

Full Research Article

Electrochemical Behavior of Epinephrine at a Glassy Carbon Electrode Modified by Electrodeposited Films of Caffeic Acid

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Abstract: A stable electroactive thin film of poly(caffeic acid) has been deposited on the surface of a glassy carbon electrode by potentiostatic technique in an aqueous solution containing caffeic acid. The voltammetric behavior of epinephrine (EP) at the poly(caffeic acid) modified glassy carbon electrode was studied by cyclic voltammetry. The poly(caffeic acid) modified electrode exhibited a promotion effect on the oxidation of EP. In a pH 7.4 phosphate buffer, the anodic current increased linearly with the concentration of EP in the range from 2.0×10^{-6} to 3.0×10^{-4} mol L⁻¹ and the detection limit for EP was 6.0×10^{-7} mol L⁻¹. The proposed method can be applied to the determination of EP in practical injection samples with simplicity, rapidness and accurate results.

Keywords: Caffeic acid, Electrodeposited films, Epinephrine, Chemically modified electrode

1. Introduction

Epinephrine (EP), also called adrenaline, is a component of neural transmission media that has an effect on the transmission of nerve impulse, and is an important hormone secreted by the medulla of the adrenal glands. It is also served as a chemical mediator for conveying the nerve pulse to efferent organs. As a medicine, EP is used to stimulate heartbeat and to treat emphysema, bronchitis, bronchial asthma and other allergic conditions, as well as in the treatment of the eye disease, glaucoma. Therefore, performing the research of EP has an important significance to medicine and life science. Previous techniques for the determination of EP have been primarily based upon liquid chromatography (LC) [1], flow injection electrogenerated chemiluminescence [2], capillary electrophoresis [3], fluorimetry [4,5], and various sensors analysis [6–8], etc. However, some of them

suffer from tedious procedure, lower sensitivity or poor selectivity, and most of these methods use an acidic system that is far from the normal pH value of human bodies.

Polymer modified electrodes have been widely used for the immobilization of mediators or enzymes by their matrix structure, electrocatalysis of molecules and construction of molecule devices, etc [9–12]. The determination and voltammetric behaviors of EP on the polymer-modified electrodes were reported before [13, 14]. To our knowledge, there is no report about the voltammetric behaviors and determination of EP at a poly(caffeic acid) modified glassy carbon electrode.

Caffeic acid, 3,4-dihydroxycinnamic acid, is a well-known natural phenol. It is very common in plants and can be found in seeds, fruits, tubers and herbaceous parts of many vegetable species [15]. Recently, there was a report about electrocatalytic oxidation of dihydronicotinamide adenine dinucleotide at caffeic acid modified glassy carbon electrode, which was fabricated by electropolymerization [16]. The objective of this work is to optimize the electropolymerization conditions, to investigate the behaviors of the poly(caffeic acid) modified electrode and to use the resulting polymer as a medium for EP redox. It was found that caffeic acid could be electropolymerized by constant-potential oxidation. The poly(caffeic acid) modified electrode exhibited a promotion effect on the oxidation of EP. Based on the fact that EP can be electrooxidated easily at the poly(caffeic acid) electrode surface, a new electrochemical method has been developed for the determination of EP.

2. Results and Discussion

2.1. Characteristics of the Deposited Film Modified Electrode

Cyclic voltammograms of the poly(caffeic acid) modified glassy carbon electrode at various scan rates in phosphate buffer (pH 7.4) in the potential range of -0.6 to 0.6 V are shown in Figure 1. A couple of redox peaks appeared at 0.065 and -0.052 V, respectively, at a scan rate of 150 mV s^{-1} , and the peak currents were directly proportional to the scan rates in the range below 500 mV s^{-1} , indicating a surface-confined redox couple.

The modified electrode exhibited a high stability whether it was placed in dry state or in phosphate buffer. No loss of electroactivity of the electrode was found for the continuously cyclically sweep for 8 hours. The modified electrode was also not deteriorated even for two months.

2.2. Electrochemical Oxidation of EP at the Poly(caffeic acid) Modified Electrode

One of the objectives of this work was the development of a modified electrode capable of the electrochemical oxidation of EP. In order to test the electrochemical activity of poly(caffeic acid) film, the cyclic voltammograms were obtained in the absence and presence of 1.0×10^{-4} mol L^{-1} EP, and the curves are shown in Figure 2. In the absence of EP (Figure 2a), a couple of redox reaction for the poly(caffeic acid) film on the electrode can be observed. When 1.0×10^{-4} mol L^{-1} EP was added, there was a dramatic enhancement of the anodic current, whereas the cathodic current peak was enhanced little, which is very characteristic of an electrochemical oxidation process. Simultaneously, the potential of anodic current peak shifts toward a positive direction after addition of EP. The anodic peak potential for the oxidation of EP on the poly(caffeic acid) modified electrode is about 0.22 V, while EP is oxidized at about 0.27 V (Figure 2d) at an unmodified glassy carbon electrode under identical

conditions. The oxidation peak current of EP at the modified electrode is obviously higher than that at the unmodified glassy carbon electrode. Furthermore, a decrease of 50 mV in overpotential is observed at the modified electrode. Therefore, the poly(caffeic acid) shows a promotion to the electrooxidation of EP.

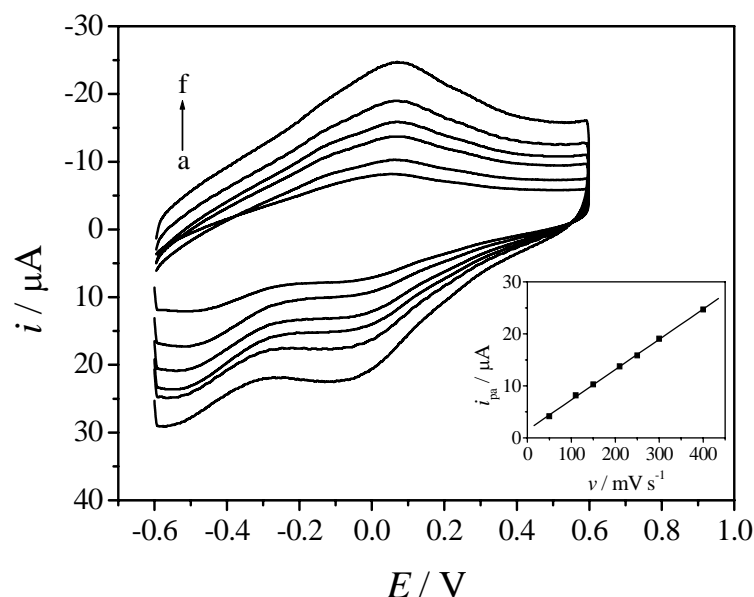


Figure 1. Cyclic voltammograms at the composite polymer modified electrode in pH 7.4 buffer at the scan rate (mV s^{-1}): (a) 110; (b) 150; (c) 210; (d) 250; (e) 300; (f) 400. Inset: plot of anodic peak current vs. the scan rate.

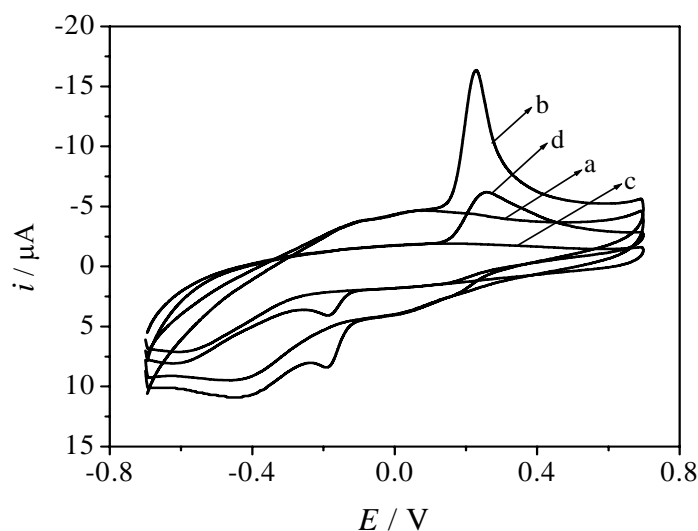


Figure 2. Cyclic voltammograms of the poly(caffeic acid) modified electrode (a, b) and the bare glassy carbon electrode (c, d) at 50 mV s^{-1} in 0.15 mol L^{-1} phosphate buffer (pH 7.4). (a) and (c), in the absence of EP; (b) and (d), in the presence of 0.1 mmol L^{-1} EP.

2.3. Optimum Conditions for the Electrochemical Deposition of Caffeic Acid on the Glassy Carbon Electrode

The effects of the preparation conditions (such as the deposition potential, deposition time, pH and the concentration of caffeic acid) of the poly(caffeic acid) modified electrode on the electrochemical activity for EP were investigated. Figure 3 illustrates the effect of different experimental conditions on the anodic peak current for EP on the poly(caffeic acid) modified electrode. The peak current for EP increases with the increasing of deposition potential. When the deposition potential exceeds 2.0 V, the peak current almost does not enhance. The effect of deposition time on the anodic peak current has been investigated and the results indicated that the peak current reached the maximum when the deposition time was 30 s. The poly(caffeic acid) film on the glassy carbon electrode may scale off when the deposition time exceeds 30 s, resulting in the peak current decrease. Figure 3c shows that the maximum anodic peak current appears at pH 7.0. As can be seen in Figure 3d, the peak current increased with increasing concentration of caffeic acid up to $2.0 \times 10^{-4} \text{ mol L}^{-1}$, but then leveled off for larger concentrations. Therefore, the optimum polymerization conditions were selected as follows: The glassy carbon electrode was immersed in phosphate buffer (pH 7.0) solution containing $2.0 \times 10^{-4} \text{ mol L}^{-1}$ caffeic acid; the potential of the electrode was maintained at +2.0 V for 30 s. Before measurement, the poly(caffeic acid) modified electrode was rinsed with water.

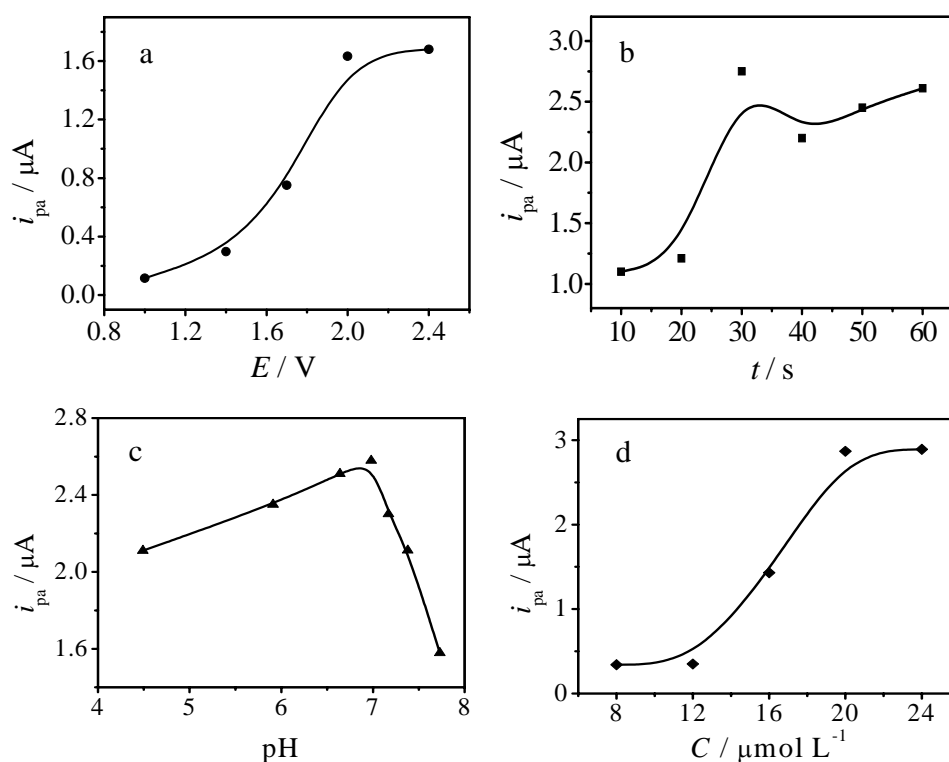


Figure 3. Variation in anodic peak of surface coverage of caffeic acid modified glassy carbon electrode with different (a) deposited potential; (b) deposited time; (c) pH; (d) concentration of caffeic acid (at 50 mV s^{-1}).

2.4. Effects of pH of EP Solution and Scan Rate

The effect of solution pH on the response of EP was investigated over the pH range of 4.49 – 9.18. Figure 4 shows that the anodic peak potential shifted toward negative direction with the increase of pH, showing that protons had taken part in the electrode process. As can be seen in Figure 4, the anodic peak potential for EP is pH dependent with a slope of 67 mV/pH unit, which shows that the uptake of electrons is accompanied by an equal number of protons.

The effect of solution pH on the anodic peak current of EP was complicated. The anodic peak current of EP increased with the increasing of solution pH until pH reached 7.38. Then the anodic peak current decreased with increasing pH. The reason is as follows: The protonated degree of EP decreased with increasing pH. In acid solution, caffeic acid is easy to be protonated. It can be easily followed that the static repulsion action between EP cation and protonated poly(caffeic acid) decreases with the increase of pH over the range from 4.49 to 6.98, thus, the anodic peak current increased with the increasing of pH. On the other hand, in neutral or close to neutral solution, caffeic acid exists mainly in nonprotonated form (neutral molecule). To study the mechanism of action, quantum chemical calculation has been carried out on the neutral caffeic acid molecule using the AM1 method from the program package Hyperchem. The results show that the oxygen atoms in the carboxyl group have large negative charge (the charge densities of O atoms in carbonyl and hydroxyl groups were -0.361 and -0.325 , respectively). Therefore, the surface functional groups of poly(caffeic acid) film were electron-rich in neutral or close to neutral solution, which could attract EP cations. As the above mentioned, the protonated degree of EP decreases with the increasing of pH, thus, the static attraction interaction between EP and poly(caffeic acid) decreased with the increasing of pH over the range from 7.74 to 9.18. So the anodic peak current decreased with the increasing of pH. Since pH 7.38 is the physiological pH value, it was chosen for the electrochemical detection of EP.

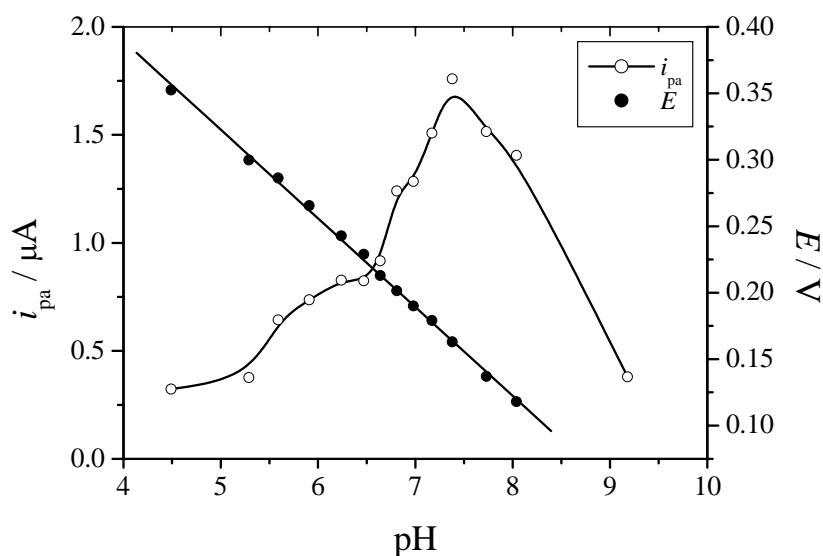


Figure 4. Effect of pH on the electrochemical oxidation of EP at the poly(caffeic acid) modified glassy carbon electrode.

Figure 5 shows the cyclic voltammograms of the caffeic acid modified glassy carbon electrode at various scan rates obtained in 0.15 mol L⁻¹ phosphate buffer (pH 7.4) containing 0.1 mmol L⁻¹ EP. The peak current for the anodic oxidation of EP is proportional to the square root of scan rate in the range of 30 – 190 mV s⁻¹ with a correlation coefficient of 0.9998, indicating that the catalytic reaction is controlled by diffusion. And the anodic potential shifted positively with the increase of scan rate, indicating the quasi-reversible nature of the electrode reaction.

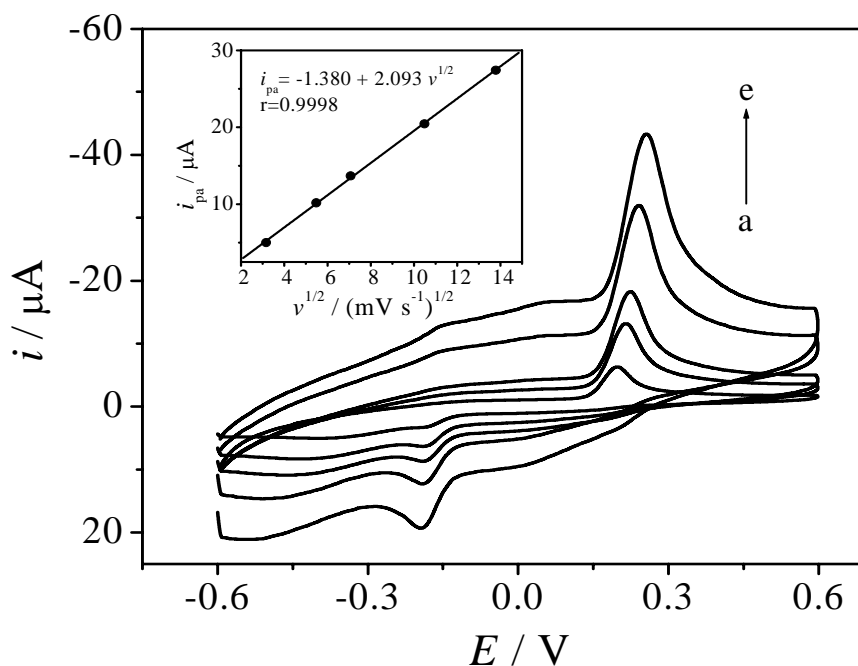


Figure 5. Dependence of the cyclic voltammetric response at the poly(caffeic acid) modified glassy carbon electrode on sweep rate in 0.15 mol L⁻¹ phosphate buffer (pH 7.4) containing 0.1 mmol L⁻¹ EP. Scan rates: (a) 10; (b) 30; (c) 50; (d) 110; (e) 190 mV s⁻¹. Inset: plot of anodic peak current vs. $v^{1/2}$

2.5. Calibration Curve

The effect of the EP concentration on the voltammetric response of the poly(caffeic acid) modified electrode was investigated. The cyclic voltammograms were obtained in a series of concentration of EP (Figure 6). Upon the addition of EP, there was a dramatic enhancement in the anodic current. The dependence of peak current on the concentration of EP was a linear relationship in the range from 2.0×10^{-6} to 3.0×10^{-4} mol L⁻¹. The linear regression equation was expressed as $i_{pa} = 0.1778 + 0.5517 C$ (correlation coefficient, $r = 0.9998$) (i_{pa} , μA ; C , 10^{-6} mol L⁻¹). The detection limit for EP was 6.0×10^{-7} mol L⁻¹, and the relative standard deviation of the results was 4.2 % for six successive determinations at 5.0×10^{-5} mol L⁻¹ EP.

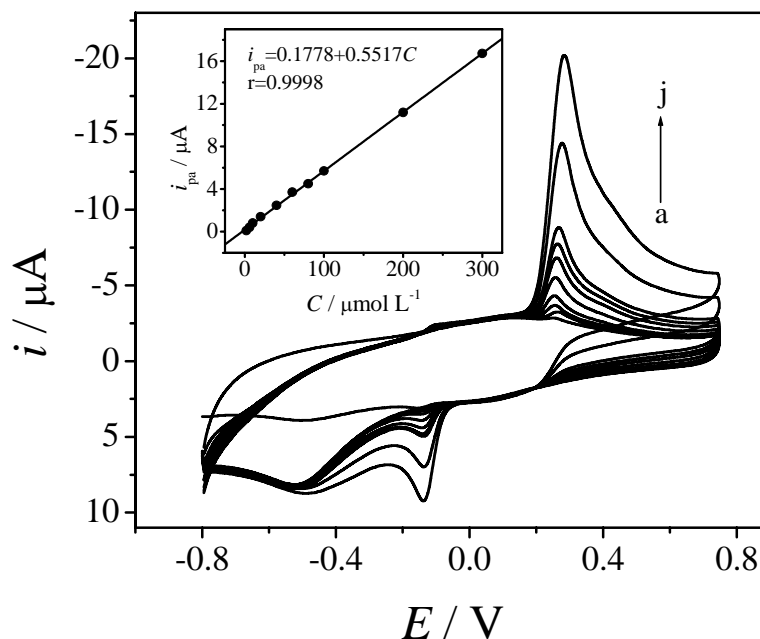


Figure 6. EP concentration dependence of the cyclic voltammetric response of the poly(caffeic acid) modified glassy carbon electrode in 0.15 mol L^{-1} phosphate buffer (pH 7.4) at 50 mV s^{-1} . EP concentrations are: (a) 2; (b) 6; (c) 10; (d) 20; (e) 40; (f) 60; (g) 80; (h) 100; (i) 200; (j) $300 \mu\text{mol L}^{-1}$. Inset: plot of anodic peak current vs. EP concentration.

2.6. Interference Study

The influence of various foreign species on the determination of $1.0 \times 10^{-5} \text{ mol L}^{-1}$ EP was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances that caused an approximately $\pm 5\%$ relative error in the determination. The tolerated ratio of foreign substances to $1.0 \times 10^{-5} \text{ mol L}^{-1}$ EP was 2000 for Na^+ , Cl^- , and K^+ ; 1000 for Zn^{2+} ; 200 for L-lysine and chitosan; 100 for glycine and glutamic acid; 50 for L-asparagine and glucose; 20 for L-cystine and Mg^{2+} ; 5 for Ca^{2+} and uric acid; 2 for ascorbic acid and dopamine, respectively.

2.7. Analytical Application

The developed method was applied to the determination of EP in EP injection. The determination results are listed in Table 1. Differential standard concentrations of EP were added to the diluted EP injection ($9.1 \times 10^{-6} \text{ mol L}^{-1}$), respectively. The results are listed in Table 2. The recoveries were between 94.7 and 102.4%. The recovery and RSD were acceptable, showing that the proposed method could be efficiently used for the determination of EP in injections.

Table 1. Determination of EP in injection of EP.

No.	Specified* (mg mL ⁻¹)	Found (mg mL ⁻¹)				RSD (%)
1	1.00	0.98	0.97	0.98	1.04	3.2
2	1.00	1.00	0.98	0.96	1.03	2.9

*From Southwest pharmaceutical Co. Ltd, Chongqing, China.

Table 2. Assays for recovery of the determination of EP.

No.	Specified (10 ⁻⁶ mol L ⁻¹)	Found (10 ⁻⁶ mol L ⁻¹)	Add (10 ⁻⁶ mol L ⁻¹)	Found (10 ⁻⁶ mol L ⁻¹)	Recovery (%)
1	9.10	8.88	50.00	60.31	102.4
2	9.10	8.79	80.00	86.12	96.3
3	9.10	8.90	100.00	106.66	97.6
4	9.10	9.37	50.00	58.47	98.7
5	9.10	8.87	80.00	84.86	94.7
6	9.10	8.67	100.00	105.29	96.2

3. Experimental Section

3.1. Reagents and Apparatus

0.15 mol L⁻¹ phosphate buffers with various pHs were prepared by mixing the stock solutions of 0.15 mol L⁻¹ NaH₂PO₄ and Na₂HPO₄. Caffeic acid, and epinephrine (EP) were from Sigma. EP and caffeic acid solutions were prepared just prior to use and all experiments were carried out at room temperature (23 ± 2 °C). All the chemicals were of analytical-reagent grade, and doubly distilled water was used throughout.

The electrochemical experiments were carried out using a CHI 660B Electrochemical Station (CH Instruments). A glassy carbon disk electrode with a diameter of 3 mm was used for working electrode. A platinum wire was employed as the counter electrode and a Ag/AgCl electrode was served as the reference electrode. All potentials were given with respect to the Ag/AgCl electrode.

A pHs-3B pH meter (Dazhong, Shanghai) was used for measuring pH.

3.2. Electrode Preparation

Prior to each experiment, the glassy carbon electrode was first polished with 0.05 μm alumina in a water slurry using a polishing cloth and rinsed with 1:1 HNO₃, acetone and water. After rinsing the electrode with water, the electropolymerization of caffeic acid was carried out in phosphate buffer (pH 7.0) containing 0.2 mmol L⁻¹ caffeic acid at +2.0 V for 30 s.

4. Conclusion

The oxidation of caffeic acid on the glassy carbon electrode gave rise to stable redox-active electropolymerized films. The optimum conditions for the preparation of poly(caffeic acid) modified electrode, such as the concentration of caffeic acid, the potential for deposition, the time of deposition, and the pH values of caffeic acid solution have been optimized. The poly(caffeic acid) film exhibited promotion effect on EP oxidation. The oxidation current of EP at poly(caffeic acid) was used for the determination of EP, and a linear EP concentration range from 2.0×10^{-6} to 3.0×10^{-4} mol L⁻¹ with a correlation coefficient of 0.9998 and a detection limit equal to 6.0×10^{-7} mol L⁻¹ was obtained. The proposed method can be applied to the detection of EP in samples.

Acknowledgements

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