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Quantum dot-enhanced chemiluminescence detection for simultaneous determination of dopamine and epinephrine by capillary electrophoresis

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ABSTRACT

A sensitive method based on quantum dot (QD)-enhanced capillary electrophoresis–chemiluminescence (CE–CL) detection was developed for simultaneous determination of dopamine (DA) and epinephrine (E). In this work, CdTe QD was added into the running buffer of CE to catalyze the post-column CL reaction between luminol and hydrogen peroxide, achieving higher CL emission. Negative peaks were produced due to the inhibitory effects on CL emission from DA and E eluted from the electrophoretic capillary. The decrease in CL intensity was proportional to the concentration of DA and E in the range of 8.0×10^{-8}–5.0×10^{-6} M and 4.0×10^{-8}–5.0×10^{-6} M, respectively. Detection limits for DA and E were 2.3×10^{-8} M and 9.3×10^{-9} M, respectively. Using this method, the levels of DA and E in human urine from healthy donors were determined.

1. Introduction

Dopamine (DA) and epinephrine (E) occur naturally in the human body and serve as neurotransmitters or hormones in the sympathetic nervous system [1]. The measurement of these biogenic monoamine neurotransmitters in biological fluids has an essential role in the diagnostics of hypertension, multiple sclerosis, Parkinson's disease and various mental diseases [2]. As a consequence, simple, sensitive and accurate analytical methods in the quantitation of DA and E would be useful for physiological investigations and disease diagnosis. This has prompted the development of the methods for the determination of these catecholamines in human plasma, urine and pharmaceutical preparations. In the past decade, a number of methods have been reported for the quantitative analysis of catecholamine, such as high performance liquid chromatography (HPLC) with electrochemical [3], fluorescence [4,5] and chemiluminescence (CL) detection [6], the capillary electrophoresis (CE) with laser-induced fluorescence (LIF) [7], electrochemiluminescence (ECL) [8], mass spectrometry [9] and electrochemical detection [10,11], and flow-injection analysis [12,13].

Recent advances in the development of luminescent semiconductor nanocrystals (also called quantum dots, QDs) open up new possibilities for biological applications. QDs have several distinctive advantages including broad excitation spectrum, sharp and symmetrical emission spectra, high quantum yield, good chemical and photostability, and size-dependent emission-wavelength tunability. They are very suitable materials for developing sensitive analysis technology [14]. In recent years, QDs have been used in biosensors [15–17], immunoassay [18,19] and biomacromolecule labeling [20–24]. QDs-catalyzed electrochemiluminescence reactions were also investigated, and applications in chemical analysis were demonstrated [25–28].

This study related to CdTe QDs-catalyzed CL reaction of luminol with hydrogen peroxide. Trace DA and E inhibited the CL emission which led to the development of a novel CE–CL detection method for selective and sensitive quantification of DA and E. QDs added into running buffer enhanced the sensitivity of the detection. Using present method, determination of trace DA and E in human urine was demonstrated.

2. Experimental

2.1. Chemicals and solutions

DA, E and luminol were purchased from Sigma (St. Louis, USA). Hydrogen peroxide (H_2O_2) was obtained from Taopu Chemicals (Shanghai, China). All other chemicals used in this work were of analytical grade. Water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout

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the work. The electrophoretic buffer was 25 mM borate solution (pH 9.8, adjusted with 1 M NaOH solution) containing 0.6 mM CdTe QDs (mM Cd^{+2}) and 0.3 mM luminol. The post-column oxidizer solution was 1 mM NaOH solution containing 25 mM H_2O_2. Stock solutions of 1.7 mM DA and 1.3 mM E were prepared in 20 mM borate buffer (pH 9.8). All solutions were filtered through 0.45 μm membrane filters before use.

2.2. CE–CL system

A laboratory-built CE–CL system described previously was used for the determination of DA and E [29]. Briefly, a high-voltage supply (0–30 kV, Beijing Cailu Science Instrument, Beijing, China) was used to drive the electrophoresis. Uncoated fused silica capillaries (75 μm i.d. × 50 cm effective length, Hebei Optical Fiber, China) were used for the separation. The polyimide on the 5-cm end section of the capillary was burned and removed. After etching with 10% hydrogen fluoride (HF) for 1 h, this end of capillary was inserted into the reaction capillary (320 μm i.d., Hebei Optical Fiber). A four-way Plexiglass joint held a separation capillary and a reaction capillary in place. The CL solution was siphoned into a tee. The grounding electrode was put in one joint of the tee. The CL solution flowed down to the detection window, which was made by burning 2 cm of the polyimide of the reaction capillary and was placed in front of the photomultiplier tube (PMT, R374 equipped with a C1556-50 DA-type socket assembly, Hamamatsu, Shizuoka, Japan). CL emission was collected by a PMT and was recorded and processed with a computer using a CT-21 Chromatography Data System (Beijing Cailu Science Instrument, Beijing, China).

2.3. Preparation of CdTe QDs

The procedure used for preparing mercapto-propyl acid (MPA) capped CdTe QDs was similar to that described by Li et al. [30] with minor modifications. Ar-saturated cadmium chloride solution was added to NaHTe solution, which was prepared by the reaction between $NaHB_4$ and tellurium powder in the presence of MPA. The concentration of Cd^{2+} was 2 mM, and the molar ratio of Cd^{2+}:Te^{2-}:MPA was maintained at 1:0.5:2.5. After mixing, the solution was heated with microwave at different reaction time to prepare different-sized QDs. The prepared CdTe QDs were purified by selective precipitation with isopropanol and re-dispersed in Milli-Q water. The CdTe QDs were further purified by dialysis using a dialysis membrane with molecular weight cut off 7000 in 10 mM NaOH. The concentration of MPA-capped CdTe QDs was defined by the number of cadmium atoms contained in the sample. Particle size distribution was obtained by a NaNo-ZS90 particle size and Zeta potentiometer analyzer.

2.4. Human urine sample preparation

The human urine samples were obtained from healthy volunteers, which was stored at 4 °C for 2 h. Then, a 500 μL volume of human urine sample was centrifuged for 10 min at 2000 rpm. The supernatant was transferred into a 1.5 mL vial and diluted to 1.0 mL with electrophoretic buffer. The solution was filtered through a 0.45 μm membrane filter and diluted 2-fold with electrophoretic buffer before injecting the solution into the CE system.

2.5. CE procedure

The new separation capillary was preconditioned by flushing with 1.0 M NaOH for 30 min before the first use. Between two consecutive injections, the separation capillary was rinsed sequentially with 0.1 M NaOH, water and running buffer for 2 min each. The reaction capillary was rinsed using the oxidizer solution for 2 min between two consecutive injections. Samples were injected into the separation capillary by hydrodynamic flow at a height differential of 20 cm for 10 s. Running voltage was 14 kV.

3. Results and discussion

3.1. CL of CdTe QDs in CE

As a preliminary experiment, using CdTe QDs to enhance the CL reaction of luminol with hydrogen peroxide was confirmed on the CE as follows. CdTe QDs was injected directly into separation capillary and analyzed using an analytical procedure similar to that described in Section 2. The peak of CdTe QD was detected within 6 min with good reproducibility of CL intensities, which can be taken into consideration for developing a CE–CL system for the determination of DA and E because of inhibiting effect of DA and E to this CL reaction. Further, we investigated the influence of the QDs with diameters of 2.31 nm, 2.93 nm, 3.82 nm, 4.08 nm and 4.43 nm on the CL intensities. The results indicted that the CL signal increased with the increase of QDs diameter. Therefore, the QD with diameters of 4.43 nm was used for the following experiments.

3.2. CL detection of DA and E

CdTe QD was added into the running buffer of CE to catalyze the post-column CL reaction between luminol and hydrogen peroxide, achieving higher CL emission. When the catalytic process was inhibited, CL intensity was reduced resulting in an inverted peak. The degree of CL suppression was proportional to DA and E concentration, which formed the basis for DA and E quantification. To maximize the sensitivity of the DA and E assay, the effects of luminol, hydrogen peroxide, QDs and NaOH concentration on the inhibition of CL intensity were investigated. In these experiments, a 1.0×10^{-6} M of DA and E solution was injected into the CE–CL system, and the CL intensity was recorded.

3.2.1. Effect of luminol concentration

The effect of luminol concentration was investigated in the range of 0.04–0.5 mM. With the increase of lumilol concentration from 0.04 to 0.3 mM, the ΔCL for both DA and E also increased. Further increasing the luminol concentration resulted in the decreased of ΔCL because of background noise augmentation. Thus, the concentration of 0.3 mM luminol was elected for this CL reaction.

3.2.2. Effect of hydrogen peroxide concentration

As oxidizer, the concentration of hydrogen peroxide played an important role in the CL reaction. Therefore, the effect of hydrogen peroxide concentration was investigated in the range of 5–40 mM. With the increase of hydrogen peroxide concentration from 5 to 25 mM, the ΔCL for both DA and E also increased. Over 25 mM, almost constant ΔCL value was observed. So the concentration of 25 mM hydrogen peroxide was used in this experiment.

3.2.3. Effect of CdTe QDs concentration

Concentration of CdTe QD in the running buffer affected CL emission, which will result in the change of ΔCL values for DA and E. Therefore, the effects of CdTe QD concentration in the range of 0.1–0.9 mM on the ΔCL were investigated. The results indicated that the DA and E had the maximal ΔCL values when the concentration of QD was 0.6 mM. Then a CE running buffer containing 0.6 mM CdTe QDs was used for further studies.

3.2.4. The influence of NaOH concentration in post-column oxidizer solution

The luminol reacted with hydrogen peroxide emitting CL in alkaline medium. NaOH solution was selected as the reaction medium.

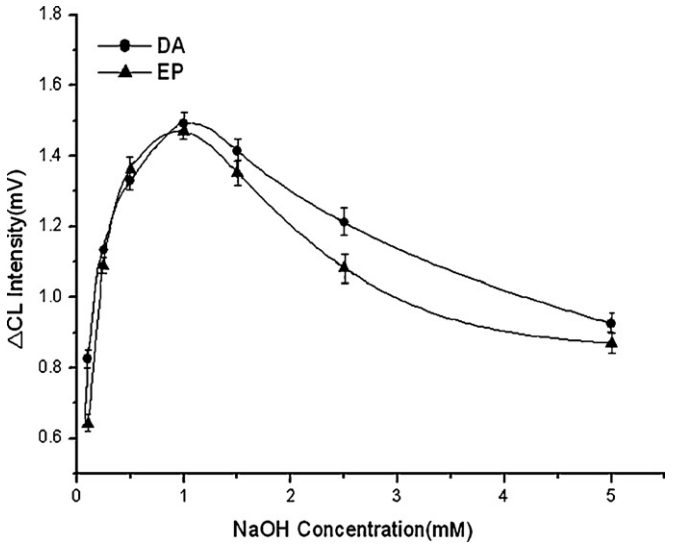

Fig. 1. Effect of NaOH concentrations on ΔCL. CE–CL conditions: the electrophoretic buffer was 25 mM borate solution (pH 9.8) containing 0.6 mM CdTe QDs and 0.3 mM luminal; the post-column oxidizer solution was NaOH solution at different concentrations containing 25 mM H_2O_2. The concentrations of DA and E were 1.0×10^{-6} M.

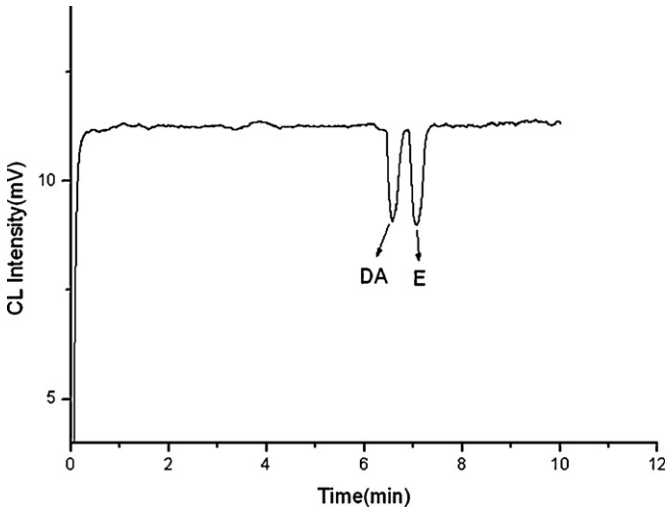

Fig. 2. The electropherogram of DA and E standard solution. CE–CL conditions: the electrophoretic buffer was 25 mM borate solution (pH 9.8) containing 0.6 mM CdTe QDs and 0.3 mM luminal; the post-column oxidizer solution was 1 mM NaOH solution containing 25 mM H_2O_2. Separation capillary was 75 μm I.D. × 50 cm effective length; voltage applied was 14 kV. The concentrations of DA and E were 1.5×10^{-6} M.

However, since the volume of the eluent from the separation capillary is very small compared with the volume of the post-column oxidizer solution, the acidity environment of the CL reaction is mainly dependent on the post-column oxidizer solution. It was found that NaOH concentration in oxidizer solution affected greatly CL intensity. In the range of 0.25–5 mM, the ΔCL for both DA and E increased with increasing NaOH concentration up to 1.0 mM, where maximum ΔCL was reached. Further increasing the NaOH concentration result in the decrease of ΔCL (Fig. 1). Thus, an oxidizer solution containing 1.0 mM NaOH was selected for the post-column CL reaction.

3.3. CE separation of DA and E

To separate DA and E, the CE conditions such as electrophoretic buffer pH and separated voltage were investigated to achieve an effective separation. The electrophoretic buffer was 25 mM borate solution containing 0.6 mM QDs and 0.3 mM luminol. The effects of electrophoretic buffer pH values from 9.0 to 10.2 were examined using the same background electrolyte composition. Best separation was achieved at a pH value of 9.8. Separation voltage also affected the resolution. It was examined over the range of 12–18 kV. As expected, increasing the separation voltage may increase electroosmotic flow and decrease the time of migration, however, it will result in excessive Joule heating that ruins the resolution and repeatability. Generally, higher resolution should be achieved when longer time is available for the separation of DA and E, while longer migration time should also cause peak broadening. Thus, a 14 kV of applied voltage is optimum considering the appropriate migration time and acceptable peak shape.

3.4. Analytical figures of merit

Under optimized conditions, a mixture solution containing 1.5×10^{-6} M DA and E was separated. Fig. 2 shows the electrophogram. As can be seen from Fig. 2, two analytes were baseline-separated within 8 min. The CE–CL method for the determination of DA and E was validated by examining the response linearity, the limit of detection and the reproducibility of peak height. The linearity between analytes concentration and CL intensity (negative peak height) was examined by injecting six different concentrations of DA and E standard solutions. The analytical characteristics of the method are summarized in Table 1. As can be seen, the limits of detection (signal/noise = 3) of E and DA were estimated to be 9.3×10^{-9} M and 2.3×10^{-8} M, respectively. Based on the results from a literature survey, the proposed indirect chemiluminescence (ICL)-based detection coupled with CE is 10–100 times more sensitive than the other ICL detection schemes used in CE [31–33]. Compared with the CE–LIF methods reported for DA and E analysis, the sensitivity of the present assay was nearly same [34]. The reproducibility was investigated by injecting a mixture solution containing 1.0×10^{-6} M DA and E eight times. The CL intensity of analytes was reproducible. For E and DA, relative standard deviations (RSDs) of peak heights were 2.6% and 3.3%, respectively.

Robustness was examined by evaluating the influence of small variation of method variables such as the concentration of luminal, QD, hydrogen peroxide and borate, and separation voltage on the performance of the proposed method. It was found that none of these variables significantly affect the method. Ruggedness was tested by applying the proposed method to the assay of E and DA using the same operational conditions but at different elapsed times. Results obtained from day-to-day variation, were found to be reproducible, as RSDs did not exceed 4%.

3.5. Analysis of human urine samples

Human urine samples from five adult healthy volunteers were analyzed. A typical electropherogram obtained from separations of human urine sample is shown in Fig. 3A. The peak corresponding to DA and E can be well identified. To verify the peak identification, the sample was spiked with E and DA at 5.0×10^{-7} M, and again measured. The electropherogram obtained is shown in Fig. 3B. As can be seen by comparing Fig. 3A with Fig. 3B, only the DA and E peak increased in peak height and size without any other major changes in the electropherograms. The analytical results from analyzing all the human urine samples are summarized in Table 2. The concentrations of DA and E in human urine were found to be in the range of $9.70–12.14 \times 10^{-7}$ M and $0.92–1.34 \times 10^{-7}$ M, respectively. These results are in accordance with those reported in literature [35–37] that are obtained by a high performance liquid chromatography procedure. The precision of the detection results was evaluated by repeatedly analyzing each human urine sample for four times

Table 1
Calibration curves and detection limits for the detection of DA and E.

Analyte	Linear regression equation	Linear ranges (M)	R	RSD of slope ($n=4$)	RSD of intercept ($n=4$)	LOD (M)
DA	$\Delta H = 0.0128 \times 10^8 C + 0.1465$	8.0×10^{-8}–5.0×10^{-6}	0.9974	0.23	0.82	2.3×10^{-8}
E	$\Delta H = 0.0132 \times 10^8 C + 0.1637$	4.0×10^{-8}–5.0×10^{-6}	0.9986	0.18	0.53	9.3×10^{-9}

R, linear correlation coefficient; ΔH, peak height (relative chemiluminescence intensity); C, concentration of analytes (M).

Table 2
Analytical results of DA and E in human urine.

Sample number	Catecholamines	Found (nM)	Added (nM)	Total found (nM)	Recovery (%)	RSD (%, $n=4$)
1	DA	1022	1000	2042	102	2.1
	E	126	1000	1106	98	3.2
2	DA	970	1000	1980	101	3.9
	E	92	1000	1102	101	3.7
3	DA	1214	1000	2184	97	2.9
	E	116	1000	1146	103	4.8
4	DA	1086	1000	2066	98	3.4
	E	134	1000	1154	102	4.1
5	DA	998	1000	2038	104	3.8
	E	108	1000	1088	98	2.4

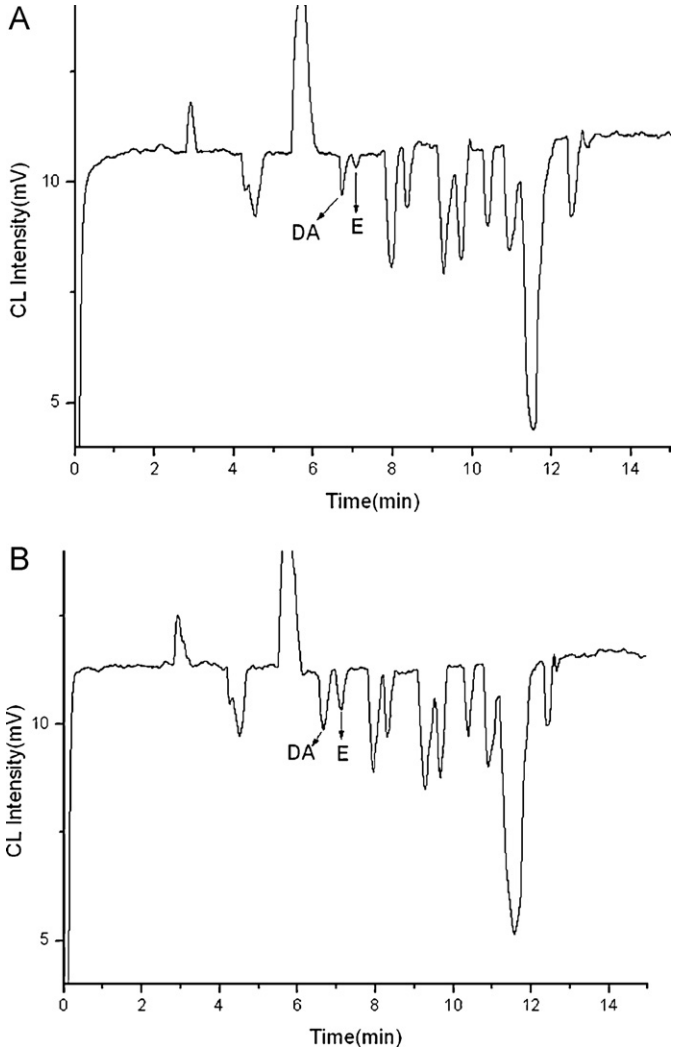

Fig. 3. Electropherograms obtained from the separation of a human urine sample (A) and the human urine sample spiked with DA and E at 5.0×10^{-7} M (B). CE–CL conditions were as in Fig. 2.

within one working day. The RSDs were between 2.1% and 4.8%. Recoveries of DA and E from these urine samples were also studied. DA and E were added to these urine samples, and an appropriate amount of the sample solution was again detected. The recoveries of DA and E were found to be in the range of 97–104% and 98–103%, respectively.

4. Conclusions

A new CE–CL method has been developed for the simultaneous determination of DA and E. It was proved in this work that the concept that CdTe QDs could be used successfully to enhance the performances of classical CE–CL tests, achieving higher sensitivities. And the method is suited for completing the analysis of DA and E in human urine samples. Low running cost, short separation time (less than 10 min), and sufficient sensitivity (detection limit of 10^{-8}–10^{-9} M) are the important features of presented method.

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References

[1] D.A. Chambers, R.L. Cohen, R.L. Perlman, Neurochem. Int. 22 (1993) 95.
[2] J. Bergquist, A. Sciubisz, A. Kaczor, J. Silberring, J. Neurosci. Methods 113 (2002) 1.
[3] K.A. Sagar, M.R. Smyth, J. Pharm. Biomed. Anal. 22 (2000) 613.
[4] X.L. Zhu, P.N. Shaw, D.A. Barrett, Anal. Chim. Acta 478 (2003) 259.
[5] T. Yoshitake, J. Kehr, S. Yoshitake, K. Fujino, H. Nohta, M. Yamaguchi, J. Chromatogr. B 807 (2004) 177.
[6] E. Nalewajko, A. Wiszowata, A. Kojło, J. Pharm. Biomed. Anal. 43 (2007) 1673.
[7] M. Du, V. Flanigan, Y.F. Ma, Electrophoresis 25 (2004) 1496.
[8] J. Kang, X.B. Yin, X. Yang, E. Wang, Electrophoresis 26 (2005) 1732.
[9] Q.F. Weng, G.W. Xu, K.L. Yuan, P. Tang, J. Chromatogr. B 835 (2006) 55.
[10] T.L. Paxon, P.R. Powell, H.G. Lee, K.A. Han, A.G. Ewing, Anal. Chem. 77 (2005) 5349.
[11] M. Chicharro, A. Sïanchez, A. Zapardiel, M.D. Rubianes, G. Rivas, Anal. Chim. Acta 523 (2004) 185.
[12] L.K. Abdulrahman, A.M. Al-Abachi, M.H. Al-Qaissy, Anal. Chim. Acta 538 (2005) 331.
[13] H. Yao, Y.Y. Sun, X.H. Lin, J.H. Cheng, L.Y. Huang, Luminescence 21 (2006) 112.
[14] R. Gill, M. Zayats, I. Willner, Angew. Chem. Int. Ed. 47 (2008) 7602.
[15] K.E. Sapsford, T. Pons, I.L. Medintz, H. Mattoussi, Sensors 6 (2006) 925.
[16] V. Pavlov, Y. Xiao, B. Shlyahovsky, I. Willner, J. Am. Chem. Soc. 126 (2006) 11768.

[17] J. Liu, Y. Lu, Angew. Chem. 118 (2006) 96.
[18] G.D. Liu, J. Wang, J. Kim, M.R. Jan, G.E. Collins, Anal. Chem. 76 (2004) 7126.
[19] E.R. Goldman, E.D. Balighian, H. Mattoussi, M.K. Kuno, J.M. Mauro, P.T. Tran, G.P. Anderson, J. Am. Chem. Soc. 124 (2002) 6378.
[20] H.Q. Yao, Y. Zhang, F. Xiao, Z.Y. Xia, J.H. Rao, Angew. Chem. 119 (2007) 4424.
[21] M.G. Sandros, D. Gao, D.E. Benson, J. Am. Chem. Soc. 127 (2005) 12198.
[22] B.P. Aryal, D.E. Benson, J. Am. Chem. Soc. 128 (2006) 15986.
[23] R. Gill, R. Freeman, J.P. Xu, I. Willner, S. Winograd, I. Shweky, U. Banin, J. Am. Chem. Soc. 128 (2006) 15376.
[24] R. Gill, L. Bahshi, R. Freeman, I. Willner, Angew. Chem. 120 (2008) 1700.
[25] S.K. Poznyak, D.V. Talapin, E.V. Shevchenko, H. Weller, Nano Lett. 4 (2004) 693.
[26] N. Myung, Z.F. Ding, A.J. Bard, Nano Lett. 2 (2002) 1315.
[27] G.Z. Zou, H.X. Ju, Anal. Chem. 76 (2004) 6871.
[28] H. Jiang, H.X. Ju, Anal. Chem. 79 (2007) 6690.
[29] S.L. Zhao, C. Xie, X. Lu, Y.R. Song, Y.M. Liu, Electrophoresis 26 (2005) 1744.
[30] J. Li, X. Hong, Y. Liu, D. Li, Y.W. Wang, J.H. Li, Y.B. Bai, T.J. Li, Adv. Mater. 17 (2005) 163.
[31] Y. Hu, X. Li, Z. Pang, J. Chromatogr. A 1091 (2005) 194.
[32] Y. Zhang, B. Huang, J.K. Cheng, Anal. Chim. Acta 363 (1998) 157.
[33] H.C. Tsai, C.W. Whang, Electrophoresis 20 (1999) 2533.
[34] H.T. Chang, E.S. Yeung, Anal. Chem. 67 (1995) 1079.
[35] W.J. Burke, H.D. Chung, S.W. Li, Anal. Biochem. 273 (1999) 111.
[36] M. Yoshitake, H. Nohta, S. Ogata, K. Todoroki, H. Yoshida, T. Yoshitake, M. Yamaguchi, J. Chromatogr. B 858 (2007) 307.
[37] T.J. Panholzer, J. Beyer, K. Lichtwald, Clin. Chem. 45 (1999) 262.