Hindawi Publishing Corporation
Journal of Spectroscopy
Volume 2013, Article ID 147128, 9 pages
http://dx.doi.org/10.1155/2013/147128

Research Article

Fluorescent Properties of Hymecromone and Fluorimetric Analysis of Hymecromone in Compound Dantong Capsule

Huanhuan Zhi,[1,2] Jingdan Wang,[2] Shujing Wang,[2] and Yongju Wei[1,2]

[1] *College of Life Science, Hebei Normal University, Shijiazhuang 050024, China*
[2] *College of Chemistry and Material Science, Hebei Normal University, Shijiazhuang 050024, China*

Correspondence should be addressed to Yongju Wei; weiyju@126.com

Received 5 September 2013; Accepted 21 October 2013

Academic Editor: Yizhuang Xu

Fluorescence spectra of hymecromone (4MU) aqueous solutions are investigated at different pHs. Two fluorescent species of 4MU, neutral molecular form and anion form, are considered to be the main fluorescent forms. Quantum yields of the two forms are measured to be 0.74 at pH 5.98 and 0.95 at pH 9.75, respectively. The ionization constant of 7-hydroxyl proton of 4MU is determined to be $pK_a = 7.85 \pm 0.03$ by a pH-fluorescence method. Addition of methanol into 4MU aqueous solution leads to a blue shift of maximum emission wavelength from 445 nm to 380 nm, and a decrease in fluorescence intensity. 3D fluorescence spectra of Chinese patent drug Compound Dantong Capsule (CDC) and its four component herbal drugs are also investigated. Based on their fluorescent properties, a novel fluorimetric method is proposed for the selective determination of 4MU in CDC without preseparation. The new method is suitable for the routine quality evaluation of CDC.

1. Introduction

Coumarins, both natural and synthetic, are of interest because of their multiple biological and photodynamic activities. They are extensively used as medicine and analytical reagent [1–7]. Hymecromone (4-Methyl-7-hydroxycoumarin, 7-Hydroxy-4-methylcoumarin, 4-Methylumbelliferone, abbreviated as 4MU) is a synthetic coumarin compound. It is claimed that 4MU and its derivatives or metal complexes possess diverse pharmacological properties, such as antiviral [8], antifungal and antioxidative [9], anticancer [10–13], anticoagulant, and spasmolytic activities [14, 15]. The fluorescence of 4MU had been observed early [16], a water-soluble polymeric fluorescent probe for measurement of near-neutral pH was synthesized based on the fluorescent properties of 4MU [17], and the effect of pH on fluorescence was restudied recently [18]; however, no comprehensive and detailed fluorescence spectra and quantum yield have been found in the literature.

Compound Dantong Capsule (CDC, also named Fufang Dantong Capsule) is a Chinese proprietary medicine [19]. It is effective in cure cholecystitis, cholangitis, intercurrent infection of gallbladder, and biliary tract concretion [19, 20]. The chemical composition of CDC includes 4MU (also named Dantong) and four kinds of Chinese herbal medicines, including Herba Isodonis Lophanthoidis (Xihuangcao), Herba Artemisiae Scopariae (Yinchen), Herba Andrographis (Chuanxinlian), and Rhei Radix et Rhizoma (Dahuang). The content of 4MU is one of the main quality indexes of CDC [21]. Earlier, the spectrophotometric method was applied for the determination of 4MU in CDC according to the absorbance of 4MU at 372 nm [19]. Later, since the interference of coexistent components, high performance liquid chromatographic methods (HPLC) were developed for the determination of 4MU in CDC [21–23]. So far, no fluorimetric method was reported for the determination of 4MU in CDC and other medicinal samples.

Fluorimetry has been recognized as one of the most useful analytical methods owing to its high sensitivity, good selectivity, simplicity, speediness, and low cost. It can be suitable for analysis of some complex samples such as traditional Chinese medicine containing a fluorescent component, especially in the case of a routine analysis. Three-dimensional fluorescence spectra (3D fluorescent fingerprint) have been used in our lab for qualitative identification of Chinese herbal medicines

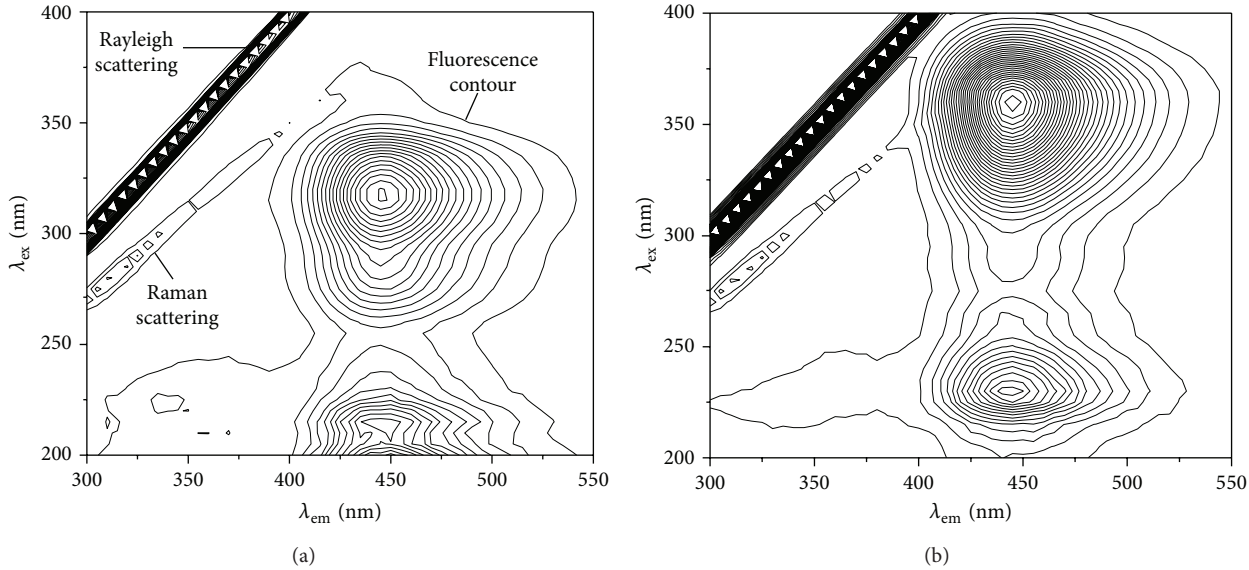

Figure 1: 3D fluorescence spectra of 4MU aqueous solution at different pH. c_{4MU}: 18.5 ng mL^{-1}. (a) pH 5.98; (b) pH 9.75. Contour interval: *R*.

[24]. Moreover, a number of fluorimetric methods have been developed for quantitative determination of active components in medicinal materials, such as paeonol in Cynanchi Paniculati Radix (Xuchangqing) [25], camptothecin in common *Camptotheca* fruit (Xishuguo) [26], and arctiin in Arctii Fructus (Niubangzi) [27]. In this study, the fluorescent properties of 4MU are investigated, and its quantum yield and 7-hydroxyl proton ionization constant are measured. The fluorescent properties of CDC and its four consisting Chinese herbal medicines are also studied. Based on the spectral differences between 4MU and other components in CDC, a fluorimetric method is proposed for the determination of 4MU in CDC sample without preseparation.

2. Experimental

2.1. Apparatus. Fluorescence measurements were performed on a Hitachi (Tokyo, Japan) F-7000 spectrofluorimeter equipped with a xenon lamp, 1 cm quartz cell, and a UV-29 filter placed into the emission light path to remove secondary spectrum. The excitation and emission slits (band pass) 5 nm/5 nm were used throughout the work. Absorption spectra were recorded using a Shimadzu (Kyoto, Japan) UV-2501PC recording spectrophotometer with 1 cm quartz cell. An Orion (Beverly, USA) 868 pH/ISE meter was used for pH measurement.

2.2. Chemicals and Materials. Hymecromone (4MU, serial no. 100241-200503, a reagent for quantitative analysis, molecular weight: 176.17) and Chinese herbal medicine (comparison drug for qualitative identification) Xihuangcao (serial no. 121488-200501), Yinchen (serial no. 120950-200305), Chuanxinlian (serial no. 121082-200302), and Dahuang (serial no. 120984-200301) were purchased from the National Institute for the Control of Pharmaceuticals and Biological Products of China (Beijing, China). 4MU stock solution was prepared by dissolving 3.61 mg reagent in 100 mL methanol (TEDIA, HPLC grade) and diluted to appropriate concentration with methanol as needed. Compound Dantong Capsule (CDC, product lot no. 100601) was produced by National Tsing Hua Pharmaceutical Co., Ltd. Hubei-day Saint, China. Quinine bisulphate (HPLC grade) was acquired from J&K Scientific Ltd. (Beijing, China); its solution was prepared by dissolving 391.47 mg reagent in 500 mL 0.05 mol L^{-1} H_2SO_4 and diluted to appropriate concentration with 0.05 mol L^{-1} H_2SO_4 when it was used. Britton-Robinson buffer solution was a mixture of phosphoric acid, boric acid, and acetic acid (each 0.02 mol L^{-1}) and adjusted to an appropriate pH by addition of 0.1 mol L^{-1} NaOH solution. All the buffer chemicals were of analytical grade. The water used throughout the study was doubly-deionized and verified to be free from fluorescence.

2.3. General Procedure for Spectral Measurement. A series of 10 mL volumetric flasks was added appropriate amount of 4MU or CDC and buffer solutions. The mixtures were diluted to the mark with water and mixed well. Fluorescence or absorption spectra were measured at room temperature. Meanwhile, Raman scattering of water at excitation wavelength of 350 nm was measured (expressed as *R*, for calibration of 3D fluorescence spectra) [24].

2.4. Determination of Ionization Constant. A number of solutions containing the same concentration of weak acid HB and different pH were prepared. Fluorescence intensity *F* and pH of each solution were measured. Then the ionization constant pK_a was calculated according to the following equation [28]:

$$\mathrm{p}K_a = \mathrm{pH} - \lg\frac{F_{\mathrm{HB}} - F}{F - F_{\mathrm{B}}}, \quad (1)$$

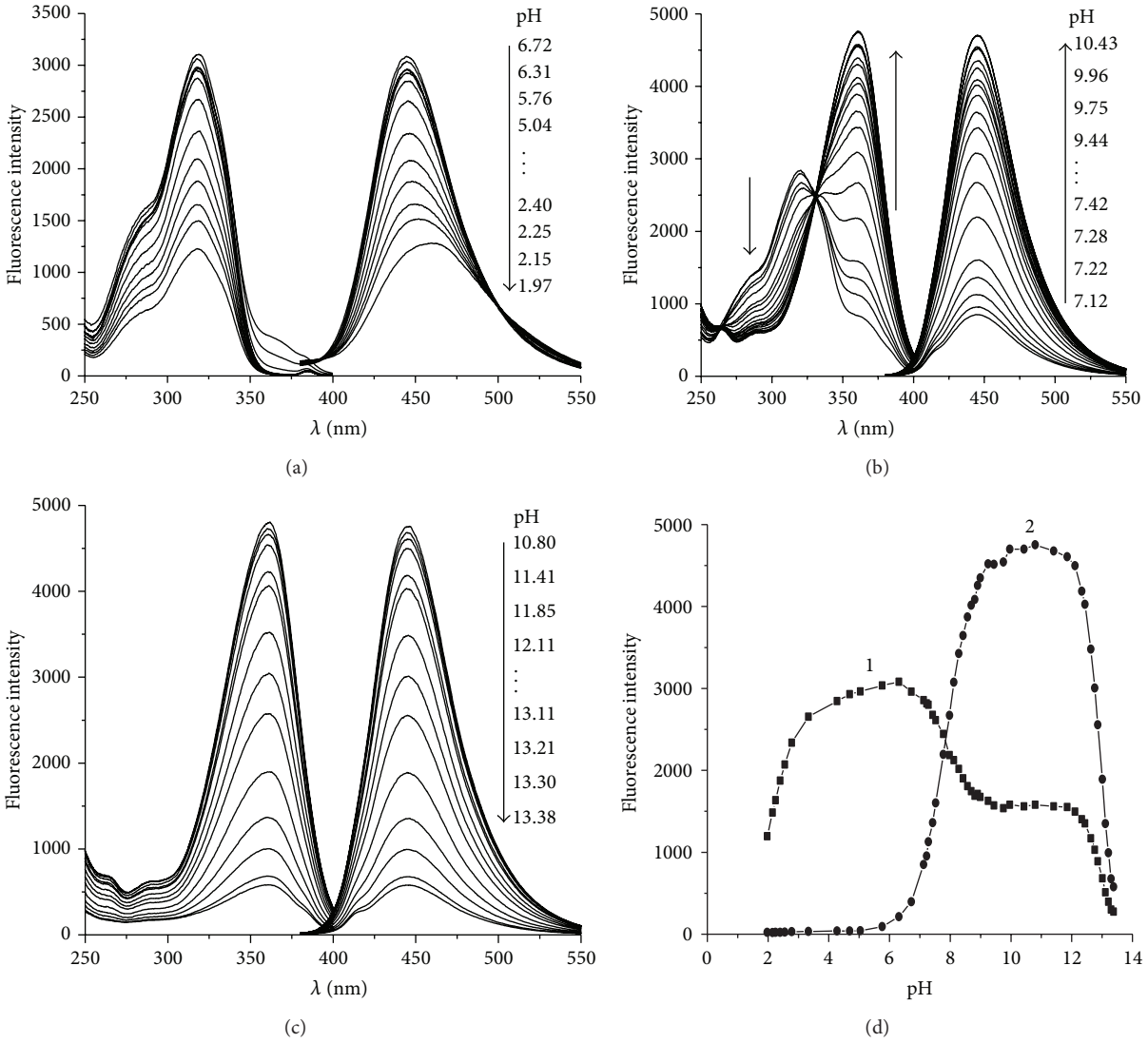

FIGURE 2: (a), (b) and (c) Fluorescence excitation and emission spectra of 4MU at different pH and (d) relationship between fluorescence intensity and pH. c_{4MU}: 18.5 ng mL^{-1}; $\lambda_{ex}/\lambda_{em}$: (a) and (d(1)): 320 nm/445 nm, (b), (c) and (d(2)): 360 nm/445 nm.

where F_{HB} and F_B were the fluorescence intensity of HB and its conjugate base B; they can be measured at sufficient acidic pH where all HB species exist in the neutral molecular form and at sufficient alkaline pH where all HB species exist in the anion form, respectively.

2.5. Measurement of Fluorescence Quantum Yield. Quinine bisulphate was used as a reference (quantum yield 0.55 at excitation wavelength of 313 nm) in measuring quantum yield of 4MU. For the measurement, quinine bisulphate and 4MU solutions were prepared in proper concentration so that the absorbance (A) of the two solutions was similar and not larger than 0.05. Absorption and fluorescence spectra were recorded, and then quantum yields were calculated according to the following equation [28, 29]:

$$Y_u = Y_r \cdot \frac{F_u}{F_r} \cdot \frac{A_r}{A_u}, \quad (2)$$

where Y_u and Y_r were the fluorescence quantum yield of unknown and the reference, F_u and F_r were the integral fluorescence intensity of unknown and reference solutions, and A_u and A_r were the absorbance of unknown and reference solutions at their excitation wavelengths, respectively.

2.6. Sample Preparation. Dissolve 9.21 mg CDC powder in 100 mL methanol and dilute to 0.921 μg mL^{-1} with methanol as sample solution.

3. Results and Discussion

3.1. Fluorescence Properties of 4MU

3.1.1. 3D Fluorescence Spectra of 4MU. 3D (three-dimensional) fluorescence spectra of solvent blank and 4MU aqueous solutions at pH 5.98 and at pH 9.75 were measured as shown in Figure 1. The spectra indicated that the solvent used in

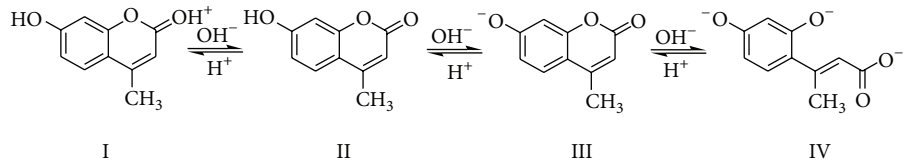

Figure 3: Proton ionization and hydrolysis process of 4MU.

this study was basically no fluorescence. 4MU can produce strong fluorescence in near neutral condition with maximum excitation wavelength (λ_{ex}) of 320 nm and maximum emission wavelength (λ_{em}) of 445 nm, while in weak alkaline condition, the fluorescence intensity enhanced, λ_{ex} red shifted from 320 nm to 360 nm, but λ_{em} remained constant. Figure 1 revealed that pH has an important influence on fluorescence.

3.1.2. Effect of pH on Fluorescence. As shown in Figure 2, effect of pH on fluorescence excitation and emission spectra of 4MU at various pHs were studied. In acidic conditions (pH 1.97–6.72), along with the decrease in pH, fluorescence intensity declined, λ_{ex} centered at 320 nm, and λ_{em} red shifted slightly from 445 nm to 455 nm. In the range of pH 7.12–10.43, along with the increase in pH, fluorescence emission at 445 nm enhanced, but the excitation band centered at 320 nm declined, meanwhile a new excitation band centered at 360 nm emerged and an iso-fluorescence point [30] formed at 330 nm. In the range of pH 10.80–13.38, along with the increase in pH, fluorescence emission at 445 nm gradually quenched while λ_{ex} and λ_{em} remained constant.

The relationship between pH and fluorescence intensity was summarized in Figure 2(d).

The variation of fluorescence spectra implies that the solution equilibrium or molecular structure of 4MU changed along with the change in pH. According to Figure 2, we suppose that the proton dissociation and hydrolysis process of 4MU is as shown in Figure 3.

The structure of 4MU contains a benzene ring (linked with 7-OH) and a lactonic ring (include lactone bond and 2-C=O). In strong acid conditions, the 2-carbonyl oxygen protonated [28, 30] to form a cationic species (type I), leading to a decrease in fluorescence intensity and a red shift in emission wavelength. In near neutral conditions (pH 4.0–7.0), 4MU exist mainly as molecular form (type II) which have strong fluorescence with λ_{ex} of 320 nm and λ_{em} of 445 nm. In weak alkaline conditions (pH 9.0–12.0), 7-hydroxyl proton dissociated and 4MU exist mainly as anion form (type III) which have stronger fluorescence with λ_{ex} of 360 nm and λ_{em} of 445 nm. In strong alkaline conditions (pH > 12.0), the hydrolysis of lactone bond take place [28, 31], leading to a fluorescence quenching.

3.1.3. Ionization Constant of 7-Hydroxyl Proton. With the above discussion, ionization constant of 7-OH proton can be determined in suitable conditions. Using the pH-fluorescence method described in Section 2.4, the ionization constant of 7-hydroxyl proton was determined to be $pK_a = 7.85 \pm 0.03$ (Table 1).

Table 1: Determination of ionization constant of 4MU.

pH	F	$\lg[(F_{HB} - F)/(F - F_B)]^*$	pK_a	Average pK_a
7.52	1601	−0.30	7.82	7.85 ± 0.03
7.77	2195	−0.07	7.84	
7.98	2672	0.11	7.87	
8.12	3074	0.26	7.86	
8.28	3427	0.41	7.87	

$^*F_{HB} = 21.18$, $F_B = 4753$.

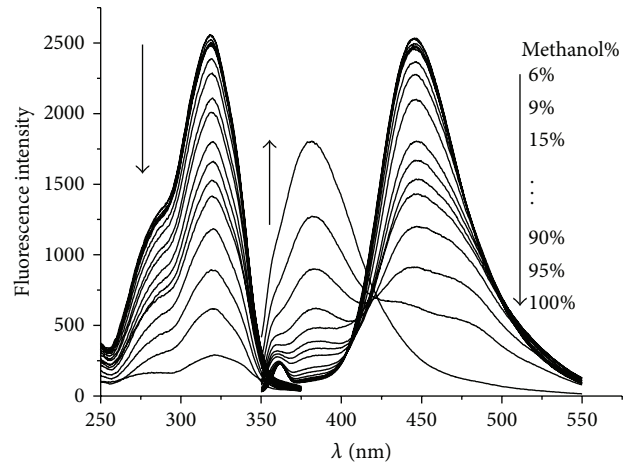

Figure 4: Fluorescence spectra of 4MU in aqueous solutions containing different amounts of methanol. c_{4MU}: 18.5 ng mL^{-1}; pH: 5.98; $\lambda_{ex}/\lambda_{em}$: 320 nm/445 nm.

In addition, according to the relationship between fluorescence intensity and pH, we can graphically estimate the ionization constant. In Figure 2(d), the abscissa of the intersection of curve (a) and curve (b) is about 7.85, which is just the pK_a.

The ionization constant determined above is close to the ionization constant of umbelliferone; $pK_a = 7.61 \pm 0.03$ [28]. The little difference between the two constants reflects the impact of 4-methyl on the acidity of 7-hydroxyl proton.

3.1.4. Effect of Solvent on Fluorescence. Solvent polarity usually has a profound effect on the emission spectral properties of fluorophores and further influences the sensitivity of a fluorimetric method. Figure 4 shows the fluorescence spectra of 4MU in aqueous solutions containing different amounts of methanol. Along with the increase percentage of methanol, fluorescence emission band centered at 445 nm gradually

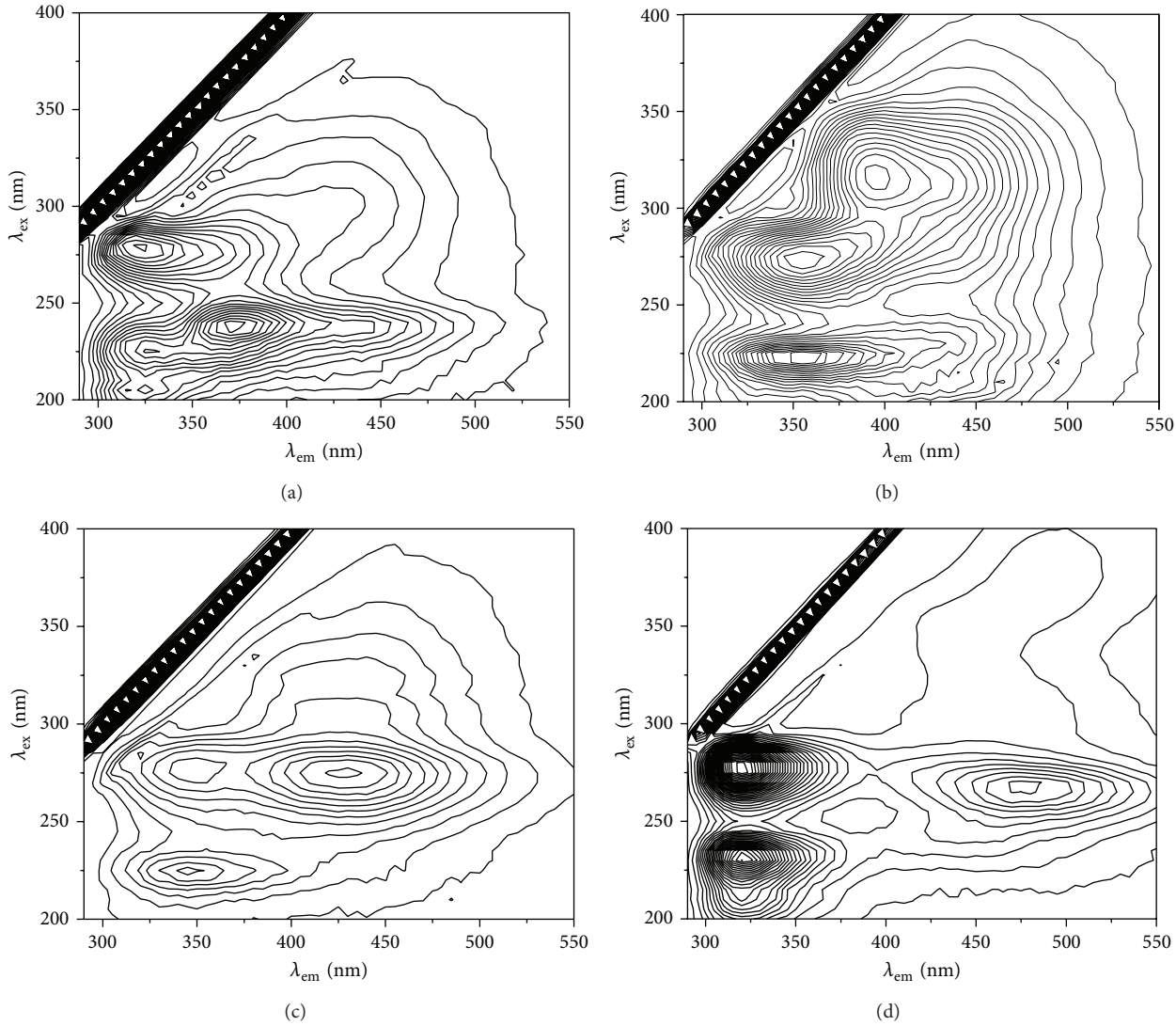

Figure 5: 3D fluorescence spectra of four kinds of Chinese herbal medicines in CDC. (a) Xihuangcao, *c*: 400 μg mL^{-1}; (b) Yinchen, *c*: 400 μg mL^{-1}; (c) Chuanxinlian, *c*: 400 μg mL^{-1}; (d) Dahuang, *c*: 100 μg mL^{-1}. Contour interval: *R*.

declined, while a new emission band centered at 380 nm emerged. When the percentage of methanol reached 100%, the λ_{em} blue shifted to 380 nm; however, the fluorescence intensity was less than that in aqueous solutions. Therefore, we choose water as the appropriate solvent in establishing a fluorimetric method in the following study, and control the methanol percentage in aqueous solution no more than 20% to obtain sensitive and stable fluorescence.

3.1.5. Measurement of Fluorescence Quantum Yield of 4MU. Using the method described in Section 2.5, quantum yields of 4MU in near neutral (pH 5.98) and weak alkaline (pH 9.75) conditions were measured, as shown in Table 2. In near neutral solution, 4MU exist as molecular form; its quantum yield at excitation wavelength 320 nm was measured to be 0.74. In weak alkaline solution, 4MU exist as anion form; its quantum yield at excitation wavelength 360 nm was measured to be 0.95. These results indicate that 4MU is an excellent fluorophore. A fluorimetric method for determination of 4MU in Chinese patent drug should be very sensitive either in near neutral or in weak alkaline conditions.

3.2. Determination of 4MU in Compound Dantong Capsule

3.2.1. 3D Fluorescence Spectra of Four Kinds of Chinese Herbal Medicines in CDC. The key point for developing a fluorimetric method is to understand the fluorescent properties of the analyte and other components in the sample, then find a way to avoid interference of the coexistent components on the fluorescence of the analyte. For these reason, 3D fluorescence spectra of four kinds of Chinese herbal materials in CDC were measured and shown in Figure 5.

From Figure 5 we showed that all the four kinds of Chinese herbal medicines contain fluorescent components. The interference of these components on the fluorescence of 4MU seems to be a serious problem. However, we noted

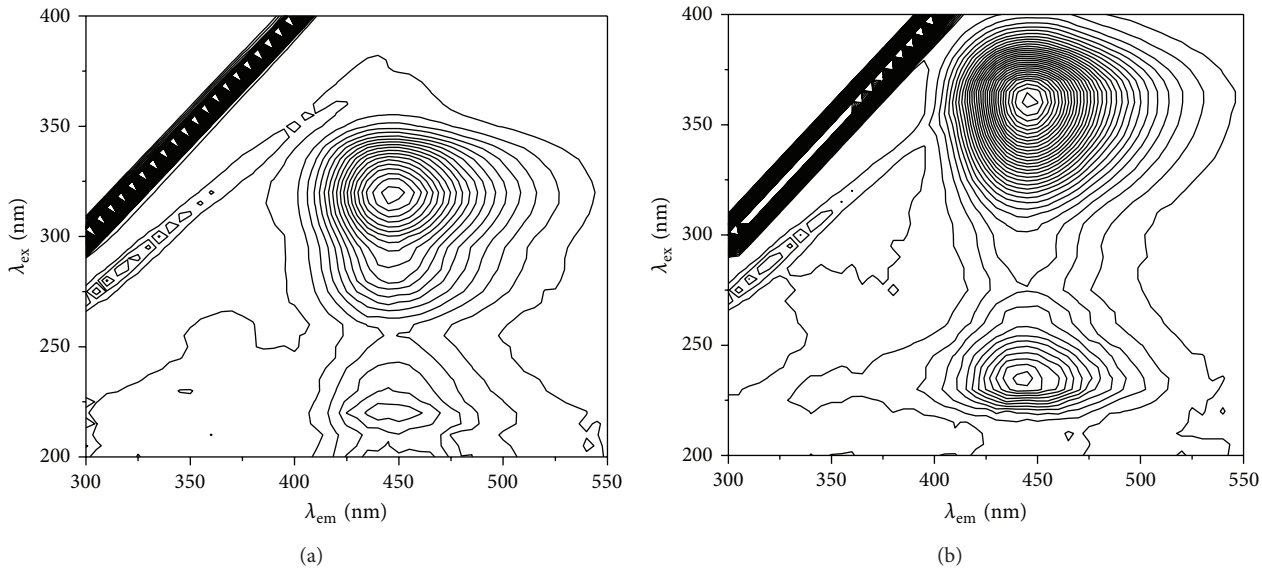

Figure 6: 3D fluorescence spectra of CDC solution at different pH. c_{CDC}: 55.3 ng mL^{-1}. (a) pH 5.85; (b) pH 9.22. Contour interval: *R*.

Table 2: Measurement of fluorescence quantum yield of 4MU in different condition.

λ_{ex}/nm	Quinine bisulphate			4MU (pH 5.98)			4MU (pH 9.75)		
	A	F^*	*Y*	*A*	F^*	*Y*	*A*	F^*	*Y*
313	0.0365	105566	0.55	0.0440	178748	0.77			
320	0.0391	104878	0.51	0.0488	190213	0.74			
350	0.0411	123457	0.57				0.0440	220646	0.95
360	0.0320	95986	0.57				0.0481	239780	0.95

F^* wavelength range of integral fluorescence intensity: 380 nm~600 nm.

that the concentrations of these herbal medicines in Figure 5 (400 μg mL^{-1} ~100 μg mL^{-1}) were much higher than the concentration of 4MU in Figure 1 (18.5 ng mL^{-1}). So, whether these herbal medicines in CDC interfere with the fluorescence of 4MU should be considered further from the view point of concentration.

3.2.2. 3D Fluorescence Spectra of CDC. Figure 6 shows the 3D fluorescence spectra of CDC dilute solutions at pH 5.85 and at pH 9.22. Comparing Figure 6 to Figure 1, we figured out that the two pictures are basically the same. The fluorescence peaks presented in Figure 5 are not emerged in Figure 6. This result indicated that the fluorescence of those four herbal medicines in CDC is too weak to be seen, either because of their low concentration or due to their poor luminous ability. So, we can conclude that the coexistent components in CDC do not interfere with the fluorescence of 4MU. The content of 4MU in CDC can be determined simply by a fluorimetric method.

From Figures 6 and 2, we also know that the fluorimetric method may be performed at near neutral or at weak alkaline pH. For simplicity, we prefer the method to be performed at near neutral pH so that the fluorescence intensity of CDC water solution can be measured directly without using a buffer solution.

3.2.3. Determination of 4MU in CDC by a Standard Curve Method. A series of standard aqueous solutions containing different amounts of 4MU, from 1.55 to 31.0 ng mL^{-1}, were prepared. Fluorescence spectra, as shown in Figure 7(a), and fluorescence intensity at measuring wavelength of $\lambda_{ex}/\lambda_{em}$ = 320 nm/445 nm of each solution were measured. A linear calibration curve of fluorescence intensity against concentration was plotted, as shown in Figure 7(b). The regression equation obtained was $I_F = 5.5 + 104.1c$ (ng mL^{-1}), with correlation coefficient $R = 0.9998$ ($n = 13$).

A number of solutions containing different amounts of CDC were prepared and their fluorescence intensities at wavelength of $\lambda_{ex}/\lambda_{em}$ = 320 nm/445 nm were recorded. Using the calibration curve, the 4MU content in CDC sample was determined to be 33.35 ± 0.10%, as shown in Table 3.

3.2.4. Determination of 4MU in CDC by a Standard Addition Method. As shown in Figure 8, a standard addition method was employed to verify the result obtained above. The regression equation obtained was $I_F = 636.9 + 105.3c$ (ng mL^{-1}), with correlation coefficient $R = 0.9998$ ($n = 5$). The content of 4MU in CDC sample was calculated to be 33.3%, which was coincident with the result obtained by standard curve method.

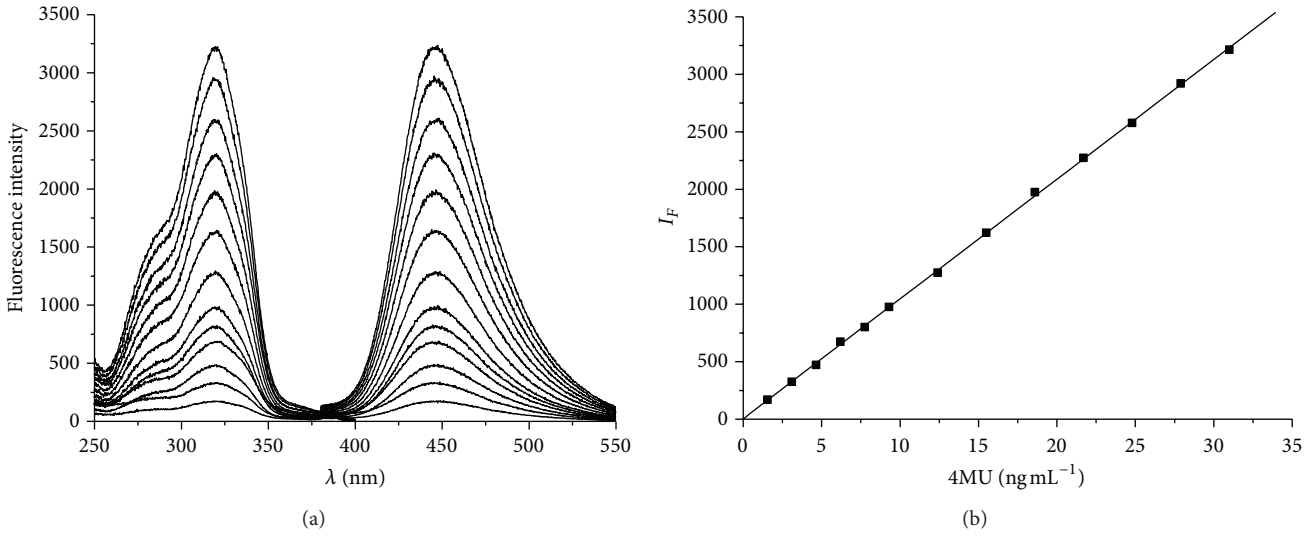

Figure 7: (a) Fluorescence spectra of 4MU in different concentrations; (b) plot of fluorescence intensity versus concentration. pH: 5.98; $\lambda_{ex}/\lambda_{em}$: 320 nm/445 nm.

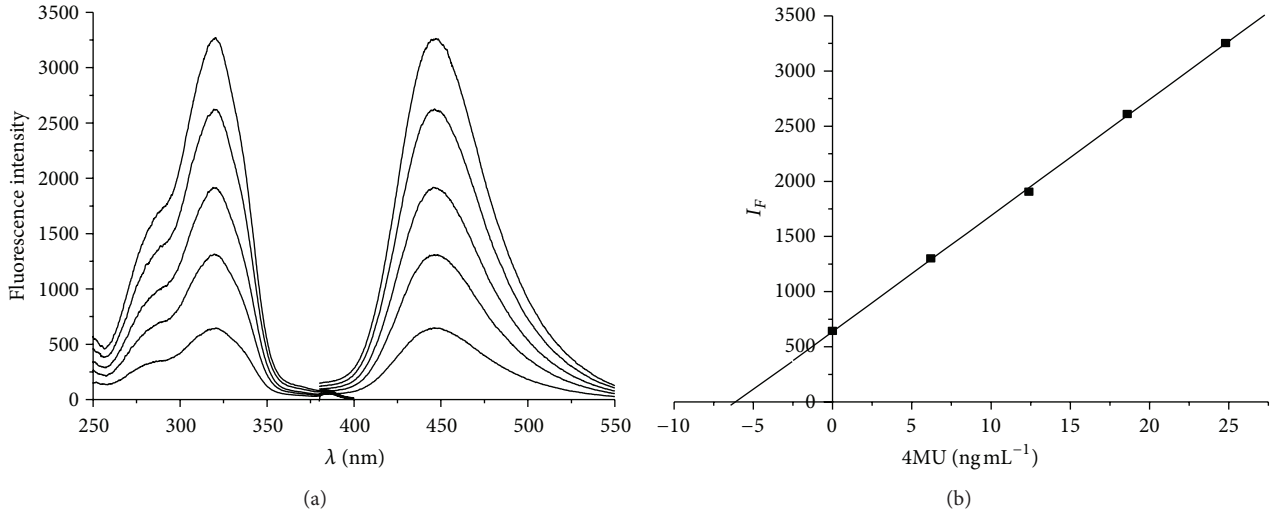

Figure 8: (a) Fluorescence spectra and (b) plot of fluorescence intensity *versus* concentration of a standard addition method for the determination of 4MU in CDC sample. pH: 5.98; $\lambda_{ex}/\lambda_{em}$: 320 nm/445 nm.

Table 3: Determination of 4MU in CDC by standard curve method.

No.	1	2	3	4	5
CDC added (V/mL)	0.20	0.40	0.60	0.80	1.00
Fluorescence intensity (I_F)	643.5	1285	1924	2561	3217
4MU determined (ng mL^{-1})	6.13	12.3	18.4	24.5	30.8
4MU content in CDC (%)	33.3	33.4	33.3	33.3	33.5
Average content (%)	33.4 ± 0.1				

According to the CDC drug label, the content of 4MU in CDC is 100 mg per grain (0.3 g). It is evident that the analytical result of this method agrees with the drug label.

4. Conclusion

Hymecromone (4MU) is an excellent fluorophore. Acidity and solvent have important influence on its fluorescence. In near neutral aqueous solutions, 4MU exist mainly as neutral molecular form which can be produced strong fluorescence at 445 nm. In weak alkaline solutions, 4MU exist mainly as anion form which can be produced stronger fluorescence at 445 nm. In methanol solution, the fluorescence peak at 445 nm blue shifted to 380 nm and the fluorescence intensity declined in a certain extent. There is a good linear relationship between fluorescent intensity and 4MU concentration. The 3D fluorescence spectra of Chinese patent drug CDC dilute solutions are very similar to the 3D fluorescence spectra of 4MU, indicating that the main fluorescent component in

CDC is 4MU. The coexistent components in CDC do not interfere with the fluorescence of 4MU because of their low concentration or poor luminous ability. So, the content of 4MU in CDC can be determined simply by fluorimetric method without preseparation.

Abbreviations

CDC: Compound Dantong Capsule (Fufang Dantong Capsule)
λ_{ex}: Maximum excitation wavelength
λ_{em}: Maximum emission wavelength.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (nos. 20675025, 20975029, and 81173496).

References

[1] Z. Zhou, N. Li, and A. Tong, "A new coumarin-based fluorescence turn-on chemodosimeter for Cu^{2+} in water," *Analytica Chimica Acta*, vol. 702, no. 1, pp. 81–86, 2011.

[2] L. Y. Wang, H. H. Li, and D. R. Cao, "A new photoresponsive coumarin-derived Schiff base: chemosensor selectively for Al^{3+} and Fe^{3+} and fluorescence " turn-on" under room light,," *Sensors and Actuators B*, vol. 181, pp. 749–755, 2013.

[3] K. Li, N. Li, X. Chen, and A. Tong, "A ratiometric fluorescent chemodosimeter for Cu(II) in water with high selectivity and sensitivity," *Analytica Chimica Acta*, vol. 712, pp. 115–119, 2012.

[4] H. X. Xu, X. Q. Wang, C. L. Zhang, Y. P. Wu, and Z. P. Liu, "Coumarin-hydrazone based high selective fluorescence sensor for copper(II) detection in aqueous solution," *Inorganic Chemistry Communications*, vol. 34, pp. 8–11, 2013.

[5] X. B. Huang, Y. Dong, Q. W. Huang, and Y. X. Cheng, "Hydrogen bond induced fluorescence recovery of coumarin-based sensor system," *Tetrahedron Letters*, vol. 54, pp. 3822–3825, 2013.

[6] N. Chattopadhyay, A. Mallick, and S. Sengupta, "Photophysical studies of 7-hydroxy-4-methyl-8- (4′-methylpiperazin-1′-yl) methylcoumarin: a new fluorescent chemosensor for zinc and nickel ions in water," *Journal of Photochemistry and Photobiology A*, vol. 177, no. 1, pp. 55–60, 2006.

[7] P. Mladěnka, K. Macáková, L. Zatloukalová et al., "In vitro interactions of coumarins with iron," *Biochimie*, vol. 92, no. 9, pp. 1108–1114, 2010.

[8] M. Mazzei, E. Nieddu, M. Miele et al., "Activity of Mannich bases of 7-hydroxycoumarin against Flaviviridae," *Bioorganic and Medicinal Chemistry*, vol. 16, no. 5, pp. 2591–2605, 2008.

[9] B. Sarkanj, M. Molnar, M. Cacic, and L. Gille, "4-Methyl-7-hydroxycoumarin antifungal and antioxidant activity enhancement by substitution with thiosemicarbazide and thiazolidinone moieties," *Food Chemistry*, vol. 139, pp. 488–495, 2013.

[10] H. Morohashi, A. Kon, M. Nakai et al., "Study of hyaluronan synthase inhibitor, 4-methylumbelliferone derivatives on human pancreatic cancer cell (KP1-NL)," *Biochemical and Biophysical Research Communications*, vol. 345, no. 4, pp. 1454–1459, 2006.

[11] S. S. Bhattacharyya, S. Paul, S. K. Mandal, A. Banerjee, N. Boujedaini, and A. R. Khuda-Bukhsh, "A synthetic coumarin (4-Methyl-7 hydroxy coumarin) has anti-cancer potentials against DMBA-induced skin cancer in mice," *European Journal of Pharmacology*, vol. 614, no. 1–3, pp. 128–136, 2009.

[12] K. Harada, H. Kubo, Y. Tomigahara et al., "Coumarins as novel 17β-hydroxysteroid dehydrogenase type 3 inhibitors for potential treatment of prostate cancer," *Bioorganic and Medicinal Chemistry Letters*, vol. 20, no. 1, pp. 272–275, 2010.

[13] A. Kultti, S. Pasonen-Seppänen, M. Jauhiainen et al., "4-Methylumbelliferone inhibits hyaluronan synthesis by depletion of cellular UDP-glucuronic acid and downregulation of hyaluronan synthase 2 and 3," *Experimental Cell Research*, vol. 315, no. 11, pp. 1914–1923, 2009.

[14] I. P. Kostova, I. I. Manolov, I. N. Nicolova, and N. D. Danchev, "New metal complexes of 4-methyl-7-hydroxycoumarin sodium salt and their pharmacological activity," *Il Farmaco*, vol. 56, no. 9, pp. 707–713, 2001.

[15] I. Kostova, I. Manolov, I. Nicolova, S. Konstantinov, and M. Karaivanova, "New lanthanide complexes of 4-methyl-7-hydroxycoumarin and their pharmacological activity," *European Journal of Medicinal Chemistry*, vol. 36, no. 4, pp. 339–347, 2001.

[16] L. D. Li and G. Z. Jin, "Luminescence properties of 4-Methyl-7-hydroxy-coumarin," *Chinese Journal of Analytical Chemistry*, vol. 22, no. 5, pp. 440–444, 1994.

[17] S. Dong, H. Ma, X. Li, M. Sun, and X. Duan, "Synthesis of a new water-soluble polymeric probe and its fluorescent properties for ratiometric measurement of near-neutral pH," *Analytical Letters*, vol. 37, no. 14, pp. 2937–2948, 2004.

[18] I. Jones, J. Hamilton, R. Srivastava, and P. Galloway, "Effect of neutral and acid pH on the fluorescence of 4-methylumbelliferone and the implications for dry blood spot assays," *Molecular Genetics and Metabolism*, vol. 108, no. 2, article S51, 2013.

[19] Pharmacopoeia Committee of Chinese Ministry of Health, *Traditional Chinese Medicine Drugs Preparation*, vol. 5, 1991.

[20] A. Abate, V. Dimartino, P. Spina et al., "Hymecromone in the treatment of motor disorders of the bile ducts: a multicenter, double-blind, placebo-controlled clinical study," *Drugs under Experimental and Clinical Research*, vol. 27, no. 5-6, pp. 223–231, 2001.

[21] L. Zhang, B. Y. Li, and A. L. Yang, "Studies on the quality standard for Compound Dantong Capsule," *Tianjin Pharmacy*, vol. 16, no. 5, pp. 15–17, 2004.

[22] L. Zhang and J. Zhu, "Determination of the content and dissolution of hymecromone in Compound Dantong Capsules by HPLC," *Traditional Chinese Drug Research & Clinical Pharmacology*, vol. 17, no. 3, pp. 222–224, 2006.

[23] L. R. Zheng, D. Liang, and Y. Chen, "Determination of 7-hydroxy-4-methylcoumarin in Compound Dantong Capsule by HPLC," *Pharmaceutical and Clinical Research*, vol. 16, no. 4, pp. 333–334, 2008.

[24] Y. J. Wei, *3D Fluorescent Fingerprint of Chinese Herbal Medicine*, Science Press, Beijing, China, 2012.

[25] F. Yang, C. G. Liu, and Y. J. Wei, "Fluorimetric analysis of paeonol in Chinese herbal medicine Cynanchi Paniculati Radix by aluminum ion-sensitized fluorescence," *Acta Pharmaceutica Sinica B*, vol. 2, no. 3, pp. 294–299, 2012.

[26] L. P. Wang and Y. J. Wei, "Fluorimetric analysis of camptothecin in Chinese herbal medicine common Camptotheca fruit," *Acta Pharmaceutica Sinica*, vol. 47, no. 10, pp. 1370–1374, 2012.

[27] S. J. Wang, Q. Zhang, L. P. Wang, and Y. J. Wei, "Fluorescent properties of arctiin and arctigenin and fluorimetric analysis of arctiin in traditional Chinese medicine Arctii Fructus," *Journal of Instrumental Analysis*, vol. 33, 2014.

[28] J. Zhang, C. Liu, and Y. Wei, "Fluorescence quantum yield and ionization constant of umbelliferone," *Huaxue Tongbao*, vol. 74, no. 10, pp. 957–960, 2011.

[29] J. G. Xu and Z. B. Wang, *Fluorimetry*, Science Press, Beijing, China, 3rd edition, 2007.

[30] C. G. Liu, Y. Z. Xu, Y. J. Wei et al., "Fluorescence spectra and protonation of ofloxacin," *Spectroscopy and Spectral Analysis*, vol. 25, no. 4, pp. 584–587, 2005.

[31] L. Y. Kong, *Coumarin Chemistry*, Chemical Industry Press, Beijing, China, 1st edition, 2008.