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SHORT COMMUNICATION

Polymethylthiophene/Nafion-modified glassy carbon electrode for selective detection of dopamine in the presence of ascorbic acid

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Abstract The possible use of an electrode modified with electroactive conductive poly(3-methylthiophene) (PMeT)/Nafion as a chemical sensor was investigated for the voltammetric analysis of Dopamine (DA). The electrochemical behavior of dopamine was examined by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. By using a PMeT-modified glassy carbon (GC/PMeT) electrode, DA and Ascorbic Acid (AA) signals could be separated but the AA at high concentrations still caused significant interference by overlapping the DA peak. In comparison to the GC/PMeT electrode, the glassy carbon (GC/Nafion/PMeT) electrode modified with hybrid film Nafion/PMeT was found to permit a superior separation by shifting the oxidation of AA peak toward the less positive potential. The DPV curves for a mixture of DA and AA at an GC/Nafion/PMeT electrode in a 0.1 M H_2SO_4 solution showed peaks of DA and AA, at 0.45 and 0.21 V, respectively, indicating that the difference in the oxidation potential was 240 mV. In the 0.1 M H_2SO_4 solution, the oxidation peak current on the differential pulse voltammograms for the GC/PMeT electrode increased linearly with the concentration of DA in the range 1×10^{-6} to 1×10^{-3} M, and the oxidation peak current on the differential pulse voltammograms for the GC/Nafion/PMeT electrode in the range 5×10^{-7} to 2×10^{-4} M. The DA detection sensitivity of the GC/Nafion/PMeT electrode (26.7 $\mu A\ \mu M^{-1}\ cm^{-2}$) was 22 times higher than that of the GC/PMeT electrode (1.21 $\mu A\ \mu M^{-1}\ cm^{-2}$).

Keywords Ascorbic acid · Dopamine · Electrochemical · Nafion · Polymethylthiophene

1 Introduction

Dopamine (DA) plays an important role as a neurotransmitter in the activities of the central and peripheral nervous systems. Extreme abnormalities of DA levels are symptoms of several diseases, such as Parkinsonism and schizophrenia [1, 2]. The most commonly used methods for the determination of this biogenic amine are fluorometric [3], radioenzymatic [4], HPLC [5], and voltammetric assays [6, 7]. The major problem during the detection of DA is the interference of ascorbic acid (AA), which is also contained in neurons at high concentration. The concentration of AA in vivo is usually higher than that of DA by 2–3 orders of magnitude and AA is oxidized at nearly the same potential as DA on a bare electrode [8, 9]. Therefore, the detection of DA in the presence of AA is a challenge in electroanalytical research. Recently, there has been an increasing demand for more sensitive and simpler analytical methods. Cyclic voltammetry (CV) and different pulse voltammetry (DPV) techniques are very useful and popular for trace analysis because these techniques are compact, efficient, and sensitive [10, 11]. Various voltammetric techniques have been shown to have the low detection limit required for dopamine analysis, depending on the working

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electrode system. Among the various approaches used, polymer modified electrodes offer several advantages in terms of the ease of preparing stable and adherent films and the possibility of manipulating the selectivity and sensitivity through the incorporation of functional groups. Polymer modified conventional electrodes, such as glassy carbon, platinum, gold, carbon paste, etc. [12, 13], have attracted great attention because of their good stability and reproducibility. Among the electronically conducting polymers, poly(3-methylthiophene) (PMeT) has been widely investigated. It can be easily electrodeposited onto an electrode surface by the electro-oxidation of its monomer. Until now, there have been relatively few reports about the use of PMeT composites for the selective measurement of dopamine. The work that has been done has included electrochemically synthesized poly(3-methylthiophene)/γ-cyclodextrin (PMeT/γ-CD) [14], electrochemically synthesized poly(3-methylthiophene)/single-walled carbon nanotubes/Nafion (P3MT/SWNTs/Nafion) [15]. Other studies have shown that a negatively charged film, such as poly(styrene sulfonic acid) [10], poly(2-picolinic acid) [11], sodium dodecyl sulfate [13], or polypyrrol/ferrocyanide film [16], could respond sensitively to DA and eliminate the interference from AA. Other approaches have shown that the use of carbon nanotubes can enhance the electron transfer from electroactive species to the surface of an electrode, and the inverse transfer, to improve sensitivity [10, 15, 17–19].

In our previous studies, polythiophene, a polythiophene derivation, and polypyrrol have been reported for use as gas sensors [20–22]. Modification of the sensor film surface was believed to be a key factor for sensing a target [20]. In the case of DA, at pH values of less than 7.0, it exists predominantly in the cationic form ($\mathrm{p}K^{+}_{\mathrm{a,DAH}} = 8.93$) [23]. Therefore, the use of Nafion, a cation-exchanger polymer [24], is suitable to improve the sensitivity and selectivity in the detection of DA. A Nafion film has proved to be suitable for the preparation of modified electrodes, where fundamental studies have been made in relation to improvements in both the charge transport dynamics and the ion-exchange reaction thermodynamics. Nafion film modified electrodes can be easily prepared by solvent casting the polymer directly on the electrode surface. In this way, Nafion film [25] and Nafion in conjunction with carbon nanotubes [15] and nano-structured platinum were used for the selective and sensitive determination of dopamine [26].

In this study, a glassy carbon electrode modified with a composition of Nafion and PMeT was first prepared, where the Nafion film was prepared on the surface of a GC electrode before the preparation of the PMeT film. The Nafion/PMeT modified GC electrode showed a high sensitivity and selectivity for DA detection.

2 Experimental

2.1 Reagents

Ascorbic acid (AA), 3-methylthiophene (MeT), and Nafion® 117 were purchased from Fluka (Switzerland) and used without further purification. Dopamine hydrochloride (DA) and tetrabutylammonium tetrafluoroborate (TBATFB) were purchased from Sigma (Germany). Other chemicals were purchased from Merck (Germany). All of the aqueous solutions were prepared with twice distilled water.

2.2 Apparatus

Electropolymerization was carried out with a 750A Electrochemical analyzer (Tokyo, Japan) in a three electrode cell (Bioanalytical Systems, USA) consisting of a Ag/AgCl (3 M NaCl) reference electrode (Bioanalytical Systems, USA), a platinum coil auxiliary electrode (Bioanalytical Systems, USA), and a glassy carbon (GC) disk electrode (2 mm i.d., Metrohm, Switzerland) used as the working electrode. All electrochemical measurements were performed in a standard cell (Tokyo, Japan).

2.3 Fabrication of modified glassy carbon electrode

Four kinds of electrode were used in this study (Table 1). The GC electrodes were pretreated using the following process. First, the surface of a GC electrode was polished with alumina slurry (0.05 μm), washed with distilled water, and placed in a water-filled ultrasonic bath for 30 s. Each GC electrode was subsequently subjected to cyclic voltammetry in 1.0 M sulfuric acid between −0.1 and +1.6 V with a scan rate of 100 mV s^{-1} for five cycles, washed, and allowed to dry at room temperature in a desiccator. Electrochemical polymerization was carried out in a one compartment cell containing a nitrogen-purged solution of 100 mM TBATFB and 150 mM MeT in acetonitrile. The PMeT film was grown for 20 s at a constant potential of 1.8 V vs. Ag/AgCl. After electropolymerization, the

Table 1 Conditions for preparation of modified electrode

No.	Electrode	Contents
1	GC[a]	Bare
2	GC/Nafion	Nafion was deposited on GC
3	GC/PMeT[b]	PMeT was electrochemically deposited on GC
4	GC/Nafion/PMeT	PMeT was polymerized on surface of Nafion-coated GC

[a] *GC* glassy carbon

[b] *PMeT* poly(3-methyl thiophene)

polymer film was kept at the reduction potential (−0.2 V) for 5 min. The preparation of a GC/Nafion/PMeT electrode required an additional step prior to the polymerization of the PMeT film on the electrode. A 0.5% Nafion solution in ethanol was prepared from a 5% Nafion® 117 solution. Five μl of the 0.5% Nafion solution was carefully deposited on the electrode surface using a 25 μL syringe (SGE, Australia). The electrode was then left in a desiccator for 5 min to evaporate the solvent to create a thin film. The average thickness of the Nafion film was estimated using a recast density of 1.98 g cm^{-3} [25].

2.4 Scanning electron microscopy

To take SEM images, the Nafion film and Nafion/PMeT film were prepared on indium tin oxide (ITO)-coated glass substrates (BAS Inc., Tokyo, Japan), instead of GC electrodes. All of the films were prepared on the ITO electrodes using the same conditions as for the GC electrodes. These films were sputtered with Pt and observed with a scanning electron microscope (S-3500, Hitachi Co. Ltd.).

2.5 Electrochemical measurement

Electrochemical experiments were performed in a 0.1 M H_2SO_4 solution containing specific concentrations of DA and AA and deoxygenated by purging with high-purity nitrogen. All of the CVs and DPVs were recorded in a suitable potential range. All of the experiments were performed at room temperature and under an air atmosphere in a standard cell.

3 Results and discussion

3.1 Electrochemical response of DA and AA at modified electrodes

A comparison was made in the cyclic voltammetric behavior of DA in the presence of AA at four kinds of modified electrodes (Table 1). The CVs of mixture solutions containing 1 mM DA and 1 mM AA in 0.1 M H_2SO_4 at four different electrodes are shown in Fig. 1. At the bare GC electrode (curve 1), the response was very poor and only one peak derived from DA could be observed because of the same peak positions for DA and AA on the bare electrode [8, 9]. The modification of the GC electrode by Nafion resulted in an increase in the DA-anodic peak current (curve 2), indicating that the Nafion, with its negatively charged sulfonic group, was able to enhance the adsorption of DA. In the case of the GC/PMeT electrode (curve 3), the DA and AA peaks were separated from each other and the peak current was higher than that of the bare GC electrode. The reason for the higher peak current may originate from the larger surface area of the PMeT film as compared with the bare GC electrode, along with the electronic conductivity of the PMeT. The Nafion and PMeT composite was designed to modify the electrode based on the above results. It was expected that the polymerization of the PMeT after the Nafion modification of the GC electrode would demonstrate both the Nafion and PMeT functions in reducing the effect of AA. The GC/Nafion/PMeT electrode showed two peaks of anodic current (~0.55 V, 0.35 V), with a high peak current as expected (curve 4).

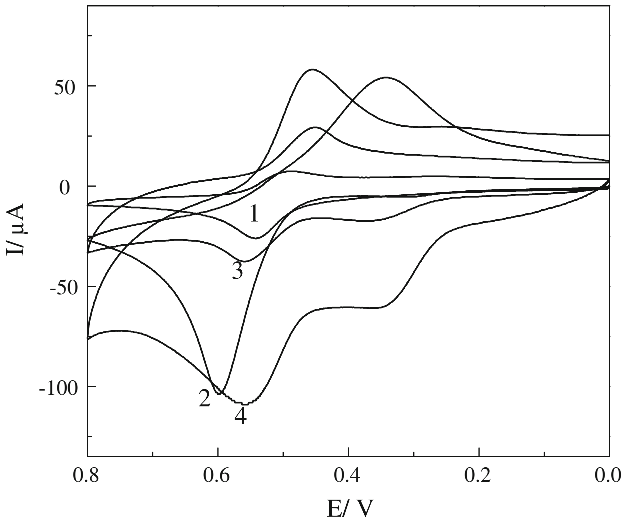

Fig. 1 Cyclic voltammograms of mixture solution containing 1 mM DA and 1 mM AA at different electrodes: (1) bare GC, (2) GC/Nafion, (3) GC/PMeT, and (4) GC/Nafion/PMeT. Electrolyte H_2SO_4 0.1 M, scan rate 50 mV s^{-1}, measurement temperature 25 °C

The GC/Nafion/PMeT electrode was selected prior to the detection of the DA in the presence of AA. The above results show a potential application of the GC/Nafion/PMeT electrode for DA detection in the presence of AA.

3.2 Observation of hybrid film using SEM

The surface properties of the electrode are a key for the selective detection of DA. The surface structures of electrodes modified with polymer films were observed. PMeT and Nafion were used for the modification of an ITO glass electrode. Figure 2a–c show the SEM images of a bare ITO electrode, an ITO/Nafion electrode mimicking the GC/Nafion electrode, and an ITO/Nafion/PMeT electrode mimicking the GC/Nafion/PMeT electrode, respectively. The surface of a bare ITO electrode was found to have a slight roughness (Fig. 2a). However, it became smoother when the surface of the electrode was coated with Nafion film (Fig. 2b). The results indicated that there was Nafion

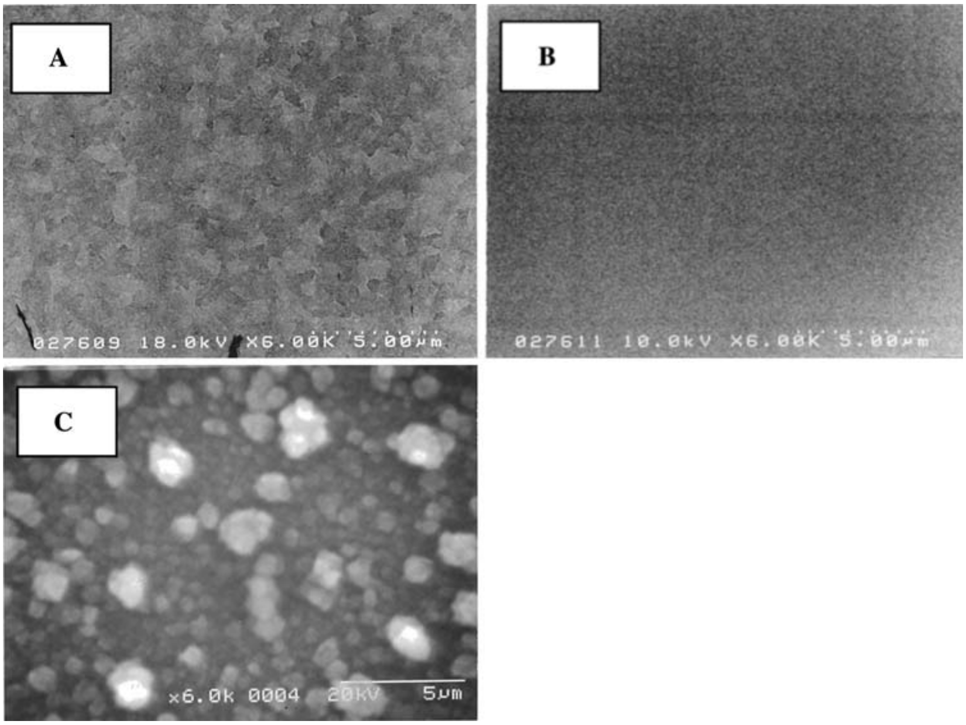

Fig. 2 SEM images of **a** bare ITO electrode, **b** Nafion modified ITO electrode, and **c** Nafion/PMeT modified ITO electrode

film on the surface of the ITO electrode. In the case of the Nafion/PMeT film, a compact spherical grain of polymer was observed on the electrode surface (Fig. 2c), resulting in an increase in the surface area and the peak current.

3.3 Effect of scan rate on the peak currents for DA at the GC/Nafion/PMeT electrode

The effect of scan rate on the peak current for DA was investigated in a 0.1 M H_2SO_4 solution containing 1 mM DA. As seen in Fig. 3a, both the cathodic and anodic peak currents increased with increase in scan rate from 10 to 200 mV s^{-1}, and the peak potential shifted slightly.

The anodic or cathodic peak currents were proportional to the scan rates from 10 to 200 mV s^{-1} and the curves were linear (see Fig. 3b), suggesting that the electrode reaction of DA at the Nafion/PMeT modified GC electrode was a typical adsorption-controlled process.

3.4 Effect of Nafion film

3.4.1 Effect of average thickness of Nafion film on DA response

At pH values below 7.0, DA (pK_a = 8.93) [23] exists predominantly in the cationic form. Because of this, the DA signal can be enhanced by improving the cation exchange capacity of the conducting polymer layer. The proton exchange polymer Nafion was used. The addition of Nafion to the GC electrode was shown to improve the DA signal since Nafion has a negatively charged ion-exchange group (SO_3^-), which enhances the adsorption of DA via the following equation [25]:

$$\mathrm{Nafion}(\sim SO_3^-)(H^+)^{\mathrm{film}} + (DA^+)^{\mathrm{solu}} \rightleftharpoons \mathrm{Nafion}(\sim SO_3^-)(DA^+)^{\mathrm{film}} + (H^+)^{\mathrm{solu}} \qquad (1)$$

When $[H^+] \gg [DA^+]$, the partitioning behavior of DA, described above, can be simply described by $(DA^+)^{\mathrm{solu}} \rightleftharpoons (DA^+)^{\mathrm{film}}$ and the partition coefficient of DA has been reported to be given by $K = C_{\mathrm{DA}}^{\mathrm{film}}/C_{\mathrm{DA}}^{\mathrm{solu}} = 401$ in the range $C_{\mathrm{DA}}^{\mathrm{solu}} < 0.1$ mM [25]. In our condition, DA was considered to preferably partition into the Nafion film.

The degree of improvement also depended on the amount of Nafion. In order to estimate the effect of the amount of Nafion (corresponding to the number of ion-exchange sites), five GC/Nafion/PMeT electrodes were prepared with different volumes of Nafion coated on the GC surface. Other than the Nafion content, all the PMeT films were prepared under the same conditions. As shown in Fig. 4b, the peak height increased as the average thickness of the Nafion film increased to 4.9 μm. The peak current decreased when the average thickness of the Nafion film became higher than 4.9 μm. Nafion has the ability both to attract DA due to its high affinity to cations (partition coefficient = 401 [25]) and to reduce the mass transfer rate of both DA and electrons [15]. Therefore, the addition of a small amount of Nafion forms a thin film resulting in poor sensitivity to DA, while a larger amount

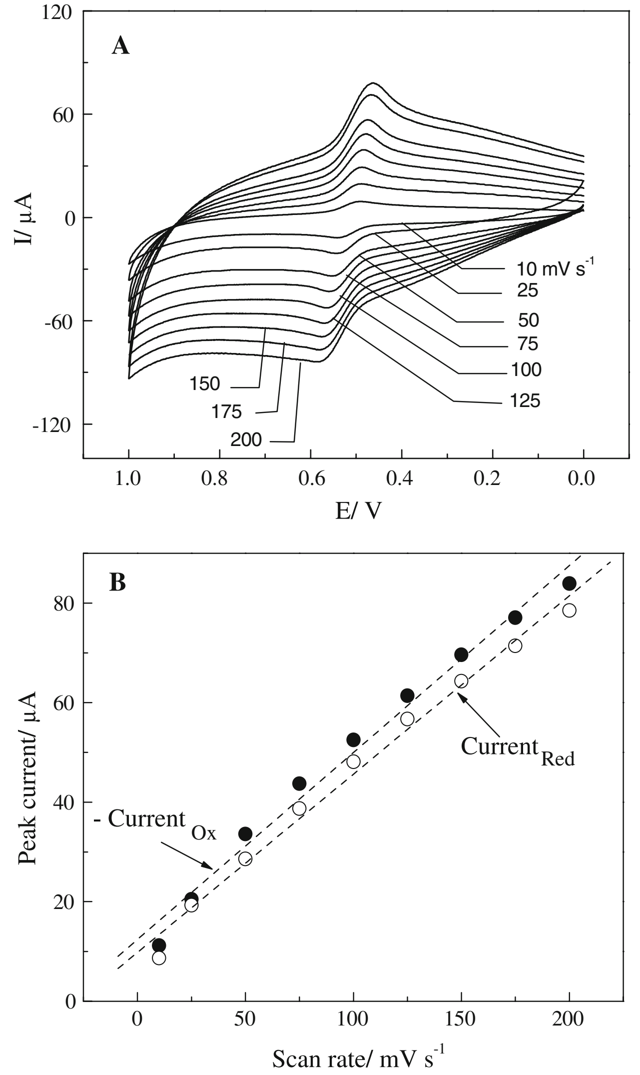

Fig. 3 **a** Electrochemical response of 1 mM DA in 0.1 M H_2SO_4 solution at Nafion/PMeT modified GC electrode with different scan rates, from 10 to 200 mV s^{-1} and **b** the plot of the peak current against the scan rate. Measurement temperature 25 °C

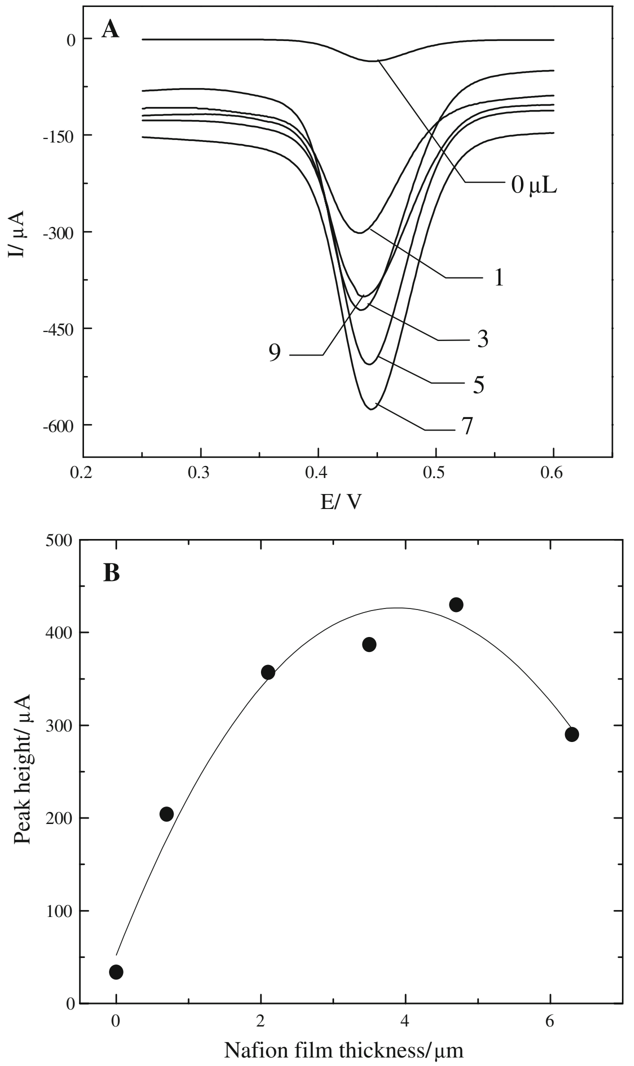

Fig. 4 Effect of amount of Nafion on measurement of DA. **a** DPV voltammograms of DA on electrodes were prepared from different volumes of Nafion (0–9 μl). **b** Effect of the average Nafion film thickness on the oxidation peak height of DA. Measurement temperature 25 °C

of Nafion forms a relatively thick film, decreasing the mass transfer rate of DA and the transfer rate of electrons within the Nafion film. Therefore, in this study, an average Nafion film thickness of 3.5 μm (corresponding to 5 μL of the 0.5% Nafion solution) was chosen for electrode modification in all further experiments.

3.4.2 Separating the DPV peaks of DA and AA

It is well known that AA ($pK_{a1} = 4.10$) [27] coexists with DA in vivo and that its concentration is much higher than that of DA, causing AA to be the major cause of interference in DA detection. The interference by AA was investigated with both GC/PMeT and GC/Nafion/PMeT electrodes [8, 9]. Figure 5a shows the DPVs of 100 μM DA in the presence of various AA concentrations (0–4,000 μM) at a PMeT electrode. By using GC/PMeT electrodes, the DA and AA signals could be separated, but AA still interfered at higher concentrations. As compared to the GC/PMeT electrode, the GC/Nafion/PMeT electrode permitted a superior separation by further shift of the AA peak toward a less positive potential (Fig. 5b–d). Figure 5b shows DPVs at various DA concentrations in the presence of ascorbic acid on a Nafion/PMeT modified GC electrode. DA and AA peaks were clearly observed at 0.45 and 0.21 V, respectively, resulting in a difference in oxidation potentials of 240 mV. The anodic peak current increased with increase in DA concentration, while the anodic peak current of AA remained nearly constant. Figure 5c shows DPVs for DA in the presence of large AA concentrations

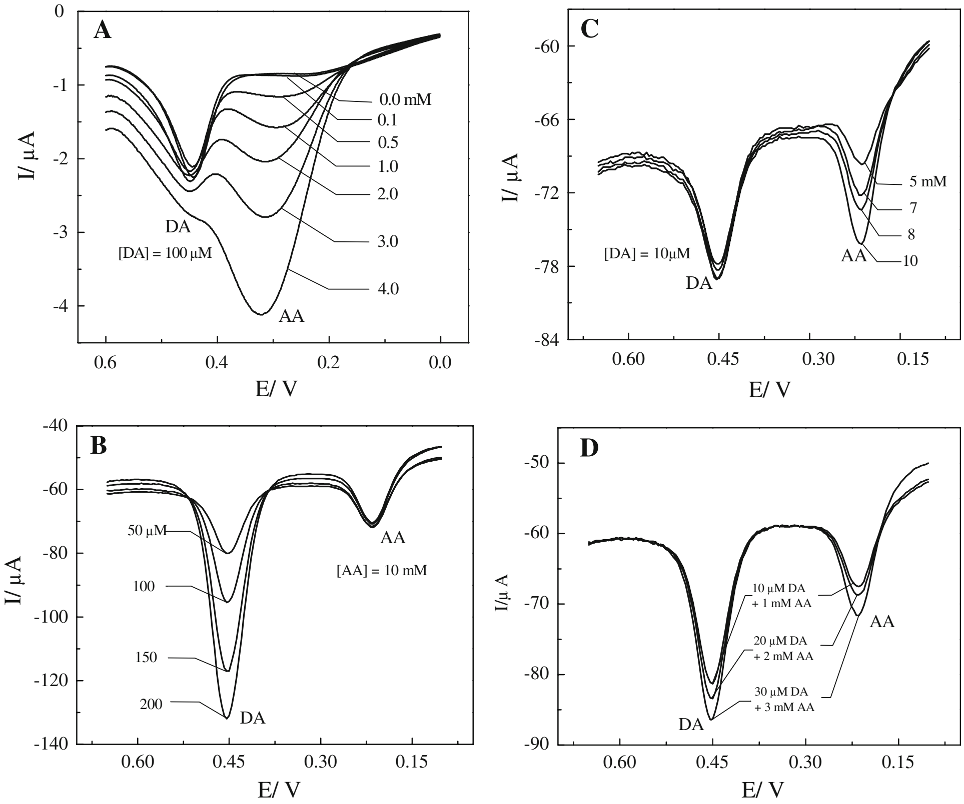

Fig. 5 **a** DPV voltammograms for 100 µM DA in the presence of different concentrations of AA, from 0 to 4 mM, at the GC/PMeT electrode. **b** DPV voltammograms of solutions containing DA with different concentrations (50–200 µM) and 10 mM AA. **c** DPV voltammograms of solutions containing 10 µM DA in the presence of different AA concentrations (5–10 mM). **d** DPV voltammograms of solutions containing DA and AA with different concentrations at a GC/Nafion/PMeT electrode. Measurement temperature 25 °C

(AA concentrations that were 500–1,000 times higher than that of DA). The anodic current peak for the AA also increased with the AA concentration and the anodic current peak for the DA decreased only slightly. As a result, AA had no interference with DA measurement. When the DA and AA concentrations both increased, the anodic current peaks for DA and AA also increased (Fig. 5d). Therefore, the GC/Nafion/PMeT electrode has the ability to selectively determine DA in the presence of a large amount of AA.

3.5 Dynamic voltammetry response of DA at modified GC electrodes

The determination of DA was finally performed with the DPV method and the height of the peak was selected as the analytical signal. Figure 5 shows the dependency on the DA concentration of the peak current measured with the GC/Nafion/PMeT and GC/PMeT electrodes in a 0.1 M H_2SO_4 solution.

3.5.1 Detection limit

The detection limit for the modified GC electrodes was found to be 0.1 µM. On the other hand, detection limits for DA in the previous report were 16 nM to 5 µM using hybrid films of a conducting polymer with poly(styrene sulfonic acid) [10], sodium dodecyl sulfate [13], Nafion [15], and carbon nanotubes [17]. The electrodes used in this study showed a detection limit that was similar to those of the previous studies.

3.5.2 Sensitivity

In 0.1 M H_2SO_4 solution, the voltammetric response of DA on the GC/PMeT electrode showed linearity from 1 to 1,000 µM (Fig. 6a). The linear regression equation was $I(\mu A) = 0.739 + 0.038 \times C$ (µM) with a correlation coefficient of 0.999. In the case of the GC/Nafion/PMeT electrode, the linear regression equation was $I(\mu A) = 7.23 + 0.845 \times C(\mu M)$ with a correlation coefficient of 0.999 in the range 0.5–200 µM (Fig. 6b). The slopes representing the sensitivities of two electrodes were 1.21 and 26.7 µA μM^{-1} cm^{-2}, respectively. It has been previously reported that the DA response by square wave voltammetry was 9.65 µA μM^{-1} cm^{-2} using PMeT/γ-CD [14]. The above results indicate that the amplification effect of Nafion is larger than that of γ-CD. Bouchta et al. mentioned that the hydrophobic cavity of γ-CD contributed to the enhancement of DA sensitivity [14]. On the other hand, the doping of a poly(pyrrole) film of self assembled membranes by a thiol-derivative on copper particles resulted in a more hydrophobic electrode surface but did not succeed in improving the sensitivity to the target molecule (ammonium), even though it succeeded in

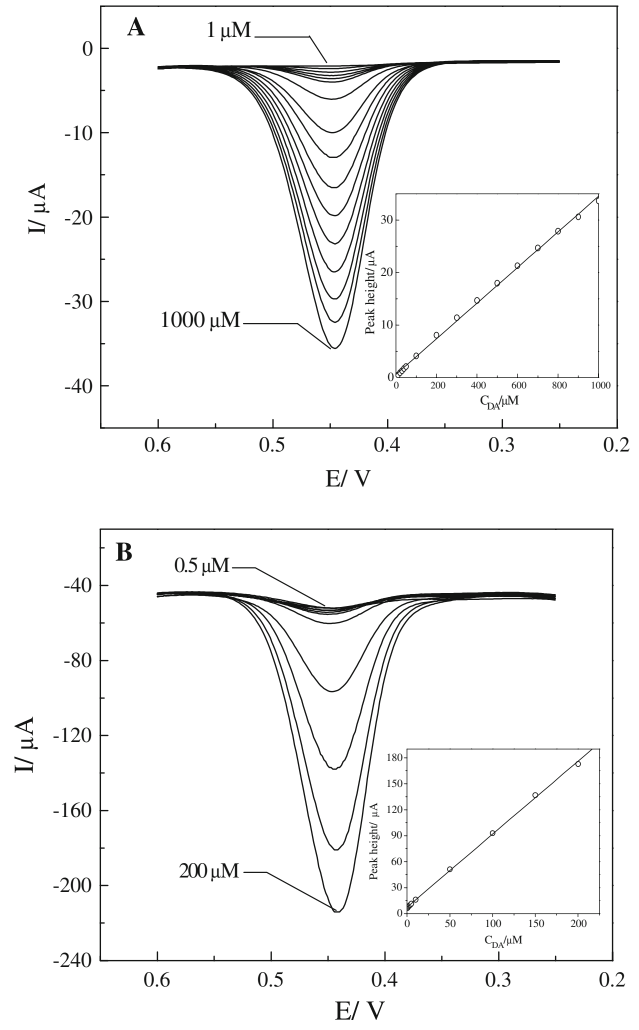

Fig. 6 DPV voltammograms for various DA concentrations in a 0.1 M H_2SO_4 solution. **a** DA concentration 1–1,000 μM at GC/PMeT electrode and **b** DA concentration 0.5–200 μM at GC/Nafion/PMeT electrode. Inset figures show the relationship between the height of the peak current and the concentration of DA. Measurement temperature 25 °C

extending the detection limit to ammonium gas [20]. Therefore, the utilization of the electrostatic interaction between Nafion film and DA was found to be effective for the improvement of the sensitivity to some extent.

Therefore, this modification was prior to the selective detection of DA in the presence of AA and for the improvement of the sensitivity (with a slope of 26.7 μA μM^{-1} cm^{-2}), rather than the improvement of the detection limit. Our results suggested the possibility of a simultaneous multi-detection system based on the DPV method.

3.5.3 Analytical applications

In order to confirm the selective property of GC/Nafion/PMeT, injections of DA in the presence of different AA concentrations were analyzed by the standard addition method and using the relationship between the height of the peak current and the concentration of DA, the DA concentration were calculated and the results are shown in Table 2. The detection of 10 μM DA at GC/Nafion/PMeT was almost unaffected by the presence of AA with a concentration 600 times higher (error $< 5\%$).

Table 2 Determination of DA in injection of dopamine hydrochloride in the presence of different concentrations of AA

No.	C_{DA} (μM)	C_{AA} (mM)	% Recovery[a]
1	10	1	104.4
2	10	2	102.3
3	10	3	100.5
4	10	4	98.4
5	10	5	97.3
6	10	6	95.7
7	10	7	94.6
8	10	8	93.0
9	10	9	92.4
10	10	10	91.9

[a] Found by the method in article

4 Conclusions

The electrochemical behavior of dopamine at GC/PMeT was compared with that at a GC/Nafion/PMeT electrode in terms of the signal amplitude and signal separation from ascorbic acid. The GC/Nafion/PMeT electrode showed superior characteristics over GC/PMeT and good selectivity over AA in the experimental conditions (AA $\gg$ DA). By using a GC/Nafion/PMeT electrode, the separation of the current peaks for DA and AA oxidation reached about 240 mV. The heights of the current peaks for DA oxidation were not affected by the presence of a large amount of AA and the oxidation peak currents on differential pulse voltammograms increased linearly with a DA concentration in the range 5×10^{-7} to 2×10^{-4} M.

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