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Selective Determination of Epinephrine in the Presence of Ascorbic Acid and Dopamine Using a Glassy Carbon Electrode Modified with Valine

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Abstract

A glassy carbon electrode (GCE) modified with valine was used for the sensitive volt metric determination of epinephrine (EP). The electrochemical response characteristics of the modified electrode toward EP, ascorbic acid (AA) and dopamine (DA) were investigated by cyclic voltammetry (CV). The results show an efficient catalytic activity of the electrode for the electro-oxidation of EP, which leads to an improvement to the reversibility of the electrode response. The effect of pH and potential sweep rate on the mechanism of the electrode process was investigated. The modified electrode exhibits an efficient electron mediating behavior along with well-separated oxidation peaks for EP, AA, and DA. Under the optimum pH of 7.0 in phosphate buffer solution(PBS), the CV anodic peak current showed a linear relation versus EP concentration in the range of 4.5×10^{-6}~1.0×10^{-5}mol·L^{-1} and 1.0×10^{-5}~1.4×10^{-4} mol·L^{-1}, with correlation coefficients of 0.9997 and 0.9942. The detection limit is 7.6×10^{-7} mol·L^{-1}. High sensitivity and selectivity, submicromolar detection limit, high reproducibility, along with ease of preparation and regeneration of the electrode surface by simple polishing make this method suitable for the determination of EP in pharmaceutical and clinical preparations.

Keywords: Chemically modified electrode, Valine, Epinephrine

1. Introduction

Epinephrine (EP), often called adrenaline, is one of the most important neurotransmitters in mammalian central nervous systems and exists in the nervous tissue and body fluids. EP controls the nervous system in its performance for a series of biological reactions and nervous chemical processes (Qimin, 1978, P.102). Many diseases are related to changes of the EP concentration in mammals. It also serves as a chemical mediator, converting the nerve pulse to different organs. Medically, EP has been used as a common emergency health care medicine (Deftereos, 1993, PP. 627–632). Also, low levels of EP have been found in patients with Parkinson's disease (Dayton, 1980, PP. 946–950). Quantitative

determination of EP is a significant thing in developing nerve physiology, diagnosis and controlling medicine (Pihel, 1994, PP. 4532–4537). In recent years, many methods have been reported for the determination of EP, such as high-performance liquid chromatography (HPLC) (FuNan, 2007, PP. 942-946), spectrophotometry(Sotomayor, 2002, P.215), thermallens microscopy(Sorouraddin, 2001, P.91), fluorimetry(Sorouraddin, 1998, P.105), and electrochemical method (Yokotani, 2007, P. 223) etc. However, most of these methods are complicated because they need derivatization or combination with various detection methods. Also, some of them suffer from low sensitivity and low specificity. Determination of EP in the presence of AA and DA, because of their coexistence in human fluids, is attractive to biological and analytical researchers. Individual determinations of these compounds have been reported in many references; however, simultaneous determination of them has always been considered as a serious challenge in these studies, an enormous amount of research has been devoted to the development of new modified electrodes for monitoring EP. The electrochemical detection of EP on the surface of bare (unmodified) electrodes has some fundamental problems, mainly high potential and sluggishness of the kinetics of the electrode process, which result in weak electrochemical responses Hernandez, 2001, PP.985–991).Thus,in this paper, we described the preparation and suitability of valine modified glassy carbon electrode modified as a new electrocatalyst in the electrocatalysis and determination of EP in an aqueous buffer solution, then we evaluated the analytical performance of the modified electrode in quantification of EP in the presence of AA and DA.

In the experiment, valine was used as a new electrocatalyst; the scheme is shown in figure 1.

2. Experimental

2.1 Chemicals and reagents

Epinephrine and D-Valine and L-ascorbic acid were purchased from National Institute for the Control of Pharmaceutical and Biological Products. Dopamine was purchased from Sigma. Phosphate buffer solutions (PBS) were prepared by mixing KH_2PO_4, which were purchased from Beijing Chemical Reagent Company. All other chemicals not mentioned here were of analytical reagent grade and were used as received. Double distilled water was used throughout.

2.2 Apparatus

Electrochemical experiments were performed with an electrochemical work station-CHI660C (CH Instruments, Shanghai Chenhua Instrument Corporation, China). A conventional three-electrode system was used throughout. The working electrode was a bare or a poly (valine) film-modified GCE (3.8mm in diameter), the auxiliary electrode was a Pt eletrode and a saturated calomel electrode was employed as reference electrode. PHS-3B (Shanghai Precision Scientific Instrument Co.,Ltd), KQ-100 ultrasonic cleaner (Kunshan Ultrasonic Instrument Factory), and Field emission scanning electron microscope (FE-SEM) images were obtained on an S-4800 field emission scanning electron microanalyser (Hitachi, Japan).

2.3 Electrode preparation and modification

Glassy carbon electrode was polished before each experiment with gold sand paper and 0.05μm alumina powder respectively, rinsed thoroughly with doubly distilled water between each polishing step, then washed successively with 1:1 nitric acid, acetone and doubly distilled water in ultrasonic bath and dried in air. The glassy carbon electrode is the working electrode and was treated in PBS (pH 9.0) by repetitive scanning in the potential range between 1.0 V and 2.2 V for 9 cycles at a scan rate of 50 $mV\cdot s^{-1}$and then rinsed with doubly distilled water.

2.4 Determination of epinephrine

Cyclic voltammetric experiments were performed on an electrochemical workstation at 25℃. A three-electrode system was used, including a GCE modified with valine as working electrode, a platinum electrode as counter electrode and a saturated calomel electrode (SCE) as reference electrode. The glassy carbon electrode was treated in PBS (pH 7.0) by repetitive scanning in the potential range between -0.4V and 0.6 V at a scan rate of100 $mV\cdot s^{-1}$.

3. Results and discussion

3.1 The optimization of electrochemical polymerization

The types of buffer solution and pH lever are the most important condition for membrane formation. We compared the buffer solution of KH_2PO_4-$Na_2B_4O_7\cdot 10H_2O$ with Na_2HPO_4–$C_6H_8O_7\cdot H_2O$ and Na_2HPO_4–NaOH, and found the buffer solution of KH_2PO_4- $Na_2B_4O_7\cdot 10H_2O$ was the best. So we chose the buffer solution of KH_2PO_4- $Na_2B_4O_7\cdot 10H_2O$ as Polymerization medium.

We also found that the glassy carbon electrode was treated in PBS (pH 9.0) by repetitive scanning in the potential range between 1.0 and 2.2 V for 9 cycles at a scan rate of 50 $mV\cdot s^{-1}$, which has a good electrochemical response to EP.

In the present work, we investigated a glassy carbon-electrode surface modified with valine. A scanning electron microscope (SEM) is shown in Fig. 2. It can be clearly seen that there are many three-dimensional bumps dispersed on the surface of GCE, which means that valine monomers are polymerized together and form a film.

3.2 Electrochemical oxidation of EP

The electrochemical behavior of EP at the GCE was investigated using cyclic voltammetry and the cyclic voltammograms are shown in Fig.3 at the bare GCE and the modified GCE. At bare GCE, the voltammogram of 1.0×10^{-5} mol·L^{-1} EP showed poor electrochemical response [Fig.3 (curve1)]. However, the Volta metric response was apparently improved at the modified GCE in PBS of pH 7.0 [Fig.3 (curve 2)]. The results of the enhancement of the peak current showed the excellent catalytic ability of valine.

3.3 Effect of pH

The effect of the medium's pH on the electrochemical signal was analyzed. The pH of the epinephrine solutions was changed using buffer solutions ranging from pH 2.0–8.0 (Fig.4). The measurements were made with a variable accumulation potential in accordance with the solution pH, at 90s accumulation time, 100 mV·s^{-1} scan rate and 1.0×10^{-5} mol·L^{-1} concentration of epinephrine.

It shows the voltammograms recorded for below pH 9. The epinephrine had no oxidation wave in experimental conditions above pH 9. This is not the observed behavior for other catecholamine. This behavior is due to the deprotonation of epinephrine (p*K*a around 8); this prevents its adsorption to the electrode surface, and removes the oxidation wave.

There was also a shift in the peak potential when the pH was changed. The potential shifted to lower values as the pH increased according to the equation:

E_{pa}=0.66 –0.059pH, r=0.9977. The intervention of H^+ in the electrochemical reaction correlates with the mechanism proposed by other authors (Yixin, 2006, pp: 156–161) [18] for the oxidation of catecholamine (Fig.5):

The best relationship between peak current and its resolution is found at a buffer concentration of pH 7.0. We took this as the optimum pH and used it in the subsequent experiments because it can get rid of the possibility interference of dopamine and ascorbic acid.

After selecting the working pH, the accumulation potential was analyzed. A solution of epinephrine was used in a phosphate medium at pH 7.0, varying the accumulation potential from-0.4V to 0.6V with a 90 s accumulation time and 100 mV·s^{-1} scan rate since we did not find any significant variation in the peak intensity below -0.4V and above 0.6V.

The study of the variation of accumulation time enabled us to ascertain the level of epinephrine adsorption on the electrode surface. To accomplish this, we varied the accumulation time between 50 and 120 s for 1.0×10^{-5} mol·L^{-1} epinephrine, an equilibration between substance concentration on the surface of the electrode and epinephrine was reached with shaking.

This occurs at shorter times when the epinephrine concentration is increased. In light of these results, we chose 90 s as the accumulation time for subsequent analyses since apex current reaches max with a 90 s accumulation time.

3.4 Effect of dopamine concentration

The concentration effect of EP was studied at modified GCE in 0.1 mol·L^{-1} phosphate buffer of pH 7.0; it shows the dependence of the Volta metric response of EP. The CV anodic peak current showed a linear relation versus EP concentration in the range of 4.5×10^{-6}~1.0×10^{-5} mol·L^{-1} and 1.0×10^{-5}~1.4×10^{-4} mol·L^{-1} with a correlation coefficient as follows. i_{pc}(A) = 4.43×10^{-6}+0.76C, r = 0.9997i_{pc} (A) = 1.30×10^{-5}+0.11C, r = 0.9942; A detection limit is 7.6×10^{-7} mol·L^{-1}.

3.5 Interference studies

Interference of DA or AA. Both AA and DA often coexist with EP in physiological fluids and possess close oxidation potentials to EP at a bare electrode, resulting in the electrochemical response of EP being almost overlaid by that of AA or DA. In this study, it was found that the above problem could be resolved by using the poly valine-modified GCE. Thus, it provides a possible method for the selective determination of EP in the mixture solution containing EP, AA and DA. In order to confirm the availability of poly valine-modified GCE to the separated determination of EP in the presence of AA and DA, we scanned a mixture solution containing1.0×10^{-5} mol·L^{-1} EP, 1.0×10^{-3} mol·L^{-1}AA, and 5.0×10^{-4} mol·L^{-1}DA in 50 m mol·L^{-1} PBS (pH 7.0) by CV.

As is shown in Fig. 6, the results demonstrate that the coexisting AA could produce no obvious peak currents and potentials in pH 7.0 PBS. Separations of the cathode peak potentials for EP-DA are about -220 and 170 mV, which also identifies that the accompanying DA could produce no effect on the assay of EP in pH 7.0 PBS at the poly (valine) film-modified electrode.

Based on the above discussion, one can conclude that either DA or AA is unlikely to bring large interference for the determination of EP in the presence of DA and AA at the poly(valine) film-modified electrode.

Interference of the other possible coexisting materials. The effects of the other substances that often accompany EP in

various pharmaceutical preparations were studied by analyzing a standard solution of EP ((1.0×10^{-5} mol·L^{-1}). If a foreign species caused a relative error of less than ±5% during the determination of 1.0×10^{-5} mol·L^{-1} EP, it was considered no interference. No interference has been found when including up to 1000 μmol·L^{-1} of K^+, Na^+, Ca^+, NH_4^+, Mg^{2+}, Cl^-, β-alanine, and 100μmol·L^{-1} of glucose and tartaric acid.

4. Applications

The present method was used to the determination of EP in pharmaceutical injection (Harvest Pharmaceutical Co. Ltd., Shanghai, China). To validate the practicability of the purposed method for the selective determination of EP in mixtures including AA and DA, we prepared three samples by dissolving amounts of EP, AA and DA into a mixture (C_{DA}/ C_{EP}=50, C_{AA}/ C_{EP}=100). The diluted solution was determined by CV at the poly (valine) film modified GCE. The results are listed in Tab.1. These results indicate that the method provides a potential tool for the separated determination of EP in the presence of AA and DA in their mixture.

5. Conclusions

The poly (valine) film-modified GCE exhibits highly electrocatalytic activity to the oxidation of EP and provides higher selectivity in Volta metric assay of EP in the presence of AA and DA. With the good selectivity and practicability, the proposed method has been applied to the determination of EP in real samples with satisfactory results.

Acknowledgements

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Table 1. Determination of EP in Injection (n=6)

Sample	Content 10^3mg·mL^{-1}	Added 10^3 mg·mL^{-1}	Found 10^3 mg·mL^{-1}	Recovery (%)
1	4.07	4.00	8.18	102.7
2	4.16	4.00	8.07	97.7
3	4.10	4.00	8.24	103.5

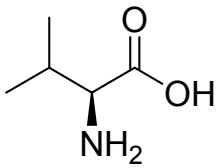

Figure 1. The structure of Valine

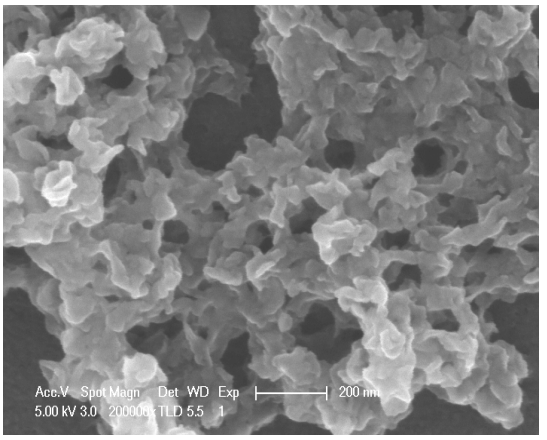

Figure 2. SEM images of valine/GCE

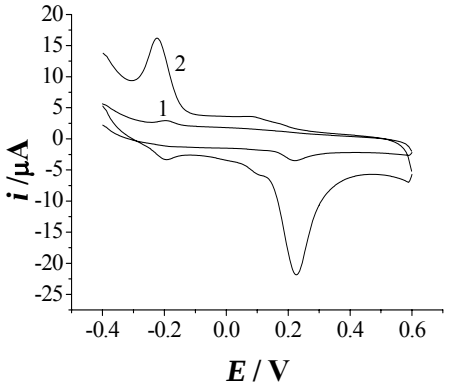

Figure 3. Cyclic voltammograms of 1.0×10^{-5} mol·L^{-1} EP obtained at the bare GCE and modified GCE at scan rate 50 mV/s in 0.1 mol·L^{-1}PBS (pH. 7.0)

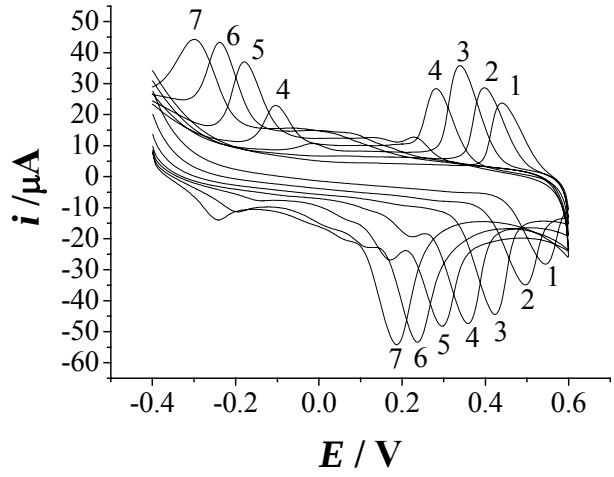

Figure 4. Cyclic voltammograms of 1.0×10^{-5} mol·L^{-1} of epinephrine at different pH levels.
pH (1-7):2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0

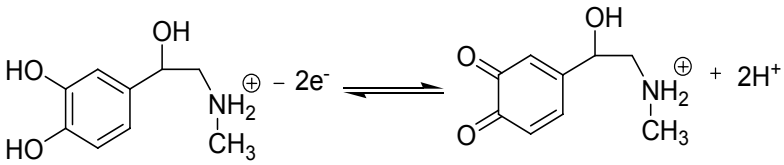

Figure 5. The mechanism of EP

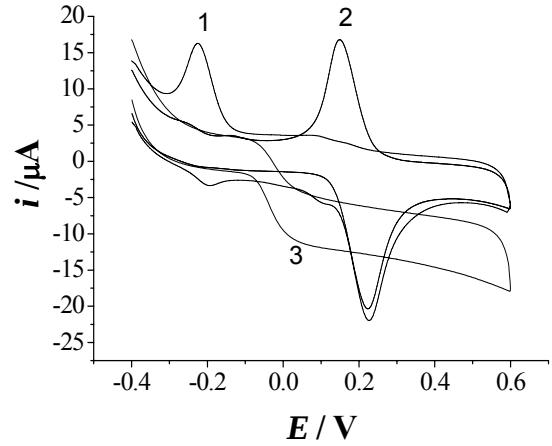

Figure 6. Cyclic voltammograms of 1.0×10^{-5} mol·L^{-1} epinephrine
(1) 5.0×10^{-4} mol·L^{-1} dopamine (2) and 1.0×10^{-3} mol·L^{-1} ascorbic acid (3) in PBS (pH7.0)