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ELECTROCATALYTICAL OXIDATION AND DETERMINATION OF DOPAMINE AT REDOX POLYMER/NAFION MODIFIED ELECTRODES

Keywords: Glassy carbon electrode, dual-layer membrane modified electrodes, $[Os(bpy)_2(PVP)_{10}Cl]Cl$, Nafion, dopamine.

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ABSTRACT

The modified glassy carbon electrodes prepared by simultaneously covering with $[Os(bpy)_2(PVP)_{10}Cl]^+$ redox polymer and Nafion film exhibited excellent electrocatalytic activity for the oxidation of dopamine (DA). Dual linear regions between 1.0×10^{-8}-1.8×10^{-5} M and 1.8×10^{-5}-4.0×10^{-4} M with correlation coefficients of 0.998 and 0.995, respectively, were obtained for log-log plots of catalytic current versus DA concentration. The detection limit for DA determination

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was ca. 5 nM with 3σ. The dual-film modified electrodes eliminated efficiently the interference from AA presence in a 1000-fold concentration ratio and showed excellent reproducibility for the determination of DA. The modified electrodes have been used to determine DA concentration with both cyclic voltammetric and chronoamperometric techniques. Electrocatalytic kinetics have been studied using a rotating disk electrode. Both the addition of Nafion film and an increase in DA concentration resulted in a decrease in the electrocatalytic rate constant. An apparent Michaelis-Menten constant of 1.3 mM and maximum catalytic current of 88μA were evaluated from the chronoamperometric measurements.

INTRODUCTION

Dopamine (DA) is a very important neurotransmitter in mammalian central nervous systems. A loss of DA-containing neurons may result in some serious diseases. Thus, its determination is important. This molecule is electroactive and has been determined using various electrochemical methods[1-4]. However, a major problem in these analyses is the coexitence of ascorbic acid (AA) in high relative concentrations. Usually, the concentration of DA is 10^{-8}-10^{-6} M and AA is as high as 10^{-4} M in biological systems. Moreover, at almost all electrode materials DA and AA are oxidized at nearly the same potential, which causes a serious interference in the voltammetric determination of DA. Methods have been developed to separate the electrochemical response of DA and AA by using an electrochemical pretreatment of carbon-based electrodes at high potentials[5] and electrode modification with a negatively charged polymer membrane or self-assembled monolayer to result in an electrostatic repulsion between electrode surface and AA or other negatively charged species[6-9]. However, in these reports the sensitivity and the linear range for DA determination were unsatisfactory. Recently, Cosnier et al reported on a linear response to DA from 5×10^{-8} to 8×10^{-5} M at the electrode modified with polyphenol oxidase in polypyrrole[10]. Zen improved the voltammetric method for DA determination in the presence of a high concentration of AA by using an electrochemically preanodized nontronit clay

modified electrode[11]. More recently, Ciszewski prepared a polyeugenol modified Pt electrode to detect DA with the detection limit of 0.1 μM[12]. Our group reported several methods to determine DA based on a nickel-hexacyanoferrate/Nafion modified microdisk platinum electrode[13] and then a poly(indole-3-acetic acid) modified glassy carbon electrode[14]. We are interested in both sensitivity and selectivity for DA determination. In our first report, however, the detection limit was only 0.10 mM [13]. We subsequently succeeded in detecting 60 nM DA [14]

In the present work, we describe the preparation of $[Os(bpy)_2(PVP)_{10}Cl]^+$ (Os-$(PVP)_{10}$) (bpy = 2,2'-bipyridine, PVP = poly-vinylpyridine and PVI = poly-vinylimidazole) and Nafion film co-modified glassy carbon electrodes. The modified electrodes showed an electrocatalytic activity for the oxidation of DA with a higher sensitivity and could eliminate the interference of AA. This suggested potential application of Os-$(PVP)_{10}$ /Nafion modified electrodes for selective detection of DA in the presence of AA.

EXPERIMENTAL

Chemicals and Materials

Synthesis and characterization of $[Os(bpy)_2(PVP)_{10}Cl]Cl$ (Os-$(PVP)_{10}$) were carried out according to literature methods[15, 16]. Dopamine hydrochloride (DA) was obtained from Sigma (USA) and used as received. A 5 wt % solution of Nafion EW 1100 was purchased from Aldrich (USA); its dilution was prepared in ethanol. Ascorbic acid was from the Shanghai Biochemical Reagent Company (China). All other chemicals were of analytical grade. All solutions were prepared with twice distilled water. 0.1 M phosphate buffer solutions (PBS) 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 NaOH and H_3PO_4. The solutions of both DA and AA were prepared with pH 7.0 PBS.

Apparatus

Electrochemical measurements were performed with a BAS 100B electrochemical analyzer (BAS Inc. USA) using a three-electrode system with a

SCE, a platinum wire and an Os-$(PVP)_{10}$ polymer modified glassy carbon electrode as reference, counter and working electrode. A modified rotating disk electrode and Model 636 RDE system (EG&G, USA) were used in the study of catalytic kinetics and the timebase (chronoamperometric) measurements.

Procedures

Prior to modification, the glassy carbon electrodes and the RDE (5.0 mm diameter) were polished successively with 0.3 μm and 0.05 μm Al_2O_3 slurry on microcloth pads (Buehler), followed by rinsing with distilled water and removal of traces of alumina from the surface by brief sonicating in doubly distilled water. The electrodes were then pretreated electrochemically by continuous cyclic sweeping between 0 and +1.4 V at 50 mV s^{-1} in pH 7.0 PBS until a constant background was observed. The pretreated electrodes were modified with a 3 μl 2 mg ml^{-1} $[Os(bpy)_2(PVP)_{10}Cl]Cl$ ethanol solution by droplet evaporation in air for 30 min. The electrodes were further modified with a 2 μl 0.5% Nafion ethanol solution and dried for 30 min again to prepare the Nafion/$[Os(bpy)_2(PVP)_{10}Cl]^+$ modified electrodes.

All electrochemical measurements were carried out in a 20 ml measuring cell with 5 ml solutions which was deaerated by purging with pure nitrogen and kept under nitrogen atmosphere at room temperature. Timebase experiments were performed with a potential step from 0 to +0.4 V at a rotating rate of 1000 rpm.

RESULTS AND DISCUSSION

Electrochemical Characteristics of $[Os(bpy)_2(PVP)_{10}Cl]^+$ Modified Electrodes

The cyclic voltammograms of an Os-$(PVP)_{10}$ modified glassy carbon electrode in pH 7.0 PBS display well-defined oxidation and reduction peaks are shown in FIG. 1. The difference between the redox peaks is about 95 mV at 100 mV/s. This couple of peaks corresponds to the Os(II/III) redox couple, yielding an

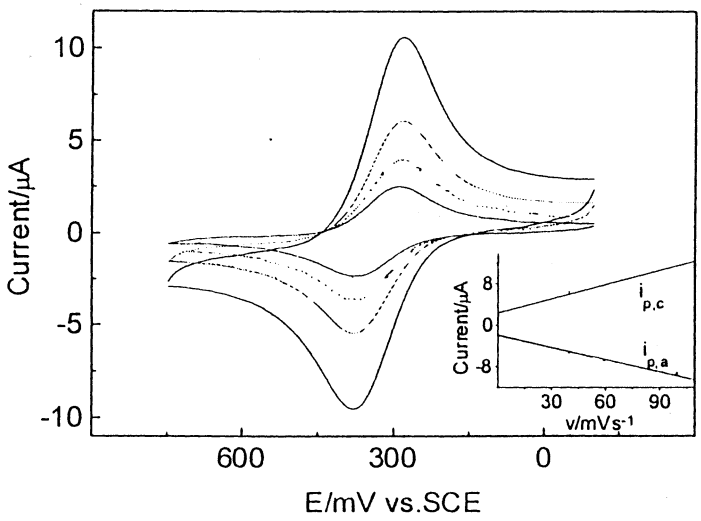

Fig. 1: Cyclic voltammograms of Os-$(PVP)_{10}$ modified electrode in pH 7.0 PBS at 100, 40, 20 and 10 mV s^{-1}, inset: plots of peak current vs. scan rate.

average formal potential of +329 mV (vs.SCE). The ratio of oxidation peak current to reduction peak current is near unity and both peak currents increased in direct proportion to the scan rates at below 100 mV s^{-1}, as expected for an electrode modified with a redox polymer film. The peak separation is rather large for a surface process, but it greatly decreases with the decreasing pH value of solution. An average Os(II/III) surface coverage of $(4.7\pm0.8)\times10^{-10}$ mol cm^{-2} were evaluated by integrating the area under the slow scan cyclic voltammetric peaks. Continuous cyclic voltammetric sweeping showed that the Os-$(PVP)_{10}$ modified electrode was very stable to these electrochemical measurements.

Electrochemical Oxidation of Dopamine at Bare and Os-$(PVP)_{10}$ Modified Electrodes

The voltammetric detection of DA is usually carried out with a carbon electrode because of problems associated with fouling of metal electrode surfaces. At a bare glassy carbon electrode, however, the electrochemical response is poor. The electrode process is irreversible and the oxidation current quickly decreases upon continuous sweeping, as a result of the electrochemical polymerization of

DA[17]. Following modification of the electrode surface with Os-$(PVP)_{10}$, a well-defined electrocatalytic voltammogram was observed in the solution of DA (not shown). The oxidation of DA occurred in the potential range of Os(II/III) redox couple. The catalytic peak current was stable and was maintained upon continuous cyclic voltammetric sweeping.

If an electrode modified with a film of PVP was used, no electrocatalytic current was observed, the oxidation currents of DA were smaller and the reversibility was worse than those observed at a bare electrode (curve a and c in FIG.2A), possibly due to restricted access of DA to the electrode surface.

The electroanalytical determination of biomolecules was often hampered by the presence of interfering electroactive species. Various methods have been used to eliminate this interference[6-9]. Films of Nafion, a sulfonated fluoropolymer with anion expulsion properties, on electrode surfaces have been successfully used to eliminate the interference of anionic species[6,7,18]. At the Nafion/PVP modified electrode, the voltammetric response of DA was less than that at the PVP coated electrode (curve c and d in FIG.2A).

Electrocatalytic Oxidation of Dopamine at Nafion/Os-$(PVP)_{10}$ Modified Electrodes

At Nafion/Os-$(PVP)_{10}$ modified electrodes, upon addition of DA to the electrochemical cell containing 0.1 M pH 7.0 PBS the oxidation peak current increased while the reduction peak current decreased, and the oxidation peak potential shifted in a positive direction for about 25 mV at the DA concentration of 0.2 mM (curve b and c in FIG.2B), indicating that the electrocatalytic activity of the Os-$(PVP)_{10}$ to the oxidation of DA was maintained. Comparing with that at a Os-$(PVP)_{10}$ modified electrode, the catalytic peak current decreased slightly and the peak potential moved a little towards more positive value.

At the same concentration of DA as in FIG.2B, the maximum anodic current of DA was only 0.8 μA, while the electrocatalytic peak current (the difference between the anodic peak currents of curve c and b in FIG.2B) was about 2.8 μA, displaying an electrocatalytic response of the Nafion/Os-$(PVP)_{10}$ modified

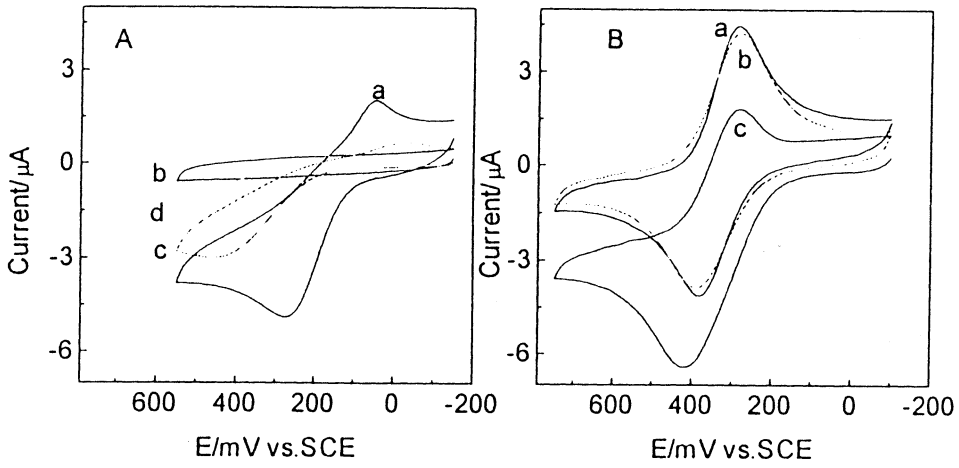

Fig. 2: Cyclic voltammograms of PVP (A) and Os-$(PVP)_{10}$ modified electrodes (B) at 40 mV s^{-1}. A): bare (a), PVP coated (b, c) and Nafion/PVP coated (d) electrodes in pH 7.0 PBS (b) and containing 0.20 mM DA (a, c,d). B): without (a)/with (b, c) Nafion coated electrodes in pH 7.0 PBS (a,b) and containing 0.20 mM DA (c);

electrode to the oxidation of DA. The electrocatalytic process could be described as follows:

$$[Os(bpy)_2(PVP)_{10}Cl]^+ - e \leftrightarrow [Os(bpy)_2(PVP)_{10}Cl]^{2+} \quad (1)$$

$$2[Os(bpy)_2(PVP)_{10}Cl]^{2+} + DA_{red} \rightarrow 2[Os(bpy)_2(PVP)_{10}Cl]^+ + DA_{ox} \quad (2)$$

The Os-$(PVP)_{10}$ membrane, with a positive charge, would attract AA, causing a strong electrocatalytic oxidation for this analyte (as shown in FIG.3). As is well known, AA is an important interfering material in the determination of dopamine. In order to reduce the interference of AA with the determination of DA, the Os-$(PVP)_{10}$ modified electrodes were further coated with the Nafion film, at which the response of AA could be eliminated completely even at relatively high concentrations of AA.

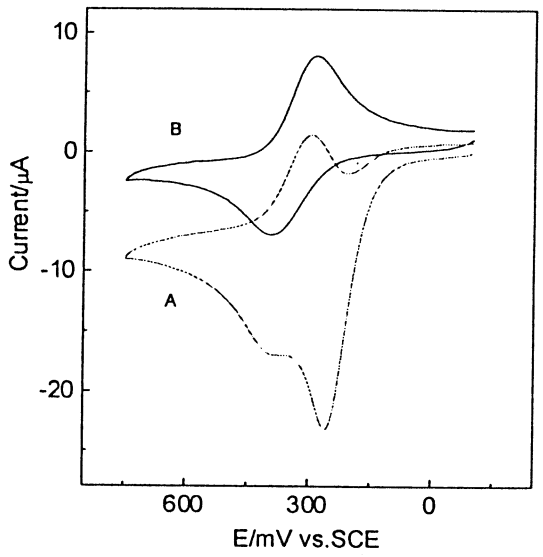

Fig. 3: Cyclic voltammograms of 1.0 mM ascorbic acid (pH 7.0) at Os-$(PVP)_{10}$ (A) and Nafion/Os-$(PVP)_{10}$ (B) modified electrodes at 100 mV s^{-1}.

FIG.4 shows the effect of scan rate on the cyclic voltammogram of Nafion/Os-$(PVP)_{10}$ modified electrode in DA solution. The catalytic oxidation current is proportional to the square root of scan rate, indicating that the catalytic reaction rate is rather large and that the electrode process is controlled by the diffusion of DA in solution[19].

The effect of pH on the electrocatalytic process is quite complicated. A maximum catalytic current was observed at about pH 7.0, near to that of biological system, probably due to a mixture of the penetration of DA into the Os polymer film and the driving force (thermodynamics) for electrocatalysis.

Determination of Dopamine

Using a standard addition method, the determination of DA concentration was carried out at a Nafion/Os-$(PVP)_{10}$ modified electrode. An aliquot of μl volume of standard solution of DA was added into 10.0 ml 0.1 M pH 7.0 PBS. The catalytic peak currents ($i_{cat} = i_2 - i_1$, i_1 and i_2 is the oxidation peak current of modified electrode in buffer without/with DA) increase with increasing DA concentration.

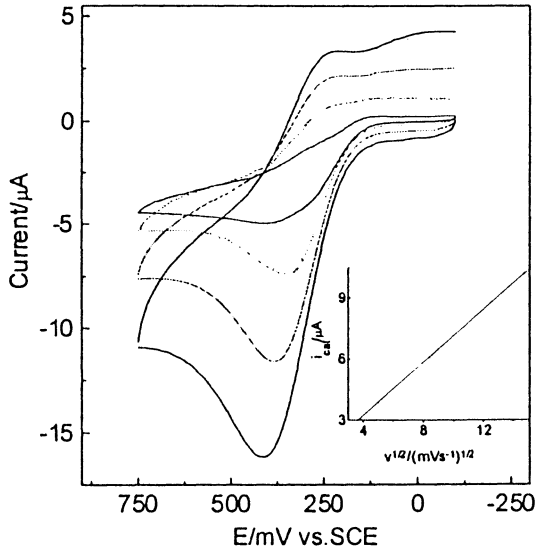

Fig. 4: Cyclic voltammograms of Nafion/Os-$(PVP)_{10}$ modified electrode in 1.0 mM DA solution (pH 7.0) at 200, 100, 40 and 10 mV s^{-1}, inset: plot of catalytic current vs. the square root of scan rate.

Log-log plots of the catalytic current versus the DA concentration exhibited dual-linear regions in the concentration ranges of 1.0×10^{-8} - 1.8×10^{-5} and 1.8×10^{-5} - 4.0×10^{-4} M with the correlation coefficient of 0.998 and 0.995, respectively. With a 3σ error, the detection limit was ca. 5 nM. The relative standard deviation of results was 2.2% and 1.0% for 8 successive determinations at 5.0×10^{-7} and 5.0×10^{-5} M DA concentrations, respectively, exhibiting an excellent reproducibility.

After AA was added into the cell containing DA, the electrocatalytic response did not change up to at least 1000-fold DA concentration at 1.0×10^{-6} M DA and 400-fold DA concentration at 1.0×10^{-5} M DA, indicating AA did not interfere in the determination of DA.

The stability of the modified electrode was also investigated. Its electrochemical activity did not change upon storage in air or in a PBS for one month or when cyclically scanned in PBS for over two hours.

With a RDE electrode, the timebase experiments were carried out at the operating voltage of +0.4 V upon the addition of DA. FIG.5 shows the chronoamperometric curves at a rotating rate of 1000 rpm. The current reached a steady value at an electrolysis time of about 5 seconds, indicating a very fast electrocatalytic response. The steady state catalytic current increased linearly with increasing DA concentrations from 0.8 to 44 μM with the correlation coefficient of 0.999 (inset A in FIG.5). With further increase in DA concentrations, a Michaelis-Menten type response could be observed. At 9.0 μM DA concentration, the relative standard deviation of results was 1.6% and 1.9% and the average value of obtained DA concentration was 8.92 μM and 9.16 μM for 5 successive determinations by cyclic voltammetry and chronoamperometry, respectively. Thus, the results obtained from the two techniques in their linear ranges were similar.

Electrocatalytic Kinetics of Dopamine at Nafion/Os-(PVP)$_{10}$ Modified Electrode

The results shown in FIG.5 exhibit a kinetic mechanism of Michaelis-Menten for the electrocatalytic process. Thus, the equation (2) may be described as follows[20,21]:

$$Os(III) + DA_{Red} \underset{k_{-1}}{\overset{k_{+1}}{\leftrightarrow}} Os(III)\cdot DA_{Red} \xrightarrow{k_{+2}} Os(II) + DA_{Ox} \qquad (3)$$

$$\frac{1}{i_{cat}} = \frac{1}{i_m} + \frac{K_m}{i_m c} \qquad (4)$$

with the apparent Michaelis-Menten constant, $K_m=(k_{-1} + k_{+2})/k_{+1}$. From the data analysis of catalytic current vs. DA concentration (inset B in FIG.5), and using equation (4), where c is DA concentration, an average K_m of (1.3±0.1) mM and maximum catalytic current of (88±5) μA were obtained.

Changing the rotating rate of RDE, the catalytic reaction dynamics of DA could also be studied by using Koutecky-Levich plots. The experimental results showed that the catalytic oxidation current increased with the increase of rotating rate ω and tended to a steady value, indicating that the thickness of the Levich layer decreased

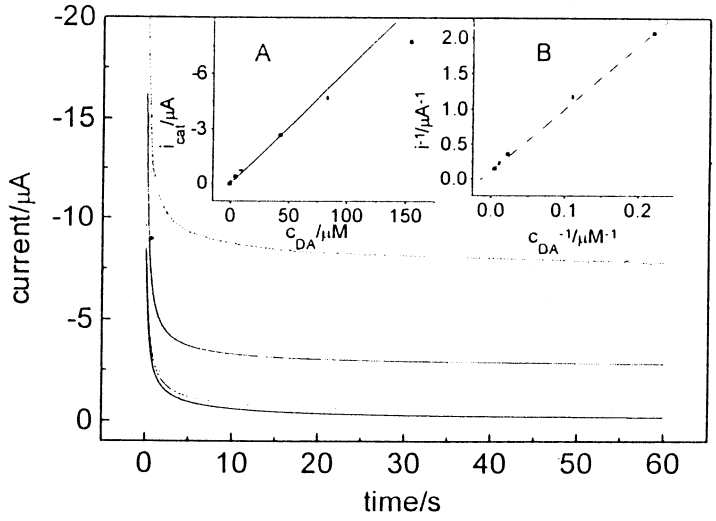

Fig. 5: Chronoamperometric curves at Nafion/Os-$(PVP)_{10}$ modified electrode with an operating voltage of +0.4 V in pH 7.0 PBS containing 0, 4.5, 9.0, 44, 84 and 160 μM DA (from bottom to top), inset: A) plot of catalytic current vs. DA concentration; B) data analysis of catalytic current vs. DA concentration.

and the electrode process was controlled by the catalytic reaction rate of DA with Os(II/III) redox couple at high values of ω. The Koutecky-Levich equation of the limiting current i_{lim} of catalytic reaction is as follows [22, 23]:

$$\frac{1}{i_{lim}} = \frac{1}{i_k} + \frac{1}{i_{lev}} = \frac{1}{nFAk_{ch}\Gamma c} + \frac{1}{0.62nFA\nu^{-1/6}D^{2/3}c\omega^{1/2}} \qquad (5)$$

where k_{ch} ($M^{-1}s^{-1}$) is the rate constant of the catalytic oxidation, D is the diffusion coefficient of DA in sample solution. FIG. 6 shows the Koutecky-Levich plots in different conditions. The linearity and similar slopes indicate that the system obeys equation (5). From the intercept of these plots and the surface coverage of 4.9×10^{-10} mol cm^{-2} of Os(II/III) redox couple, the rate constant (k_{ch}) for the electrocatalytic reaction of DA could be evaluated. The rate constants were $(2.7\pm0.4)\times10^4$ and $(2.0\pm0.3)\times10^4$ $M^{-1}s^{-1}$ at the DA concentrations of 50 μM and 0.10 mM, respectively. These rate constants are rather high, indicating a fast electrocatalytic process. As is usually the case[13], the value of k_{ch} increased with decreasing concentration of DA.

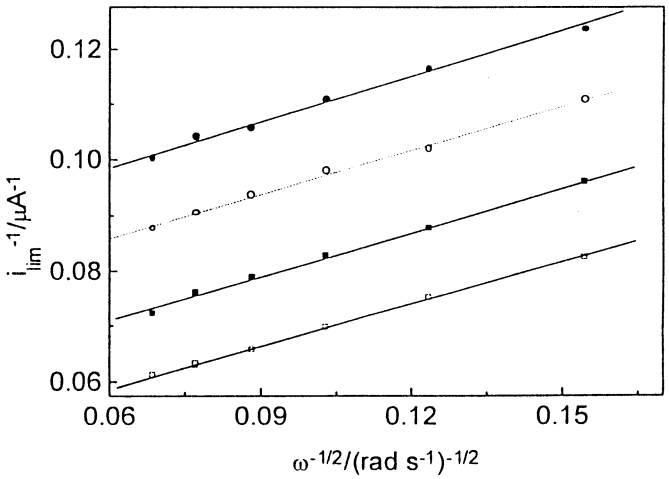

Fig. 6: Koutecky-Levich plots for electrocatalytic oxidation of DA at RDE modified with Os-$(PVP)_{10}$ (○, □) and Nafion/ Os-$(PVP)_{10}$ (●, ■) in pH 7.0 PBS containing 50 (○, ●) and 100 μM (□, ■) DA.

With electrolysis under above conditions, rate constants of $(3.1\pm0.5)\times10^4$ and $(2.5\pm0.4)\times10^4$ M^{-1} s^{-1} at the DA concentrations of 50 μM and 0.10 mM were obtained at the Os-$(PVP)_{10}$ modified electrode without Nafion film. It can be seen that the Nafion film overlaid onto the Os-$(PVP)_{10}$ polymer results in a decrease in the rate constant possibly because of an increase in the resistance to diffusion of DA across the modifying film to come into contact with the electrocatalytic sites.

CONCLUSION

The redox couple of Os(III/II) in $[Os(bpy)_2(PVP)_{10}Cl]^+$ redox polymer shows very sensitive electrocatalytic activity to the oxidation of both DA and AA. A dual-film modified electrode constructed by $[Os(bpy)_2(PVP)_{10}Cl]^+$ coated with Nafion, not only eliminates efficiently the interference of AA in the determination of DA, but also excellently keeps the electrocatalytic activity to DA oxidation in a wide concentration range. Thus, it can be used to determine DA with a very high sensitivity, wide linear range and good reproducibility. The electrocatalytic rates is

rather fast. As was our experience with other Nafion modified electrodes, however, our experiments indicate that it is difficult to rule out completely the influence of other neurotransmitter cations and some electroinactive cations at Nafion modified electrode due to their near distribution coefficients in Nafion film[7]. Fortunately, for a practical detection process the concentrations of other interference cations are constant, thus it is possible to determine DA sensitively using our modified electrode with excellent properties.

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