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ORIGINAL PAPER

Dopamine sensor based on molecularly imprinted electrosynthesized polymers

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Abstract Molecularly imprinted polymers (MIPs) have been applied as molecular recognition elements to chemical sensors. In this paper, we combined the use of MIPs and electropolymerization to produce a sensor which was capable of detecting dopamine (DA). The MIP electrode was obtained by electrocopolymerization of *o*-phenylenediamine and resorcinol in the presence of the template molecular DA. The MIP electrode exhibited a much higher current response compared with the non-imprinted electrode. The response of the imprinted sensor to DA was linearly proportional to its concentration over the range 5.0×10^{-7}-4.0×10^{-5} M. The detection limit of DA is 0.13 μM (S/*N*=3). Moreover, the proposed method could discriminate between DA and its analogs, such as ascorbic acid and uric acid. This method was successfully applied to the determination of DA in dopamine hydrochloride injection and healthy human blood serum. These results revealed that such a sensor fulfilled the selectivity, sensitivity, sped, and simplicity requirements for DA detection and provided possibilities of clinical application in physiological fields.

Keywords Dopamine · Molecularly imprinted polymers · Electropolymerization · Sensor

Introduction

Dopamine ((3,4-dihydroxyphenyl) ethylamine, DA), plays a significant role in the functioning of central nervous, renal, and hormonal systems [1]. It is essential to develop simple and rapid methods for dopamine detection [2, 3]. Various methods have been applied to detecting DA, such as spectrophotometry [4], HPLC [5], and ion chromatography [6] and so on. Although these methods are highly specific and sensitive, they require sophisticated and expensive instrumentation, and are time-consuming. Electrochemical methods are widely used for the reason that they offer a simple, rapid, on-line, and sensitive way of detecting DA [7]. The major problem of electrochemical determination is the interference from ascorbic acid (AA) and uric acid (UA), which largely coexist with DA. The oxidation potentials of DA, AA, and UA were nearly the same on solid electrodes, resulting in an overlapping voltammetric response. Recently, various chemical-modified electrodes have been used to improve the selectivity of the DA detection [8, 9].

Molecularly imprinted polymers (MIPs) have been the subject of vigorous research in the last two decades [10, 11]. Molecular imprinting technique is an approach to synthesizing a polymer matrix with molecular recognition sites, which are specific in shape and size to the target molecular, showing specific high binding behaviors to the target molecules [12]. Due to their mechanical and chemical stability, high affinity and outstanding substrate recognition ability, and low cost of preparation, MIPs have been successfully applied in the chemical sensing area [13, 14].

In the application of MIPs as sensing elements for chemical sensors, the majority of reports on MIPs synthesis refer to radical polymerization with acrylic or vinylic types of monomers [15, 16]. Less work has been carried out to study the formation of MIPs by electropolymerization. This method has many advantages such as simple preparation, easy control of the film thickness and good reproducibility

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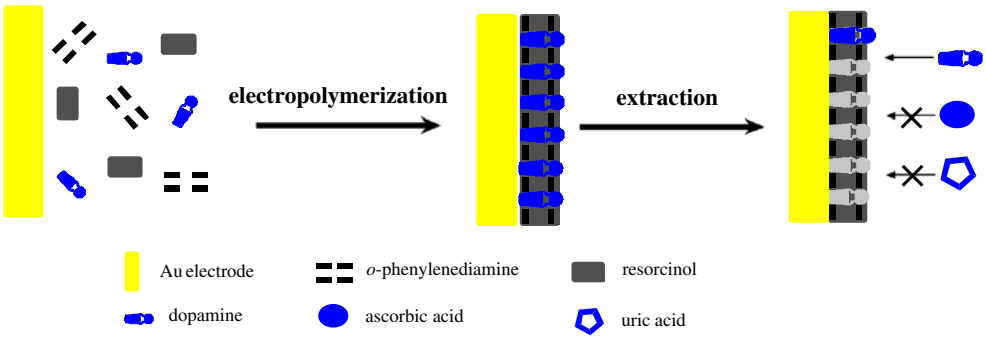


Fig. 1 Schematic for the preparation of the MIP electrode

of uniform polymer films [17, 18]. Besides, electropolymerization could construct multilayer structures with low thickness, fabricating fast responding sensors with reduced interferences [19, 20]. What's more, the template molecular in the imprinted film can be removed rapidly and thoroughly. The extraction procedure can be shortened to a large extent.

Weetall et al. produced a sensing electropolymerized electrode using an aqueous solution equimolar in resorcinol/*o*-phenylenediamine and in the presence of the template molecular, which showed higher affinity for template molecular 2,4-dichlorophenoxyacetic acid than for a non-template dye [18]. Li et al. successfully synthesized DA-imprinted *o*-aminophenol membranes, which showed high selectivity, sensitivity, and reproducibility to the determination of DA [21]. As so far, less study reported the combination of electrochemical method and molecular imprinting technology for DA detection. In this paper, considering the potential permselectivity [22] of poly(*o*-phenylenediamine) and the advantages of MIP, we chose an equal mixture of *o*-phenylenediamine and resorcinol as matrix polymer, successfully fabricated a rapid and convenient DA-imprinted sensor. The preparation conditions for the MIP electrode, suitable operating conditions, calibration curve, detection limit, and selectively in DA detection were presented and discussed. The modified electrode not only exhibited an excellent sensibility and sensitivity for determination of DA in the presence of high concentration AA and UA, but also can be used to detect DA in dopamine hydrochloride injection and healthy human blood serum, with satisfied result.

Experimental

Chemicals and apparatus

DA, AA, and UA were bought from Sigma. Resorcinol and *o*-phenylenediamine were obtained from Sinopharm Chemical Reagent Co. Dopamine hydrochloride injection was bought from Harvest Pharmaceutical Co. Phosphate buffer solution (0.1 M, formed by mixing NaH_2PO_4 with Na_2HPO_4 solutions, PBS) was used as supporting electrolyte solution. All chemicals were of analytical grade and used without further purification. All solutions were prepared with twice-distilled water.

Instruments

Electropolymerization and voltammetric techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI660C electrochemical

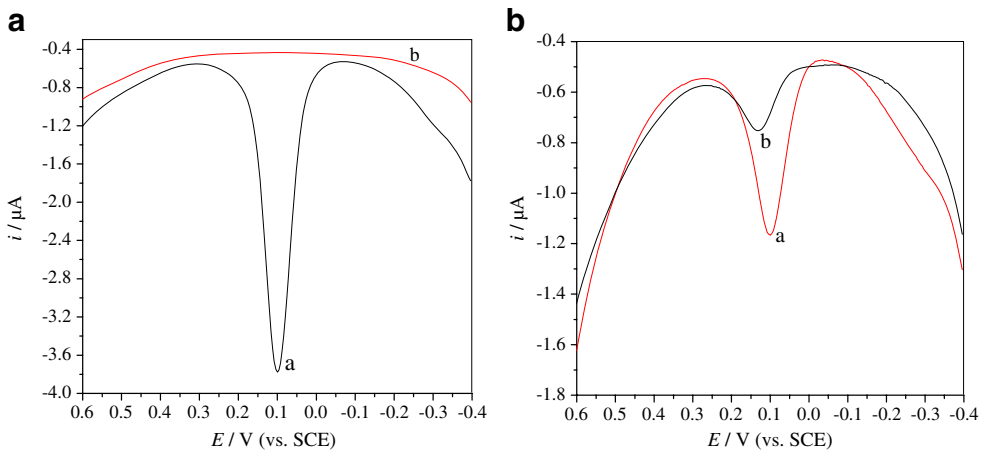


Fig. 2 **a** DPVs of dopamine-imprinted film before *a* and after *b* dopamine removal in 0.1 M PBS (pH7.4). **b** DPVs of MIP electrode *a* and NIP electrode *b* in 0.1 M PBS containing 15 μM DA

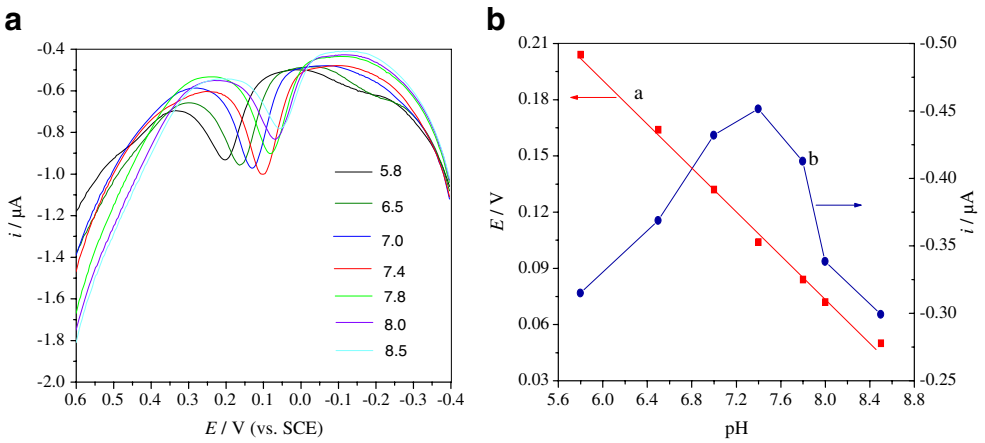


Fig. 3 **a** Dependence of DPVs on the value of pH for the MIP electrode in 0.1 M PBS with 10 μM DA at 5.8, 6.5, 7.0, 7.4, 7.8, 8.0, and 8.5. **b** The influence of solution pH on the peak potential and peak current of DA

workstation (Shanghai Chenhua, China). All electrochemical experiments were carried out in a conventional three-electrode system using a modified Au electrode as working electrode, a platinum wire as counter electrode, and a saturated calomel electrode as reference electrode. Electrochemical solutions were thoroughly deoxygenated by N2 bubbling before use and maintained N_2 atmosphere throughout the experiment. All experiments were carried out at room temperature.

Preparation of the MIP-modified electrode

The surface of the Au electrode was polished with 1.0, 0.3, and 0.05 μm alumina slurry, respectively, sonicated with distilled water after each polishing step. Then the electrode was subjected to cyclic potential sweeps between 0.2 and 1.5 V in 1.0 M H_2SO_4 until a stable cyclic voltammogram was obtained.

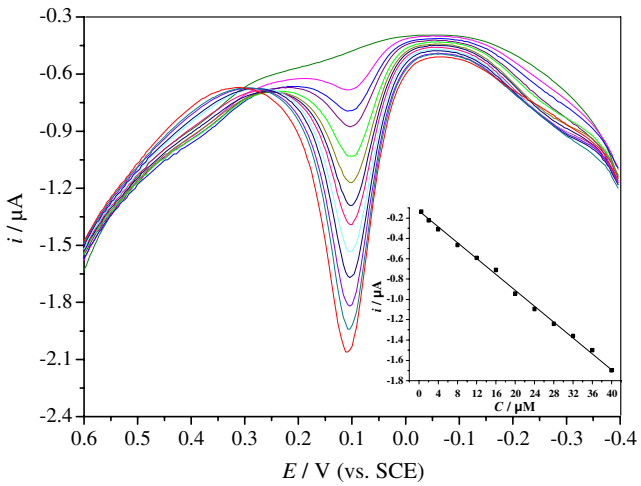


Fig. 4 DPVs of DA at MIP electrode in 0.1 M pH7.4 PBS. DA concentrations (from 0.5 to 40 μM). *Inset* is the linear relationship between peak currents and the concentration of DA

Electropolymerization was performed by cyclic voltammetry (20 cycles) in the potential range of 0-0.8 V at a scan rate 50 mV/s in a solution of *o*-PD (5 mM) and resorcinol (5 mM) with NaAc-HAc (0.1 M, pH6.5). For the preparation of imprinted polymerization, dopamine was also added at a concentration of 20 mM. Then, the electrode was rinsed several times in 0.1 M PBS (pH7.4) to remove the template entrapped in the polymeric matrix. A control electrode (non-imprinted polymer electrode, NIP electrode) was obtained in the same way but without being added the dopamine template.

In fabricating the MIP electrode, dopamine was imprinted and formed specific sites on the Au electrode by electrosynthesis of *o*-phenylenediamine and resorcinol. The schematic of the MIP electrode preparation was shown in Fig. 1.

Electrochemical measurements

Electrochemical measurements were carried out on the three-electrode system which was immersed in a cell containing 10 mL PBS (pH7.4). CV were performed from 0.8 to −0.4 V. DPV were performed from 0.6 to −0.4 V, the pulse amplitude was 50 mV, the pulse width was 50 ms, the pulse period was 0.2 s, and the potential increment was 4 mV.

Results and discussion

Electrochemical responses of the MIP electrode

Figure 2a showed the DPV of the DA-imprinted film before and after removal of DA template with 0.1 M PBS (pH7.4). Since the template molecular DA is electroactive, the electrode showed an apparent peak at 0.100 V before extraction (a in Fig. 2a). After extracting the template with

Table 1 Comparison of the MIP electrode with other modified electrodes

Modified electrode	Detection range (mol/L)	Detection limit (mol/L)	Reference
Fc-SWNTs	5.0×10^{-6}-3.0×10^{-5}	5.0×10^{-8}	[9]
Poly (caffeic acid)	1.0×10^{-6}-3.5×10^{-5}	2.0×10^{-7}	[25]
Poly(4-(2-Pyridylazo)-Resorcinol)	5.0×10^{-6}-3.0×10^{-5}	2.0×10^{-7}	[30]
PtAu hybrid film	2.4×10^{-5}-3.8×10^{-4}	2.4×10^{-5}	[31]
MIP	5.0×10^{-7}-4.0×10^{-5}	1.3×10^{-7}	Proposed method

PBS, there was no current response observed in the DPV measurements (b in Fig. 2a), which also confirmed the successfully removal of the template in the buffer solution. The rebinding ability of the imprinted film was also tested by DPV experiments. After adding 15 μM DA into the PBS, the MIP electrode (a in Fig. 2b) exhibited a much higher current response, the ratio of peak current on MIP electrode increased by a factor of 3 compared to the NIP electrode (b in Fig. 2b), indicated that the template DA imprinted procedure was successful. The oxidation potential of DA at the NIP electrode was 132 mV, while at the MIP electrode the potential of DA shifted toward negatively potentials. The enhanced peak current and a shift in the oxidation potential of DA by about 32 mV clearly illustrated catalytic effect of the MIP electrode towards to the oxidation of DA.

Effect of scan rate

The effect of the scan rate on peak current of DA was investigated in pH7.4 PBS containing 10 μM DA. The anodic and cathodic peak currents were proportional to the scan rate in the range of 20-120 mV/s. The linear regression equations were s and $i_{pc}(\mu A) = 0.0126 \times +1.008$, with the correlation coefficient R, 0.996 and 0.981, respectively. It illustrated that the process of the electrode reaction is controlled by the adsorption of DA [23, 24].

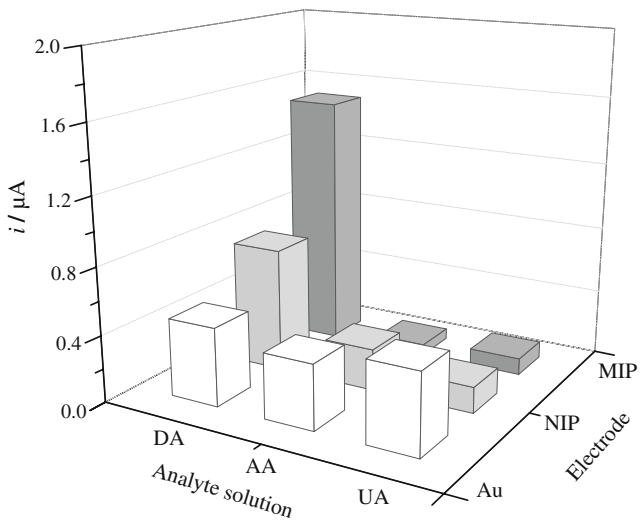


Fig. 5 Peak currents of 15 μM DA, AA, UA at Au electrode, MIP electrode and NIP electrode, respectively

Effect of pH

The pH of the supporting electrolyte has a significant influence on the DA oxidation at modified electrodes by affecting both peak currents and peak potentials [25]. Figure 3a showed the pH effect on DPVs at the MIP electrode. The peak potentials of DA oxidation shifted negatively at a slope of −57 mV per pH unit in the range of 5.8-8.5 (a in Fig. 3b), the magnitude of the dE_p/dpH was very close to the theoretical Nernstian value of −59 mV/pH, indicating equal number of electrons and protons involved in the oxidation [7, 26]. The linear regression equation was obtained as: $E_{pa}(V) = 0.533 - 0.057$ pH (R=0.991). As shown in b of Fig. 3b, the peak current increased with increasing solution pH until it reached to 7.4. The maximum catalytic current was obtained at the value of 7.4. Since pH7.0 is the physiological pH value, PBS at pH 7.4 was chosen for use in all experiments.

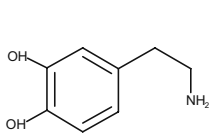
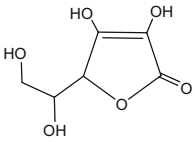
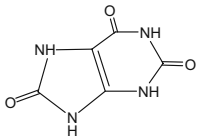
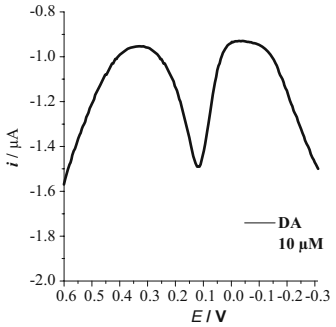
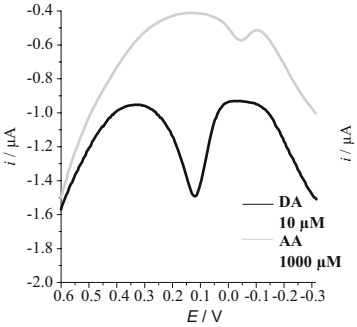
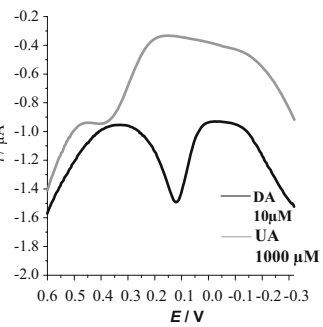
Determination of DA

The determination of DA concentration using the MIP electrode was performed by DPV (Fig. 4). The response time of the electrode to achieve the steady-state current was within 3 s. The results showed that plot of the anodic peak current (i_{pa}) as a function of concentration gave a linear line in the range from 5.0×10^{-7}-4.0×10^{-5}M with a correlation coefficient of 0.997. The linear regression equation is expressed as: $i_{pa}(\mu A) = -0.140 - 0.0386$ c (μM). The detection limit was determined to be 0.13 μM at a signal-to-noise ratio of 3. This value is comparable to values reported by other research groups for electrocatalytic oxidation of DA at the surface of electrodes chemical modified by other mediators (Table 1).

Interference study

A molecularly imprinted sensor should show a minimum of affinity towards molecules having structures

Table 2 Selectivity of MIP electrode to DA

Structure			
DPV			
k_{pc} (1:100)	1	0.16	0.18

Response-selectivity coefficient of solutions containing 10 μM DA and 1,000 μM interferents

closely related to the imprinted molecules. We compared the electrochemical response of Au electrode, MIP electrode, and NIP electrode to the same concentration of DA, AA, and UA (15 μM). As shown in Fig. 5, the peak currents of the three analytes on the Au electrode were almost as high as each other, while on the MIP electrode, the current of DA was apparently higher than the current of AA and UA, which showed an excellent selectivity of the MIP electrode.

In order to further examine the selectivity of the designed MIP electrode, the response of the modified electrode was tested in a solution containing 10 μM DA and 100-fold amounts of potential interfering substances such as AA and UA. The response-selectivity coefficient $k_{pc}=i_p/i_c$ [20] was calculated to demonstrated the selectivity of the MIP sensor, where *i*p is the peak intensity response of the sensor to 10 μM DA and *i*c is the peak intensity response caused by 1,000 μM interferents. The results of an interference study, undertaken with related analyte, were shown in Table 2. It was found that AA and UA produced negligible peak currents even when the DA-interferent ratio of 1:100 was used. This illustrated that the binding sites of the DA MIP could recognize the DA molecular by means of shape selection and the size of functional groups. The MIP had bulky negatively charged functional groups which had strong electrostatic attraction toward DA cations while repelled AA and UA anions. Also the functional group (−NH2, −OH) can form strong hydrogen bonding [27] with "imprint" molecular (DA). So the polymer can be used as a selective binding medium for the "imprint" molecular

Table 3 The results for the determination of DA in dopamine hydrochloride injection and healthy human blood serum

No.	DA in dopamine hydrochloride injection				DA in healthy human blood serum			
	Content (mg mL^{-1})	Found (mg mL^{-1})	R.S.D. (%) *n* =5	Recovery (%)	Spiked (mg mL^{-1})	Found (mg mL^{-1})	R.S.D. (%) *n* =5	Recovery (%)
1	10	10.04	1.87	100.4	10	9.99	0.95	99.9
2	10	9.93	2.46	99.3	10	9.92	2.31	99.2
3	10	10.06	1.09	100.6	10	9.67	1.60	96.7

(DA). Therefore, satisfactory selectivity of DA was obtained by such a kind of sensor.

Real samples analysis

In order to verify the performance and feasibility of the method in pharmaceutical product, the MIP electrode was applied to the determination of DA in dopamine hydrochloride injection (standard concentration of DA 10 mg/mL, 2 mL/injection). A 4.7-μL sample from dopamine hydrochloride injection was taken with a microinjection, and added into 10 mL (0.1 mol/L) PBS. After solution was mixed, the oxidation current of DA was tested. The concentration of dopamine was calculated with the linear regression equation. However, most of the previously reported modified electrodes were applied to the detection of DA content in dopamine hydrochloride injection, a very few of reports applied for assessment of DA in human blood serum [28]. In this paper, the recovery of DA in a spiked healthy human blood serum sample was also measured at the same electrode by DPV. Interfering proteins in serum were removed by precipitation with four volumes of ice-cold acetone containing trichloroacetic acid (10%, *w*/*v*) [29] and centrifugation for 15 min at 3,000 r/min. The serum samples were diluted 1:10 with PBS (7.4), and stored at −20 °C until used. The satisfactory results were listed in Table 3. The recovery and relative standard deviations (R.S.D) were acceptable, showing that the proposed method could be efficiently used for the analysis of DA in real samples.

Stability and reproducibility

The storage stability of the sensor was tested, after being stored it in refrigerator (4 °C) in a dry state for 30 days, the MIP electrode retained 94% of the initial current response. The fabrication of five electrodes, made independently, showed an acceptable reproducibility with the RSD of 3.5% for the current determination of 10 μM DA.

Conclusions

A sensitive and selective electrochemical sensor was fabricated for detection of the DA via electropolymerization to form a thin MIP film on the Au electrode. The excellent performance of the MIP electrode towards DA can be ascribed to the functional monolayer with electrochemical catalytic activities and the porous MIP film with plentiful selective binding sites. The peak current was linear to the concentration of DA from 5.0×10^{-7}-4.0×10^{-5}M with the detection limit of 0.13 μM. Moreover, as observed, the sensor possessed selective response to DA without influence of interferents commonly existed. On this basis, we suggest the proposed convenient and inexpensive chemical sensor could find application in the measurement of DA level in clinical samples as well as in pharmaceutical industry.

References

1. Venton BJ, Wightman RM (2003) Anal Chem 75:414a–421a
2. Luczak T (2008) Electrochim Acta 53:5725–5731
3. Vasantha VS, Chen SM (2006) J Electroanal Chem 592:77–87
4. Li QM, Li J, Yang ZJ (2007) Anal Chim Acta 583:147–152
5. Muzzi C, Bertocci E, Terzuoli L, Porcelli B, Ciari I, Pagani R, Guerranti R (2008) Biomed Pharmacother 62:253–258
6. Heidbreder CA, Lacroix L, Atkins AR, Organ AJ, Murray S, West A, Shah AJ (2001) J Neurosci Methods 112:135–144
7. Jo S, Jeong H, Bae SR, Jeon S (2008) Microchem J 88:1–6
8. Yan W, Feng XM, Chen XJ, Li XH, Zhu JJ (2008) Bioelectrochemistry 72:21–27
9. Jiao SF, Li MG, Wang C, Chen DL, Fang B (2007) Electrochim Acta 52:5939–5944
10. Toth B, Pap T, Horvath V, Horvai G (2007) Anal Chim Acta 591:17–21
11. Shoji R, Takeuchi T, Kubo I (2003) Anal Chem 75:4882–4886
12. Gupta R, Kumar A (2008) Biotechnol Adv 26:533–547
13. Holthoff EL, Bright FV (2007) Anal Chim Acta 594:147–161
14. Xu XL, Zhou GL, Li HX, Liu Q, Zhang S, Kong JL (2009) Talanta 78:26–32
15. Blanco LMC, Lobo CMJ, Miranda OAJ, Tunon BP (2004) Trac Trends Anal Chem 23:36–48
16. Kindschy LM, Alocilja EC (2005) Biosens Bioelectron 20:2163–2167
17. Feng L, Liu Y, Tan Y, Hu J (2004) Biosens Bioelectron 19:1513–1519
18. Weetall HH, Rogers KR (2004) Talanta 62:329–335
19. Ulyanova YV, Blackwell AE, Minteer SD (2006) Analyst 131:257–261
20. Gomez CA, Goicolea MA, Barrio RJ (2005) Analyst 130:1012–1018
21. Li JP, Zhao J, Wei XP (2009) Sens Actuators B 140:663–669
22. Rothwell SA, Killoran SJ, Neville EM, Crotty AM, O'Neill RD (2008) Electrochem Commun 10:1078–1081
23. Kumar SA, Tang CF, Chen SM (2008) Talanta 76:997–1005
24. Lin XQ, Kang GF, Lu LP (2007) Bioelectrochem 70:235–244
25. Li NB, Ren W, Luo HQ (2007) Electroanalysis 19:1496–1502
26. Hu GZ, Zhang DP, Wu WL, Yang ZS (2008) Colloids Surf B 62:199–205
27. Yu C, Mosbach K (1997) J Org Chem 62:4057–4064
28. Wang HS, Li TH, Jia WL, Xu HY (2006) Biosens Bioelectron 22:664–669
29. Chen YY, Lin SY, Yeh YY, Hsiao HH, Wu CY, Chen ST, Wang AHJ (2005) Electrophoresis 26:2117–2127
30. Chen JH, Zhang J, Lin XH, Wan HY, Zhang SB (2007) Electroanalysis 19:612–615
31. Thiagarajan S, Chen SM (2007) Talanta 74:212–222