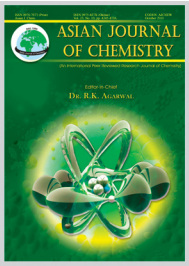
Asian Journal of Chemistry; Vol. 24, No. 8 (2012), 3715-3718

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



L-Cysteine-capped CdTe Quantum Dots Based Fluorescence Quenching Method for Determination of Chloroamphenicol

YONGQIANG MAO[1,2], NA LI[2], YE HAN[1], BAOAN NING[3], ZHIXIAN GAO[3,*] and ZHIJIANG ZHOU[1,*]

[1]School of Chemical Engineering and Technology, Tianjin University, Tianjin, P.R. China
[2]College of Science, Liaoning Technical University, Fuxin, P.R. China
[3]Tianjin Key Laboratory of Risk Assessment and Control Technology for Environment and Food Safety, Institute of Health and Environmental Medicine, Tianjin, P.R. China

*Corresponding authors: E-mail: zzjtju@126.com; gaozhx@126.com

(*Received*: 14 September 2011; *Accepted*: 23 March 2012) AJC-11223

L-Cysteine-capped CdTe quantum dots (QDs) with a narrow, symmetric emission spectrum was synthesized in aqueous medium. Based on the fluorescence quenching of CdTe quantum dots, a simple, rapid and sensitive method was developed for the quantitative determination of chloroamphenicol. Under optimum conditions, the calibration plot of the relative fluorescence intensity with the concentration of chloroamphenicol was linear in the range from 5 to 40 μg mL^{-1} with the correlation coefficient of 0.9963. The limit of detection was 50 ng mL^{-1}. The content of chloroamphenicol in pharmaceutical tablet were determined by the proposed method and the results agreed with the claimed value. In addition, the possible fluorescence quenching mechanism was also discussed.

Key Words: CdTe Quantum dots, Chloroamphenicol, Determination, Fluorescence quenching.

INTRODUCTION

Chloramphenicol (CAP) is an effective broad-spectrum antibiotic widely used in veterinary practices for the prevention and treatment of various infections[1,2]. However, recent research demonstrated that chloramphenicol might lead to serious toxic side effects on humans and animals, such as bone marrow depression, aplastic anemia, hypoplastic anemia and thrombocytopenia[3]. Therefore, the clinical use of chloramphenicol was generally restricted to the treatment of serious infections. In addition, most countries, including China, the United States of America and European Union countries, have strictly prohibited the use of chloramphenicol in food-producing animals. Nevertheless, due to its low cost and steady antibiosis effectiveness, chloramphenicol was illegally used in livestock production. Thus, it is important to develop a sensitive method for determining chloramphenicol in the areas of clinical medical assay, nutrition and pharmaceutical formulations. Up to date, many analytical methods such as microbiological assay[4], immunological assay[5], enzymatic assay[6], chromatographic assay[7] and biosensor and microarray techniques[8,9] was reported for effectively monitoring chloramphenicol. However, the above methods involved complicated and time-consuming pretreatment procedures and required expensive instruments or special apparatus.

Quantum dots (QDs) have attracted increaseing interest in the fields of biolabeling and bioimaging in the past decades[10,11]. Compared with traditional organic dyes and fluorescence proteins, quantum dots exhibits broad excitation wavelength range, narrow emission spectrum, size-tunable emission peak, high quantum yields and stability against photobleaching[12]. As a kind of fluorescent probes, quantum dots was widely used in biology and medicine, especially in analytical chemistry. It was found that Pb^{2+}, Cu^{2+}, Ag^{+} and Hg^{2+} could dramatically affect the fluorescence intensity of quantum dots and the methods for the determination of these inorganic cations were investigated[13-16]. Furthermore, based on the fluorescence quenching or enhancement methods, quantum dots was also applied in the quantitative determination of pharmaceutical molecules, such as ciprofloxacin, edaravone, methimazole and berberine[17-20]. To our best of knowledge, the method was not applied to the quantitative analysis of chloramphenicol so far.

In the present paper, we investigated the interaction between CdTe quantum dots and chloramphenicol. The results showed that the fluorescence intensity of CdTe quantum dots was quenched in the presence of chloramphenicol. The quenched fluorescence intensity of CdTe quantum dots was linearly proportional to the concentration of chloramphenicol. Based on this observation, a novel method for the determination

of chloramphenicol was developed. The method was applied to the determination of chloramphenicol in commercial tablets and satisfactory results were obtained. In addition, the possible mechanism of reaction was also discussed.

EXPERIMENTAL

Tellurium powder, $CdCl_2 \cdot 2.5H_2O$, L-cysteine and $NaBH_4$ were purchased from Tianjin No.1 Chemical Reagent Plant. Chloroamphenicol was obtained from Sigma Chemical Co. Chloroamphenicol tablets were bought from local drug store. All the other chemicals used were of analytical reagents grade. Double deionized water was used throughout the experiment.

The UV-VIS absorption spectrum was acquired on a Hitachi U-3010 Spectrophotometer. All fluorescence measurements and the syschronous fluorescence spectra were made with a Hitachi F-4500 fluorescence spectrophotometer. Transmission electron microscopy (TEM) images of the quantum dots were performed on a JEOL-2010 transmission electron microscope. The pH values were measured with a PHS-3C meter.

Synthesis of L-cysteine-capped CdTe quantum dots: The L-cysteine-capped CdTe quantum dots colloidal solution was prepared according to the reported method with some slight modifications[21]. The molar ratio of Cd^{2+}: NaHTe: L-cysteine was set at 1: 0.5: 2.4. Briefly, 1.2×10^{-4} mol L^{-1} of fresh NaHTe aqueous solution, which was prepared by reaction of 160 mg Te powder and 100 mg $NaBH_4$ in 2 mL double deionized water at 0 ºC for 8 h, was injected into 200 mL oxygen-free aqueous solution containing 6.0×10^{-3} mol L^{-1} of L-cysteine and 2.5×10^{-3} mol L^{-1} of $CdCl_2 \cdot 2.5H_2O$ at pH 11.0. Then the mixture solution was heated and further refluxed until the colour of solution changed from pale yellow to deep red. The crude solution was precipitated by acetone and centrifuged to remove excess precursors. Finally, the resultant precipitate were redispersed in double deionized water and then kept in dark at 4 ºC for further use.

Analytical procedures: 400 µL 0.1 mol L^{-1} *Tris*-HCl buffer solution, 100 µL 3.6×10^{-4} mol L^{-1} CdTe quantum dots solution and various amounts of the chloramphenicol standard solution or sample solution were sequentially added into a series of 5.0 mL calibrated test tubes, then diluted to the mark with double deionized water. The mixture was mixed thoroughly and equilibrated waiting for 20 min. The fluorescence intensity was measured with the excitation wavelengths of 350 nm and the emission wavelengths of 554 nm. Both excitation and emission were performed with a slit width of 5 nm.

RESULTS AND DISCUSSION

Characterization of CdTe quantum dots: Fig. 1 showed the UV-VIS absorption spectrum of CdTe quantum dots with a shoulder centered at 495 nm. In addition, the maximum emission appeared at 554 nm. Moreover, as shown in Fig. 2, the average size of CdTe quantum dots was 5.1 nm, which revealed much less agglomerated spherical particles.

Effect of the CdTe quantum dots concentration: It was well known that the concentration of quantum dots affected not only the fluorescence intensity but also the sensitivity of the assay[22]. When the concentration of CdTe quantum dots was too high, the changes of the fluorescence intensity was limited as well as low sensitivity. However, if the concentration of CdTe quantum dots was too low, the fluorescence intensity changed significantly, which might result in the narrow linear range. Taking these factors into account, 7.2×10^{-6} mol L^{-1} of CdTe quantum dots solution was recommended.

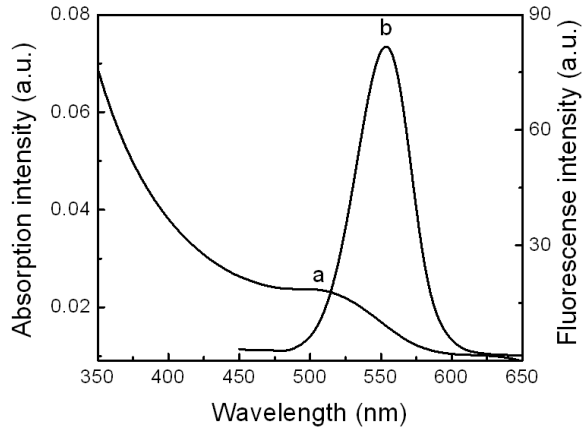


Fig. 1. UV-VIS absorption (a) and fluorescence (b) spectra of CdTe quantum dots

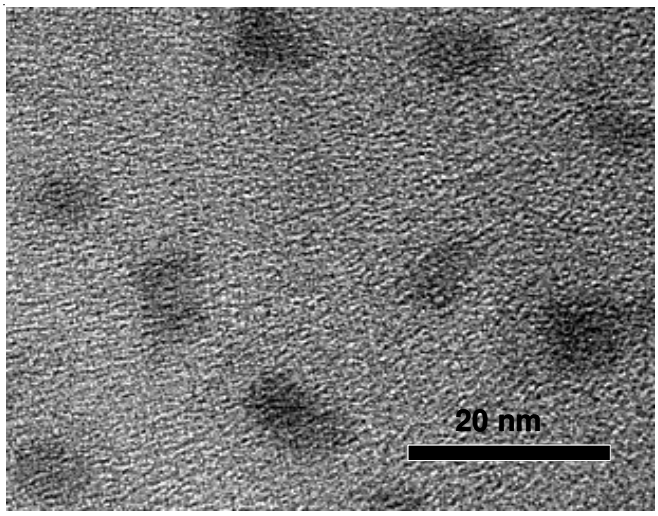


Fig. 2. TEM image of CdTe quantum dots scale bar is 20 nm

Effect of the buffer pH: The effects of different pH buffers on the fluorescence intensity of CdTe quantum dots was shown in Fig. 3. It was observed that the fluorescence intensity reached a maximum value in the pH range of 7. With a further decrease in pH, the fluorescence intensity decreased dramatically. Therefore, pH 7 was suitable for application in the following assay.

Effect of reaction time: According to initial experiments, the reaction between CdTe quantum dots and chloramphenicol was slow. As shown in Fig. 4, the relative fluorescence intensity (F_0/F) reached equilibrium within 20 min and the signals remained stable for more than 30 min. Hence, the fluorescence intensity were recorded after reaction for 20 min.

Calibration and sensitivity: The emission spectra of CdTe quantum dots in the absence and presence of various concentrations of chloramphenicol were recorded. The result

TABLE-1
EFFECT OF COEXISTING SUBSTANCES ON THE FLUORESCENCE INTENSITY OF CdTe QUANTUM DOTS[a]

Coexited substance	Coexited concentration (μg mL^{-1})	Change of fluorescence intensity (%)	Coexited substance	Coexited concentration (μg mL^{-1})	Change of fluorescence intensity (%)
Starch	Saturated solution	2.14	Ba^{2+}, NO_3^-	10	2.14
Magnesium stearate	Saturated solution	0.62	Mn^{2+}, Cl^-	10	2.19
Glucose	10	2.96	Fe^{3+}, NO_3^-	5	2.92
Sucrose	50	2.99	Al^{3+}, Cl^-	5	4.25
Lactose	10	1.34	Zn^{2+}, SO_4^{2-}	5	2.58
Citric acid	10	1.41	Mg^{2+}, Cl^-	5	3.50
Sorbitol	10	2.91	Ni^{2+}, SO_4^{2-}	5	3.92
Phthalic acid	10	1.48	Co^{2+}, Cl^-	5	4.50
Mannitol	10	2.63	Fe^{2+}, Cl^-	5	3.89
SDS	10	2.44	Pb^{2+}, CH_3COO^-	0.01	1.47
K^+, Cl^-	100	2.19	Ag^+, NO_3^-	0.01	1.34
Na^+, Cl^-	100	2.20	Cu^{2+}, SO_4^{2-}	0.01	1.90
Ca^{2+}, Cl^-	10	2.34	Hg^{2+}, Cl^-	0.01	3.31

[a]CAP, 25.0 μg mL^{-1}; CdTe QDs, 7.2×10^{-6} mol L^{-1}; pH 7.0

was shown in Fig. 5. With increasing the concentrations of chloramphenicol, the fluorescence intensity of CdTe quantum dots decreased significantly. Under optimum conditions, the quenching effect of chloramphenicol on the fluorescence intensity of CdTe quantum dots could be described by equation: $F_0/F = 0.01345C_{CAP} + 0.99077$. Where F_0 and F represent the fluorescence intensity of the CdTe quantum dots in the absence and presence of chloramphenicol, respectively and C_{CAP} is the concentration of chloramphenicol (μg mL^{-1}). A good linear relationship between F_0/F and the concentration of chloramphenicol ranging from 5 to 50 μg mL^{-1} was observed with the correlation coefficient of 0.9963. The detection limit of chloramphenicol was 50 ng mL^{-1}. The standard deviation for six replicate measurements of solution containing 25 μg mL^{-1} chloramphenicol was 1.4 %.

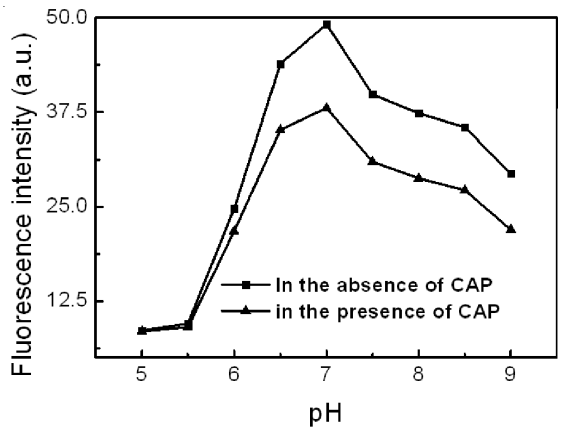


Fig. 3. Effect of the buffer pH on the fluorescence intensity of CdTe quantum dots. CdTe quantum dots, 7.2×10^{-6} mol L^{-1}; chloramphenicol, 25.0 μg mL^{-1}

Effect of coexisting foreign substances: Many compounds have potential to quench the fluorescence signal of quantum dots[23]. In order to further assess the possibility of practical application in determination of pharmaceutical preparation, the interference from some metal ions and excipients contained in tablets was tested. As shown in Table-1, most of the tested substances could be allowed at relatively high concentrations. Whereas, some heavy metal ions, such as Hg^{2+}, Ag^+, Pb^{2+} and Cu^{2+} could be tolerated at relatively low concentration levels. In fact, their contents in drugs and pharmaceutical excipients are strictly limited, so that they could hardly cause any interference to the fluorescence intensity.

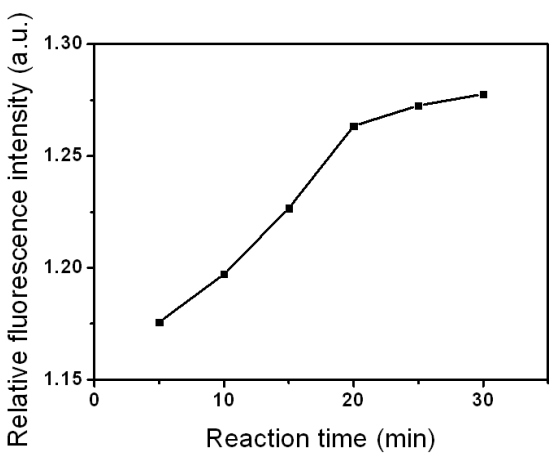


Fig. 4. Effect of reaction time on the relative fluorescence intensity (F_0/F) of CdTe quantum dots. CdTe quantum dots, 7.2×10^{-6} mol L^{-1}; chloram-phenicol, 25.0 μg mL^{-1}

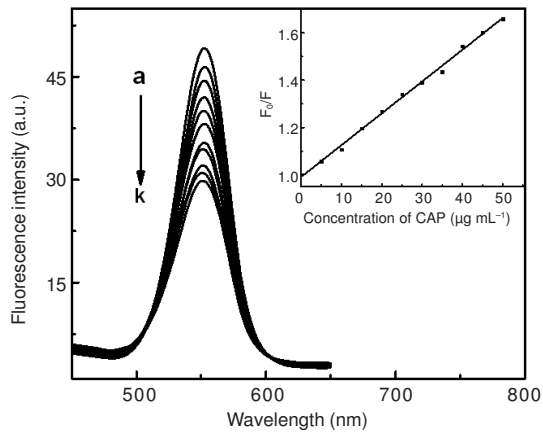


Fig. 5. Fluorescence spectra of CdTe in absence and presence of various concentrations of chloramphenicol. Chloramphenicol concentration from (a) to (k): 0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0 and 50.0 μg mL^{-1}; CdTe quantum dots, 7.2×10^{-6} mol L^{-1}. Inset: The linear regression curves of chloramphenicol concentration dependence of the relative fluorescence intensity (F_0/F) of CdTe quantum dots

TABLE-2
DETERMINATION OF CHLORAMPHENICOL IN THE COMMERCIAL CHLORAMPHENICOL TABLETS

Sample	Labeled values [a] (μg mL^{-1})	CAP found [b] (μg mL^{-1})	R.S.D.% (n = 6)	CAP added (μg mL^{-1})	Total found [b] (μg mL^{-1})	Recovery (%)
1	5	4.9	1.6	10	15.6	104.0
2	50	49.6	1.5	10	57.5	95.8

[a]Based on the content of chloramphenicol given by the manufacturer; [b]Mean of six determinations, CAP = Chloramphenicol.

Analytical applications: To investigate the possibility of practical application, chloramphenicol in commercial chloramphenicol tablets and chloramphenicol tablets with adding a known concentration of chloramphenicol were directly determined by the present method. The results were listed in Table-2. A satisfactory recovery of 95.8-104.0 % were obtained, suggesting that the proposed method was reliable and practical.

Quenching mechanism: To elucidate the interaction between CdTe quantum dots and chloramphenicol, the UV-VIS absorption and synchronous fluorescence spectra of CdTe quantum dots were investigated in the absence and presence of chloramphenicol. Fig. 6 showed that no conspicuous absorption band was observed in the wavelength range from 400 to 700 nm for chloramphenicol, so the quenching effect of chloramphenicol on the fluorescence of CdTe quantum dots was not attributed to an inner filter resulting from the absorption of the emission wavelength by chloramphenicol. In addition, it could be observed in Fig. 7 that the presence of chloramphenicol slightly enhanced the fluorescence signal of CdTe quantum dots in the 300-600 nm wavelength range, which implied that the CdTe quantum dots went through an aggregation process and little larger particles formed in the solution[24].

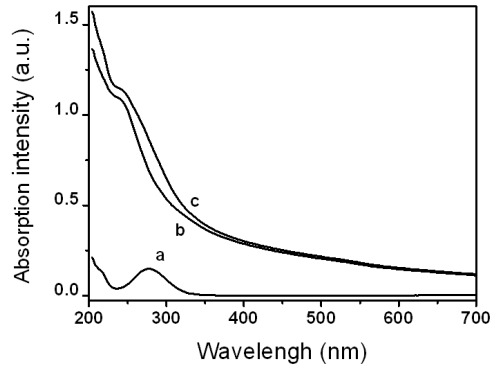


Fig. 6. UV-VIS absorption spectra of chloramphenicol (a), CdTe quantum dots (b) and CdTe quantum dots adding chloramphenicol (c)

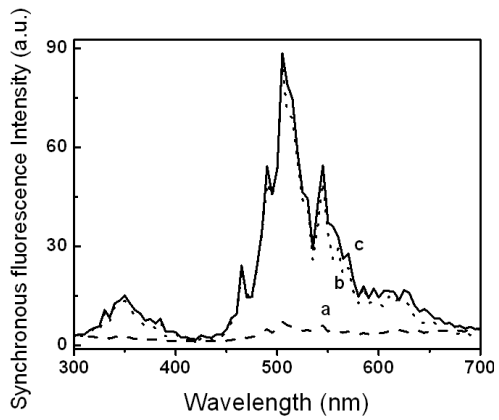


Fig. 7. Synchronous fluorescence spectra of chloramphenicol (CAP) (a), CdTe quantum dots (QDs) (b) and CdTe QDs adding CAP (c)

Conclusion

In summary, a simple, rapid, sensitive and selective method for the determination of chloramphenicol was established based on the fluorescence quenching of CdTe quantum dots. Under optimum conditions, a good linear relationship between the fluorescence intensity of CdTe quantum dots and the concentration of chloramphenicol in the range of 5-40 μg mL^{-1} was achieved and the limit of detection was 50 ng mL^{-1}. The concentration of chloramphenicol in the commercial chloramphenicol tablets was detected by the proposed method and the results were consistent with the claimed value. The quenching mechanism might be due to little large particles formed in the solution when chloramphenicol was added.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the financial supports by Key Project in the National Science and Technology Pillar Program (2009BADB9B03), Tianjin Applied Basic and Cutting-edge Technology Reaserch Program (09JCZDJC17700) and Science and Technology Program of Tianjin (09ZCKFSH02700).

REFERENCES

1. K. Vivekanandan, M.G. Swamy, S. Prasad and R. Mukherjee, *Rapid Commun. Mass Spectrom.*, **19**, 3025 (2005).
2. L. Wang, Y. Zhang, X. Gao, Z.J. Duan and S. Wang, *J. Agric. Food Chem.*, **58**, 3265 (2010).
3. P. Mottier, V. Parisod, E. Gremaud, P.A. Guy and R.H. Stadler, *J. Chromatogr. A*, **994**, 75 (2003).
4. J.L. Thomas, P.T. Francis, G.B. John and L.G. Sherwood, *Antimicrob. Agents Chemother.*, **9**, 874 (1976).
5. G. Scortichini, L. Annunziata, M.N. Haouet, F. Benedetti, I. Krusteva and R. Galarini, *Anal. Chim. Acta*, **535**, 43 (2005).
6. S. Yamato, H. Sugihara and K. Shimada, *Chem. Pharm. Bull.*, **38**, 2290 (1990).
7. J.Z. Shen, X. Xia, H.Y. Jiang, C. Li, J.C. Li, X.W. Li and S.Y. Ding, *J. Chromatogr. B*, **877**, 1523 (2009).
8. T. Fodey, G. Murilla, A. Cannavan and C. Elliott, *Anal. Chim. Acta*, **592**, 51 (2007).
9. P. Zuo and B.C. Ye, *J. Agric. Food Chem.*, **54**, 6978 (2006).
10. W.C.W. Chan and S.M. Nie, *Science*, **281**, 2016 (1998).
11. J.K. Jaiswal, H. Mattoussi, J.M. Mauro and S.M. Simon, *Nat. Biotechnol.*, **21**, 47 (2002).
12. X. Michalet, F.F. Pinaud, L.A. Bentolila, J.M. Tsay, S. Doose, J.J. Li, G. Sundaresan, A.M. Wu, S.S. Gambhir and S. Weiss, *Science*, **307**, 538 (2005).
13. H.M. Wu, J.G. Liang and H.Y. Han, *Microchim. Acta*, **161**, 81 (2008).
14. Y.S. Xia and C.Q. Zhu, *Analyst*, **133**, 928 (2008).
15. J. Wang, J.G. Liang, Z.H. Sheng and H.Y. Han, *Microchim. Acta*, **167**, 281 (2009).
16. J.L. Chen, Y.C. Gao, Z.B. Xu, G.H. Wu, Y.C. Chen and C.Q. Zhu, *Anal. Chim. Acta*, **577**, 77 (2006).
17. P. Liao, Z.Y. Yan, Z.J. Xu and X. Sun, *Spectrochim. Acta A*, **72**, 1066 (2009).
18. D. Li, Z.Y. Yan and W.Q. Cheng, *Spectrochim. Acta A*, **71**, 1204 (2008).
19. F. Dong, K.W. Hu, H.Y. Han and J.G. Liang, *Microchim. Acta*, **165**, 195 (2009).
20. M. Cao, M.G. Liu, C. Cao, Y.S. Xia, L.J. Bao, Y.Q. Jin, S. Yang and C.Q. Zhu, *Spectrochim. Acta A*, **75**, 1043 (2010).
21. N. Gaponik, D.V. Talapin, A.L. Rogach, K. Hoppe, E.V. Shevchenko, A. Kornowski, A. Eychmüller and H. Weller, *J. Phys. Chem. B*, **106**, 7177 (2002).
22. Y.Q. Wang, C. Ye, Z.H. Zhu and Y.Z. Hu, *Anal. Chim. Acta*, **610**, 50 (2008).
23. A.Y. Nazzal, L.H. Qu, X.G. Peng and M. Xiao, *Nano. Lett.*, **3**, 819 (2003).
24. C.Z. Huang and Y.F. Li, *Anal. Chim. Acta*, **500**, 105 (2003).