Determination of Hyoscine Butylbromide with Ag^+ and Dihalogenated Fluorescein Dyes in Capsules by Resonance Rayleigh Scattering Method Coupled with Flow Injection Analysis Technique†

Song, Yanqi[a,b](宋彦琪) Liu, Shaopu[a](刘绍璞)
Liu, Zhongfang[a](刘忠芳) Hu, Xiaoli*,[a](胡小莉)

[a] *School of Chemistry and Chemical Engineering, Key Laboratory on Luminescence and Real-Time Analysis, Ministry of Education, Southwest University, Chongqing 400715, China*
[b] *Chongqing Station, National Water Quality Monitoring Net of City Water Supply, Chongqing 400013, China*

In pH 4.2—5.2 HOAc-NaOAc buffer solution, Ag^+ reacted with dihalogenated fluorescein (DHF) dyes to form a 1∶2 anionic complex. This anionic complex could further react with hyoscine butylbromide (HBB) to form 1∶1 ion-association complex, which resulted in the significant enhancement of resonance Rayleigh scattering (RRS) intensity. Therefore, a novel method for the determination of HBB by resonance Rayleigh scattering (RRS) coupled with flow injection analysis (FIA) technique has been established. The present method had been applied to determine HBB in capsules and the results were in good agreement with those obtained by the literature method.

Keywords resonance Rayleigh scattering, flow injection analysis, hyoscine butylbromide, Ag^+, dihalogenated fluorescein

Introduction

Hyoscine butylbromide (HBB), an antimuscarinic antispasmodic, is a hydrophilic quaternary ammonium compound. It acts to block the action of acetylcholine at parasympathetic sites in smooth muscle and in secretory glands, stopping spasms in these areas. HBB is specifically indicated for the treatment of bladder or intestinal spasms. It has been used in the treatment of irritable bowel syndromes and other gastro-intestinal conditions, as well as the immediate treatment of gastro-intestinal distress. However, if inappropriately used, HBB can cause many side effects, such as constipation, dry mouth, trouble urinating and nausea.[1-3] In order to take full advantage of the effect of HBB and to decrease its toxicity, it is very important to quantitatively determine HBB in clinical analysis. Nowadays, HBB has been determined in pharmaceutical preparations and biological sample by titrimetric method,[4] spectrophotometry,[5] liquid chromatography,[6] capillary electrophoresis[7] and electrochemical methods.[8,9] In Chinese Pharmacopoeia, the HBB content was measured by titrimetric method with cumbersome operations and low accuracy.[4] In the pretreatment of spectrophotometry,[5] organic solvent was used as extract liquid and the determination procedure included extraction, drying, concentration and so on, which was too trivial and time consuming to satisfy the need for a rapid determination. The aim of present work is to develop a facile, rapid, selective, sensitive and reproducible new method for the determination of HBB and pharmaceutical samples. Resonance Rayleigh scattering (RRS) has been widely applied to the analytical application field such as nucleic acids,[10] proteins,[11] organic substances[12] and inorganic substances.[13] Furthermore, RRS technique has been increasingly used in ion-association complex systems lately.[14-16] Because of the high sensitivity of resonance Rayleigh scattering and the high precision of flow injection analysis, the technique of flow injection analysis coupled to resonance Rayleigh scattering detector could not only speed up the analysis to realize the automatization of RRS, but also enhance the sensitivity and precision of the analysis results. Up to now, there are a small number of reports describing the FIA-RRS technique (FIA-RLS).[17-21]

Our experiment discovered that in a HOAc-NaOAc buffer medium, HBB reacts with Ag^+ and dihalogenated fluorescein (DHF) dyes such as dichlorofluorescein (DCF), dibromofluorescein (DBF) or diiodofluorescein (DIF) to form an ion-association complex, which resulted in the greatly enhancement of RRS in-

* E-mail: xiaolihu@swu.edu.cn
Received May 27, 2011; revised July 6, 2011; accepted July 29, 2011.
Project supported by the National Natural Science Foundation of China (No. 20875078) and Chongqing Municipal Key Laboratory on Luminescence and Real-Time Analysis (No. CSTC 2006CA8006).
† Dedicated to Professor Weiyuan Huang on the occasion of his 90th birthday.

tensity. The scattering intensities at their maximum scattering peaks were proportional to the concentration of HBB in certain range. The established method exhibited high sensitivity and excellent reproducibility, and provided a new technology for the determination of HBB in capsules.

Experimental

Apparatus and reagents

A Hitachi F-2500 spectrofluoremeter with a 90 μL quartz flow cell (Tokyo, Japan) was used throughout as the detector. The determination parameters were the slit (ex/em) of 5.0 nm/5.0 nm and PMT voltage of 400 V. The flow system used consisted of a peristaltic pump (Shanghai Qingpu Huxi Instrument Factory, Shanghai) and an eight-way rotary valve with exchangeable sample loop polytetrafluoroe thylene (PTFE) with 1.0 mm internal diameter tubing, which was used to connect all components in the flow system. A UV-8500 spectrophotometer (Tianmei, Shanghai) was used to record the absorption spectra and measure absorbance intensity.

Hyoscine butylbromide (HBB) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The stock concentration of HBB was 100.0 μg/mL and a series of working concentrations were prepared by diluting the stock solutions with water before use.

The capsules of HBB were purchased from Guangzhou Hanfang Pharmaceutical Company Limited (Guangzhou, China).

Dichlorofluorescein (DCF), dibromofluorescein (DBF) and diiodofluorescein (DIF) were purchased from J&K Chemical Ltd. (Beijing, China). The stock concentration of dihalogenated fluorescein dyes were 5.0×10^{-4} mol/L, and the working solutions were prepared by diluting the stock solutions with water.

HOAc-NaOAc buffer solution: prepared by mixing 0.1 mol/L HOAc and 0.1 mol/L NaOAc according to suitable proportion and adjusting pH values with pH meter.

All reagents were of analytical reagent grade (A.R.), and doubly distilled water was used throughout.

General procedure

The measurements were obtained using the time scan pattern of fluorospectrophotometer. The mixed solution, including pH 4.5 HOAc-NaOAc buffer solution, 1.0×10^{-4} mol/L $AgNO_3$ and 2.5×10^{-5} mol/L DHF dyes (DCF, DBF or DIF), as carrier solution was continuously pumped through the system until a stable baseline was obtained. The HBB solutions of different concentration were injected into the flow system by an eight-way injection valve. The RRS intensity (I) was detected at $\lambda_{ex}=\lambda_{em}$. $\Delta I=I-I_0$, I and I_0 are the RRS intensities of the carrier in presence and absence of HBB, respectively. Typically, three repeated injections of standards and sample were made and the results are reported as the mean value. In the present work, the major flow-through parameters were 80.0 μL, 2.5 mL/min and 85.0 cm for the injection volume, carrier flow rate and reactor length, respectively. A scheme of the FIA feature was presented in Figure 1.

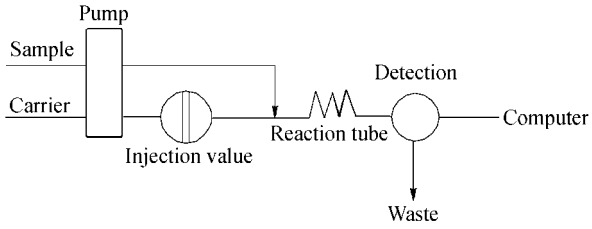

Figure 1 Schematic diagram of FIA system with RRS detector.

Sample preparation

Two capsules were combined. An amount of powder equivalent to about 0.5 mg HBB was accurately weighed and diluted with water into a 50 mL volumetric flask as sample solution for the experiment.

Results and discussion

RRS spectra

RRS spectra of DHF, Ag^+, HBB, Ag^+-DHF, Ag^+-HBB, DHF-HBB and Ag^+-DHF-HBB were obtained by wavelength scanning mode when the maximum RRS peak in flow cell was detected. It could be seen that the RRS of DHF, Ag^+, HBB, Ag^+-DHF, Ag^+-HBB and DHF-HBB was very weak. When the DHF, Ag^+ and HBB reacted to form an ion-associate complex, which led to a remarkable enhancement of RRS intensity and the maximum RRS peak was at 322 nm (DCF), 320 nm (DBF) and 335 nm (DIF). Figure 2 shows the RRS spectrum of DIF-Ag^+-HBB system.

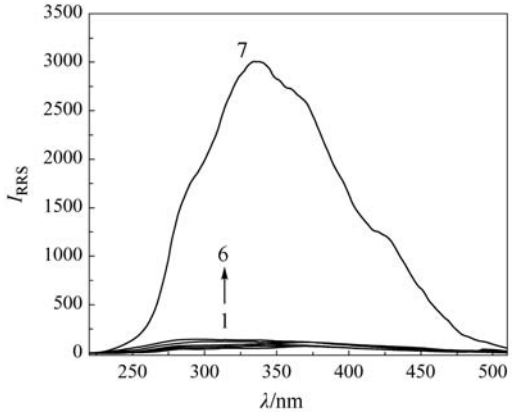

Figure 2 RRS spectra of DIF-Ag^+-HBB system. (1) DIF; (2) Ag^+; (3) HBB; (4) Ag^+-DIF; (5) Ag^+-HBB; (6) DIF-HBB; (7) DIF-Ag^+-HBB. c(HBB)=2.0 μg/mL; $c(Ag^+)=1.0\times10^{-4}$ mol/L; c(HBB)$=2.5\times10^{-5}$ mol/L; pH=4.5.

Optimum reaction conditions

Effects of DHF concentrations The experiment showed that RRS intensity reached the maximum and changed little when the ranges of DHF concentration

were 1.0×10^{-5}—8.0×10^{-5} mol/L. So, 2.5×10^{-5} mol/L was chosen as a suitable DHF concentration.

Effects of Ag^+ concentrations The effect of the concentration of Ag^+ was tested. The results were shown in Figure 3. It could be seen that when the concentration of Ag^+ was 0.6×10^{-4}—1.5×10^{-4} mol/L, the intensities reached the maximum and changed little. So, 1.0×10^{-4} mol/L was chosen as a suitable Ag^+ concentration.

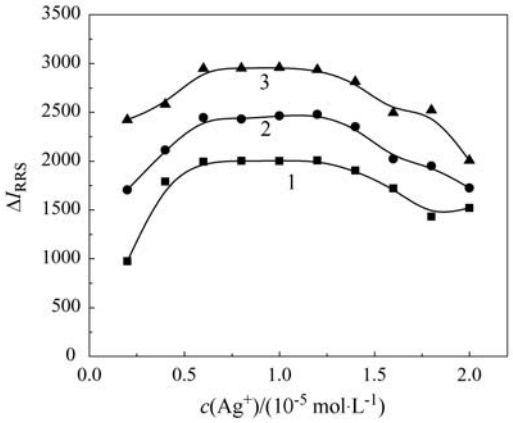

Figure 3 Effect of Ag^+ concentrations. (1) DCF-Ag^+-HBB; (2) DBF-Ag^+-HBB; (3) DIF-Ag^+-HBB. c(HBB) = 2.0 μg/mL; c(DHF)=2.5×10^{-5} mol/L; pH=4.5.

Effect of acidity of the carrier The influences of different buffer solution on the RRS intensities were tested with Britton-Robison, citrate sodium-HCl and HOAc-NaOAc. The results showed that HOAc-NaOAc was better than other buffer solutions. The optimum pH range for the determination of HBB was 4.2—5.2. The RRS intensities would decrease if the acidity was beyond the range (Figure 4). Therefore, pH 4.5 was chosen as the reaction acidity. The appropriate volume of buffer solution was found in the range 0.5—2.0 mL, hence 1.0 mL was chosen for the reaction.

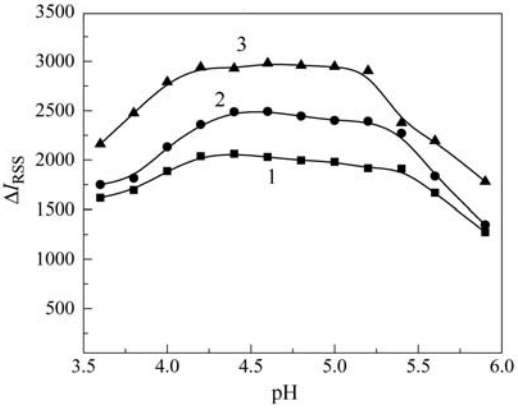

Figure 4 Effect of acidity. (1) DCF-Ag^+-HBB; (2) DBF-Ag^+-HBB; (3) DIF-Ag^+-HBB. c(HBB)=2.0 μg/mL; c(Ag^+)=1.0×10^{-4} mol/L; c(DHF)=2.5×10^{-5} mol/L.

Effect of ionic intensity The effect of ionic strength on the RRS intensities for this system was investigated using 1.0 mol/L $NaNO_3$ solution. The results showed that a large amount of $NaNO_3$ has little effect. ΔI_{RRS} hardly changed when the $NaNO_3$ concentration $\leqslant$0.6 mol/L, which meant that over a wide range of ionic strengths the methods could be applied to the determination of HBB accurately.

Stability of the reaction systems Within 60 min the RRS intensities were recorded every 5 min. As the intensities changed little, it showed that the stability of the reaction systems was good.

Influence of FIA variables The injection volume, the carrier flow rate and the reactor length were investigated with the same concentration of HBB and the best concentration of the carrier. The results showed as follows.

(1) Injection volume. Samples of different volumes were injected. The results showed that the higher the injection volume, the higher the peak height. Nevertheless, the RRS intensity increased slowly when the injection volume was over 80.0 μL. Moreover, the uniformity of reaction and the poor reproducibility would be caused by the increase of reagent. So a volume of 80.0 μL was selected as a reasonable compromise.

(2) Carrier flow rate. The determination was affected heavily by the carrier flow rate. The velocity of flow which was too low or too high would make the peak appeare before or after the flow cell, which was unfavorable for detecting the RRS signal completely by photomultiplier tube. And a too low velocity of flow not only reduced sample throughput but also broadened the peak. Although the response of the RRS signal was better with the increase of flow rate, the intensity was instable. The experimental results showed if the carrier flow rate was too quick, both the accuracy and reproducibility of the determination of HBB were negatively influenced by it. Considering the above factors, a flow rate of 2.5 mL/min was chosen.

(3) Reactor length. The length of reactor influenced the diffusion coefficient and the analysis rate of the sample in pipelines directly as well as the reaction of HBB with the carrier. The interaction was incomplete when the reaction coils length was too short, whereas the diffusion coefficient would augment and the analysis rate would fall when length was too long. A reasonable compromise was a length of 85.0 cm. The range of the variable FIA parameters were studied and their optimum values are listed in Table 1.

Table 1 Optimization of FIA parameters

Variable	Studied range	Optimum value
Injection volume/μL	60.0—100.0	80.0
Carrier flow rate/(mL•min^{-1})	1.0—3.5	2.5
Reactor length/cm	50.0—110.0	85.0

Relation between RRS intensity and HBB concentrations

HBB with different concentrations reacted with the

carrier and their RRS intensities were measured, and there was a linear regression equation of HBB with different concentrations. Figure 5 showed that the RRS intensity of DIF-Ag^+-HBB system was proportional to the concentration of HBB. The correlation coefficient, linear ranges and detection limits (3σ) for the calibration curves are shown in Table 2. It could be seen that these three methods had high sensitivity. The detection limits of these methods were in the range of 6.2—11.6 ng/mL. The order of sensitivity was DIF＞DBF＞DCF.

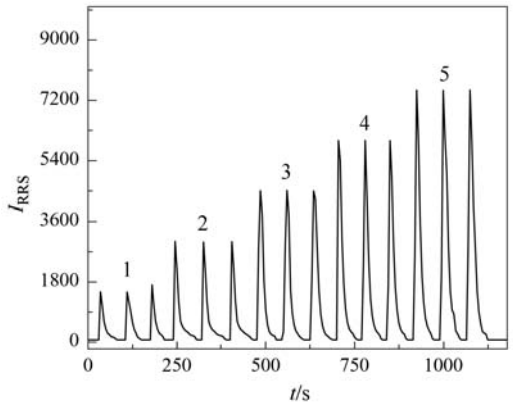

Figure 5 Calibration peak signals for the determination of HBB by FIA-RRS method. DIF-Ag^+-HBB (1—5): c(HBB): 1.0, 2.0, 3.0, 4.0, 5.0 μg/mL. $c(Ag^+)=1.0\times10^{-4}$ mol/L, c(DIF)$=2.5\times10^{-5}$ mol/L, pH＝4.5.

Reasons for RRS enhancement

Considering the three dyes had similar structures while they also had similar properties, DBF was taken as an example to study the ion-association reaction of Ag^+ with DBF and HBB. The composition ratio of the ternary complex was established by using Job's method and molar ratio method. The results showed that the ratio of n(Ag)∶n(DBF)∶n(HBB) was 1∶2∶1. Hence, the composition of the ion-association complex might be expressed as $[Ag(DBF)_2]$•HBB. The structure of the complex and the reaction mechanism are as follows.

DBF was expressed as H_2L. In pH 4.5 aqueous solution according to its dissociation constants pKa_1＝2.8 and pKa_2＝4.9,[22] HL^- was the main existant species. The distribution fractions were 70.5% for HL^-, 28.1% for L^{2-} and 1.4% for H_2L, respectively. As a halogenated fluorescein, DBF had two electron-withdrawing groups of Br being close to OH, which reduced the charge density of oxygen atom on OH. Therefore, OH tended to dissociate more easily. Meantime, HBB dissociated as large organic cation (HBB^+) and Br^-. Under the experimental conditions, Ag^+ and HL^- reacted to form a 1∶2 anionic complex $[Ag(HL)_2]^-$. This anionic complex could further react with HBB^+ to form a 1∶1 neutral ion-association complex by the electrostatic attraction and the hydrophobic force. The schematic diagram of the ion-association complex is shown in Figure 6.

After the ion-association reaction, the RRS intensity enhanced observably. The reasons are as follows: (1) Resonance Rayleigh scattering is an absorption-rescattering process produced by the resonance between Rayleigh scattering (RS) and light absorption with equal frequency. Therefore, RRS spectrum should be closely related to the absorption spectrum. Figure 7 showed the comparison of RRS and absorption spectra of DCF system. It could be seen that RRS peak was located at its absorption band. The RRS peaks have good corresponding relationship with the absorption peaks, which results in a resonance enhanced effect and leads to great enhancement of RRS intensity. This is necessary for the production of RRS. (2) According to the Rayleigh scattering formula, if the molecular volume is difficult to estimate, the formula could be simplified as $I=kI_0Mc$,[23] that is, when the incident light intensity (I_0) and the concentration of the solution (c) are constant, the scattering intensity (I) is proportional to the molecular weight (M) of the particle. Before the reaction, the molecular weight of HBB^+ is 360.4, while after the reaction, the molecular weight of the product increases to 1313 for DCF system, 1491 for DBF system and 1679 for DIF system, respectively. Therefore, the scattering intensity is also enhanced because of the enlargement of

Table 2 Some parameters for the calibration graphs and the detection limits

System	Linear regression equation	Linear range/(μg•mL^{-1})	Correlation coefficient (r)	Detection limit/(ng•mL^{-1})
DIF	$\Delta I=-46.3+1501.8c$	0.04—6.0	0.9990	6.2
DBF	$\Delta I=23.7+1226.5c$	0.02—7.0	0.9995	7.5
DCF	$\Delta I=35.4+985.2c$	0.02—8.0	0.9993	11.6

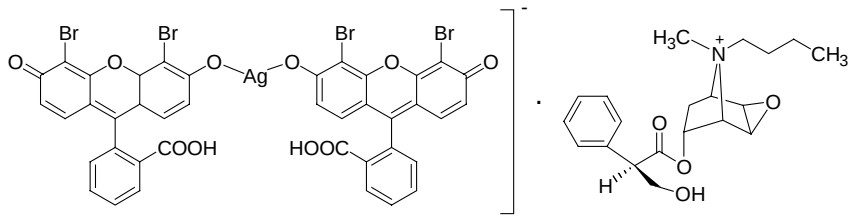

Figure 6 Schematic diagram of the $[Ag(DBF)_2]$•HBB.

molecular volume (or weight). (3) In acidic medium, the three substances could react to form the neutral ion-association complexes. These neutral association complexes could further assemble to form hydrophobic particles. They will form the hydrophobic interface with the aqueous phase. The formation of this hydrophobic interface will be advantageous to scattering enhancement.[24]

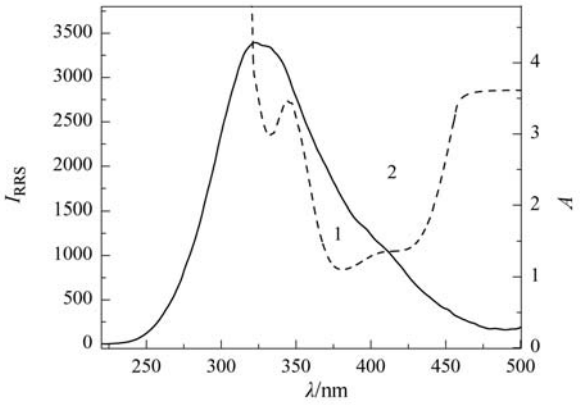

Figure 7 Comparison of absorption and RRS spectrum of DCF system. (1) Absorption spectrum; (2) RRS spectrum.

Selectivity of the method

Because of the high sensitivity of DIF system, it was selected to investigate the effects of some coexisting substances on the determination of HBB under optimum conditions. When the HBB concentration was 2.0 μg/mL and the allowable error was within ±5%, about 20 kinds of coexisting substances did not interfere with the determination. These substances were as follows: 400-fold Na_2SO_4, $ZnSO_4$, potassium sodium tartrate and VC; 200-fold $MgSO_4$, $CuSO_4$, glucose, maltose and urea; 100-fold $Pb(OAc)_2$, bovine serum albumin and lactose; 500-fold $KAl(SO_4)_2$, sodium citrate, serum albumin, tyrosine, histidine, tryptophan and amylum; 20-fold $NaNO_2$ and VB_1. Therefore, the FIA-RRS method presented could be used in real samples with highly selectivity.

Analytical application

The real samples were detected by FIA-RRS method (DIF system) and spectrophotometry.[5] The results are listed in Table 3 and agreed well with those obtained by the literature methods.[5] It could be seen from Table 3 that the method has high accuracy and good reproducibility, and the result was consistent with that specified. The RSD was 1.1%—1.4%, and the recovery was 98.6%—99.5%. From these results, it is suggested that the present RRS method with FIA detector could be applied successfully to determinate HBB concentration in pharmaceutical preparation.

Table 3 Results for the determination of HBB in capsules (n=9)

Method	Specified amount/(mg/granule)	Found/(mg/granule)	Added/(mg/granule)	Found/(mg/granule)	Recovery/%	RSD/%
FIA-RRS	10.0	10.42	2.5	12.90	99.0	1.3
			5.0	15.35	98.6	1.1
			10.0	20.38	99.5	1.4
Spectrophotometry	10.0	9.763	2.5	12.18	96.5	2.9
			5.0	14.66	97.9	3.0
			10.0	19.54	97.7	3.3

References

1 Editor Council of British Pharmacopoeia, *British Pharmacopoeia*, Her Majesty Stationery Office, London, **2001**, p. 882.
2 You, Q. D. *Pharmaceutical Chemistry*, Chemical Industry Press, Beijing, **2004**, pp. 219—220 (in Chinese).
3 Li, H. D. *Detoxification Pharmacotherapeutics*, People's Medical Publishing House, Beijing, **2001**, p. 81 (in Chinese).
4 The Pharmacopoeia Committee of the People's Republic of China, *The Pharmacopoeia of the People's Republic of China* (*Part II*), Chemical Industry Press, Beijing, **2005**, pp. 19—20 (in Chinese).
5 Ayman, A. G.; Zeineb, E. S.; Nagda, H.; Rham, E. *Spectrochim. Acta, Part A* **2008**, *70*, 785.
6 Yasser, S. E.; Fadia, H. M.; Mohamed, R.; Sonia, Z. E. *J. AOAC Int.* **2007**, *90*, 102.
7 Ding, P. L.; Yu, Y. Q.; Chen, D. F. *Phytochem. Anal.* **2005**, *16*, 257.
8 Khalil, F.; Ahmad, K. *J. Chin. Chem. Soc.* **2007**, *54*, 165.
9 El-Saharty, Y. S.; Metwaly, F. H.; Refaat, M. *Talanta* **2007**, *72*, 675.
10 Guo, M.; Wang, L. T.; Wu, X. *Molecules* **2009**, *14*, 8.
11 Dong, L. J.; Chen, X. G.; Hu, Z. D. *Talanta* **2007**, *71*, 555.
12 Xiao, X. L.; Wang, Y. S.; Chen, Z. M. *Spectrochim. Acta, Part A* **2008**, *71*, 398.
13 Kang, C. Y.; Xi, D. L.; Chen, Y. Y. *Talanta* **2008**, *74*, 867.
14 Tian, L. F.; Liu, Z. F.; Kong, L.; Hu, X. L.; Liu, S. P. *Chin. Sci. Bull.* (*Chin. Ver.*) **2011**, *56*, 396 (in Chinese).
15 Ma, Y.; Li, N. B.; Luo, H. Q. *Instrum. Sci. Technol.* **2009**, *37*, 345.
16 Li, C. X.; Liu, S. P.; Liu, Z. F.; Hu, X. L. *J. Fluorescence* **2011**, *21*, 723.
17 Li, Y.; Dong, J. J.; Wang, W. P.; Hu, Z. D.; Chen, X. G. *Anal. Biochem.* **2006**, *354*, 64.
18 Li, Y.; Dong, L. J.; Zhang, Y. H.; Hu, Z. D.; Chen, X. G.

Talanta **2007**, *71*, 109.
19 Dong, L. J.; Li, Y.; Zhang, Y. H.; Chen, X. G.; Hu, Z. D. *Microchim. Acta* **2007**, *159*, 49.
20 Hu, X. L.; Xu, D. P.; Liu, S. P.; Liu, Z. F.; Liu, S. *J. Lumin.* **2009**, *24*, 79.
21 Matveets, M. A.; Shcherbov, D. P.; Akhmetova, S. D. *Zh. Anal. Khim.* **1979**, *34*, 1049.
22 Editor Council of the Cyclopaedia in China, *Cyclopaedia in China*, *Biology II*, Cyclopaedia in China Press, Beijing, **1991**, p. 1374 (in Chinese).
23 He, Y. Q.; Liu, S. P.; Kong, L.; Liu, Z. F. *Spectrochim. Acta, Part A* **2005**, *61*, 2861.

(E1005272 Zhao, C.)

