Determination of Antibacterial Ofloxacin in Human Serum Samples by a Resonance Light Scattering Technique with Alizarin Violet 3B

Zhanguang CHEN,^{*†} Yurui PENG,^{*} Junhui CHEN,^{**} Taiyu ZHANG,^{*} and Guoliang LIU^{*}

^{*}Department of Chemistry, Shantou University, Shantou 515063, China

^{**}Medical College of Shantou University, Shantou 515031, China

A method for the determination of ofloxacin (OFL) has been developed, based on the enhancement of resonance light scattering (RLS) of OFL in the presence of alizarin violet 3B (AV3B). Under the experimental conditions, the RLS intensity of AV3B was greatly enhanced by adding OFL. At pH 5.09, the enhancement of the RLS intensity at 439.5 nm was proportional to the concentration of OFL in the range 0.10 – 2.50 µg/ml. The detection limit (3σ) was 0.013 µg/ml. At pH 6.90, the enhancement of the RLS intensity at 405.0 nm was proportional to the concentration of OFL in the range 0.05 – 3.00 µg/ml. The detection limit (3σ) was 0.021 µg/ml. The proposed method with high sensitivity, selectivity and reproducibility was satisfactorily applied to the determination of OFL in human serum.

(Received July 14, 2008; Accepted September 1, 2008; Published July 10, 2009)

Introduction

Ofloxacin (OFL), 9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-pierazinyl)-7-oxo-7H-pyrido[1,2,3-de]-1,4-benzoxazine-6-carboxylic acid, Fig. 1, is one of the third-generation members of synthetic fluoroquinolone derivatives with antibacterial activity. This synthetic antibiotic is widely used in the treatment of urinary and respiratory tract infections and other infections, which are caught by Gram-positive and Gram-negative bacteria.¹ The bactericidal activity of OFL results from inhibiting enzyme DNA gyrase required for bacterial DNA replication.² Because the OFL main excretion pathway is urinary, only trace amounts are found in plasma.³ Therefore, great attention was paid to study the possibility of exposure to low levels of this medicine.

Studies on determination of fluoroquinolones have attracted considerable interest in recent years. OFL is a synthetic fluoroquinolone, and the current analytical methods devoted to determination OFL were capillary electrophoresis,⁴ flow-injection spectrophotometry,⁵ flow-injection chemiluminescence,^{6,7} fluorescence spectrometry,⁸ thin layer chromatography,⁹ high-performance liquid chromatography (HPLC)¹⁰ and HPLC-fluorometry.¹¹ However, these methods were limited in either complicated operation or sophisticated instrumentation or poor selectivity or poor limit of detection. Therefore, the development of a reliable, rapid and accurate method for the determination of OFL is of great importance.

Resonance light scattering (RLS) is a scattering-absorption-rescattering process that occurs when the wavelength of the incident beam exists in or close to the absorption band of the aggregation of particles.^{12,13} Based on this theory, Pasternack *et al.* first established a new technique (RLS) to study the

aggregations of porphyrins by using a common fluorescence spectrometry.^{14–16} Huang *et al.* successfully applied this technique to the determination of nucleic acids at the nanogram level, and its detection limits were much lower than that of the fluorescence quenching method.^{17,18} From then on, this simple and sensitive method has been greatly used for analytical purposes.^{19,20} The RLS technique applied to the determination of nuclei acids,^{21–24} proteins,^{25–29} amino acid,³⁰ metal ions³¹ and medicine³² has already been reported. This method has proved to be promising based on the enhanced RLS intensity of the assay system. The RLS technique is becoming a novel analytical method characterized with high sensitivity, rapidity and simplicity.

In this paper, the RLS spectra of alizarin violet 3B (AV3B) in the presence and absence of OFL are discussed. A reaction between AV3B and OFL occurred, and produced fine particles. The interaction between OFL and AV3B was also studied based on the absorption spectra, scanning electron micrograph and other analytical methods. The RLS signal was derived from fine particles of the formed complex,^{13,14} and the intensity was enhanced by increasing the OFL in the system. The enhancement of the RLS intensity was proportional to the OFL concentration. Based on the linear relationship, a simple and sensitive new assay was proposed for the routine determination of OFL in

Fig. 1 The structure of OFL.

[†] To whom correspondence should be addressed.
E-mail: kqlu@stu.edu.cn

Fig. 2 RLS spectra of the OFL-AV3B system. (A) AV3B, 5.0×10^{-5} M; OFL ($\mu\text{g/ml}$), (a) 1.0 (without AV3B), (b) 0, (c) 0.5, (d) 1.0, (e) 2.5; pH 5.09. (B) AV3B, 5.0×10^{-5} M; OFL ($\mu\text{g/ml}$), (a) 1.0 (without AV3B), (b) 0, (c) 0.1, (d) 1.0, (e) 2.0; pH 6.90.

Fig. 3 Absorption spectra of AV3B in the presence of OFL. In the direction of the arrow, the absorption spectra were recorded for 0, 0.1, 0.5 $\mu\text{g/ml}$ of OFL. AV3B, 5.0×10^{-5} M; pH 5.09 (A) and pH 6.90 (B), respectively (versus water).

human serum samples. At pH values of 5.09 and 6.90 for Britton-Robinson (BR) buffer solutions, the RLS intensity of the AV3B system with OFL was rather stronger than that of a blank solution without OFL, and the enhanced RLS intensity was linear over a certain range of the OFL concentration. This assay was validated for the determination of OFL in human serum, and the results were obtained satisfactorily.

Experimental

Apparatus

All of the resonance light-scattering spectra were determined on an LS-55 spectrofluorometer (Perkin-Elmer, USA) with a 1×1 cm quartz cell. An Agilent 8453 ultraviolet spectrophotometer (Perkin-Elmer) was used to determine the absorption spectra of the different systems. The pH values of the solutions were recorded on a PHS-3C acidity meter (Leici, Shanghai). Scanning electron microscopy (SEM) images were obtained by Hitachi-600 scanning electron microscopy (Tokyo, Japan).

Materials

All chemicals used were of analytical reagent grade, unless stated otherwise, and doubly distilled water was used throughout. OFL was purchased from Sigma and ofloxacin injection, 10 ml: 0.2 g (Furen Medicine Group Co., Ltd.) was provided by Medical College of Shantou University. A stock solution of OFL was prepared by dissolving commercially purchased OFL, and the working solution was $10 \mu\text{g/ml}$ by diluting the OFL stock solution. They were all stored at approximately 4°C in a refrigerator.

Alizarin violet 3B (AV3B) was obtained from Shanghai Kayon Biological Technology Co., Ltd. Its stock solution was

prepared by dissolving the purchased produce in water, and the concentration of the working solutions was 5×10^{-4} M.

The acidity of the solutions was controlled by BR buffer. The BR buffer solution was made up of 0.04 M phosphoric acid, 0.04 M acetic acid, 0.04 M boric acid, and 0.2 M sodium hydroxide. A 0.5 M NaCl solution was used to adjust the ionic strength of the assay solutions.

General procedures

Into a 10-ml calibrated tube with a plug was added 1.0 ml of a BR buffer solution, 1.0 ml of AV3B and appropriate amounts of OFL or samples and stirred after each solution added. Then, the mixture was diluted to the mark by doubly distilled water and mixed thoroughly for determinations.

All RLS spectra were obtained by scanning simultaneously the excitation and emission monochromators ($\Delta\lambda = 0.00$ nm) from 250.0 to 700.0 nm with excitation and emission slit widths of 5.0 nm. The RLS intensity was determined at the maximum wavelength. The enhanced RLS intensity of the system by adding OFL is presented as $\Delta I_{\text{RLS}} = I_{\text{RLS}} - I_{\text{RLS}}^0$, where ΔI_{RLS} is the enhancement of RLS intensity; I_{RLS} and I_{RLS}^0 are the RLS intensity of the system with and without OFL, respectively.

Results and Discussion

Spectral characteristics

Figure 2 displays the RLS spectra of the OFL-AV3B system at pH 5.09 (A) and 6.90 (B), respectively. It can be seen that both the RLS intensity of AV3B and OFL are rather weak. However, the RLS intensity of AV3B can be greatly enhanced by adding OFL, and increases with an OFL concentration

Scheme 1 The procedure of reaction between OFL and AV3B.

Fig. 4 Scanning electron micrograph of AV3B particles in the absence of OFL (A) and in the presence of OFL at pH 5.09 (B) and pH 6.90 (C), respectively. Conditions: AV3B, 5.0×10^{-5} M; OFL, 1.0 $\mu\text{g}/\text{ml}$.

Scheme 2 The proton equilibrium procedure of OFL in aqueous solution with different pH values.

increase. At pH values of 5.09 and 6.90, AV3B has a rather weak RLS peak near to 405.0 and 439.5 nm, respectively.

Under the condition of pH 5.09, the peak at 405.0 nm gradually disappeared, and the peak at 439.5 nm greatly increased with the addition of OFL. Under the condition pH 6.90, with the addition of OFL, the RLS peak at 405.0 nm was more significant and more linear than that at 439.5 nm. This indicated that reactions occurred between OFL and AV3B and formed the OFL-AV3B complex. The characteristics of the RLS spectra in Fig. 2 have not been reported before, so the reaction mechanism of between AV3B and OFL needed further study. In the present work the reaction of these two substances was studied at pH 5.09 and 6.90, respectively. The maximum RLS peak at 439.5 nm was selected for the analytical wavelength at pH 5.09, and the maximum RLS peak at 405.0 nm was selected for the analytical wavelength at pH 6.90.

The absorption spectra of the OFL-AV3B system were determined at pH 5.09 (A) and 6.90 (B), and the results are displayed in Fig. 3. It can be found that the absorption intensity of AV3B increased with OFL addition with 3 distinct peaks located at 254, 276 and 569 nm. A blue shift at 254 nm and a red shift at 276 nm appeared with increasing OFL. The intensity of the peaks both at 254 and 276 nm increased with increasing OFL. However, there was no influence on the peak at 569 nm due to the concentration of OFL. This phenomenon indicated that an interaction between AV3B and OFL occurred, and a novel complex was produced. The reaction mechanism can possibly depict as in Scheme 1. OFL possesses two relevant ionizable functional groups: a basic piperazinyl group and a

carboxylic acid group. As an electron-donating group, the OFL molecular with a piperazinyl group can coordinate with AV3B, which is a plane-donor. In addition, the hydrogen bond and electrostatic force can also enhance the strength of the combination to form aggregations. Based on the scattering electron micrograph technique, the particles of AV3B and the complexes of the OFL-AV3B system were studied; the results are shown in Fig. 4. It can be seen that the particles in photo A uniformly distributed in the solution. However, in photos B and C, larger particles obviously appeared. This indicated that the particles produced by the AV3B and OFL mixture are larger than that of AV3B, and reactions between two materials occurred. According to the literatures,¹⁴ the RLS intensity of this system was remarkably enhanced mainly due to fine particles produced. A strong RLS signal was produced by the OFL-AV3B fine particles with large enough dimensions, which was proved by experiments discussed above.

Effect of the pH and buffers

As shown in Scheme 2, the acidity of the solution can change the molecule structure of OFL. Therefore, it can change the RLS spectra characteristics of the assay system. The effect of the solution acidity on the RLS intensity of the system is shown in Fig. 5. It can be seen that when the pH value was higher than 5.85, the RLS spectra characteristics of the system changed. Therefore, when the pH value was lower than 5.85, the RLS intensity was determined at 439.5 nm. When the pH value was higher than 5.85, the RLS intensity of the system was determined at 405 nm. As it depicted in Fig. 5, the RLS intensity of AV3B

Fig. 5 Effect of the pH on the RLS intensity of the OFL-AV3B system. Conditions: AV3B, 5.0×10^{-5} M; OFL, 1.0 $\mu\text{g/ml}$. (a) I_{RLS}^0 , (b) ΔI_{RLS} .

Fig. 6 Effect of the AV3B concentration on the RLS intensity of the OFL-AV3B system. Conditions: AV3B, 5.0×10^{-5} M; OFL, 1.0 $\mu\text{g/ml}$. (a) I_{RLS}^0 , (b) ΔI_{RLS} , pH 5.09, (c) I_{RLS}^0 , (d) ΔI_{RLS} , pH 6.90.

slightly changed with an acidity change. However, the RLS intensity of AV3B in the presence of OFL considerably changed with an acidity change. This phenomenon resulted from changing of the OFL molecule structure in the variational acidity solutions. The enhancement of the RLS intensity at 439.5 nm increased with increasing pH in a low pH level range, and reached its maximum in the pH range 4.23 – 5.85. With the pH value further increasing, the enhancement of the RLS intensity at 405.5 nm increased, and reached maximum value in the pH range 6.50 – 8.23. Henceforth, the enhancement of the RLS intensity decreased with increasing pH. Therefore, the optimum pH was selected, respectively, at 5.09 and 6.90 for further study. Under the optimum condition, the influence of different buffers, such as the Britton–Robinson buffer, hexamethylene tetramine–hydrogen chloride, and potassium biphthalate–hydrogen chloride were studied concerning the RLS signal of the determination systems. The results showed that the BR buffer was more beneficial to an enhancement of the RLS intensity. The BR buffer was employed for further study, and the optimum volume of the buffer was 1.0 ml.

Effect of AV3B concentration

The effect on the RLS intensity of different AV3B concentrations was studied, and the results are shown in Fig. 6. It can be seen that the RLS intensity gradually increased when the AV3B concentration increased. At pH 5.09, the maximum and stable RLS signal was obtained with the AV3B volume in the range of 0.6 – 1.4 ml. At pH 6.90, the maximum and stable RLS peak was obtained with the AV3B volume in the range of

Table 1 Effect of the potential foreign substances^a

No.	Foreign substance	Concentration/ $\mu\text{g ml}^{-1}$	Change in ΔI_{RLS} , %
1	L-Tyrosine	20.0	1.9
2	L-Glutamine	20.0	1.4
3	L-Aspartate	20.0	–0.8
4	L-Proline	20.0	–2.6
5	L-Cysteine	20.0	1.4
6	L-Leucine	20.0	–4.1
7	L-Lysine	20.0	–1.3
8	L-Histidine	20.0	–1.5
9	L-Phenylalanine	20.0	–0.7
10	ctDNA	30.0	2.2
11	yRNA	30.0	1.0
12	Protein, BSA ^b	30.0	–2.4
13	Protein, HSA ^c	30.0	–3.1
14	Fe^{3+} , Cl^-	20.0	2.5
15	K^+ , Cl^-	20.0	–1.8
16	Sn^{2+} , Cl^-	20.0	2.2
17	Ag^+ , NO_3^-	20.0	2.4
18	Pb^{2+} , NO_3^-	20.0	–2.1
19	Zn^{2+} , Cl^-	20.0	2.7
20	Ba^{2+} , Cl^-	20.0	–3.2
21	Na^+ , Cl^-	20.0	–1.6
22	Mg^{2+} , Cl^-	20.0	1.9
23	Ca^{2+} , Cl^-	20.0	3.3
24	Mn^{2+} , SO_4^{2-}	30.0	–1.7
25	Al^{3+} , SO_4^{2-}	30.0	2.9
26	Pb^{2+} , NO_3^-	30.0	–1.1
27	Cu^{2+} , NO_3^-	30.0	–1.6
28	Urea	60.0	2.2
29	Glucose	50.0	–2.5
30	Sucrose	50.0	–1.3
31	Lactose	50.0	–2.6
32	CTMAB ^d	30.0	–2.2

a. Conditions: OFL, 1.0 $\mu\text{g/ml}$; AV3B, 5.0×10^{-5} M.

b. Bovine serum albumin.

c. Human serum albumin.

d. Cetyltrimethylammonium bromide.

0.6 – 1.8 ml. In a further study, a 1.0-ml AV3B volume was employed both at pH 5.09 and 6.90.

Incubation time and stability

The stability of the reaction system was also studied by detecting the RLS intensity one time every 2 min at room temperature. The RLS intensity of OFL-AV3B reached a maximum for about 6 min, and remained stable for about 110 min. The result showed that AV3B reacted with OFL rapidly, and that this system has good stability at room temperature.

Interference of foreign substances on the RLS intensity

Under the optimum conditions of the general procedure, the OFL-AV3B system with the potential foreign substances was tested; the results are given in Table 1. Most amino acids, proteins, sugars and metal ions can be tolerated at high concentrations. Thus, this method has high selectivity and practicability for quantitative analysis in human serum samples.

Linear ranges and detection limits

According to the above standard procedure, calibration curves were constructed; the results were summarized in Table 2. The limits of detection were given by the equation $3S_0/K$, where 3 is the factor at the 99% confidence level, S_0 is the standard

Table 2 Analytical parameters of the assay^a

pH	$\lambda_{\max}/$ nm	Linear range/ $\mu\text{g ml}^{-1}$	Linear regression equation, $c/$ $\mu\text{g ml}^{-1}$	Detection limit, $3\sigma/$ $\mu\text{g ml}^{-1}$	Correlation coefficient, r
5.09	439.5	0.10–2.50	$Y = -3.68 + 49.72c$	0.013	0.9997 ($n = 9$)
6.90	405.0	0.05–3.00	$Y = 4.93 + 29.97c$	0.021	0.9994 ($n = 10$)

a. Condition: AV3B, 5.0×10^{-5} M.

Table 3 Comparison of various methods for the determination of OFL

Method	Detection limit, $3\sigma/\mu\text{g ml}^{-1}$	Ref.
RLS	0.013 ^a /0.021 ^b	This work
CE ^c	8.5	4
Flow-injection spectrophotometry	0.72 (2.0×10^{-6} M)	5
Flow-injection chemiluminescence	0.0398 (1.1×10^{-7} M)	7
Spectrofluorometry	0.14	8
HPLC	0.0625	10
Bioassay	0.125	10
HPLC-fluorometry	0.0252	11

a. b. Detection limit at pH 5.09 and 6.90, respectively.

c. Capillary electrophoresis.

deviation of the blank measurements ($n = 16$), and K is the slope of the calibration curve.

This method was compared with other reported methods applied to the determination of OFL in real samples; the results of the sensitivity are given in Table 3. The sensitivity of this method is much higher than that of spectrofluorometry. Also the detection limit by this method is nearly 5-times lower than that by HPLC, which has a very low detection limit. It indicated that the proposed method provided a sufficient sensitivity for the quantification of trace OFL in human serum. Therefore, this investigation will be valuable for studying OFL and conducting practical tests.

Analytical applications

Rate of recovery and precision. Under the optimal experimental conditions, the validity of the method was confirmed by applying the standard addition method technique to determination the recovery and precision of OFL in human serum. A volunteer was a healthy student from Shantou University; his heart, liver and kidney were examined and shown to be well before testing. And the volunteer can not take any medicines for 14 days before the testing day; 5 ml of blood was taken from the object and anticoagulated with heparin. The mixture was then centrifugated (20 min at 4000 rpm) to obtain serum, which was transferred into a 100-ml flask, and diluted to the mark with water and stored at 4°C. A suitable volume of the solution appropriately spiked with the standard OFL solution was taken, and three different samples with different OFL concentrations were obtained for analysis. The relative standard deviation (RSD) was calculated and is given in Table 4. The recovery levels of the method were close to 100% in all cases and the RSD was just between 1.0 and 4.2%.

Analytically, the proposed method with high accuracy and good precision are suitable for a trace determination of OFL in human serum.

Measurement of real samples. Under the optimal experimental

Table 4 Day-to-day and within-day precision and accuracy study for determination of OFL

Added/ $\mu\text{g ml}^{-1}$	Day-to-day ($n = 5$)			Within-day ($n = 5$)		
	Found $\pm$ SD/ $\mu\text{g ml}^{-1}$	RSD	Rec., %	Found $\pm$ SD/ $\mu\text{g ml}^{-1}$	RSD	Rec., %
pH 5.09						
0.50	0.50 ± 0.01	2.0	100.0	0.48 ± 0.02	4.1	96.0
1.50	1.47 ± 0.05	3.4	98.0	1.47 ± 0.03	2.0	98.0
2.00	1.93 ± 0.04	2.1	96.5	1.94 ± 0.05	2.6	97.0
pH 6.90						
0.50	0.48 ± 0.02	4.2	96.0	0.48 ± 0.01	2.1	96.0
1.50	1.51 ± 0.04	2.6	100.7	1.47 ± 0.05	3.4	98.0
2.00	1.98 ± 0.03	1.5	99.0	1.97 ± 0.04	1.0	98.5

Table 5 Results for the determination of OFL in human serum

Added/ $\mu\text{g ml}^{-1}$	Found $\pm$ SD/ $\mu\text{g ml}^{-1}$	RSD	Recovery, % ($n = 5$)
0	0.98 ± 0.04	4.1	—
1.50	2.41 ± 0.07	2.9	97.2
0.50	1.46 ± 0.03	2.1	98.6

conditions, the proposed method was carried out for the determination of the OFL in serum samples. The unit dose drug was injected into a vein of a volunteer at a constant velocity for about half an hour. Four hours after using medicine, 5 ml blood was taken from the object and analyzed following the procedure described in section *Rate of recovery and precision*. The obtained results are given in Table 5. This method was applied to analysis of OFL in the human serum with satisfactory results.

Conclusion

A novel, sensitive, simple, practical and selective method for the determination of trace amounts of OFL has been developed. It is based on a remarkable enhancement of the RLS intensity, which results from the reaction between OFL and AV3B, and large particles are produced. Under the optimum condition, the determinations of all systems, including the real samples, were investigated and satisfactory results were obtained. The simplicity of the procedure was just applied on a common spectrophotofluorometer. The reagent AV3B was very cheap. Compared to other methods, the sensitivity of the proposed method was much higher, and the detection limits reached to 0.013 $\mu\text{g/ml}$ at pH 5.09 and 0.021 $\mu\text{g/ml}$ at pH 6.90, respectively. In addition, as for the tolerance of foreign substances, the proposed method supplied a high selectivity for the determinations of real samples. Therefore, the present study indicates that the RLS technique is a promising and practical tool for developing a quantitative method in biochemistry analytical and clinical fields.

Acknowledgements

All of the authors express their sincere thanks for support from the National High Technology Research and Development Program (863) Foundation of China (No. 2003AA2Z3162) and the Municipal Science Foundation of Shantou (No. S2002023).

References

1. N. Ichihara, H. Tachizawa, M. Tsumura, T. Une, and K. Sato, *Chemotherapy*, **1984**, 32, 118.
 2. K. Sato, U. Matsuura, M. Inone, T. Ueno, Y. Osada, H. Ogawa, and M. Mitsuhashi, *Antimicrob. Agents Chemother.*, **1982**, 22, 548.
 3. J. A. Murillo, A. Alañón Molina, A. Muñoz de la Peña, I. Durán Merás, and A. Jiménez Girón, *J. Fluoresc.*, **2007**, 17, 481.
 4. S. S. Zhang, H. X. Liu, Y. J. Wu, and C. L. Yu, *Analyst*, **2001**, 126, 441.
 5. M. Soledad García, M. Isabel Alberro, C. Sánchez-Pedreño, and M. S. Abuherba, *Eur. J. Pharm. Biopharm.*, **2005**, 61, 87.
 6. H. W. Sun, L. Q. Li, and X. Y. Chen, *Anal. Sci.*, **2006**, 22, 1145.
 7. Y. D. Liang, J. F. Song, and X. F. Yang, *Anal. Chim. Acta*, **2004**, 510, 21.
 8. O. Ballesteros, J. L. Vilchez, and A. Navalón, *J. Pharm. Biomed. Anal.*, **2002**, 30, 1103.
 9. S. Thangadurai, S. K. Shukla, and Y. Anjaneyulu, *Anal. Sci.*, **2002**, 18, 97.
 10. L. R. Zhang, N. N. Cheng, B. Y. Chen, and Y. M. Wang, *Chin. J. New Drugs Clinic Remed.*, **2003**, 22, 201.
 11. A. Espinosa Mansilla, A. Muñoz de la Peña, D. González Gómez, and F. Salinas López, *Talanta*, **2006**, 68, 1215.
 12. C. Z. Huang and Y. F. Li, *Anal. Chim. Acta*, **2003**, 500, 105.
 13. Y. F. Li, C. Z. Huang, and X. L. Hu, *Chin. J. Anal. Chem.*, **1998**, 26, 1508.
 14. R. F. Pasternack, C. Bustamante, P. J. Collings, A. Giannetto, and E. J. Gibbs, *J. Am. Chem. Soc.*, **1993**, 115, 5393.
 15. R. F. Pasternack, K. F. Schaefer, and P. Hambright, *Inorg. Chem.*, **1994**, 33, 2062.
 16. R. F. Pasternack and P. J. Collings, *Science*, **1995**, 269, 935.
 17. C. Z. Huang, K. A. Li, and S. Y. Tong, *Anal. Chem.*, **1996**, 68, 2259.
 18. C. Z. Huang, K. A. Li, and S. Y. Tong, *Anal. Chem.*, **1997**, 69, 514.
 19. J. Ling, C. Z. Huang, Y. F. Li, Y. F. Long, and Q. G. Liao, *Appl. Spectrosc. Rev.*, **2007**, 42, 177.
 20. W. Lu, B. S. Fernández Band, Y. Yu, Q. G. Li, J. C. Shang, C. Wang, Y. Fang, R. Tian, L. P. Zhou, L. L. Sun, Y. Tang, S. H. Jing, W. Huang, and J. P. Zhang, *Microchim. Acta*, **2007**, 158, 29.
 21. P. Bao, A. G. Frutos, C. Greef, J. Lahiri, U. Muller, T. C. Peterson, L. Warden, and X. Y. Xie, *Anal. Chem.*, **2002**, 74, 1792.
 22. Z. G. Chen, W. F. Ding, F. L. Ren, J. B. Liu, and Y. Z. Liang, *Anal. Chim. Acta*, **2005**, 550, 204.
 23. Z. G. Chen, W. F. Ding, F. L. Ren, Y. L. Han, and J. B. Liu, *Anal. Lett.*, **2005**, 38, 2301.
 24. Z. G. Chen, T. Y. Zhang, X. M. Chen, Y. L. Han, and L. Zhu, *Microchim. Acta*, **2007**, 157, 107.
 25. S. P. Liu, R. Yang, and Q. Liu, *Anal. Sci.*, **2001**, 17, 243.
 26. Z. G. Chen, J. B. Liu, Y. Z. Liang, and F. L. Ren, *J. Biomol. Screen.*, **2006**, 11, 400.
 27. Z. G. Chen, T. Y. Zhang, F. L. Ren, and W. F. Ding, *Microchim. Acta*, **2006**, 153, 65.
 28. Z. G. Chen, J. B. Liu, and Y. L. Han, *Talanta*, **2007**, 71, 1246.
 29. Z. G. Chen, J. B. Liu, T. Y. Zhang, and Y. L. Han, *Anal. Sci.*, **2007**, 23, 1305.
 30. Z. G. Chen, J. B. Liu, Y. L. Han, and L. Zhu, *Anal. Chim. Acta*, **2006**, 570, 109.
 31. S. P. Liu, Z. F. Liu, and C. Z. Huang, *Anal. Sci.*, **1998**, 14, 799.
 32. Z. G. Chen, Y. R. Peng, J. H. Chen, and L. Zhu, *J. Pharm. Biomed. Anal.*, **2008**, 48, 946.
-