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Amperometric determination of epinephrine with an osmium complex and Nafion double-layer membrane modified electrode

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Abstract

The $[Os(bpy)_2(PVP)_{10}Cl]Cl$ polymer and Nafion have been covered on a glassy carbon electrode by drop-coating to fabricate a double-layer membrane modified electrode. The modified electrode showed an excellent electrocatalytical activity for the oxidation of the neurotransmitter epinephrine (EP). Under usual physiological pH conditions, the logarithm of the catalytic current of EP vs. its concentration showed a linear relation in the range of 4.0×10^{-9}–5.4×10^{-6} M and 5.4×10^{-6}–3.1×10^{-4} M with correlation coefficient of 0.995 and 0.996, respectively. The modified electrode could eliminate efficiently the interference of ascorbic acid, glucose and uric acid at 200-, 8600- and 20-fold concentration of EP (at 5.0×10^{-5} M), and it has been satisfactorily used for the amperometric determination of EP. The electrocatalytical rate constant k_{ch} was evaluated with a rotating disk electrode. Both the presence of a Nafion film and the increase in EP concentration resulted in a decrease of k_{ch}. The apparent Michaelis–Menten constant is about 0.51 mM. The modified electrodes exhibited a good reproducibility, sensitivity and stability for the determination of EP. © 1999 Published by Elsevier Science B.V.

Keywords: Glassy carbon electrode; Double-layer membrane modified electrode; $[Os(bpy)_2(PVP)_{10}Cl]Cl$; Nafion; Epinephrine; Electrocatalytical oxidation

1. Introduction

Epinephrine (EP) is an important compound for the message transfer in the mammalian central nervous system, existing as an organic cation in the nervous tissue and biological body fluid. Many diseases are related to changes of its concentration, thus, it is necessary to develop a quantitative determination method of trace EP for studying its physiological function and the diagnosis of some diseases in clinical medicine. Usually, the determination of EP is carried out by using liquid chromatography (LC) [1,2], visible spectrophotometry [3] and fluorimetric flow injection [4]. EP is an electroactive molecule, its electrochemical behaviors have been studied [5–7] and some

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methods have also been reported for its electrochemical determination [8–10]. A major problem in the determination is the lack of resolving power between EP and its coexisting electroactive species such as ascorbic acid (AA) and uric acid. AA with a higher concentration in biological systems causes a serious interference in the determination of EP. A variety of discriminative coatings, based on their different transport properties, can be used to control the access to the electrode surface [11–16]. Using these coatings, some chemically modified electrodes have been prepared for the determination of EP [14,15,17–19]. One of the most common routes is to cover the electrode surface with a negatively charged Nafion ionomer, which can repel AA and other negatively charged species, to eliminate the interference of these species to EP determination [14,20]. In these reports, however, both the sensitivity and the linear range were unsatisfactory. It is necessary to further develop a new modified electrode for the determination of EP. To this end, our group prepared some modified electrodes with poly-(indole-3-acetic acid) [21] and methylene green [22] and presented several methods for the determination of EP with detection limits of 4.0×10^{-8} and 1.0×10^{-8} M, respectively. However, their linear ranges were very poor, only from 1.0×10^{-7} to 6.0×10^{-6} M and 1.0×10^{-7} to 5.0×10^{-4} M.

Recently, there has been much interest in the electron transfer behavior and the electrocatalytical application of osmium bis(bipyridine) complex polymers [23–25]. The high molecular weight backbone makes the polymers to adhere strongly to the surface of electrode and gives excellent stability. Some modified electrodes have been prepared with $[Os(bpy)_2(PVP)_{10}Cl]Cl$ and $[Os(bpy)_2(PVI)_nCl]Cl$ (bpy=2,2′-bipyridine, PVP=poly-(vinylpyridine) and PVI=poly-vinylimidazole) films at the carbon electrodes [24–27]. These redox polymers can be used as electron transfer mediator between enzymes [24,25], NADH [27] or NO_2^- [28] and the electrode surface. Here, we describe the fabrication of $[Os(bpy)_2(PVP)_{10}Cl]Cl$ and Nafion double-layer membrane modified glassy carbon electrodes. Our purpose is to utilize these films to improve both selectivity and sensitivity for the determination of EP. This work indicated that the modified electrodes possess ideal electrochemical behavior and excellent electrocatalytical activity for the oxidation of EP with high sensitivity, and can eliminate the interference of AA and uric acid.

2. Experimental

2.1. Chemicals and materials

$[Os(bpy)_2(PVP)_{10}Cl]Cl$ was synthesized and characterized according to literatures [26,29]. Epinephrine, grade(II), was obtained from Sigma (USA) and used as received. Uric acid was obtained from British Drug Houses (UK). Both ascorbic acid and glucose were purchased from the Shanghai Biochemical Reagent Company (China). A 5 wt% solution of Nafion (EW 1100) was obtained from Aldrich (USA). All other chemicals were of analytical grade. All solutions were prepared with distilled water. 0.1 M phosphate buffer solution (PB) with various pHs were prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4, and then adjusting the pH with 0.1 M H_3PO_4 and NaOH. The solutions of epinephrine, AA, uric acid and glucose were prepared with pH 6.9 PB, respectively.

2.2. Apparatus

Electrochemical measurements were performed with a BAS 100B electrochemical analyzer (Bioanalytical Systems, USA) with a SCE reference electrode, against which all potentials were quoted in our results, a platinum wire counter electrode and a modified glassy carbon (without or with Nafion film) working electrode. The modified rotating disk glassy carbon electrode was used for the study of catalytic kinetics and the timebase measurements on ring-disk electrode (RDE) system, Model 636 (EG&G, USA).

2.3. Preparation of modified glassy carbon electrodes

Prior to modification, the glassy carbon electrodes as well as the RDE (5.0 mm diameter) were polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on microcloth pads (Buehler), and then rinsed with distilled water and briefly sonicated in acetone and twice in distilled water. After the electrodes were pretreated electrochemically by a continuous cyclic sweep between 0 and +1.4 V at 50 mV s^{-1} in pH 6.9 PB until a constant background cyclic voltammetry (CV) curve, they were modified with a 3 μl, 2 mg ml^{-1} $[Os(bpy)_2(PVP)_{10}Cl]Cl$ ($Os\text{-}(PVP)_{10}$) ethanol solution or further with a 2 μl 0.5% Nafion ethanol solution by droplet evaporation in air for 30 min to prepare the $Os\text{-}(PVP)_{10}$ or $Os\text{-}(PVP)_{10}$/Nafion double-layer membrane modified electrodes.

All electrochemical measurements were carried out in a deaerated solution under nitrogen atmosphere at room temperature. Time base experiments were performed with a potential step from 0 to +0.4 V at the rotating rate of 1000 rpm.

3. Results and discussion

3.1. Electrocatalytical oxidation of EP at a Os-$(PVP)_{10}$ polymer

At a bare glassy carbon electrode, the electrochemical response of EP is very poor. Its cyclic voltammogram exhibits an irreversible oxidation peak and the peak current quickly decreases with a continuous cyclic sweep (Fig. 1). So, it is impossible to determine the EP concentration with this peak.

Fig. 2 shows the cyclic voltammograms of an Os-$(PVP)_{10}$ polymer modified electrode in pH 6.9 PB at different scan rates. The cyclic voltammograms display a couple of peaks which correspond to the Os(II/III) redox couple with an average formal potential of +(325±3) mV. The ratio of oxidation to reduction peak currents is near to one and both peak currents increase in direct proportionality to the scan rate at scan rates less than 200 mV s^{-1}, as expected for a surface-immobilized redox couple. At lower scan rate the peak-to-peak potential separation is almost constant. With a continuous cyclic voltammetric sweep, both the peak shape and the peak currents are steady. From the peak area an average surface coverage Γ of $(2.78\pm0.74)\times10^{-10}$ mol cm^{-2} is obtained.

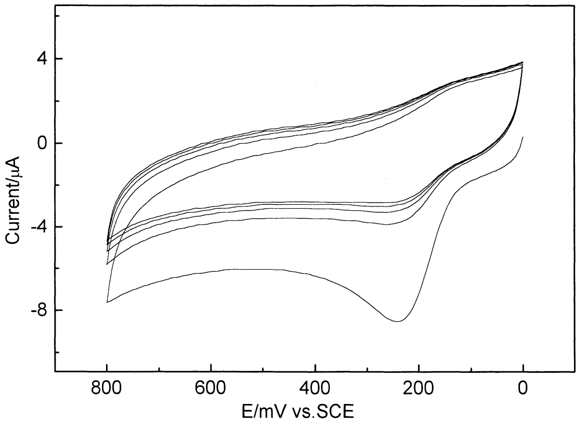

Fig. 1. Repetitive cyclic voltammogram of 1.1 mM EP at a bare glassy carbon electrode in 0.1 M pH 6.9 PB at 40 mV s^{-1}.

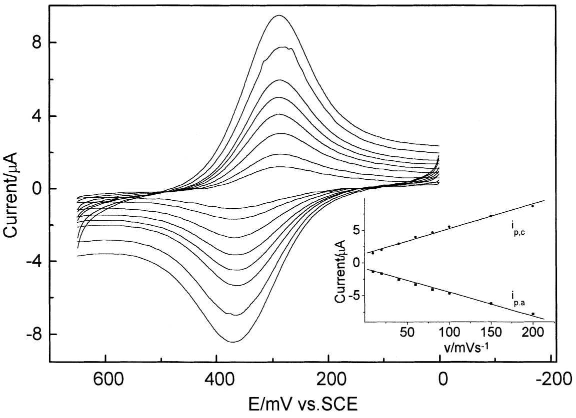

Fig. 2. Cyclic voltammograms of $Os\text{-}(PVP)_{10}$ polymer modified electrode in pH 6.9 PB at 200, 150, 100, 80, 60, 40, 20, and 10 mV s^{-1} (from external to internal). Inset: plot of peak currents vs. scan rates.

After EP is added to the PB, a well-shaped electrocatalytic voltammogram can be observed. The oxidation of EP occurred in the potential range of Os(II/III) redox couple. With increasing EP concentration the oxidation peak current increases clearly and the reduction peak current decreases and vanishes at a higher EP concentration. Different from that at a bare electrode, the oxidation peak current is at a constant value with both continuous sweep and repetitive measurements. The effects of scan rate on the cyclic voltammogram and the catalytic peak current are shown in Fig. 3. The catalytic peak current is directly proportional to the square root of the scan rate, indicating a fast electrocatalytic reaction and the electrocatalytic process is controlled by the diffusion of redox species [30]. The electrode process could be described as follows:

$$[Os\text{-}(PVP)_{10}]^{+} \Leftrightarrow [Os\text{-}(PVP)_{10}]^{2+} + e \qquad (1)$$

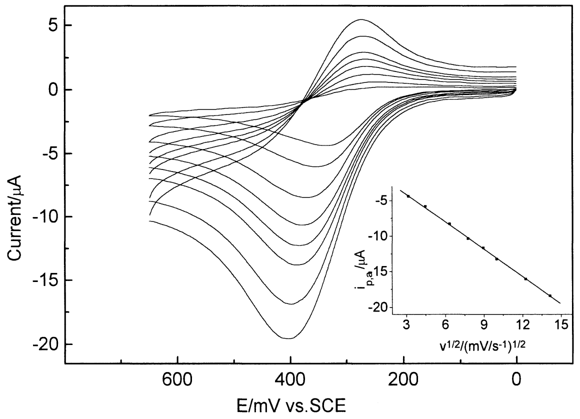

Fig. 3. Cyclic voltammograms of Os-(PVP)$_{10}$ modified electrode in 1.2×10^{-4} M EP (pH 6.9) at 200, 150, 100, 80, 60, 40, 20 and 10 mV s^{-1} (from external to internal). Inset: plot of anodic peak current vs. the square root of scan rate.

$$2[\text{Os-(PVP)}_{10}]^{2+} + \text{EP}_{\text{red}} \rightarrow 2[\text{Os-(PVP)}_{10}]^{+} + \text{EP}_{\text{ox}} \qquad (2)$$

3.2. Electrochemical characteristics of Os-(PVP)$_{10}$/ Nafion modified electrodes

The cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified electrode in pH 6.9 PBS also displays a couple of well-defined redox peaks (Fig. 4). Their shape is similar to that of the voltammograms of the Os-(PVP)$_{10}$ modified electrode (curve A and B in Fig. 5) and the peak currents slightly decrease. Both oxidation and reduction peak currents are directly proportional to the scan rate, indicating that the existence of Nafion film does not change the electrochemical characteristics of the Os(III/II) redox couple.

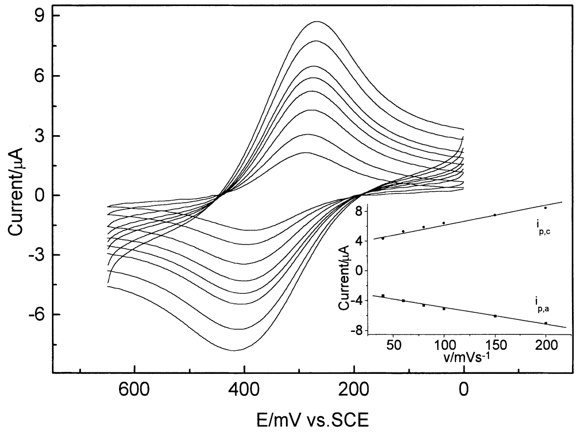

Fig. 4. Cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified electrode in pH 6.9 PB at 200, 150, 100, 80, 60, 40, 20 and 10 mV s^{-1}. Inset: plot of peak currents vs. scan rates.

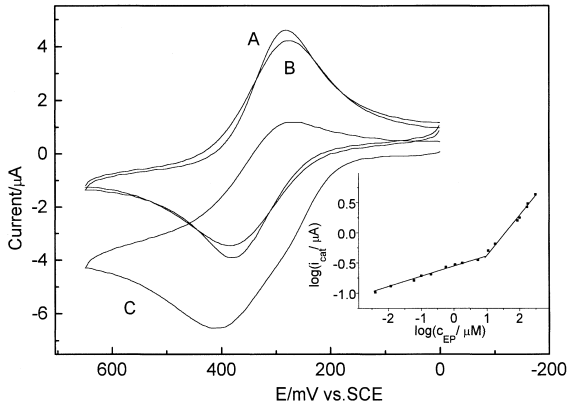

Fig. 5. Cyclic voltammograms of Os-(PVP)$_{10}$ (A) and Os-(PVP)$_{10}$/Nafion (B, C) modified eletrodes in pH 6.9 PB (A, B) and (B)+1.0×10^{-4} M (C) at 40 mV s^{-1}. Inset: plot of logarithm of catalytic current vs. EP concentration.

3.3. Electrocatalytical oxidation of EP at an Os-(PVP)$_{10}$/Nafion double-layer membrane modified electrode

The AA and uric acid, which are electroactive, often coexist with neurotransmitters in biological system, and they can be oxidized at a potential near to that of neurotransmitters at most solid electrodes, resulting in an overlapping voltammetric response. The positively charged osmium polymer membrane attracts negatively charged AA and uric acid, causing a strong electrocatalytic oxidation. Coating of Nafion has successfully been used to eliminate the interference of AA in the determination of primary neurotransmitters [14,20,31]. After the Os-(PVP)$_{10}$ modified electrodes were further coated with the Nafion film, a transport channel only for cations could be provided. At Os-(PVP)$_{10}$/Nafion modified electrodes the response of AA could be eliminated completely even at a higher concentration of AA and uric acid (see the following text). At the same time the electrocatalytic oxidation of EP was kept (Fig. 5 for curve B and C). Fig. 6 shows a proportional relation between the electro-

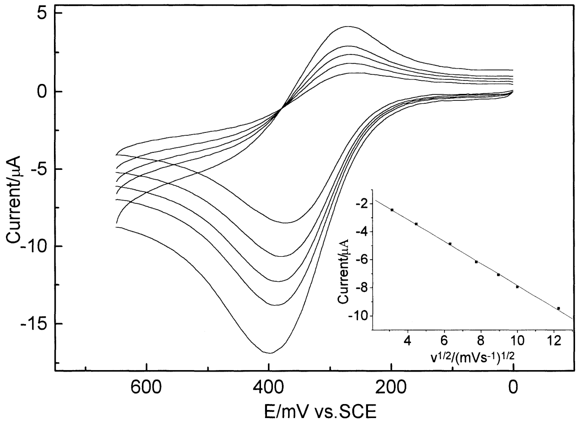

Fig. 6. Cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified electrode in 1.2×10^{-4} M EP (pH 6.9) at 150, 100, 80, 60 and 40 mV s^{-1} (from external to internal). Inset: plot of anodic peak current vs. the square root of scan rate.

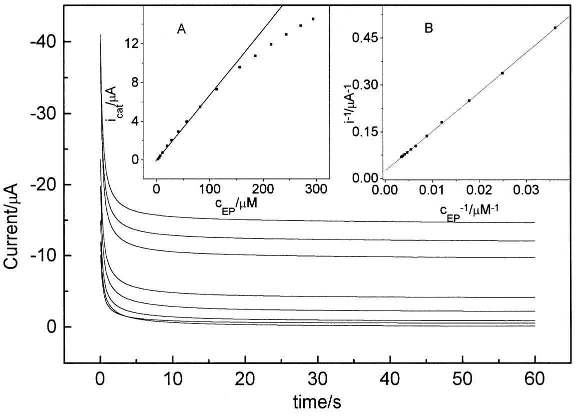

Fig. 7. Chronoamperometric curves with a potential step from 0 to +0.4 V at Os-(PVP)$_{10}$/Nafion modified electrode in pH 6.9 PB containing 0, 6.5, 11, 28, 56, 156, 215 and 294 μM (from bottom to top). Inset: (A) plot of catalytic current vs. EP concentration; (B) data analysis of catalytic current vs. EP concentration.

catalytic current and the square root of scan rate, also indicating that the electrocatalytic reaction rate is fast.

With the increase of solution acidity both the oxidation and reduction peak currents of the Os(II/III) redox couple increase, resulting from the protonation of the pyridine group in the polymer chain, whilst the oxidation current of EP decreases due to the participation of two protons in the oxidation process. An optimum pH of 6.9 was obtained for the electrochemical determination of EP, which is just within the physiological pH range value.

3.4. *Determination of epinephrine with an Os-(PVP)$_{10}$/Nafion modified electrode*

The determination of EP concentration at the Os-(PVP)$_{10}$/Nafion modified electrodes was carried out with both cyclic voltammetry and time-based technique (chronoamperometry) by using a standard addition method. After 9 μl volumed standard solution of EP was added into 10.0 ml of 0.1 M pH 6.9 PB and the solution was stirred for 5 min, the cyclic voltammogram was recorded. The plateau anodic current increased with increasing EP concentration. Their logarithmic curves exhibited a linear relation in the concentration range 4.0×10^{-9}–5.4×10^{-6} M or 5.4×10^{-6}–3.1×10^{-4} M with a correlation coefficient of 0.995 and 0.996, respectively (inset in Fig. 5). The relative standard deviations were 1.2% and 0.67% for 10 successive determinations at 2.0 and 100 μM EP, respectively, exhibiting an excellent reproducibility. The modified electrode showed good stability. Its electrochemical activity did not change after storage in air or in a PB solution for more than one month or cyclically scanned in PB solution.

After AA, glucose or uric acid was added into the cell containing 5.0×10^{-5} M EP, the electrocatalytical response did not change up to 200-, 8600- or 20-fold of the concentration of EP, respectively, indicating that AA, glucose or uric acid did not interfere. The modification of the electrode can efficiently eliminate their interference.

The time-based experiments were carried out upon the addition of EP at a RDE with a rotation rate of 1000 rpm (Fig. 7). After an electrolysis time of about 5 s, the oxidation reached a steady value, indicating a fast electrocatalytic response. The steady-state current increased linearly with the concentration of EP at lower concentrations (inset in Fig. 7). The linear range was from 2.18 to 113 μM with a correlation coefficient of 0.998. On further addition of EP, a Michaelis–Menten type response could be observed.

3.5. *Electrocatalytical kinetics of EP at Os-(PVP)$_{10}$/Nafion modified electrode*

3.5.1. *Apparent Michaelis–Menten constant K_m*

Fig. 7 exhibits a kinetic mechanism according to Michaelis–Menten for the electrocatalytic process.

From the data analysis of the catalytic oxidation current vs. EP concentration by using Eq. (3) (inset (B) in Fig. 7) an average Michelis–Menten constant K_m of $(5.07\pm0.01)\times10^{-4}$ M and an average maximum catalytic current i_m of (40.1 ± 1.0) μA were obtained.

$$1/i_k = 1/i_m + K_m/i_m c \qquad (3)$$

where c is the concentration of EP.

3.5.2. Electrocatalytic rate constant k_{ch}

The electrocatalytic rate constant can be determined by using Koutecky–Levich plots at a modified rotating disk electrode. The experimental results showed that the catalytic current increased linearly with increasing $\omega^{1/2}$, and then tends to a steady value, indicating that the current was controlled by the transport of EP through the rather thick Levich layer at lower rotating rate ω. At higher ω the thickness of a Levich layer decreased, the electrode process will be controlled by the catalytic reaction rate, k_{ch} ($M^{-1}\ s^{-1}$), between EP and the Os(II/III) redox couple. The Koutecky–Levich equation of the limiting current i_{lim} of the catalytic reaction is as follows [32]:

$$1/i_{lim} = 1/i_k + 1/i_{lev} = 1/nFAk_{ch}\Gamma c + 1/0.620nFAcD^{2/3}\nu^{-1/6}\omega^{1/2}. \qquad (4)$$

The Koutecky–Levich plots are shown in Fig. 8. From the intercepts and the average surface coverage of 2.78×10^{-10} mol cm^{-2} of the Os(III/II) redox couple, the k_{ch} values of $(3.01\pm0.02)\times10^4$ and $(2.34\pm0.02)\times10^4\ M^{-1}\ s^{-1}$ at the EP concentrations of 50 and 100 μM, respectively, were obtained. The rate constants are rather high, indicating a fast electrocatalytic process. The value of k_{ch} increased with decreasing concentrations of EP. Under the same experimental conditions, the rate constants k_{ch} of $(4.62\pm0.03)\times10^4$ and $(3.45\pm0.04)\times10^4\ M^{-1}\ s^{-1}$ at the EP concentrations of 50 and 100 μM, respectively, were obtained at an Os-$(PVP)_{10}$ modified electrode without Nafion. It can be seen that the coating of Nafion film resulted in a decrease in the rate constant because the transfer of EP molecule in Nafion film was more difficult than that in solution.

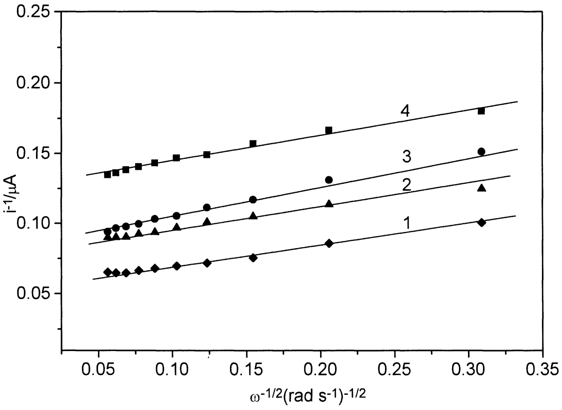

Fig. 8. Koutecky–Levich plots for electrocatalytical oxidation of EP at rotating disk electrodes modified with Os-$(PVP)_{10}$ (1, 3) and Os-$(PVP)_{10}$/Nafion (2, 4) in pH 6.9 PB containing 50 μM (3, 4) and 100 μM (1, 2) EP.

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