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ORIGINAL PAPER

Determination of Paracetamol Based on its Quenching Effect on the Photoluminescence of CdTe Fluorescence Probes

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Abstract L-Cysteine capped CdTe nanoparticles (NPs) were synthesized in aqueous medium, and their application as fluorescence probes in the determination of paracetamol was studied. The L-cysteine capped CdTe NPs were characterized by transmission electron microscopy, X-ray diffraction spectrometry, spectrofluorometry, ultraviolet-visible and Fourier transform infrared spectrometry. Based on the distinct fluorescence quenching of CdTe fluorescence probes in the presence of paracetamol, a simple, rapid and specific method for paracetamol determination was presented. Under optimum conditions, the relative fluorescence intensity of CdTe NPs was linearly proportional to paracetamol concentration from 1.0×10^{-8} mol/L to 1.6×10^{-7} mol/L with a detection limit of 4.2×10^{-9} mol/L. The proposed method was applied to detect paracetamol in commercial tablets with satisfactory results.

Keywords CdTe nanoparticles · Fluorescence probe · Paracetamol

Introduction

Paracetamol (PAR) or acetaminophen is a commonly used analgesic and antipyretic drug [1]. PAR products give relief for all kinds of mild to moderate pain, including migraine headache, muscular aches, backache, neuralgia, rheumatic pains, pains from minor injuries and all the everyday aches of normal life [2–4]. It is also used for the reduction of fever of bacterial or viral origin [4]. Generally, PAR does not exhibit any harmful side effects, but at higher doses in few cases it will lead to the formation of some liver and nephrotoxic metabolites [5]. Given that PAR is being used increasingly for therapeutic purposes, its quantitative analysis is of vital importance.

Several analytical techniques have been employed for the determination of PAR, such as thin layer chromatography [6], high performance liquid chromatography [7], liquid chromatography tandem mass spectroscopy [8, 9], capillary electrophoresis [10], electrochemical analysis [4, 11], chemiluminescence [12], spectrophotometry [13], Raman spectrometry and Fourier transform infrared spectrometry [14]. However, these methods have several drawbacks such as environmentally unfriendly solvents, requirement for sample pretreatment, expensive instrumentation and in some cases long analysis time that makes them unsuitable for routine analysis [4, 15]. Compared with the above-mentioned methods, the fluorescence probes method appears to be a promising alternative for PAR detection because of its appealing advantages including simple and fast experimental process, low cost, easy operation, and high sensitivity for different fluorescent emission wavelengths corresponding to different molecular structures [16]. In addition, to our knowledge, there is no report on the analytical application of fluorescence probes to the determination of PAR up to now.

Semiconductor nanoparticles (NPs), also named quantum dots (QDs) or semiconductor nanocrystals, exhibit some unique optical and electronic properties based on the strong confinement of excited electrons and their corresponding holes within their structures [17]. Compared with traditional organic dyes, QDs have broad excitation wavelength range, narrow and symmetrical emission spectrum, size-tunable emission peak, longer fluorescence

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lifetime, high photobleaching threshold and excellent photostability [18–20]. Since the first application of QDs in biological system demonstrated by Nie's and Alivisatos's groups independently in 1998, the use of QDs as optical probes in analytical field has been extensively exploited [21]. It relies on changes of the fluorescence intensity when the target analyte interacts with the surface of QDs. Interactions occurring at the surface of QDs influence the efficiency of the radiative recombination, leading to either photoluminescence activation or quenching [22]. The achievements of QDs study also drive us to fabricate fluorescence probes for analytical use.

In the present paper, a simple and sensitive assay of PAR was proposed based on the quenching of the fluorescence intensity of L-cysteine capped CdTe NPs in aqueous solution. The CdTe fluorescence probes were prepared through a straightforward process by using low-cost inorganic salts as precursors and safe L-cysteine as the stabilizer. Under the optimum conditions, the relative fluorescence intensity versus PAR concentrations gave a linear response according to Stern–Volmer equation. Interference tests showed that some foreign substances, such as most ions and biomolecules had little interference. The method has been applied to the determination of PAR in real samples and satisfactory results were obtained.

Experimental Section

Reagents and Solution

Tellurium powder (99.999%), sodium borohydride (96%), $CdCl_2 \cdot 2.5H_2O$ (analytical purity), L-cysteine (analytical purity), paracetamol (analytical purity), and other routine chemicals were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd. (China) and used without any further purification. Doubly distilled water (DDW) was used throughout the work. Fen An Ka Min tablets purchased from local drugstore were ground to powder, dissolved in DDW, and filtered for further use. The phosphoric buffer solution (PBS) was prepared by adjusting 0.1 mol/L K_2HPO_4, KH_2PO_4, H_3PO_4 or NaOH accordingly.

Apparatus

JEOL 200 CX transmission electron microscope (TEM) was used to observe the appearance and size of nanoparticles. X-ray powder diffraction (XRD) spectra were taken on a rigaku D/max 2550 X-ray diffractometer with Cu Kα radiation (λ=0.15418 nm). The absorption spectrum was acquired on an UV-2501PC spectrometer. Fourier transform infrared (FTIR) spectra were recorded on an AVATAR 370 spectrometer. All fluorescence measurements were made with RF-5301PC spectrofluorometer equipped with a 1 cm quartz cell. The pH values were measured with a pHS-3C pH meter. All optical measurements were carried out at room temperature under ambient conditions.

Synthesis of Water-Soluble L-Cysteine Capped CdTe Fluorescence Probes

The L-cysteine modified CdTe NPs were directly synthesized in aqueous solution according to the method [23] described previously with some minor modifications. Briefly, 91.3 mg $CdCl_2 \cdot 2.5H_2O$ was dissolved in 100 mL DDW in the presence of 121.2 mg L-cysteine as the stabilizing agent. Then the pH of the solution was adjusted to 11.2 by stepwise addition of 1.0 mol/L NaOH solution, and the solution was N_2-saturated for 30 min. Subsequently, the fresh NaHTe solution, prepared previously by dissolving 10.2 mg Te powder and 80 mg $NaBH_4$ in 5 mL oxygen-free DDW, was quickly injected into the flask under vigorous stirring. The reaction between Cd^{2+} and NaHTe took place immediately upon the injection of NaHTe solution. The molar ratio of Cd^{2+} : Te^{2-}: L-cysteine was set at 1: 0.2: 2.5 in our experiment. The resulting mixture was heated at 95 °C and further refluxed for 20 min to promote the growth of CdTe NPs. Monodisperse CdTe NPs were purified by precipitation, centrifugation, decantation, and re-dispersed in DDW before keeping in the dark for further use.

Determination of Paracetamol with L-Cysteine Capped CdTe Fluorescence Probes

To determine the concentrations of PAR, a series of solutions prepared as follows were used to monitor the change of the relative fluorescence intensity by means of spectrofluorometer. A set of 25 mL calibrated flasks were successively loaded with 500 μL of L-cysteine capped CdTe NPs stock solution and different amounts of freshly prepared PAR standard solutions, then the resulting solutions were diluted to the mark with PBS and mixed thoroughly. Ten minutes later, the fluorescence spectra were measured and recorded, respectively. Excitation was performed with a slit width of 5 nm and a wavelength of 330 nm, and the emission slit was set at 5 nm.

Results and Discussion

Characterization of L-Cysteine Capped CdTe Fluorescence Probes

The morphology and structure of L-cysteine capped CdTe NPs were characterized by TEM and XRD. As

shown in Fig. 1a, the CdTe NPs are irregularly spherical and dispersed well in aqueous solution. As the linkages between the NPs are visible, it indicates that the L-cysteine has already modified the surface of NPs, because L-cysteine is a multifunctional ligand, which can afford thiol group to strongly bond Cd^{2+} on the NPs surface by chemical bond, besides, hydrophilic amine and carboxyl group could serve as the points of hydrogen bond attachment to each other or the PAR molecules [24]. The XRD spectrum (Fig. 1b) which scans over the 2 theta (θ) range of 10–80° shows diffractive peaks at 23.67° and 42.70°, corresponding to the crystal planes 002 and 103, confirming that it has a hexagonal wurtzite crystalline structure.

The optical properties of L-cysteine capped CdTe NPs were also characterized by the UV–Vis and fluorescence spectra (Fig. 2). It can be seen that the CdTe fluorescence probes have a wide range of absorption with a shoulder centers at about 490 nm. The line width of the fluorescence spectrum is relatively narrow, and the maximum emission wavelength is 551 nm.

To identify the conjugation mode between L-cysteine and CdTe NPs, the FTIR spectra of pure L-cysteine and L-cysteine capped CdTe NPs were measured and shown in Fig. 3. The resemblance in both spectral features and several peak positions proves a successful chemical attachment of L-cysteine onto the surface of CdTe NPs.

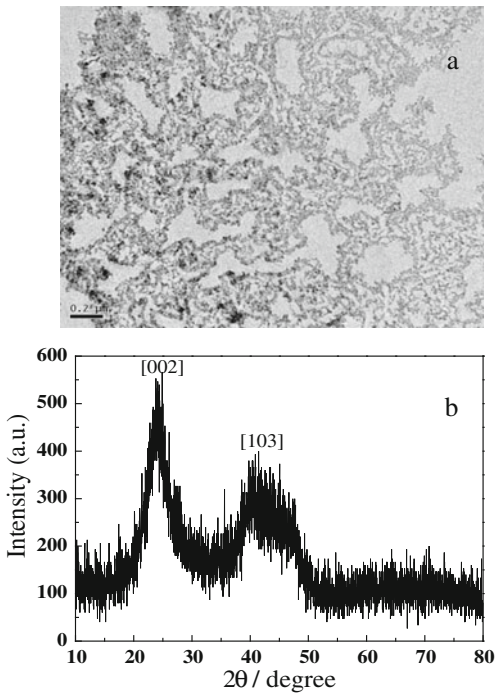

Fig. 1 **a** TEM image and **b** XRD pattern of the as-prepared L-cysteine capped CdTe nanoparticles

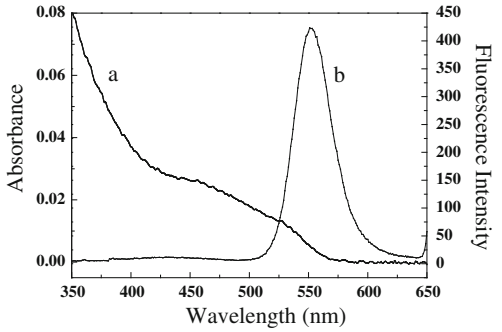

Fig. 2 **a** Absorption and **b** fluorescence emission spectra of L-cysteine capped CdTe nanoparticles

For instance, the peaks at 2,900–3,425 cm^{-1} indicate $–NH_2$ group, and the peaks around 1,550–1,600 cm^{-1} represent the carboxyl group [23, 25]. On comparison of the two spectra, the most significant point to be noted is that the band at 2,552 cm^{-1}, corresponding to –SH stretching mode [26], is absent in the spectrum of L-cysteine capped CdTe NPs. It can be attributed to the cleavage of S–H bonds and formation of Cd–S bonds, which further reveals that the L-cysteine has already been bound to the surface of Cd^{2+} site [26, 27].

Optimization of Determination Conditions

In this work, factors like pH and reaction time were optimized to find the better determination condition. PBS (0.1 mol/L) was used as the buffer to adjust the acidity of the aqueous medium. Fig. 4a presents the fluorescence intensities of L-cysteine capped CdTe NPs with the pH change from 4.0 to 11.0 in the absence and presence of 50 nM PAR. It is found that the fluorescence intensity of L-cysteine-CdTe-PAR increases gradually

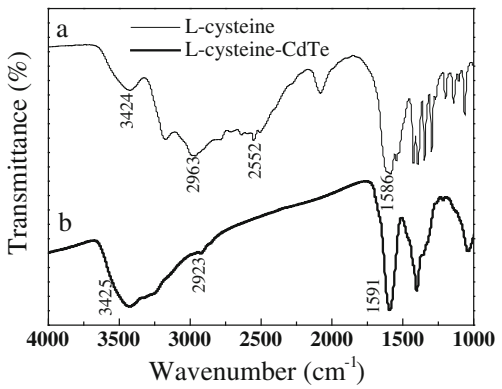

Fig. 3 FTIR spectra of **a** pure L-cysteine and **b** L-cysteine capped CdTe NPs

when the pH value varies from 4.0 to 8.0. When the pH value is higher than 8.0, the fluorescence intensity decreases at first, and then almost attains a plateau. Therefore, pH 8.0 was chosen to run the assay. Under room temperature, the effect of reaction time on the fluorescence intensity of the L-cysteine-CdTe-PAR system was tested at different time intervals. The result (in Fig. 4b) shows that the maximum fluorescence intensity is obtained when the reaction time is 10 min. Hence, we recorded the fluorescence intensity after the system had reacted for 10 min.

Fluorescence Behaviour of L-Cysteine Capped CdTe NPs in the Presence of Paracetamol

Under the fluorescence conditions described in the experimental section, good fluorescence spectra were obtained for L-cysteine capped CdTe NPs in the absence and presence of PAR. As shown in Fig. 5, with increasing amount of PAR added in the L-cysteine capped CdTe NPs solution, a significant luminescence quenching with no optical shift of emission is observed, and the fluorescence band centers at 556 nm (excitation at 330 nm). The quenching may be due to the hydrogen bond function of molecules between L-cysteine and PAR on the surface of the NPs. The hydrogen bond leads to L-cysteine molecules to be partly peeled off the surface of the NPs, and the surface changes of CdTe NPs induce the fluorescence quenching [24]. Based on this observation, it offered the possibility of developing a sensitive method for quantitative assay of PAR by using spectrofluorometry.

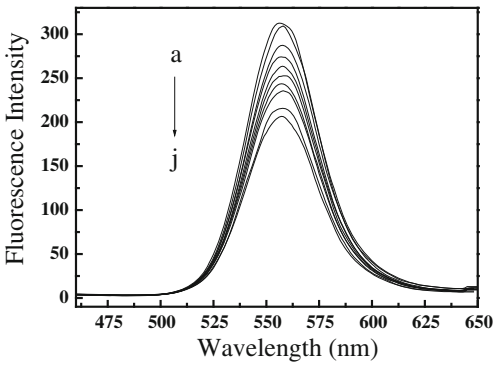

Fig. 5 Fluorescence emission spectra of L-cysteine capped CdTe NPs in the absence and presence of PAR obtained in PBS (pH= 8.0) with excitation wavelength at 330 nm. C_{PAR} (10^{-8} mol/L): (a) 0; (b) 1; (c) 2.8; (d) 4; (e) 6.4; (f) 7.6; (g) 9.4; (h) 10; (i) 14; (j) 16

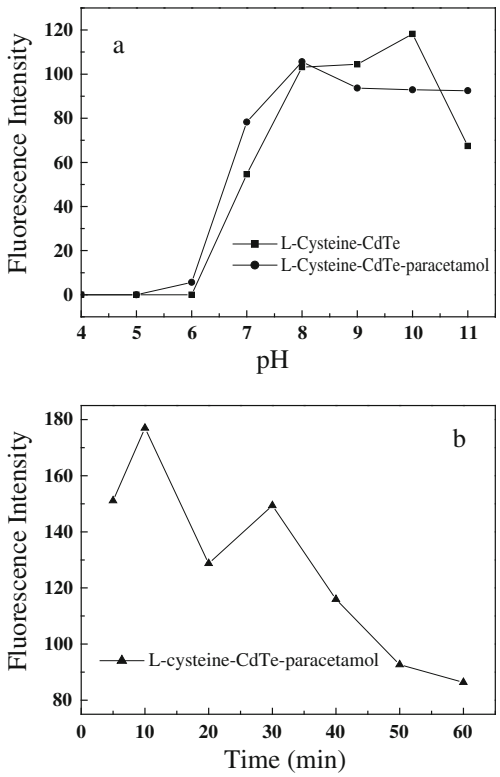

Fig. 4 **a** Effect of pH on the fluorescence intensity of L-cysteine capped CdTe NPs in the absence and presence of PAR. **b** Effect of reaction time on the fluorescence intensity of L-cysteine-CdTe-PAR system

Calibration Curve and Detection Limit

Under the optimal experimental conditions, the relative fluorescence intensity ($\Delta I = I_0/I$) of L-cysteine capped CdTe NPs increased linearly in the concentration range of 1.0×10^{-8} mol/L to 1.6×10^{-7} mol/L for PAR with the correlation coefficient (r) of 0.9988 (Fig. 6). The PAR concentration dependence of the CdTe intensity was coincident with the fluorescence quenching described

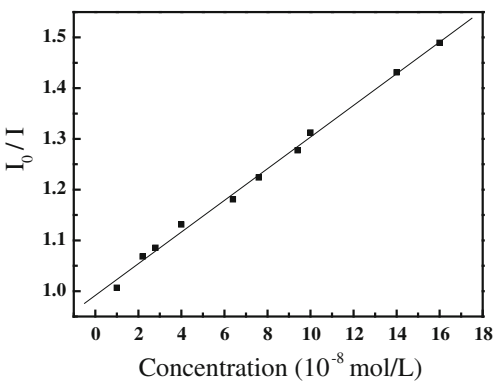

Fig. 6 Linear graph of the relative fluorescence intensity (I_0/I) versus the concentrations (C) of PAR

Table 1 Effect of coexisting foreign species

Species added	Mole ratio ($C_{species}/C_{PAR}$)	Change of fluorescence intensity to PAR (%)	Species added	Mole ratio ($C_{species}/C_{PAR}$)	Change of fluorescence intensity to PAR (%)
NH_4^+ , Cl^-	20	−0.439	Uric acid	20	−0.79
Na^+ , I^-	20	−0.197	Ascorbic acid	5	−1.45
K^+, NO_3^-	30	−1.42	Citric acid	10	+0.99
Ca^{2+} , Cl^-	10	−2.32	L-alanine	20	+0.19
Mg^{2+} , SO_4^{2-}	20	−2.42	L-leucine	10	−0.67
Zn^{2+} , SO_4^{2-}	20	+2.74	L-tryptophan	30	−4.63
Cu^{2+} , SO_4^{2-}	10	+2.14	L-glutamate	20	−2.02
Pb^{2+} , NO_3^-	5	−0.53	L-cysteine	10	−4.77
Al^{3+} , NO_3^-	20	+2.10	Uracil	20	−2.28
Co^{2+} , NO_3^-	5	−1.66	Thymine	20	−0.14
Dopamine	5	+2.03	Adenine	10	+0.24
Glucose	10	+4.28	Guanine	20	−1.52

by the Stern–Volmer equation which could be drawn as follows:

$$I_0/I = 1 + K_{SV}C \quad (1)$$

I_0 and I are the fluorescence intensities in the absence and presence of quencher concentration solution, respectively. C is the concentration of quencher and K_{SV} is the Stern–Volmer quenching constant. The calibration curve established the following equation, $I_0/I=0.992+0.031$ C (C, 10^{-8} M) for PAR. The detection limit was 4.2×10^{-9} mol/L which was determined on the basis of three times the standard deviation of ten replicate measurements of the fluorescence intensity of the blank samples. The data revealed that the proposed method could be applied to the determination of PAR.

Interference Analysis

The interference of foreign species in the presence of 5×10^{-8} mol/L PAR was conducted to evaluate the selectivity of the proposed method under the chosen conditions. The tolerance limit was defined as the maximum concentration of the foreign substance produced a determination error of less than 5%. It is found that (Table 1) most ions and biomolecules have no distinct effect on the determination. Ions like Pb^{2+}, Co^{2+} can be allowed only at a relatively low concentration, but they can be eliminated by means of necessary separation strategy such as mercapto cotton treatment [28]. Interferences from dopamine and ascorbic acid could be neglected due to the low concentration in samples and the appropriate dilution. Hence, it demonstrated that this method had a high selectivity.

Sample Determination

To discuss the feasibility for PAR assay in pharmaceutical application, this method was applied for the detection of PAR in Fen An Ka Min tablets. Samples were prepared as described in "Reagents and Solution". The content of PAR of the pharmaceutical preparation was detected (138.9 mg/tablet), which is comparable with the content of PAR provided by the manufacturer (150 mg/tablet). The result indicated that the proposed method in this work was acceptable.

Conclusions

Water-soluble CdTe NPs modified with L-cysteine were prepared to investigate their photoluminescence response to PAR. A simple and rapid method for the selective and sensitive determination of PAR based on the fluorescence quenching of L-cysteine capped CdTe NPs was proposed in this paper. Under the optimum conditions, the relative fluorescence intensity was linearly proportional to the concentrations of PAR with good correlation coefficient. The content of PAR in commercial tablets determined by the present method agreed with the claimed value. In a word, NPs have a considerable potential as fluorescence probes and to simplify the performance of analysis.

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References

1. Ozcan A, Sahin Y (2011) A novel approach for the determination of paracetamol based on the reduction of N-acetyl-p-benzoquinoneimine formed on the electrochemically treated pencil graphite electrode. Anal Chim Acta 685:9–14
2. Zhang Y, Luo LQ, Ding YP, Liu XA, Qian ZY (2010) A highly sensitive method for determination of paracetamol by adsorptive stripping voltammetry using a carbon paste electrode modified with nanogold and glutamic acid. Microchim Acta 171:133–138
3. Babaei A, Garrett DJ, Downard AJ (2011) Selective simultaneous determination of paracetamol and uric acid using a glassy carbon electrode modified with multiwalled carbon nanotube/chitosan composite. Electroanal 23:417–423
4. Goyal RN, Gupta VK, Chatterjee S (2010) Voltammetric biosensors for the determination of paracetamol at carbon nanotube modified pyrolytic graphite electrode. Sensor Actuat B-Chem 149:252–258
5. Kalimuthu P, John SA (2010) Selective electrochemical determination of paracetamol using nanostructured film of functionalized thiadiazole modified electrode. Electroanal 22:303–309
6. Gandhi SV, Ranher SS, Deshpande PB, Shah DK (2011) Simultaneous HPTLC determination of nabumetone and paracetamol in combined tablet dosage form. J Brazil Chem Soc 22:1068–1072
7. Ravisankar S, Vasudevan M, Gandhimathi M, Suresh B (1998) Reversed-phase HPLC method for the estimation of acetaminophen, ibuprofen and chlorzoxazone in formulations. Talanta 46:1577–1581
8. Lou HG, Yuan H, Ruan ZR, Jiang B (2010) Simultaneous determination of paracetamol, pseudoephedrine, dextrophan and chlorpheniramine in human plasma by liquid chromatography-tandem mass spectrometry. J Chromatogr B 878:682–688
9. Hewavitharana AK, Lee S, Dawson PA, Markovich D, Shaw PN (2008) Development of an HPLC-MS/MS method for the selective determination of paracetamol metabolites in mouse urine. Anal Biochem 374:106–111
10. Zhao SL, Bai WL, Yuan HY, Xiao D (2006) Detection of paracetamol by capillary electrophoresis with chemiluminescence detection. Anal Chim Acta 559:195–199
11. Lourencao BC, Medeiros RA, Rocha RC, Mazo LH, Fatibello O (2009) Simultaneous voltammetric determination of paracetamol and caffeine in pharmaceutical formulations using a boron-doped diamond electrode. Talanta 78:748–752
12. Easwaramoorthy D, Yu YC, Huang HJ (2001) Chemiluminescence detection of paracetamol by a luminol-permanganate based reaction. Anal Chim Acta 439:95–100
13. Sirajuddin AR, Khaskheli A, Shah ML, Bhanger AN, Mahesar S (2007) Simpler spectrophotometric assay of paracetamol in tablets and urine samples. Spectrochim Acta A 68:747–751
14. Al-Zoubi N, Koundourellis JE, Malamataris S (2002) FT-IR and Raman spectroscopic methods for identification and quantitation of orthorhombic and monoclinic paracetamol in powder mixes. J Pharmaceut Biomed 29:459–467
15. Kachoosangi RT, Wildgoose GG, Compton RG (2008) Sensitive adsorptive stripping voltammetric determination of paracetamol at multiwalled carbon nanotube modified basal plane pyrolytic graphite electrode. Anal Chim Acta 618:54–60
16. Li L, Bian RX, Ding YP, Yu ML, Yu DW (2009) Application of functionalized ZnS nanoparticles to determinate uracil and thymine as a fluorescence probe. Mater Chem Phys 113:905–908
17. Vinayaka AC, Thakur MS (2010) Focus on quantum dots as potential fluorescent probes for monitoring food toxicants and foodborne pathogens. Anal Bioanal Chem 397:1445–1455
18. Wang GL, Dong YM, Yang HX, Li ZJ (2011) Ultrasensitive cysteine sensing using citrate-capped CdS quantum dots. Talanta 83:943–947
19. Cao M, Cao C, Liu MG, Wang P, Zhu CQ (2009) Selective fluorometry of cytochrome c using glutathione-capped CdTe quantum dots in weakly basic medium. Microchim Acta 165:341–346
20. Fei XN, Gu YC, Wang J, Jia GZ, Liu ZJ (2011) Preparation and fluorescent properties of a complex probe based on inorganic QDs and organic dye. J Lumin 131:291–296
21. Xu H, Miao R, Fang Z, Zhong XH (2011) Quantum dot-based "turn-on" fluorescent probe for detection of zinc and cadmium ions in aqueous media. Anal Chim Acta 687:82–88
22. Frasco MF, Chaniotakis N (2010) Bioconjugated quantum dots as fluorescent probes for bioanalytical applications. Anal Bioanal Chem 396:229–240
23. Zhao WF, Fung YS, O W, Cheung MPL (2010) L-cysteine-capped CdTe quantum dots as a fluorescence probe for determination of cardiolipin. Anal Sci 26:879–884
24. Kuang R, Kuang X, Pan SY, Zheng XD, Duan JC, Duan YQ (2010) Synthesis of cysteamine-coated CdTe quantum dots for the detection of bisphenol A. Microchim Acta 169:109–115
25. Wang GL, Dong YM, Yang HX, Li ZJ (2011) Ultrasensitive cysteine sensing using citrate-capped CdS quantum dots. Talanta 83:943–947
26. Priyam A, Chatterjee A, Das SK, Saha A (2005) Synthesis and spectral studies of cysteine-capped CdS nanoparticles. Res Chem Intermed 31:691–702
27. Li MY, Zhou HM, Zhang HY, Sun P, Yi KY, Wang M, Dong ZZ, Xu SK (2010) Preparation and purification of L-cysteine capped CdTe quantum dots and its self-recovery of degenerate fluorescence. J Lumin 130:1935–1940
28. Diao XL, Xia YS, Zhang TL, Li Y, Zhu CQ (2007) Fluorescence-detecting cationic surfactants using luminescent CdTe quantum dots as probes. Anal Bioanal Chem 388:1191–1197