	0.22	4.747	3.23	2.30

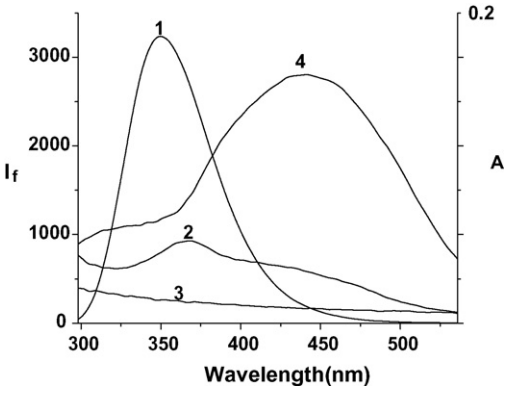

Fig. 6. Overlap between Trp (donor) emission and absorption of Eu^{3+}, CU (acceptor): (1) Trp emission, (2) CU absorption, (3) Eu^{3+} absorption and (4) Eu^{3+}–CU absorption; conditions: Trp, 5.0×10^{-6} g L^{-1}; Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

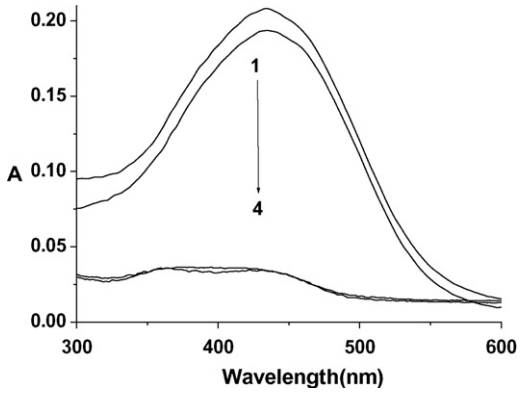

Fig. 7. The absorption of curcumin: (1) Trp–Eu^{3+}–CU, (2) Eu^{3+}–CU, (3) CU and (4) Trp–CU; conditions: Trp, 5.0×10^{-6} g L^{-1}; Eu^{3+}, 9.0×10^{-5} M; CU, 5.0×10^{-6} M; Tris–HCl, 0.1 M (pH 7.7).

$$J = \frac{\sum F_D(\nu)\varepsilon_A(\nu)\nu^{-4}\,\Delta\nu}{\sum F_D(\nu)\,\Delta\nu} \quad (5)$$

where the energy transfer efficiency E_a and the critical transfer radius R_0 (cm) are calculated, A_d and A_a are the absorbance of energy donor and acceptor, respectively, I_{ad}, I_a, I_{da} and I_d are the fluorescence intensities of the acceptor in the presence of donor, acceptor in the absence of donor, donor in the presence of acceptor and donor in the absence of acceptor, respectively. In Eq. (3), k^2 is a factor describing the relative orientation in space of the transition dipoles of donor and acceptor. In solutions of low viscosity, where rotation of the donor and the acceptor is sufficiently fast, an average value of $k^2 = 2/3$ may be assumed, ϕ_d is the fluorescence quantum of the donor (Trp, 0.13 [20]) in the absence of acceptor, n is the refractive index of the medium and $n = 1.336$ and J ($cm^6\,M^{-1}$) is the spectral overlap integral between the emission spectrum of donor (Trp) and the absorption spectrum of acceptor (CU or Eu) in the range of 298–530 nm. $F(\lambda)$ and $\varepsilon(\lambda)$ are the fluorescence intensity of the fluorescence donor (Trp) and the molar absorption coefficient of the acceptor (CU or Eu) at wavelength λ (from 298 to 530 nm), respectively. The values of E_a, R_0 and r are listed in Table 4.

It can be seen that in the binary complex of Trp–CU and Trp–Eu, the energy transfer efficiency is low. Whereas in ternary complex of Eu–CU–Trp, the energy transfer between any two substances is more efficient than that of the relevant binary complex. It is considered that the distances, Trp and CU, Eu in ternary complex are changed to 1.90 and 3.23 nm from 2.13 and 1.38 nm of binary complex, respectively. Therefore, the energy transfer efficiency between Trp and CU increases while that of Trp and Eu decreases. So, it is concluded that the curcumin molecule inserts into the midst of Trp and Eu.

5.3. The absorption of Eu^{3+}–Trp–CU system

From the absorption spectra of Eu^{3+}–Trp–CU shown in Fig. 7, it can be seen that the absorption at the range of 290–360 nm, only CU has obvious absorption. When Eu^{3+} is added into CU, the absorption of CU at 430 nm has greatly enhanced, so it is concluded that the complex Eu^{3+}–CU has formed.

Due to the electronic dipole allowed π–π^* type excitation of its extended aromatic system, curcumin exhibits an intense, round-shaped absorption band in the visible region of 350–480 nm. Upon light absorption, a π electron is excited from the ground state to the first excited state and oscillates from one end of the chromophore to the other. If a bulky substituent is present on the central methylene carbon atom and sterically prevents the molecule from adopting a planar geometry, the maximum of light absorption shifts to the near-ultraviolet (354–366 nm) region and the molecule loses its color [21] since there is no conjugation between the two feruloyl parts. It is assumed that the absorption at 430 nm enhanced in this system attribute to a larger conjugation comes from the coordination of Eu^{3+} with the oxygen on the carbonyl group of CU. while the absorption of CU has enhanced further after Eu^{3+}–Trp is added into CU. So it is also concluded that the complex Eu^{3+}–Trp–CU has formed.

The reason for the fluorescence quenching is therefore rationalized on the basis of the abovementioned results. The fluorescence quenching of Trp mainly comes from the formation of the ternary complex Eu^{3+}–Trp–CU.

6. Conclusion

In this paper, we found that the fluorescence intensity of Eu^{3+}–Trp decreased with the addition of CU. Based on this, a new fluorimetric method for the determination of curcumin had been reported. Under optimum conditions, the quenched intensity of fluorescence is in proportion to the concentration of curcumin in the range of 1.0×10^{-8} to 1.2×10^{-4} mol L^{-1}. The detection limit (S/N = 3) is 9.0×10^{-10} mol L^{-1}. The interaction mechanism is also studied.

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