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The Sensitive Fluorimetric Method for the Determination of Curcumin Using the Enhancement of Mixed Micelle

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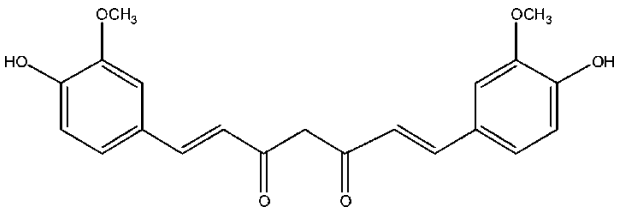
Curcumin ($C_{21}H_{20}O_6$) is a natural antioxidant, which is considered to be a very useful compound in health matters, and is employed in the treatment of cardiovascular and arthritic illnesses. It is found that the fluorescence of curcumin is greatly enhanced by mixed micelle of sodium dodecyl benzene sulfonate (SDBS) and cetyltrimethylammonium bromide (CTAB) surfactants. Based on this, a sensitive fluorimetric method for the determination of curcumin in aqueous solution is proposed. In the HOAc–NaOAc buffer, the fluorescence intensity of curcumin is proportional to the concentration of curcumin in the range of 0.00020–0.74 μg/mL and the detection limit is 0.017 ng/mL. The synthetic and actual samples are satisfactorily determined. In addition, the interaction mechanism is also studied.

KEY WORDS: Fluorescence; Curcumin; Anionic-cationic surfactant.

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 pharmaceutical functions, such as antioxygenation, antibiosis and anti-tumor. 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 aqueous solution, but their intensities is very low. This fact makes the determination of trace amount of curcumin in aqueous solution be difficult.

In this paper, a study of the fluorescence of curcumin in several of micelle systems has been carried out. It is found that the fluorescence of curcumin can be greatly enhanced by the mixed surfactant of SDBS and CTAB. Based on this, the sensitive quantitative analysis of curcumin in aqueous solution is established. This method has the advantages of high sensitivity, selectivity and stability.

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ABBREVIATIONS: CU, Curcumin; SDBS, Sodium Dodecyl Benzene Sulfonate; CTAB, Cetyltrimethylammonium bromide; CMC, Critical micelle concentration.

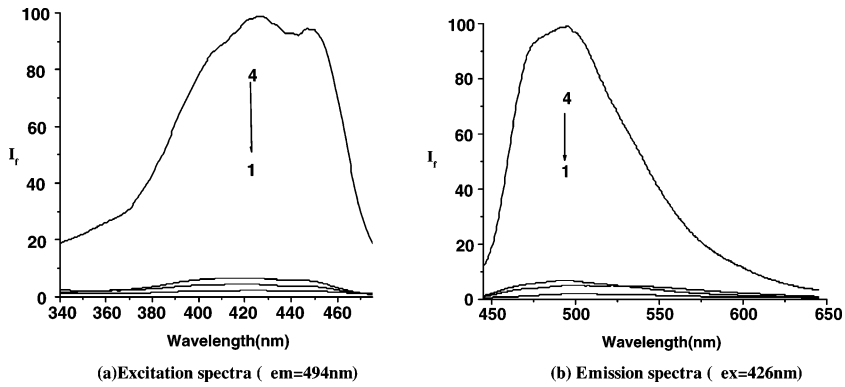

Fig. 1. Fluorescence spectra. (a) Excitation spectra ($\lambda_{em} = 494$ nm) (b) Emission spectra ($\lambda_{ex} = 426$ nm). (1) CU; (2) CU-CTAB; (3) CU-SDBS; (4) CU-SDBS-CTAB; Conditions: CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L; pH = 4.0.

EXPERIMENTAL

Apparatus

The spectra and intensity of fluorescence were measured with a PE-LS55 spectrofluorometer (Perkin-Elmer, America). An UV-4100 (Hitachi, Japan) spectrophotometer was employed in all absorption spectra recordings. The surface tension (σ) was measured with a Krűss Kizmks program surface tension instrument (Krűss GmbH) by using the suspended plate method. All pH measurements were made with a Delta320-S acidity meter (Mettler Toledo, Shanghai).

Reagents

A stock solution of curcumin (CU) (1.00×10^{-4} mol/L) was prepared by dissolving CU in ethanol, and then diluted to 1.00×10^{-5} mol/L with ethanol as the working solution. A series of HOAc–NaOAc buffer solutions (0.2 mol/L) was used for the pH adjustment. A solution of surfactant SDBS (1.00×10^{-2} mol/L) was prepared by dissolving SDBS in deionized water. A solution of surfactant CTAB (1.00×10^{-2} mol/L) was prepared by dissolving CTAB in deionized water. All the chemicals used were of analytical reagents grade and doubly deionized water was used throughout.

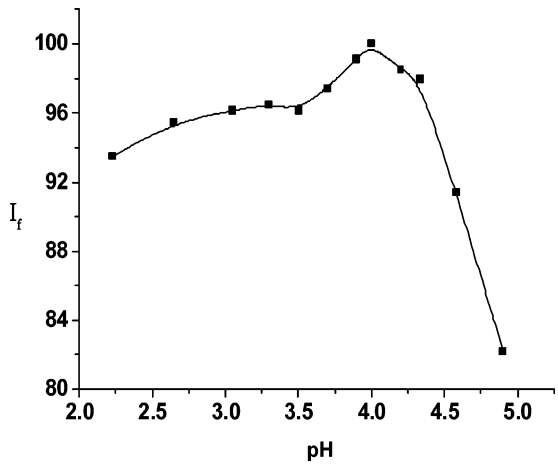

Fig. 2. Effect of pH on the intensity of fluorescence. Conditions: pH = 4.0; CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L.

Procedures

To a dry 10 ml test-tube, solutions were added as the following order: 0.5 ml HOAc–NaOAc (pH = 4.0), 2.0 ml of SDBS (1.00×10^{-2} mol/L), 1.0 ml of CTAB (1.00×10^{-2} mol/L) and definite standard CU (or sample solution). The mixture was diluted to volume with water. The fluorescence intensity is measured at $\lambda_{ex}/\lambda_{em} = 426$ nm/494 nm in a 1 cm quartz cell and with slit at 10.0 nm for the excitation and emission.

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.

Table I. Normalized Effects of the Surfactants

Surfactants	$\lambda_{ex}/\lambda_{em}$(nm)	I_f	Surfactants	$\lambda_{ex}/\lambda_{em}$(nm)	I_f
Without surfactants	425/495	3.1	SDBS+CPB	427/494	81
SDBS	426/492	30	SDS+CTAB	420/492	49
SDS	426/492	17	SDBS+OP	424/489	58
OP	423/490	26	SDS+OP	426/492	56
CPB	423/494	12	SDS+CPB	420/484	48
CTAB	418/490	26	CPB+OP	418/488	22
SDBS+CTAB	423/492	100			

Notes. Conditions: CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L; pH = 4.0

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.

RESULTS AND DISCUSSION

Fluorescence Spectra

The fluorescence spectra of the CU (1), CU-CTAB (2), CU-SDBS (3) and CU-SDBS-CTAB (4) systems are shown in Fig. 1. From this figure, it can be seen that CU in aqueous solution has very weak fluorescence with 426 nm excitation peak and 550 nm emission peak, but the intensity can be greatly enhanced near 55 times by the mixture of SDBS and CTAB, and the fluorescence peak has a blueshift to 494 nm. This indicates that CU is solubilized in mixed micelle of SDBS and CTAB.

Effects of pH and Buffers

The effect of pH on the fluorescence intensity of the system is shown in Fig. 2. It can be seen that the maximum intensity is obtained in the pH = 4.0. Experiments indicate that different buffers also have a large effect on fluorescence intensity (I_f) of the system. The I_f for HOAc–NaOAc, HMTA–HCl, $C_6H_4(COO)_2HK$ – HCl and NaOH-citric at pH = 4.0 is 100.0, 96.0, 29.0 and 31.0, respectively. The results indicate that the HOAc–NaOAc is the best in the buffers tested, so the HOAc–NaOAc buffer (pH = 4.0) is selected for the assay and the optimum volume of HOAc–NaOAc buffer is 0.5 ml.

Effect of Surfactants

The effects of the surfactants on the fluorescence intensity are tested and the results are shown in Table I, it can be seen that anionic, cationic and nonionic surfactants have all enhancement on the fluorescence intensity of CU; whereas the mixtures of both anionic-cationic and anionic-nonionic surfactants have synergistic enhancement effect, of them the enhancement caused by SDBS-CTAB is the most remarkable. Figures 3 and 4 show that 2.0×10^{-3} mol/L SDBS and 1.0×10^{-3} mol/L CTAB in the system give the maximum intensity.

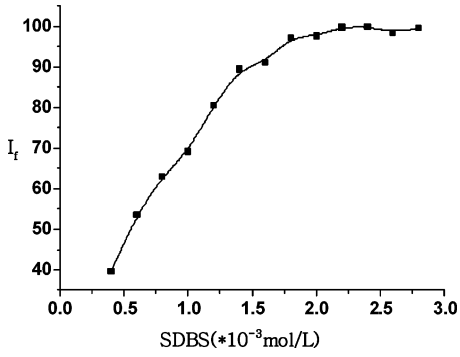

Fig. 3. Effects of the concentration of SDBS. Conditions: pH = 4.0; CU: 1×10^{-6} mol/L; CTAB: 1×10^{-3} mol/L.

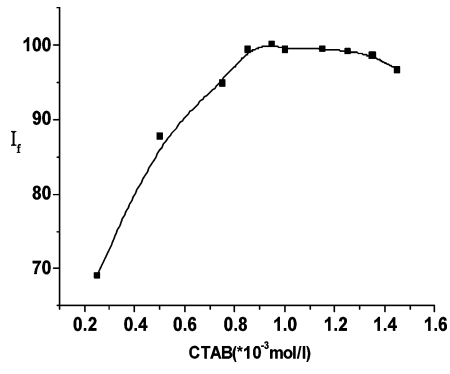

Fig. 4. Effects of the concentration of CTAB. Conditions: pH = 4.0; CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L.

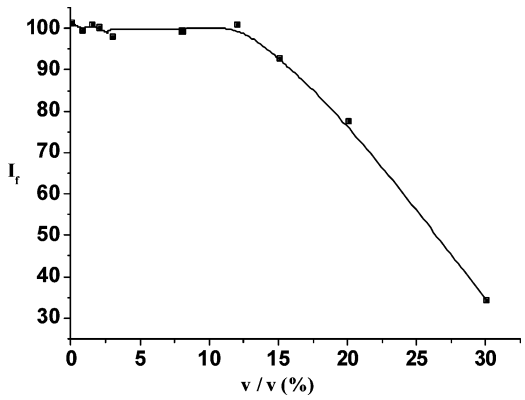

Fig. 5. Effects of ethanol. Conditions: CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L; pH = 4.0.

Effects of Ethanol

The quantity of ethanol would influence the fluorescence of the system because CU may dissolve in ethanol. The effect of ethanol on the fluorescence intensity is shown in Fig. 5. It can be seen that ethanol has little influence when the quantity of ethanol is not exceed 15%.

Stability

Tests show that the fluorescence intensity reaches a maximum rapidly after reagents is added and remains stable for at least 1 h. So the assay has good stability.

Effect of Foreign Substances

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

ANALYSIS OF SAMPLE

Analytical Parameters

Under the optimum conditions defined, a linear relationship is obtained between the fluorescence intensity and the concentration of CU. The analytical parameters for this method and other methods are given in Table III. It can be seen that the detection limit of this method is 0.017 ng/mL for CU, which is greatly less than that of other methods.

Table II. Interference from Foreign Substances

Foreign substances	Concentration (10^{-8} mol/L)	ΔI_f(%)
K^+, Cl^-	6800	−4.6
Mn^{2+}, SO_4^{2-}	27000	2.7
NH_4^+, Cl^-	116000	−3.2
Zn^{2+}, SO_4^{2-}	2000	3.2
Mn^{2+}, SO_4^{2-}	570	−3.6
Na^+, CO_3^{2-}	380000	−5.4
Al^{3+}, Cl^-	4000	3.4
Ca^{2+}, Cl^-	100	3.2
Na^+, Cl^-	1800000	−3.7
Na^+, SO_4^{2-}	500000	4.4
Ba^{2+}, Cl^-	400000	4.5
Fe^{3+}, SO_4^{2-}	500	5.4
Fe^{2+}, SO_4^{2-}	500	−4.7
D-mannose	500	4.4
Glucose	88	−5.1
Sucrose	1300	−6.1
D- fructose	780	−3.8
fsDNA[a]	7	−4.2
RNA[a]	250	−4.6
BSA[a]	62	−4.2
HSA[a]	70	−4.1

[a] 10^{-8} g/mL

Conditions: CU: 1×10^{-8} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L; pH = 4.0

Table III. The Comparison of Analytical Parameters

Methods of CU determination	Linear range (μg/mL)	Correlation coefficient	Limit of detection (ng/mL)
This method	0.00020–0.74	0.9990	0.017
β-CD spectrophotometric method [16]	0–15000	0.9991	250
Nitrile fluorimetric method [12]	0.27–1500	–	0.08

Table IV. Determination of Curcumin in Synthetic Samples

Added (μg/ ml)	HSA (μg/mL)	Mean founded (μg/mL)	Average recovery (%)	RSD (%)
0.0185	0.016	0.0187, 0.0179, 0.0187, 0.0185, 0.0182	99.5	1.9

Table V. The Results of Samples Determination

Spectrophotometric method (mg/g)	Nitrile fluorimetric method (mg/g)	This method (mg/g)	Average (mg/g)	RSD (%)
1.29	1.28	1.28, 1.27, 1.28, 1.28, 1.27	1.28	0.55

Sample Determination

The standard addition method was used for determination of CU in synthetic and actual samples. The concentration of curcumin in synthetic samples that were prepared by mixed certain amounts of human serum albumin (HSA) was determined. The results are shown in Table IV. It can be seen that the recovery ratio and relative standard deviation are 99.5 and 1.9%, respectively.

The curry sample is treated (see sample treatment section) and determined using the proposed method. The result is shown in Table V. As can be seen, the result obtained by this method agrees 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.

THE INTERACTION MECHANISM

From Fig. 1, it is apparent that SDBS-CTAB can greatly enhance the fluorescence of curcumin. Furthermore, it can be seen that the emission peak of curcumin shift to a shorter wavelength, which indicates that the microenvironment of the system occurs change. Both absorption spectra and the character of the mixed micelles can also prove this change.

Define CMC

In order to understand the present form of the surfactant in this system, the critical micelle concentration (CMC) of SDBS (or CTAB) in the presence of CU and CU-CTAB (or CU-SDBS) are measured by determining its surface tension under this experimental condition, the results are shown in Table VI.

It is known that the surfactant is easy to form spherical micelle when the concentration of the surfactant reaches CMC; furthermore the shape and the size of the micelle haven't obvious change in the concentration of 1–10 CMC. It can be seen from Table VI that SDBS and CTAB exist as spherical micelle in the CU-SDBS and CU-CTAB systems, respectively. The CMC values of anionic-cationic surfactant mixtures are much lower than those of the individual surfactants. In this paper, the optimum concentrations of SDBS and CTAB in the CU-SDBS-CTAB system are 2.0×10^{-3} mol/L and 1.0×10^{-3} mol/L, respectively, greatly larger than their CMC as shown in Table VI. Therefore, SDBS and CTAB exist as the rodlike micelle in CU-SDBS-CTAB system [17], which is also proved by the fluorescence polarization test. The experiments indicate that the fluorescence polarizations of CU in SDBS, CTAB, SDBS-CTAB systems are 0.428, 0.440 and 0.187, respectively. These results mean that SDBS-CTAB micelle is much larger than those of SDBS and CTAB, which is in agreement with that SDBS and CTAB exists as the rodlike micelle in CU- SDBS-CTAB system.

In spherical micelle of a single surfactant, there is the distance between the ionic heads of the surfactant because of coulombic repulsive force. But in the rodlike micelle of anionic-cationic surfactants, the coulombic repulsive force will be screened and replaced by the attractive force between the negative charges of SDBS and positive charges of CTAB. So the distance between two surfactant polar groups becomes small and the polarity of the inner core of the micelle decreases. Therefore, the CU is transferred into inner core of mixed micelle form aqueous solution containing 1.0% ethanol.

Absorption Spectra

It is known that there are two carbonyls, two methoxy and hydroxyl groups in the curcumin formula. Figure 6 indicates that curcumin in aqueous solution containing 1.0% ethanol exhibits an weak absorption band centered at 426 nm, which contains a π–$\pi*$ transition and an n–$\pi*$ one. With the decrease of the polarity of solvents, the

Table VI. The CMC of Surfactants

Systems	SDBS(mol/L)	CTAB(mol/L)
Single surfactant-CU system	2.4×10^{-4}	2.2×10^{-4}
Mixed surfactants-CU system	4.6×10^{-5}	7.0×10^{-5}

Note. Conditions: CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L; pH = 4.0

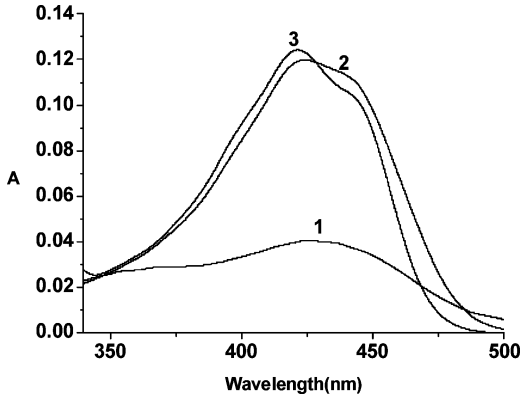

Fig. 6. The Absorption spectra of curcumin in different solvents. Conditions: (1)H_2O; (2)CH_3CH_2OH; (3)CH_3COCH_3. CU: 1×10^{-6} mol/L; pH = 4.0.

absorption of curcumin increases, and the absorption peak of π–$\pi*$ transition has a blueshift, whereas the absorption of n–$\pi*$ transition has a redshift. Therefore, the separation of two absorption bands is observed. From Fig. 7, it can be seen that the absorbance of curumin in the range of 400–460 nm is greatly enhanced by SDBS, CTAB, the mixture of SDBS and CTAB, respectively. It is considered that the carbonyl groups of curumin can bind with cationic bead group of CTAB, whereas the hydrophobic part of curumin is solubilized in the micelle of CTAB. In weak acidic solution, curcumin can undergo the protonation and combine with the sulfonic acid group of SDBS by electrostatic force, forming ion association which can be solubilized in both SDBS micelle and mixed micelle of SDBS and CTAB.

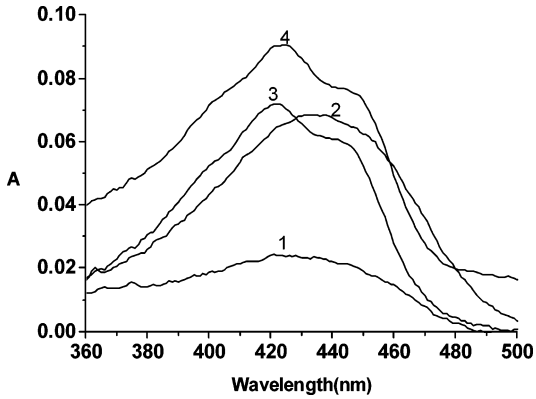

Fig. 7. Absorption spectra. Conditions: (1) CU;(2) SDBS-CU; (3) CTAB-CU;(4) SDBS-CTAB-CU. CU: 1×10^{-6} mol/L; SDBS: 2×10^{-3} mol/L; CTAB: 1×10^{-3} mol/L.

It is known that the mixed micelle have larger solubilization than single micelle for the hydrophobic molecule, which indicates that the mixed micelle of SDBS-CTAB can provide more hydrophobic microenvironment than the single micelle of SDBS or CTAB for CU. Therefore, the absorption and the fluorescence of CU is greatly enhanced by the mixture of SDBS and CTAB.

Fluorescence Quantum Yield

The fluorescence quantum yields of curcumin in both aqueous solution containing 1.0% ethanol and SDBS-CTAB micelle are determined using quinine sulphate as a reference with a known fluorescence quantum yield of 0.55 in 0.05 mol/L H_2SO_4. The results are 0.0062 in aqueous solution containing ethanol and 0.34 in SDBS-CTAB micelle. This indicates that fluorescence quantum yield of curcumin in SDBS-CTAB micelle is about 55 folds larger than that in aqueous solution containing 1.0% ethanol, which agrees with their fluorescence intensity ratio. The above result indicates that the increase in fluorescence intensity of curcumin in this system is mainly attributed to the enhanced fluorescence of curcumin caused by SDBS-CTAB micelle.

CONCLUSION

In this work, it is found that anionic-cationic surfactant mixtures can significantly enhance the intensity of the fluorescence intensity of curcumin. So, a sensitive and convenient method for the determination of curcumin is proposed. This method is used for the determination of the curcumin in curry and synthetic samples, and the results are satisfactory. In addition, the interaction mechanism between curcumin and SDBS–CTAB is also investigated. It is considered that the increase in fluorescence intensity of curcumin in this system mainly attributed to the fluorescence of curcumin caused by SDBS-CTAB micelle.

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