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[Os(bpy)$_2$(PVP)$_{10}$Cl]Cl polymer and Nafion dual-film modified graphite electrode for the amperometric determination of trace amounts of norepinephrine

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A chemically modified graphite electrode was prepared by using a dual film of [Os(bpy)$_2$(PVP)$_{10}$Cl]Cl polymer and Nafion. The modified electrode showed excellent electrocatalytical activity for the oxidation of norepinephrine (NE) and an ability to eliminate efficiently the interference of ascorbic acid and other anions. The catalytic peak currents obtained from the cyclic voltammograms increased linearly with increasing concentration of NE. A log–log plot of catalytic current *versus* NE concentration showed a dual-linear relationship in the ranges 1.8×10^{-8}–4.4×10^{-6} M and 4.4×10^{-6}–2.9×10^{-4} M with correlation coefficients of 0.990 and 0.999, respectively. The detection limit was about 18 nM (3σ). At a potential of +500 mV the chronoamperometric response showed a linear relationship between the steady state current and NE concentration in the range 1.3–130 μM. With a further increase in NE concentration a Michaelis–Menten-shaped response was observed. The apparent Michaelis–Menten constant and the maximum current were 1.7 mM and 86 μA, respectively. The modified electrode showed excellent reproducibility, sensitivity and stability for the determination of NE at trace levels.

Norepinephrine (NE), which is present mainly in mammalian central nervous systems, is one of the most important biochemical messengers. The determination of NE levels in biological fluids yields important information on its physiological functions. NE has been determined mainly using high-performance liquid chromatography,[1] gas chromatography[2] and spectrophotometry.[3] In addition, the oxidation of NE at various electrodes has been extensively studied.[4–6] The ease of NE oxidation led to the development of convenient and rapid procedures for measuring NE levels using various electrochemical methods.[7–13] However, a serious problem with these methods is that ascorbic acid (AA) often co-exists with NE in body fluids; its content is about 0.3–0.4 mg g^{-1} in wet brain tissue.[14] AA can be oxidized at potentials similar to those of NE at most solid electrodes, resulting in an overlapping voltammetric response[8] and interference in NE determinations. A variety of approaches have been explored for addressing this problem, including the use of electrochemical pre-treatment[15] and chemically modified electrodes.[16–18] The coating of electrode surfaces with polymer films is an attractive means to increase the sensitivity and selectivity in voltammetric analysis. A Nafion film, with its anion exclusion properties and high affinity for large hydrophobic cations relative to small inorganic cations, has been successfully used for the determination of primary neurotransmitters, such as dopamine and NE.[16,17] However, the lowest detection limit previously reported for NE determination was only 20 nM by using 0.5 differential stripping voltammetry with a 3 min preconcentration, and the linear range was 50 nM–0.2 mM in several steps.[14]

Recently, there has been much interest in the electron transfer properties and electrocatalytic applications of redox polymers containing osmium bis(bipyridine) complexes.[19,20] In this paper, we describe the preparation of a graphite electrode modified with polymer films of [Os(bpy)$_2$(PVP)$_{10}$Cl]Cl, where bpy is 2,2′-bipyridine and PVP is poly(4-vinylpyridine), and Nafion for the determination of NE at trace levels. The modified electrode shows electrocatalytic activity for the oxidation of NE and can eliminate the interferences from AA and uric acid (UA) in NE determinations. This work improved both the linear range and analysis time, and these electrodes could be useful in the construction of relevant amperometric sensors for the detection of NE levels.

Experimental

Chemicals and materials

[Os(bpy)$_2$(PVP)$_{10}$Cl]Cl [Os-(PVP)$_{10}$] was synthesized and characterized as reported previously.[19,21] L-Norepinephrine was obtained from Fluka (Buchs, Switzerland) and used as received. Uric acid was purchased from BDH (Poole, Dorset, UK). Ascorbic acid, urea and glucose were obtained from Shanghai Biochemical Reagents (Shanghai, China). A 5% m/v solution of Nafion EW 1100 was purchased from Aldrich (Milwaukee, WI, USA), which was diluted to 0.5% m/v with ethanol for the preparation of the modified electrodes. Spectrographic graphite rods were obtained from the Material Institute of Shanghai (Shanghai, China). Paraffin was a product of the Chemical Plant of Shanghai (Shanghai, China). We prepared 0.1 M phosphate buffer solutions (PBS) of various pHs by mixing stock standard solutions of NaH_2PO_4 and Na_2HPO_4 and adjusting the pH with 0.1 M H_3PO_4 or NaOH. Solutions of NE, AA, UA, urea and glucose were prepared in pH 7.0 PBS.

Apparatus

Electrochemical measurements were performed with a BAS 100B electrochemical analyzer (Bioanalytical Systems, West

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Lafayette, IN, USA) using a three-electrode system. An SCE was used as the reference electrode, a platinum wire as the counter electrode and a modified graphite electrode as the working electrode. All potentials are referenced to the SCE.

Preparation of Os-(PVP)$_{10}$ modified graphite electrode

A spectrographic graphite rod (about 5.7 mm in diameter) was subjected to extraction in anhydrous methanol for 24 h to remove organic contaminants, followed by paraffin impregnation. The graphite rod was then cut into a length of about 1 cm and the resulting rod was tightly sealed with epoxy resin in a glass tube to form a disk electrode. Prior to modification, the graphite electrode was successively polished with fine wet emery paper (grain size 1000) and 0.3 and 0.05 μm Al_2O_3 slurry on microcloth pads (Buehler, USA), then rinsed with doubly distilled water and ultrasonicated for 5 min to remove loose particles. The effective surface area of the graphite electrode was 0.251 cm^2, which was determined by cyclic voltammetry using potassium hexacyanoferrate(III) solution. The graphite electrode was modified with 8 μl of a 2 mg ml^{-1} Os-(PVP)$_{10}$ ethanolic solution and allowed to dry in air for 30 min. The electrodes were further coated with 4 μl of 0.5% Nafion ethanolic solution and air-dried for 30 min to prepare the Os-(PVP)$_{10}$/Nafion film modified electrodes.

All electrochemical measurements were carried out in a 20 ml measuring cell with 5 ml of solution which was deoxygenated by purging with high-purity nitrogen for at least 10 min and kept under a nitrogen atmosphere at room temperature (20 ± 1 °C). Chronoamperometric experiments were performed using a potential step from 0 to +500 mV.

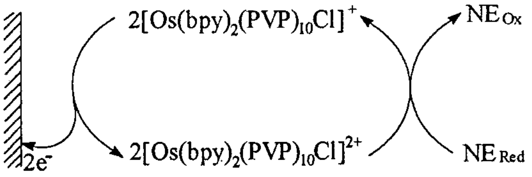
Results and discussion

Electrochemical stability of Os-(PVP)$_{10}$ modified graphite electrode

The adsorption and retention of Os-(PVP)$_{10}$ films on graphite electrode surfaces were initially investigated. A pair of well defined oxidation and reduction peaks associated with the surface-bound Os$^{2+/3+}$ redox couple were observed in pH 7.0 PBS [Fig. 1(A)]. The average osmium complex surface coverage of (1.05 ± 0.30) × 10^{-9} mol cm^{-2} was obtained by integration of the background corrected peak area of slow-scan cyclic voltammograms. An average formal potential of +341 (±3) mV was evaluated for the redox process and the cathodic and anodic peak currents scaled linearly with scan rate at lower scan rates, indicating the predominantly surface behavior of the modifier, as noted with other redox polymer modified electrodes.[22] The peak separation was about 111 mV, which was large for a surface process since the electrode was a paraffin impregnated type and the paraffin impregnation made the resistance of the electrode increase and decreased the electrochemical reversibility. Both the peak shape and peak current height were stable to continuous cyclic sweeping for 2 h. Cyclic voltammograms for the modified electrode remained unaltered following storage in air for 1 month. Hence the osmium redox polymer is strongly adsorbed on the surface of the electrode and yields stable polymer modified electrodes.

Voltammetric response of NE at Os-(PVP)$_{10}$ modified graphite electrode

The cyclic voltammogram (CV) (not shown) of NE at a bare graphite electrode displays an irreversible oxidation peak at +210 mV. The oxidation current quickly decreases upon continuous cyclic sweeping, owing to the passivation of the electrode surface by the oxidation products of NE.[23] At an Os-(PVP)$_{10}$ modified electrode, upon addition of NE to the electrochemical cell containing 0.1 M PBS (pH 7.0), electrocatalytic oxidation of NE occurs at the oxidation potential of the osmium redox polymer [Fig. 1(B)]. It is well known that the PVP polymer (without attached pendant osmium complexes) displays no electroactivity in this potential window.[24] Hence the increase in oxidation peak current and decrease in reduction peak current indicate that the Os$^{2+/3+}$ redox couple acts as an electrocatalyst. The catalytic current was directly proportional to the square root of the scan rate, indicating a fast electrocatalytic reaction and that the electrocatalytic process is controlled by the diffusion of redox species in solution,[25] which could be described as follows:



Electrochemistry of Os-(PVP)$_{10}$/Nafion modified electrodes

Cyclic voltammograms of an Os-(PVP)$_{10}$/Nafion modified electrode in 0.1 M PBS (pH 7.0) are shown in Fig. 2. The CVs

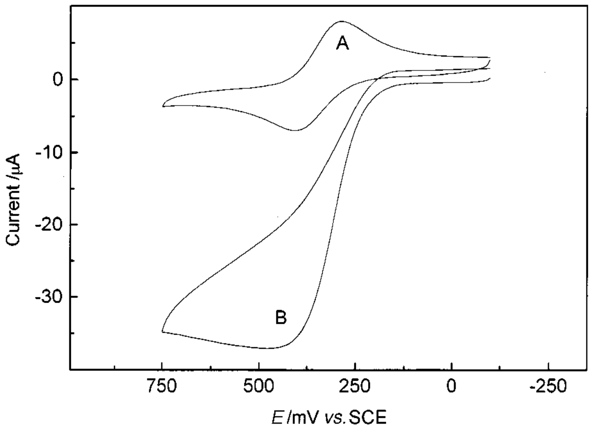


Fig. 1 Cyclic voltammograms of Os-(PVP)$_{10}$ modified electrode in 0.1 M PBS (pH 7.0). (A) without and (B) with addition of 0.39 mM NE at 30 mV s^{-1}.

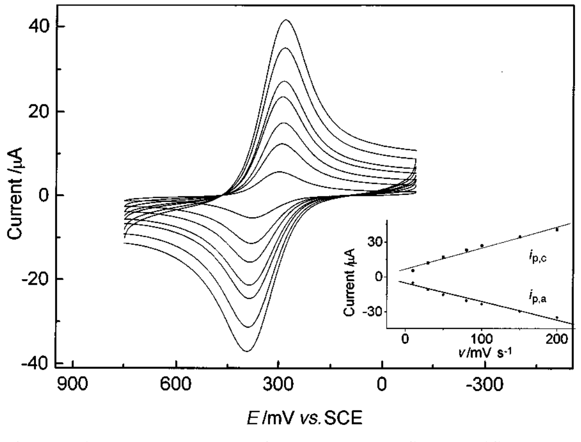


Fig. 2 Cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified electrode in 0.1 M PBS (pH 7.0) at 200, 150, 100, 80, 50, 30 and 10 mV s^{-1} (from highest to lowest peak currents). Inset: plots of peak current *versus* scan rate.

display a well defined shape similar to that of CVs without Nafion. The redox peak currents decrease slightly, with respect to the redox polymer modified electrode alone, and are still directly proportional to the scan rate. Hence the addition of a Nafion film does not alter the electrochemical process of the $Os^{2+/3+}$ redox couple.

In the pH range 9.5–5.0, the $Os^{2+/3+}$ redox couple showed almost constant formal potential and peak separation. However, when the pH was lower than 5.0, the formal potential shifted towards a more negative value and the peak separation decreased with decreasing pH. A significant increase in peak currents was also observed. These phenomena are due to the protonation of the pendant pyridine in the PVP polymer chain with a pK_a for PVP of 3.3,[26] yielding a film with a more open porous structure, making the oxidation of Os^{2+} easier and the peak separation less.

Voltammetric response of NE at Os-(PVP)$_{10}$/Nafion modified graphite electrode

The electrochemical determination of NE levels is often hampered by co-existing electroactive species such as AA and UA in biological systems. A large area of research has been devoted to the study of polymeric coatings for heterogeneous catalysis and electrochemical sensors. Among various kinds of polymer coatings, Nafion attracts special interest because of its high chemical stability and its ability to complex and retain organic cations.[27–30] Common hydrophobic electroactive cations, such as $M(bpy)_3^{2+}$ (where M = Fe, Os or Ru), are strongly retained by Nafion films but the rates of charge propagation across the film are typically sluggish.[27] Similarly to other Nafion modified electrodes,[17,31,32] when the Os-(PVP)$_{10}$ polymer was further coated with Nafion, a transport channel could be provided for cations only and the response to AA and UA could be eliminated while maintaining the electrocatalytic activity towards NE.

Cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified graphite electrodes in pH 7.0 PBS containing NE show that the coverage of Nafion does not affect the ability of the modified electrode to electrocatalyze the oxidation of NE (shown in Fig. 3). The catalytic current is proportional to the square root of the scan rate. Therefore, the rate of electrocatalytic oxidation is fast.[25]

The influence of pH on the NE electrocatalytic currents at these Os-(PVP)$_{10}$/Nafion modified electrodes is shown in Fig. 4. Oxidation of NE is a two-electron, two-proton process. Variations in the electrolyte pH will result in variations in the formal potential of NE. Therefore, the thermodynamic driving force for the catalysis will vary with pH, making the peak current different at different pH values. The optimum pH for NE determination is in the range 7.0–8.6 and pH 7.0 (near to the physiological pH value) was chosen for subsequent studies.

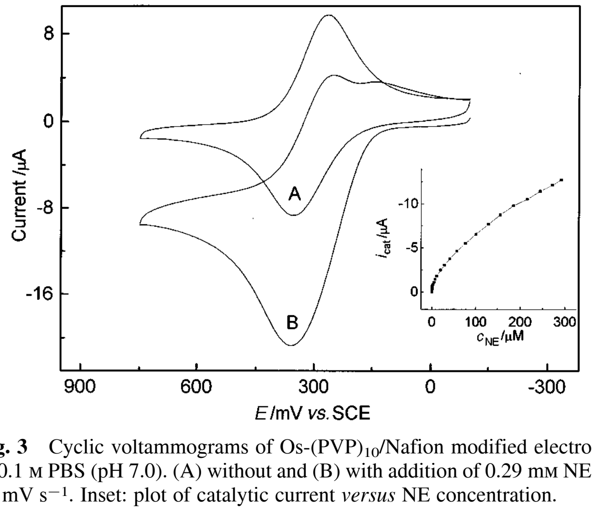


Fig. 3 Cyclic voltammograms of Os-(PVP)$_{10}$/Nafion modified electrode in 0.1 M PBS (pH 7.0). (A) without and (B) with addition of 0.29 mM NE at 30 mV s^{-1}. Inset: plot of catalytic current *versus* NE concentration.

Determination of NE levels with Os-(PVP)$_{10}$/Nafion modified graphite electrode

The determination of NE levels at the modified electrodes was carried out using a standard addition method. An aliquot (1 μl) of NE standard solution was added to 25.0 ml of PBS using a microliter syringe. The catalytic current increased with increasing NE concentration (inset in Fig. 3). The log–log plot of catalytic current *versus* NE concentration shows a dual-linear relationship in the ranges 1.8×10^{-8}–4.4×10^{-6} M ($r = 0.990$) and 4.4×10^{-6}–2.9×10^{-4} M ($r = 0.999$). The detection limit was about 18 nM (3σ). The relative standard deviations of the results were 1.8 and 0.55% for 10 successive determinations at 1.0×10^{-5} and 5.2×10^{-5} M NE levels, respectively, exhibiting excellent reproducibility.

The electrocatalytic activity of the modified electrode did not change upon cyclic scanning in PBS for 2 h or when stored in air or in pH 7.0 PBS for more than 1 month.

For analytical purposes, the chronoamperometric signal at this modified electrode is simpler for implementation in a flow or steady state system. We therefore investigated the chronoamperometric response of modified electrodes following addition of NE. According to the hydrodynamic voltammogram of the modified electrode in PBS containing 12 μM NE, we

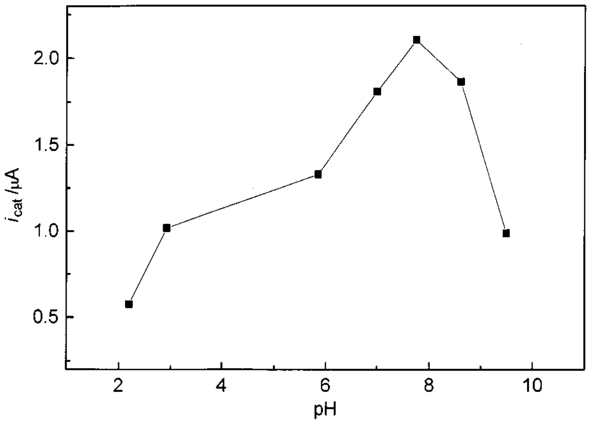


Fig. 4 Influence of pH on catalytic current in the sample solution containing 0.01 mM NE at Os-(PVP)$_{10}$/Nafion modified electrode. Scan rate: 30 mV s^{-1}.

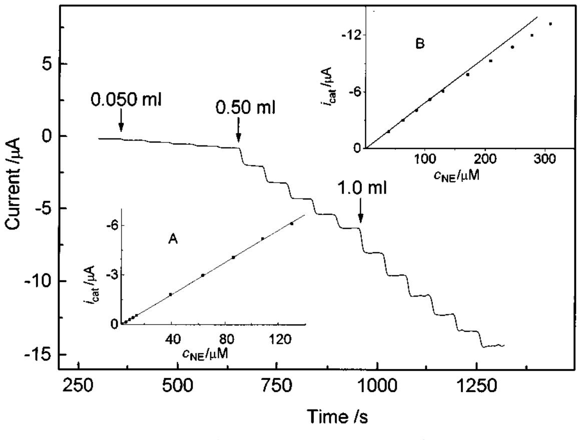


Fig. 5 Chronoamperometric response currents of NE at Os-(PVP)$_{10}$/Nafion modified electrode in 25.0 ml of pH 7.0 PBS after successive addition of 50 μl (five times), 0.50 ml (five times) and 1.0 ml (five times) of 1.3 mM NE at an applied potential of +0.5 V. Inset: plots of catalytic current *versus* NE concentration.

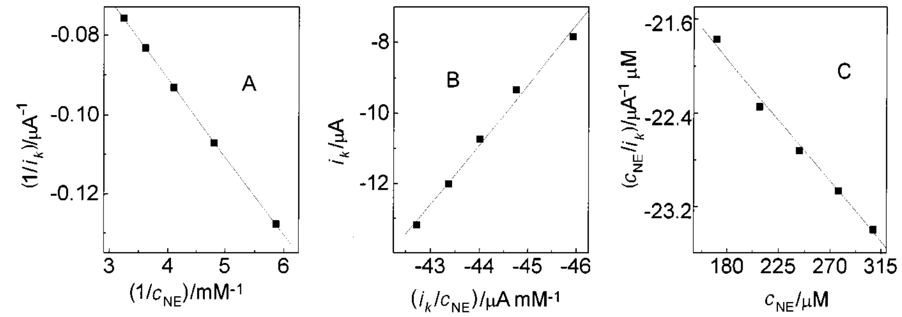


Fig. 6 Data analysis of amperometric response with (A) Lineweaver–Burke, (B) Eadie–Hoftsee and (C) Hanes transformation. Experimental conditions as in Fig. 5.

chose a step potential from 0 to +0.5 V for the experiment. At various concentrations of NE, all of the responses reached steady catalytic current values within about 5 s (Fig. 5). Furthermore, with increasing NE concentration the catalytic current increased linearly in the concentration range 1.3–130 µM (r = 0.999) (inset A in Fig. 5). When the concentration increased further, the line curved and a Michaelis–Menten-shaped curve was observed (inset B in Fig. 5).

The electrochemical response of NE did not change when up to 100-fold AA and 20-fold UA were added to the cell containing NE (5.0×10^{-5} M). In body fluids the concentration of AA is about 1.7–2.3 mM of wet brain tissue.[14] The concentration of AA in a 100-fold excess is more than the content in biological tissues. Hence AA would not interfere with the determination of NE levels in practical samples. At a concentration of 20 nM NE, AA in a 100-fold excess did not interfere. In this system, when the concentration of glucose or urea was less than 6000 or 4000 times the concentration of NE at 5.2×10^{-5} M, respectively, they also did not interfere in the determination of NE levels.

Apparent Michaelis–Menten constant for electrocatalytical oxidation of NE at the modified electrode

At higher concentrations of NE, the plot of catalytic current *versus* NE concentration displays a Michaelis–Menten shape; hence the electrocatalytic process of NE can be described by a Michaelis–Menten kinetic mechanism:

$$2\mathrm{Os(III)} + \mathrm{NE_{red}} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} 2\mathrm{Os(III)}\cdot\mathrm{NE_{red}} \xrightarrow{k_{+2}} 2\mathrm{Os(II)} + \mathrm{NE_{ox}} + 2\mathrm{H^+} \quad (1)$$

The steady state catalytic current i_k can be expressed as[33]

$$i_k = \frac{i_m c_{NE}}{K_m + c_{NE}} \quad (2)$$

where K_m is the apparent Michaelis–Menten constant and i_m is the maximum catalytic current. The apparent Michaelis–Menten constant can be evaluated using Lineweaver–Burke, Eadie–Hoftsee or Hanes transformations.[34] Linear regression analysis of the corresponding plots is shown in Fig. 6. From the slopes and intercepts of these plots, an average K_m of 1.7 ± 0.1 mM and an i_m of 86 ± 4 µA were obtained.

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