Analytical Methods

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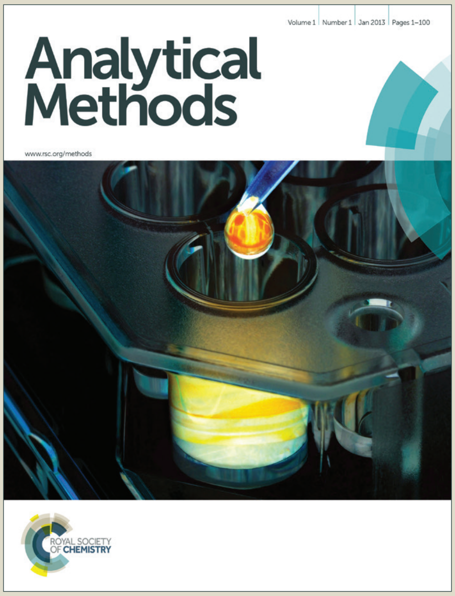

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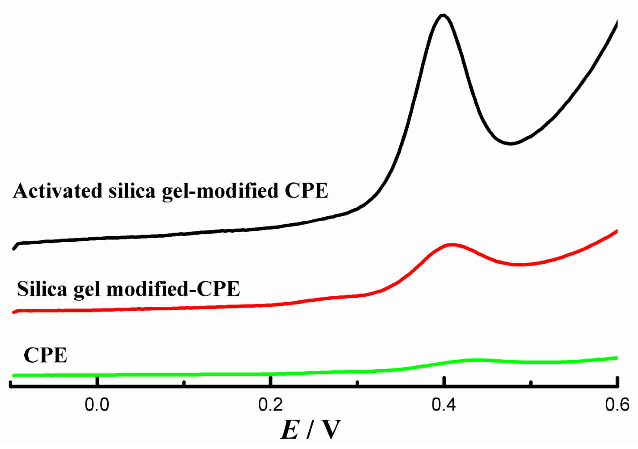

Highly-sensitive and rapid determination of protocatechuic aldehyde based on the electrochemical enhancement of activated silica gel

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Protocatechuic aldehyde (PAL) is a major polyphenolic active ingredient with a variety of biological activities. Herein, the oxidation behaviors and mechanism of PAL were studied using different electrode surface, and it was found that two-electrons were transferred. Moreover, silica gel-modified carbon paste electrode (CPE) gave rise to a higher oxidation signal to PAL relative to the unmodified CPE, and a further signal enhancement was observed on the activated silica gel-modified CPE. Based on the remarkable surface enhancement effects of activated silica gel, a new electrochemical method with high sensitivity was developed for the determination of PAL. The linear range was over the range from 5 to 250 μg L^{-1}, and the detection limit was 3.24 μg L^{-1} (23 nM) after 1-min accumulation. The proposed method was applied in Xiangdan injection samples, and the results consisted with the values that obtained by high-performance liquid chromatography.

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Introduction

Protocatechuic aldehyde (PAL) is a major polyphenolic active ingredient, and studies have demonstrated that it has a variety of biological activities. These include neuroprotection,[1] microcirculation improvement,[2] antioxidant,[3] anti-inflammatory,[4] apoptosis inhibition,[5] reduction of intravascular thrombosis,[6] and suppression of hepatitis B virus replication.[7] Therefore, it is of great importance to develop sensitive, effective and reliable analytical methods for PAL.

Until now, a number of techniques have been employed for the detection of PAL, including high-performance liquid chromatography (HPLC),[8] liquid chromatography-tandem mass spectrometry (LC-MS),[9] capillary electrophoresis-chemiluminescence (CE-CL),[10] and tyrosinase-based electrochemical sensor.[11] Although electroanalysis exhibits many advantages such as short analysis time, good handling convenience, high sensitivity and low cost, the report regarding direct electrochemical determination of PAL is very limited.

The main objective of this work is to develop a sensitive and simple electrochemical method for PAL detection. Thanks to its large surface area and strong sorption ability, silica gel was used to modify the carbon paste electrode (CPE). Compared with the unmodified CPE, the silica gel-modified CPE increased the oxidation peak currents of PAL by 3.9-fold. Interestingly, the oxidation signals of PAL further enhanced greatly when silica gel was activated using 6.0 M HCl. The remarkable signal enlargement indicates that activated silica gel has higher electrochemical reactivity towards PAL oxidation. After activation, the particle size of silica gel was found to be decreased obviously, resulting in higher response area and stronger accumulation efficiency. As a result, the oxidation signal and detection sensitivity were significantly improved for PAL.

Experimental section

Reagents

All chemicals were of analytical grade and used as received. Silica gel (200-300 mesh) was purchased from Qingdao Ocean Chemical Co. (Qingdao, China). PAL was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Stock solution of 1.0 mg mL^{-1} PAL was prepared with ethanol and stored at 4 ^{o}C. Graphite powder (spectral reagent) and paraffin oil were purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Ultrapure water (18.2 MΩ) was obtained from a Milli-Q water purification system and used throughout.

Instruments

Electrochemical measurements were performed on a CHI 660A electrochemical workstation (Chenhua Instrument, Shanghai, China) with a conventional three-electrode system. The working electrode was a modified CPE, the reference electrode was a saturated calomel electrode (SCE), and the counter electrode was a platinum wire. Scanning electron microscopy (SEM) characterization was conducted with a Quanta 200 microscope (FEI Company, Netherlands).

High-performance liquid chromatography (HPLC) determination of PAL was carried out with an Agilent 1100, coupled with UV-Vis detector. The C18 analytical column (4.6 mm × 150 mm × 5 μm) was used. Isocratic elution was employed and the mobile phase was 1% acetic acid (A) and methanol (B) (9/1). The flow rate was 1 mL min^{-1}, and the column temperature was maintained at 25 ^{o}C. The sample injection volume was 10 μL, and the detection wavelength was 281 nm.

Preparation of modified CPE

For preparation of activated silica gel, the procedure was the same as we published previously.[12] Typically, the pristine silica gel (8.0 g) was firstly refluxed for 8 h in 60 mL, 6.0 M hydrochloric acid. After that, the mixture was filtered, washed with ultrapure water to neutral, and finally dried at 70 ^{o}C for 8 h.

The preparation procedure for activated silica gel-modified CPE was shown in Fig. 1. In detail, a known amount of prepared activated silica gel and graphite powder were exactly weighed, and put in a carnelian mortar. The total mass was controlled at 1.0 g. After that, 0.25 mL paraffin oil was added, and then mixed homogeneously. The resulting carbon paste was tightly pressed into the end cavity of electrode body, and the electrode surface was polished on a smooth paper. The silica gel-modified CPE was prepared by the same procedure using silica gel, and the unmodified CPE was also prepared without addition of silica gel.

Analytical procedure

0.1 M, pH 4.6 acetate buffer solution was used as supporting electrolyte for PAL detection. After 1-min accumulation at -0.1 V, the differential pulse voltammograms were recorded from -0.1 to 0.6 V, and the oxidation peak current at 0.39 V was measured for PAL. The pulse amplitude was 50 mV, the pulse width was 50 ms, and the scan rate was 40 mV s^{-1}.

Results and discussion

Morphology and electrochemical properties

SEM was used to characterize the surface morphology of silica gel and activated silica gel. From the comparison presented in Fig. 2, it was found that the particle size of silica gel obviously reduced after activation. The decreased particle size enhances the surface area of silica gel, and consequently provides higher accumulation efficiency for PAL.

In order to compare their electrochemical properties, the active electrode area (A) of CPE, silica gel-modified CPE and activated silica gel-modified CPE was individually measured using probe of $K_3[Fe(CN)_6]$ and based on the Randles-Sevčik equation. The values of A were calculated to be 0.041, 0.045 and 0.067 cm^2 for CPE, silica gel-modified CPE and activated silica gel-modified CPE. Apparently, activated silica gel-modified CPE exhibits larger surface area.

In addition, electrochemical impedance spectroscopy (EIS) was also used to investigate the electrochemical properties of different electrodes using the redox probe $[Fe(CN)_6]^{3-/4-}$. Fig. 3 shows the Nyquist impedance diagrams of different electrodes in 0.1 M KCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$. On the surface of bare CPE (curve a), a large semicircle was observed over high frequency range. However, the semicircle lowered obviously on the surface of silica gel-modified CPE (curve b) and activated silica gel-modified CPE (curve c). In the Nyquist plot, the value of semicircle diameter corresponds to the charge transfer resistance (R_{ct}) of the electrochemical reaction. By fitting the data, the values of R_{ct} on CPE, silica gel-modified CPE and activated silica gel-modified CPE were 114.9 KΩ, 1.21 KΩ and 1.03 KΩ, respectively. Clearly, activated silica gel-modified CPE owns higher electron transfer ability. In summary, activated silica gel-modified CPE displayed superior electrochemical properties in comparison with silica gel-modified CPE and bare CPE.

Surface enhancement effects of activated silica gel

The oxidation responses of PAL on different CPEs were compared using differential pulse voltammetry (DPV). As seen in Fig. 4b, an insensitive oxidation wave at 0.43 V was observed for PAL on the bare CPE surface, indicating low oxidation activity. When using the silica gel-modified CPE (Fig. 4d), the oxidation peak of PAL increased by 3.9-fold, maybe attributed to the faster electron transfer ability as confirmed by EIS. More importantly, the oxidation wave of PAL was further raised by 3.8-fold on the surface of activated silica gel-modified CPE (Fig. 4f). From the comparison, we clearly found that activated silica gel is more active for the oxidation of PAL, and exhibits strong signal enhancement effects. SEM measurements reveal that the particle size of silica gel decreased obviously after activation. The decreased particle size undoubtedly improves the response area and accumulation efficiency. Therefore, activated silica gel displays higher electrochemical reactivity towards PAL oxidation, and greatly increases the oxidation peak currents of PAL. Additionally, the DPV responses in the absence of PAL were also studied on the CPE (Fig. 4a), silica gel-modified CPE (Fig. 4c) and activated silica gel-modified CPE (Fig. 4e). Their blank curves were featureless, suggesting that the oxidation wave in Fig. 4 was certainly due to the oxidation of PAL. In brief, conclusion can be made from Fig. 4 that the oxidation activity and detection sensitivity of PAL increase significantly on the surface of activated silica gel-modified CPE.

Oxidation mechanism of PAL

The electrochemical behaviors of PAL under different scan rates were examined using cyclic voltammetry (CV) to discuss its oxidation mechanism. As shown in Fig. 5, a pair of redox peaks was observed on the activated silica gel-modified CPE, while the oxidation peak is more sensitive than the reduction wave. As increasing the scan rate, the oxidation peak shifted to more positive potential, indicating an irreversible electrode process. Moreover, the oxidation peak currents increased linearly with the scan rate, revealing that the PAL oxidation was controlled by adsorption. According to Laviron's theory,[13] the relationship between oxidation peak potential (E_{pa}) and scan rate (v) for an irreversible and adsorption-controlled oxidation process can be described using following equation:

$$E_{\text{pa}} = E^{o}{}' + \frac{RT}{\alpha n_{\alpha} F} \ln \frac{RTk^{o}}{\alpha n_{\alpha} F} + \frac{RT}{\alpha n_{\alpha} F} \ln \nu$$

Where k^{o} is the standard rate constant, $E^{o'}$ is the formal potential, α is the electron-transfer coefficient, and other symbols have their usual significance. Apparently, the value of αn_{α} can be easily obtained from the slope value of E_{pa}-ln(ν) plot. Herein, the E_{pa} of PAL also shifted linearly with ln(ν), and the slope was 0.0284. Thus, the value of αn_{α} was calculated to be 0.904. Generally, α is considered as 0.5 for an irreversible electrode reaction. Therefore, the PAL oxidation transfers two electrons, maybe occurs at two phenolic hydroxyl groups.

Electrochemical determination of PAL

Fig. 6 depicts the influences of pH value on the oxidation signals of PAL. It was found that the oxidation peak currents of PAL on CPE (curve a), silica gel-modified CPE (curve b) and activated silica gel-modified CPE (curve c) increased gradually with improving pH from 3.6 to 4.6, and then decreased gradually as further increasing pH value. For higher sensitivity, pH 4.6 acetate buffer solution was applied for PAL detection.

The influences of accumulation potential on PAL oxidation signal were studied, and the results were displayed in Fig 7. When the potential changed from -0.2 to -0.1 V, the oxidation peak currents of PAL increased gradually. After that, the oxidation peak currents of PAL gradually decreased as continuously changing the potential to 0.3 V. To achieve higher response signal, accumulation was performed at -0.1 V.

The impacts of accumulation time were also examined, and the results were shown in Fig. 8. By extending the accumulation time from 0 to 1 min, a remarkable signal enhancement were observed for PAL oxidation, indicating that accumulation is efficient to improve the detection sensitivity. Longer accumulation time than 1 min did not enhance the oxidation peak currents obviously, suggesting that the surface amount of PAL tends to a limiting value. Considering sensitivity and working efficiency, 1 min accumulation was employed.

Fig. 9 illustrates the effects of amount of activated silica gel on the response signals of PAL. When the mass content improved from 0 to 15%, the oxidation peak currents of PAL increased sharply. The accumulation efficiency of activated silica gel-modified CPE obviously enhances with improving the amount of activated silica gel. Undoubtedly, the oxidation signals of PAL increased remarkably. As further improving the mass content to 20%, the oxidation signals leveled off, likely due to a saturation status. Hence an electrode containing 15% activated silica gel was selected for the determination of PAL.

The successive measurements using one same activated silica gel-modified CPE were investigated. Unfortunately, the oxidation signals of PAL decreased continuously, likely attributed to the strong surface adsorption and fouling. Thus, the modified electrode was employed for single measurement. The relative standard deviation (RSD) was 3.3% for eight activated silica gel-modified CPEs. The low RSD value proved that the fabrication reproducibility and detection precision were good.

The potential interferences for PAL detection were studied. It was found that 100 mg L^{-1} glucose and vitamin C; 10 mg L^{-1} glycine, tyrosine, methionine and phenylalanine; 1 mg L^{-1} tanshinol sodium, salvianolic acid A, salvianolic acid B, caffeic acid, cryptotanshinone and isoferulic acid had no influence on the determination of 100 μg L^{-1} PAL (signal change below 5%).

Under the optimized conditions, the linear range and detection limit were evaluated using DPV. As shown in Fig. 10, the oxidation peak current (I_p, μA) was proportional to the concentration of PAL (C, μg L^{-1}) over the range from 5 to 250 μg L^{-1}. The liner regression equation was $I_p = 0.00394\ C$, and the correlation coefficient was 0.999, revealing good linearity. After 1-min accumulation, the value of detection limit was estimated to be 3.24 μg L^{-1} (23 nM) based on three signal-to-noise ratio, which are lower than that of other methods (Table 1).

Practical application

To testify the practical application of the proposed method, it was used to detect PAL content in Xiangdan injection samples. The samples were purchased from a local pharmacy, and diluted by 100-fold using ultrapure water. After adding 25 μL sample solution into 10.0 mL, pH 4.6 acetate buffer solution, the DPV curves were recorded from -0.1 to 0.6 V after 1-min accumulation. Each sample underwent three parallel detections, and the RSD was lower than 5%. The PAL content was obtained by the standard addition method, and the results were shown in Table 2. Besides, the concentration of PAL was also measured using HPLC to test its accuracy. We clearly found that the results obtained by HPLC and activated silica gel-modified CPE were in good agreement, and the relative error was below 7%. Apparently, the newly developed electrochemical method for PAL detection is accurate and has promising application.

Conclusion

A highly-sensitive and rapid electrochemical method was successfully developed for the determination of PAL based on the strong signal enhancement of activated silica gel. After activation, the particle size of silica gel decreased, and in turn resulted in higher electrochemical reactivity toward PAL oxidation. On the surface of activated silica gel, the oxidation signal and the detection sensitivity of PAL were greatly improved. The low detection limit (23 nM), short accumulation time (1 min) and low relative error (7%) strongly demonstrated that the newly-proposed method was fascinating and feasible.

Acknowledgments

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Captions

Fig. 1 Schematic preparation procedure for activated silica gel-modified CPE.

Fig. 2 SEM images of silica gel (A) and activated silica gel (B).

Fig. 3 Nyquist impedance plots of CPE (a), silica gel-modified CPE (b) and activated silica gel-modified CPE (c) in 0.1 M KCl containing 5 mM $K_3/K_4Fe(CN)_6$. Frequency range: 100000 Hz to 0.1 Hz; amplitude: 10 mV.

Fig. 4 DPV curves of 100 μg L^{-1} PAL on CPE (b), silica gel-modified CPE (d) and activated silica gel-modified CPE (d). Curves (a), (c) and (e): blank curves in pH 4.6 acetate buffer solution. Accumulation time: 1 min. Mass content of modifier: 15%.

Fig. 5 CV curves of 1 mg L^{-1} PAL in pH 4.6 acetate buffer on activated silica gel-modified CPE at different can rates of 50, 100, 150, 200, 250 and 300 mV s^{-1}. Insert plot: molecular structure of PAL.

Fig. 6 Influence of pH on the oxidation peak currents of 100 μg L^{-1} PAL on CPE (a), silica gel-modified CPE (b) and activated silica gel-modified CPE (c). Accumulation time: 1 min, mass content of modifier: 10%. Error bar represents the standard deviation of triple measurements.

Fig. 7 Influence of accumulation potential on the oxidation peak currents of 100 μg L^{-1} PAL. Other conditions were the same as in Fig. 6.

Fig. 8 Relationship between accumulation time and oxidation peak current of 100 μg L^{-1} PAL. Other conditions were the same as in Fig. 6.

Fig. 9 Effects of mass content of activated silica gel on the oxidation peak currents of 100 μg L^{-1} PAL. Other conditions were the same as in Fig. 6.

Fig. 10 (A) DPV curves of PAL on activated silica gel-modified CPE with concentrations of 0 (a), 5 (b), 10 (c), 25 (d), 50 (e), 100 (f), 175 (g) and 250 μg L^{-1} (h). (B) Calibration curve for PAL.

Table 1 Performance comparison of different methods for the detection of PAL.

Table 2 Detection of PAL in Xiangdan injection samples.

Fig. 1

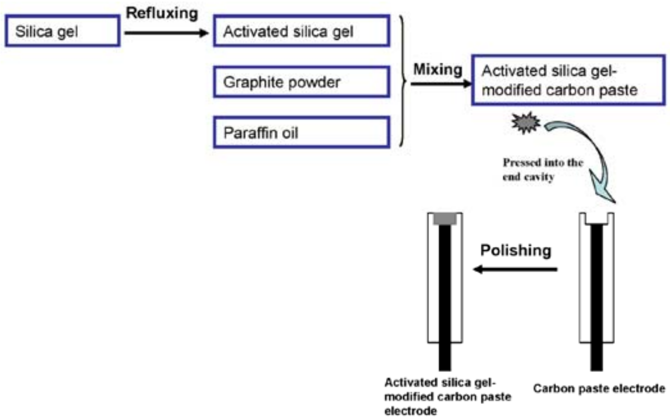
Silica gel
Refluxing
Activated silica gel
Graphite powder
Paraffin oil
Mixing
Activated silica gel-modified carbon paste
Pressed into the end cavity
Polishing
Activated silica gel-modified carbon paste electrode
Carbon paste electrode

Fig. 2

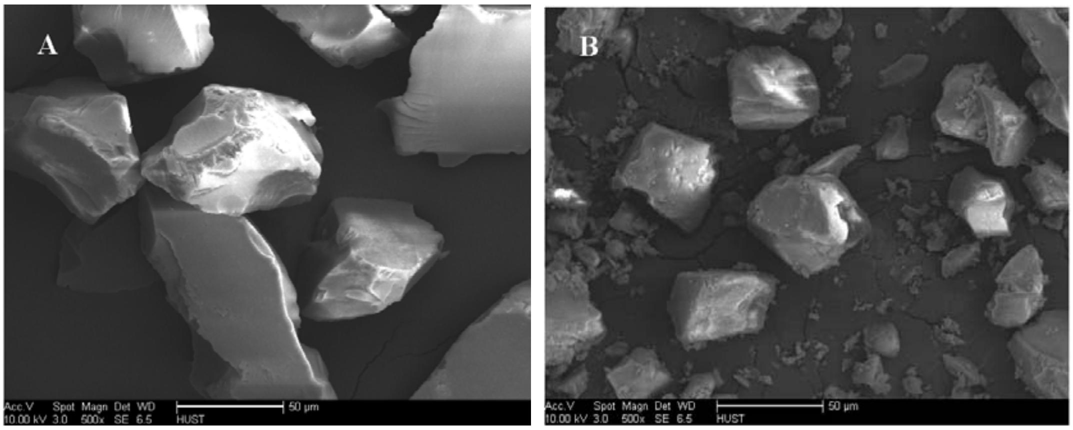
A
Acc.V Spot Magn Det WD
10.00 kV 3.0 500x SE 6.5
HUST
50 µm
B
Acc.V Spot Magn Det WD
10.00 kV 3.0 500x SE 6.5
HUST
50 µm

Fig. 3

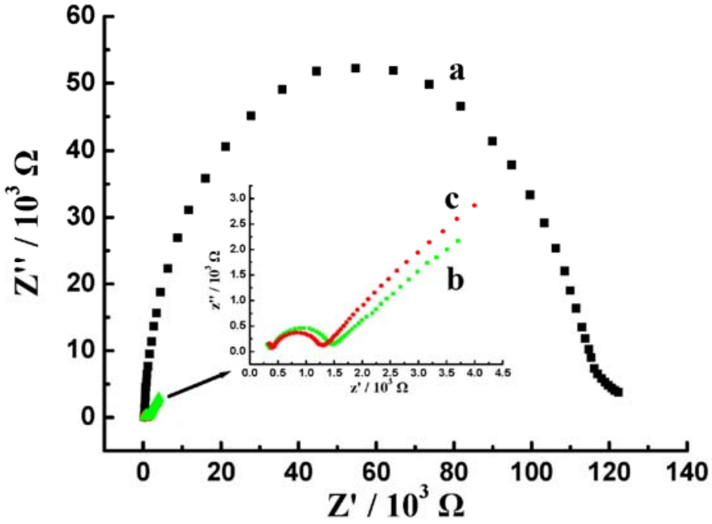
60
50
40
30
20
10
0
Z'' / 10^3 Ω
0
20
40
60
80
100
120
140
Z' / 10^3 Ω
a
c
b
3.0
2.5
2.0
1.5
1.0
0.5
0.0
z'' / 10^3 Ω
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
z' / 10^3 Ω

Fig. 4

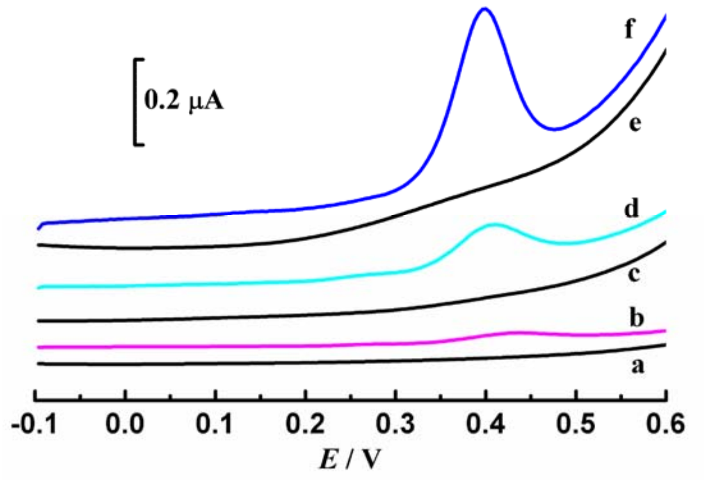

Fig. 5

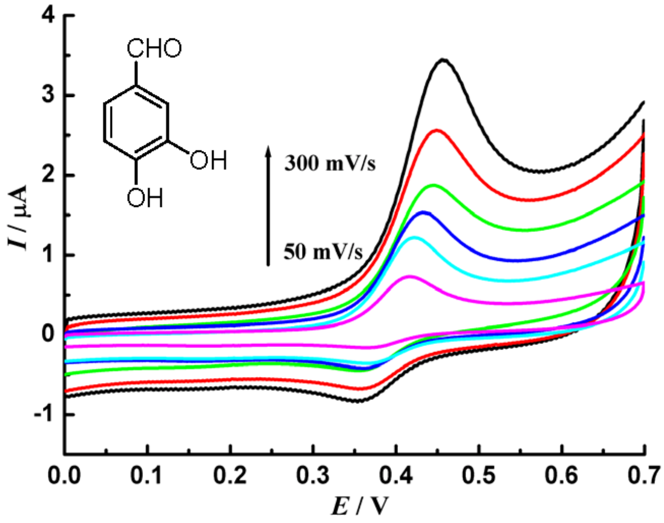

Fig. 6

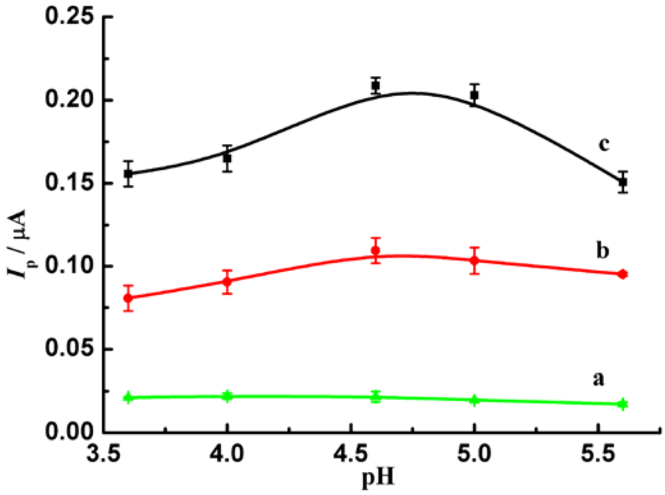

Fig. 7

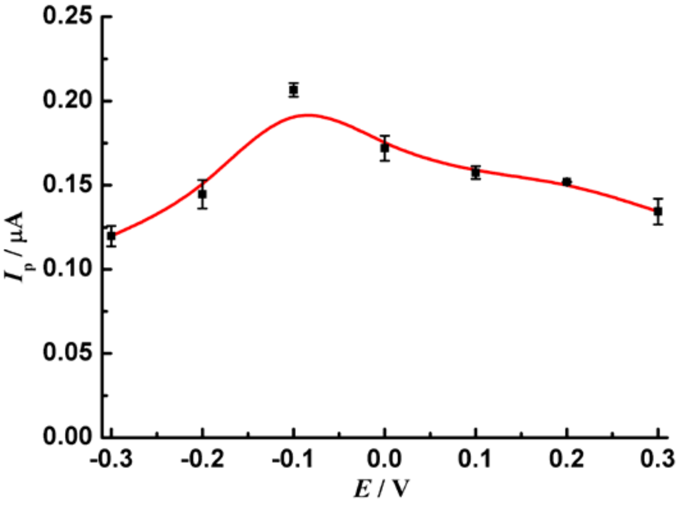

Fig. 8

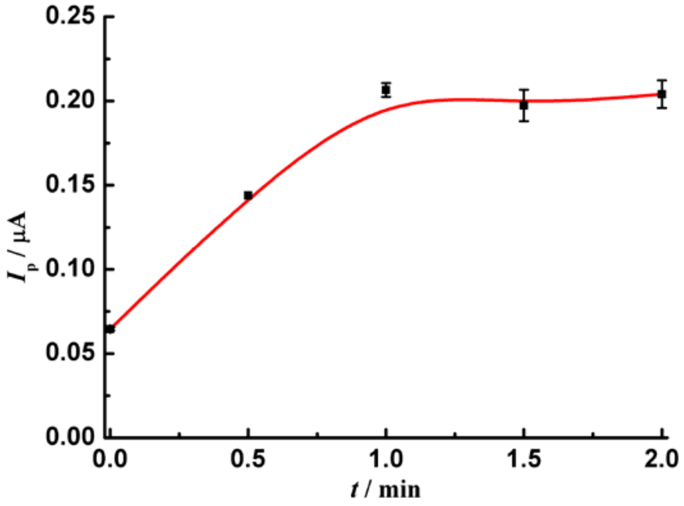

Fig. 9

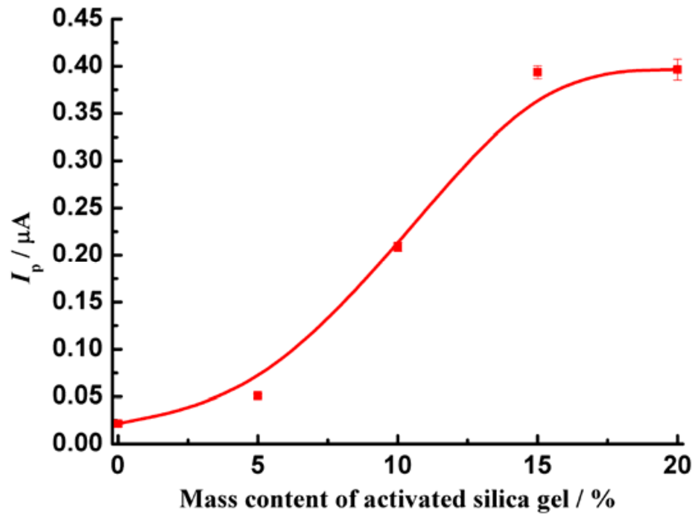
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
I_p / μA
0
5
10
15
20
Mass content of activated silica gel / %

Fig. 10

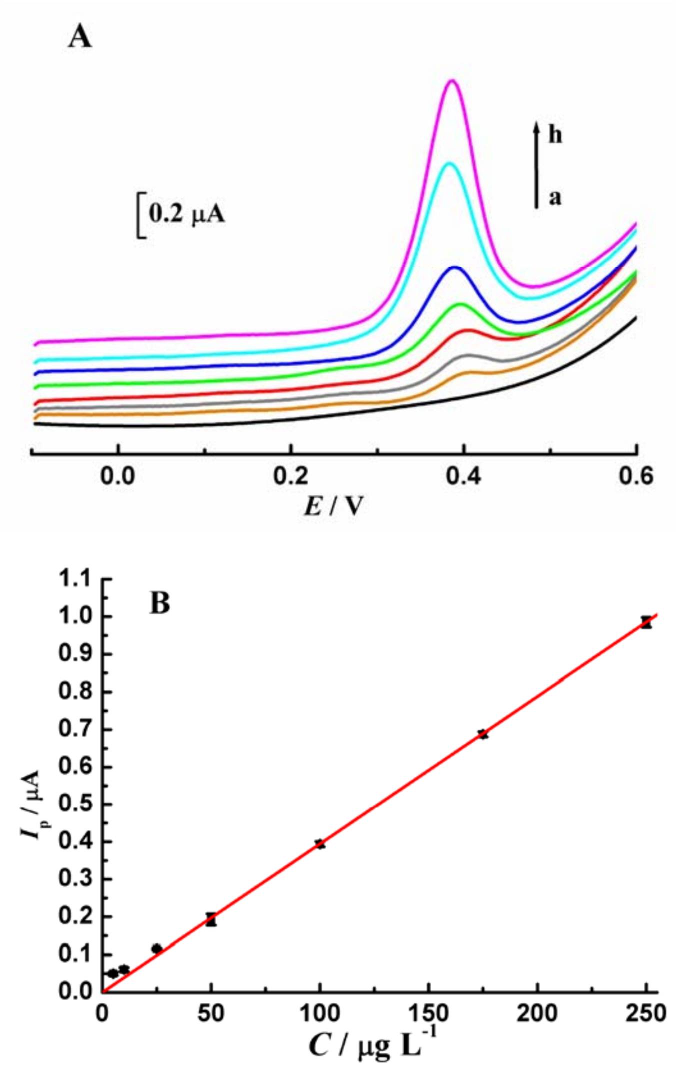
A
h
a
0.2 μA
0.0
0.2
0.4
0.6
E / V
B
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
I_p / μA
0
50
100
150
200
250
C / μg L^-1

Table 1

Methods	Linear range (μg L^{-1})	Detection limit (μg L^{-1})	Ref.
HPLC	2×10^3 to 2×10^5	200	[8]
LC-MS/MS	5×10^2 to 1×10^5	30	[9]
CE-CL	83 to 4.1×10^3	9.67	[10]
Tyrosinase-based electrochemical sensor	8.3×10^2 to 8.3×10^4	276.24	[11]
Activated silica gel-modified CPE	5 to 250	3.24	This work

Table 2

No.	by HPLC (mg mL^{-1})	by activated silica gel-modified CPE (mg mL^{-1})	RSD	Relative Error
1	0.43	0.45	2.1%	4.7%
2	0.29	0.31	5.9%	6.9%
3	0.36	0.38	6.4%	5.6%