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ORIGINAL PAPER

Electrochemical determination of estradiol using a poly(L-serine) film-modified electrode

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Abstract A thin film of poly(L-serine) was prepared via electropolymerization for the determination of trace levels of estradiol. In pH 5.0 phosphate buffer, L-serine was oxidized during the cyclic potential sweeps between −0.60 and 2.0 V, forming a thin film at the electrode surface. The electrochemical behavior of estradiol was investigated. The oxidation peak potential of estradiol shifts negatively at the poly(L-serine) film-coated glassy carbon electrode (GCE) compared with that at the bare GCE. Otherwise, the oxidation peak current greatly increases at the poly(L-serine) film-modified GCE. These phenomena suggest that the poly(L-serine) film exhibits catalytic activity towards the electrochemical oxidation of estradiol. Based on this, a sensitive, rapid and simple electrochemical method was proposed for the determination of estradiol. The limit of detection is evaluated to be 2.0×10^{-8} mol L^{-1}. Finally, this method was successfully used to determine estradiol in blood serum.

Keywords Serine · Estradiol · Electrochemistry · Determination · Modified electrode

1 Introduction

Recently, thin films of various amino acids have achieved wide application in electrochemistry [1, 2]. L-Serine is an important amino acid, which is involved in the biosynthesis of purines, pyrimidines and other amino acids. Li [3] reports that L-serine can be polymerized and form a thin film at an electrode surface by cyclic potential sweeping. This poly(L-serine) film-modified electrode shows catalytic ability to tyrosine. However, to the best of our knowledge, the application of poly(L-serine) biofilm to the electrochemical determination of estradiol has not been reported.

Estradiol (Fig. 1) is an essential steroid estrogen. It plays a very important role in female fertility and its concentration and change are closely related to human health. Therefore, determination of estradiol is very important in clinical practice. So far, different methods have been reported for the determination of estradiol such as high-performance liquid chromatography [4], liquid chromatography [5], enzyme immunoassay [6] and liquid chromatography–mass spectrometry [7]. Although the electrochemical activity of estradiol is very poor, many efforts have been made to improve its electrochemical response, and a variety of modified electrodes have been reported for the direct determination of estradiol. For example, a Nafion-modified glassy carbon electrode (GCE) combing with a surfactant enhancement effect [8], a multi-wall carbon nanotube-narion modified GCE [9], a boron-doped diamond thin film electrode [10], and a DNA aptamer immobilized gold electrode chip [11] have been employed to detect estradiol.

The main objective of this work is to fabricate a poly(L-serine) film-modified electrode and to develop an electrochemical method for the analysis of estradiol. At first, a poly(L-serine) thin film was electrochemically polymerized at a GCE surface according to previously published work [3]. The electrochemical behavior of estradiol was then examined at bare GCE and the poly(L-serine) film-coated GCE. From the comparison it is clear that the electrochemical oxidation

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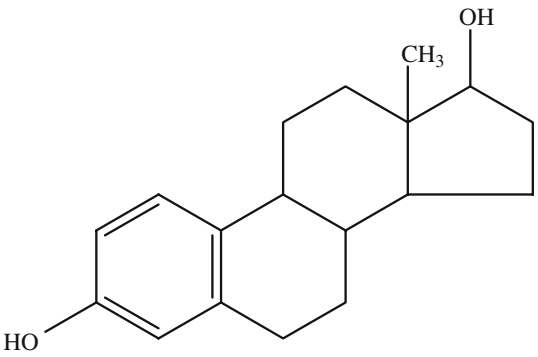

Fig. 1 Molecular structure of estradiol

of estradiol is remarkably facilitated as indicated by the obvious peak current enhancement and negative shift of the oxidation peak potential. As a result the poly(L-serine) film-coated GCE catalyzes the electrochemical oxidation of estradiol, possessing promising application for sensitive and rapid determination of estradiol.

2 Experimental

2.1 Reagent

All the chemicals were of analytical grade and were used without further purification. The water used in this work was bi-distilled. L-Serine and estradiol were purchased from Sigma (USA). 1.00×10^{-2} mol L^{-1} stock solution of estradiol was prepared by dissolving estradiol into ethanol, and then stored at 4 °C. HPLC grade methanol was purchased from Sigma-Aldrich.

2.2 Instruments

All the electrochemical measurements were carried out using a CHI 830 B Electrochemical Workstation (CH Instrument, Austin, USA). A conventional three-electrode system, consisting of a poly(L-serine) film-modified glassy carbon working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode, was employed.

High performance liquid chromatographic determination was carried out with an Agilent 1100 with UV-visible detector. The C18 analytical column (250 mm × 3.0 mm × 5 μm) was used. The mobile phase was a 23:24:53 (v/v) mixture of acetonitrile, methanol and water. Detection was accomplished at a wavelength of 225 nm.

2.3 Preparation of poly(L-serine) film-modified GCE

The poly(L-serine) film-modified GCE was prepared as follows. A GCE was polished with 0.05 μm alumium slurry, then rinsed with re-distilled water and finally sonicated in redistilled water to give a clean and mirror surface. Electropolymerization of L-serine on GCE surface was carried out using cyclic sweeps between −0.6 and 2.0 V in 0.1 mol L^{-1} phosphate buffer (pH 5.0) containing 1.00×10^{-2} mol L^{-1} L-serine. After 20 cycles, the film-coated GCE was washed with redistilled water to remove any physically adsorbed species.

2.4 Electrochemical measurement

The pH 6.5 phosphate buffer (0.1 mol L^{-1}) was used as supporting electrolyte for electrochemical measurement of estradiol. At first, the poly(L-serine) film-modified GCE underwent successive cyclic sweeps between 0.20 and 0.90 V until the voltammograms were stable. Then, an estradiol solution was added. After 2-min accumulation, the linear sweep voltammograms were recorded, and the peak current was measured at 0.60 V. After each measurement, the poly(L-serine) film-modified GCE was activated by five cyclic sweeps between 0.20 and 0.90 V in the blank phosphate buffer (pH 6.5) to remove any adsorbates and give a reproducible electrode surface.

3 Results and discussion

3.1 Electrochemical behavior of estradiol

The electrochemical behavior of estradiol at the poly(L-serine) film-modified GCE was investigated using cyclic voltammetry (CV). Figure 2 shows the cyclic voltammograms of 2.0×10^{-5} mol L^{-1} estradiol in 0.1 mol L^{-1}, pH 6.5 phosphate buffer. During the first anodic sweep

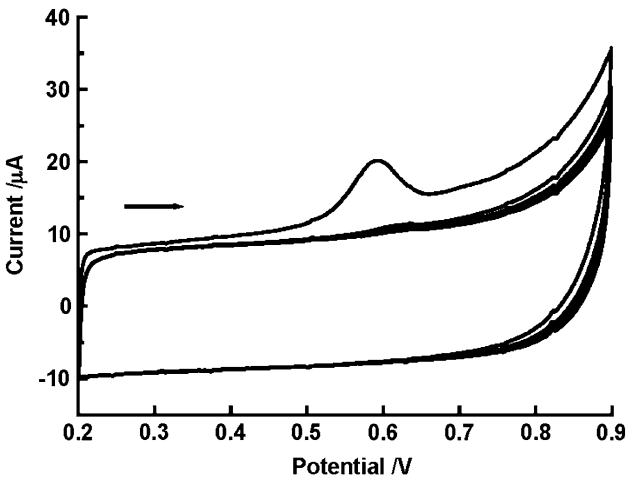

Fig. 2 Cyclic voltammograms of 2.0×10^{-5} mol L^{-1} estradiol in pH 6.5 phosphate buffer at poly(L-serine) film-modified GCE. Scan rate = 100 mV s^{-1}

from 0.20 to 0.90 V, a well-shaped oxidation peak is observed at 0.60 V. On the reverse scan from 0.90 to 0.20 V, no corresponding reduction peak is observed. Furthermore, the cyclic voltammetric responses of estradiol under different scan rates such as 10, 25, 50, 75, 150, 200, 300 and 400 mV s^{-1} were examined. At all scan rates, just an oxidation peak is observed, suggesting that the electrochemical oxidation of estradiol at the poly(L-serine) film-modified GCE is totally irreversible. Additionally, the oxidation peak current of estradiol greatly decreases during the second cyclic sweep, which is caused by the fact that the oxidative product adsorbs at the electrode surface. Therefore, the oxidation peak current in the first anodic sweep is recorded for estradiol to achieve higher sensitivity.

In order to elucidate the unique properties of poly(L-serine) film, the electrochemical responses of low concentration of estradiol at bare GCE and poly(L-serine) film-coated GCE were compared by linear potential sweep (LSV) since the electrode process of estradiol is irreversible. The results are shown in Fig. 3. At bare GCE, a negligible oxidation peak appears at 0.69 V for 1.0×10^{-6} mol L^{-1} estradiol in pH 6.5 phosphate buffer (curve a). Otherwise, the oxidation peak current of estratiol obviously increases after 2-min open-circuit accumulation (curve b), indicating that the accumulation is effective in improving the electrochemical response of estradiol.

Curve (c) depicts the electrochemical response of 1.0×10^{-6} mol L^{-1} estradiol at the poly(L-serine) film-coated GCE without accumulation. An obvious oxidation peak is observed at 0.60 V. Compared with curves (a) and (c), it is well-known that the peak current of estradiol significantly increases at the poly(L-serine) film-coated GCE. Moreover, the peak potential shifts negatively by 90 mV at the poly(L-serine) film-coated GCE. The remarkable peak current enhancement and negative shift of the oxidation peak potential clearly indicate that the poly(L-serine) film exhibits catalytic ability towards the electrochemical oxidation of estradiol. As expected, the oxidation peak current of estradiol also remarkably increases after 2-min accumulation at the poly(L-serine) film-coated GCE (curve d).

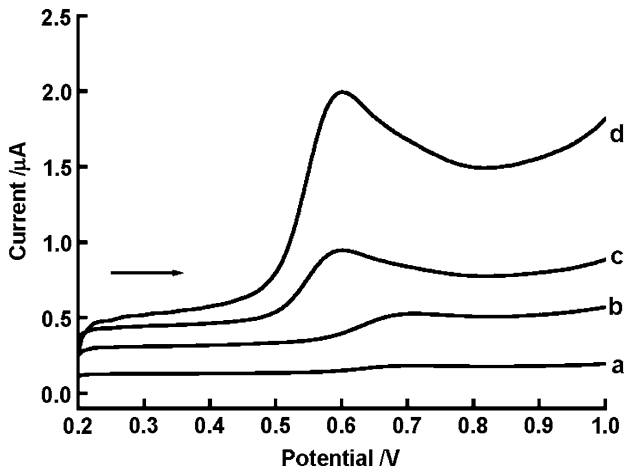

Fig. 3 Linear sweep voltammograms of 1.0×10^{-6} mol L^{-1} estradiol at bare GCE (a, b) and poly(L-serine) film-modified GCE (c, d). (b, d): After 2-min accumulation. Scan rate = 100 mV s^{-1}

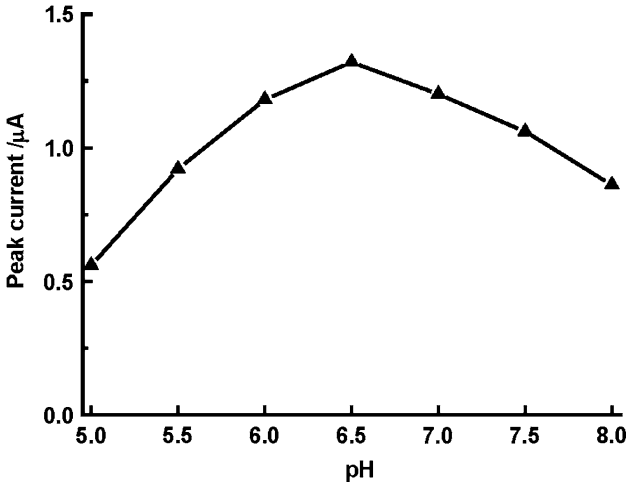

Fig. 4 Effect of pH value on the oxidation peak current of 1.0×10^{-6} mol L^{-1} estradiol

3.2 Effect of pH

The electrochemical oxidation of estradiol in 0.1 mol L^{-1} phosphate buffer with different pH values was studied using LSV. Figure 4 shows the oxidation peak current of 1.0×10^{-6} mol L^{-1} estradiol as a function of pH. When pH gradually increases from 5.0 to 6.5, the oxidation peak current of estradiol at the poly(L-serine) film-coated GCE also gradually increases. With further increase in pH from 6.5 to 8.0 the oxidation peak current decreases slightly. Thus, a pH 6.5 phosphate buffer was used as suitable supporting electrolyte for the electrochemical measurement of estradiol.

3.3 Influence of scan rate

In pH 6.5 phosphate buffer, the effect of scan rate on the oxidation peak current of estradiol were studied by LSV. It is found that the oxidation peak current of estradiol at the poly(L-serine) film-coated GCE increases linearly with scan rate over the range 20 to 400 mV s^{-1}, suggesting that the electrochemical oxidation of estradiol is controlled by adsorption.

3.4 Effect of accumulation time

Figure 5 shows the influence of accumulation time on the oxidation peak current of 1.0×10^{-6} mol L^{-1} estradiol.

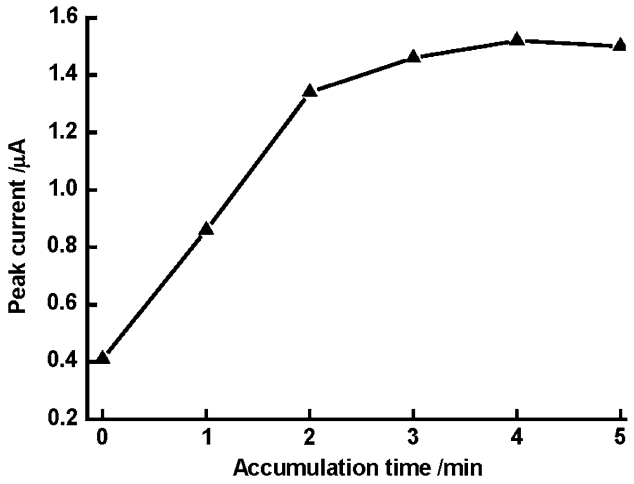

Fig. 5 Influence of accumulation time on the oxidation peak current of 1.0×10^{-6} mol L^{-1} estradiol

Table 1 Determination of estradiol in blood serum

Blood serum	By HPLC (mol L^{-1})	By this method (mol L^{-1})	RSD (%)
1	8.43×10^{-8}	8.04×10^{-8}	4.6
2	9.65×10^{-8}	9.92×10^{-8}	4.4
3	1.98×10^{-7}	1.90×10^{-7}	3.8
4	2.36×10^{-7}	2.42×10^{-7}	3.4

When the accumulation time extends from 0 to 2 min, the oxidation peak current of estradiol linearly increases. With increasing accumulation time, the amount of estradiol accumulated at the poly(L-serine) film surface also increases. So, the oxidation peak current shows a remarkable increase. However, the oxidation peak current of estradiol increases slightly when the accumulation time is longer than 2.0 min, suggesting that the amount of estradiol at the poly(L-serine) film surface tends to saturation. Considering sensitivity and working efficiency, an accumulation time of 2.0 min was employed.

3.5 Linear range, limit of detection and reproducibility

Under the optimized conditions, the relationship between oxidation peak current and concentration was examined. The oxidation peak current of estradiol is proportional to its concentration over the range from 1.0×10^{-7} to 3.0×10^{-5} mol L^{-1}. After 2-min accumulation, the limit of detection was evaluated to be 2.0×10^{-8} mol L^{-1} based on a signal-to-noise ratio of 3.

The reproducibility was evaluated by successive measuring the same 1.0×10^{-6} mol L^{-1} estradiol for 10 times using a single poly(L-serine) film-coated GCE. The relative standard deviation (RSD) of 6.5% indicates that this method has excellent reproducibility.

3.6 Interferences

The influences of foreign species on the oxidation peak current of 1.0×10^{-6} mol L^{-1} estradiol were examined. It was found that 3.0×10^{-4} mol L^{-1} progesterone, cholesterol, vitamin B_2, caffeine, vitamin E, vitamin A; 1.5×10^{-4} mol L^{-1} uric acid (UA), ascorbic acid (AA), dopamine (DA), hypoxanthine, L-lysine, L-tyrosine and L-cysteine, interfere minimally with the determination of estradiol since the peak current change was below 5%.

3.7 Analytical application

This novel modified electrode was used to determine estradiol in several blood serums, obtained from Renmin Hospital of Wuhan University. The estradiol content was determined using the standard addition method and the results are summarized in Table 1. Each sample was determined in triplicate and the RSD is below 5%. In order to confirm the accuracy of this method, the estradiol content was analyzed by HPLC. The results are in good agreement, revealing that this method possesses good accuracy.

4 Conclusion

In this work, a polymeric film of L-serine was prepared onto an electrode surface via electropolymerization. This film exhibits catalytic ability towards the electrochemical oxidation of estradiol, and has great potential application in the sensitive determination of estradiol.

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