Simultaneous Determination of Dopamine and Ascorbic Acid with Poly(3-methylthiophene)/Polypyrrole Bilayer-Coated Carbon Fiber Electrodes

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Poly(3-methylthiophene)/polypyrrole bilayer-coated carbon fiber microcylinder electrodes were prepared by electropolymerization of the respective monomers. The voltammetric characteristics of the electrodes for dopamine (DA) and ascorbic acid (AA) in phosphate buffer saline (PBS) solutions (pH 7.4) were studied. The bilayer-coated electrodes exhibited highly enhanced electrochemical reversibility for the AA oxidation. Quasi-steady-state cyclic voltammograms obtained at 10 mV/s had half-wave potentials at -50 and +125 mV vs. SCE for AA and DA, respectively, in PBS solution. It has been demonstrated the bilayer-coated electrodes can be used for simultaneous determination of DA and AA in concentrations higher than 3 µM and 50 µM, respectively, by differential pulse voltammetry.

Keywords Poly(3-methylthiophene)/polypyrrole bilayer coating, bilayer-coated carbon fiber electrode, ascorbic acid, dopamine, differential pulse voltammetry

Electrochemical determination of dopamine (DA) in the presence of ascorbic acid (AA) at physiological pH has been of considerable interest for the last decade.\textsuperscript{1-4} For this purpose, carbon electrodes such as carbon fiber, carbon paste and glassy carbon have been most widely used because of their durability in biological systems; among them, carbon fiber electrodes have been extensively used in \textit{in vivo} measurements. Efforts have been devoted to enhance the selectivity and sensitivity of the electrodes for DA and/or AA. Electrochemical pretreatments\textsuperscript{3,5-10} and laser light activation\textsuperscript{11} of carbon fiber electrodes have proved to improve greatly the sensitivity of such electrodes for DA. Coating of the electrode surface with a film of perfluorinated ionomer Nafion\textsuperscript{12-15} has been shown to enhance the selectivity of carbon fiber electrodes for neurotransmitters such as DA, norepinephrine and 5-hydroxytryptamine, which are cations at physiological pH.

On the other hand, the electrochemical oxidation of AA at untreated carbon electrodes is irreversible; the oxidation waves usually appear at about 0.3 V vs. SCE although the thermodynamic redox potential of AA is more negative than 0.0 V. Oxidation of AA at potentials close to the reversible redox potential has been attempted by various methods.\textsuperscript{5,10-18} Ewing and co-workers reported that oxidation of AA at polypyrrole coated glassy carbon electrodes occurred at potentials about 0.3 V more positive than at the bare electrodes.\textsuperscript{18} They explained that the interaction between cationic sites on polypyrrole film and anionic AA solute is responsible, to some degree, for the enhanced reversibility of AA oxidation. Another conductive polymer, poly(3-methylthiophene) was used by Wang and Li.\textsuperscript{19} They showed that the poly(3-methylthiophene)-coated glassy carbon electrodes exhibited a diminished response to AA in contrast to the polypyrrole-coated electrodes.

Modification of electrode surface with bilayer coating has proved to be effective for improvements of selectivity and sensitivity. Wang and Tuzhi\textsuperscript{20} employed Nafion/cellulose acetate bilayer-coated glassy carbon electrodes and could distinguish DA from other organic cations such as epinephrine and norepinephrine.

Though Ewing and co-workers\textsuperscript{18} showed that differential pulse voltammetric waves for AA and DA were resolved at polypyrrole-coated glassy carbon electrodes, such resolution was achieved only for solutions of DA and AA in comparable concentrations to each other, e.g., 0.5 and 0.5 mM (M=\text{mol/dm}^3), respectively. We have applied the polypyrrole-coated carbon fiber electrodes to a solution containing both 1.0 mM AA and 20 µM DA, but we could not obtain any satisfactory resolution of waves. Fortunately, however, we have found that voltammetric resolution of AA and DA which are present in different concentrations is possible using carbon fiber electrodes coated with a bilayer that consists of a thin inner poly(3-methylthiophene) layer and a thin outer polypyrrole layer. These bilayer-coated carbon fiber electrodes are easily fabricated and enable us to...
determine DA and AA simultaneously. We describe in this paper the characteristics of the bilayer-coated carbon fiber electrodes.

Experimental

Chemicals and apparatus

All chemicals used were of reagent grade and were used as received except for acetonitrile, which was distilled three times before use. Water was purified by a Millipore Milli-Q system. Phosphate buffer saline (PBS) solution (pH 7.4) was used as the base solution throughout the measurements.

Cyclic voltammetry was performed using a HECS 318 potentiostat and a HECS 321B potential sweeper (Huso Electrochemical System). A computer-controlled electrochemical system (Cypress System CS-1090) was used for potential step chronoamperometry and differential pulse voltammetry (DPV). Experimental conditions of DPV were as follows: pulse height, 50 mV; potential sweep rate, 10 mV/s; pulse width, 50 ms; and pulse interval, 1.0 s. A conventional three-electrode cell was employed. A spiral platinum wire and a saturated calomel electrode (SCE) were used as the auxiliary and the reference electrodes. Solutions were deaerated by bubbling nitrogen. All the measurements were made at room temperature.

Electrode preparation

Carbon fiber electrodes were fabricated from pitch-based carbon fibers (FORCA FT-700, Tonen Industries) and glass capillary micropipettes (Drummond Scientific) without using any adhesive, as described elsewhere.10 The electrodes were cylindrical: 9.5±0.4 µm in diameter and 2.50±0.05 mm in length. Prior to the electrode coating the carbon fiber electrodes were rinsed successively with acetone and with Milli-Q water and then dried. The bilayer coating was performed by electropolymerization in two steps as follows. First, a thin poly(3-methylthiophene) layer was formed on the carbon fiber electrode in 0.1 M NaClO4 acetonitrile solution containing 50 mM 3-methylthiophene by potential sweep electrolysis in the potential region between 0.0 V and 1.70 V at 0.5 V/s. Then the electrode was washed in acetonitrile solution and dried in air. Next an outer polypyrrole layer was formed on the poly(3-methylthiophene) coated electrode in 0.1 M NaSO4 aqueous solution containing 50 mM pyrrole (pH was adjusted to 1.0 with H2SO4) again using potential sweep electrolysis in the region between 0.0 V and 0.75 V. The upper limit potential for the potential sweep electrolysis required for formation of the poly(3-methylthiophene) layer was found to be more positive than 1.5 V and that for polypyrrole film formation ranged from 0.7 to 1.1 V. We found that the thickness of such layers could be controlled by varying the number of potential sweep cycles or the potential sweep rate as well as the upper limit of the potential range for polymerization. Because of the porous structure of the polymer coating, the bilayer-coated electrodes exhibited increased charging currents compared to those of the untreated electrodes. We chose a value of 0.75 V as the upper limit potential for polypyrrole layer formation since the electrodes obtained using this condition showed charging currents lower than those coated using more positive upper limit potential values.

Unless otherwise stated, the typical electrodes employed in the present work were prepared by two potential cycles for polymerization of 3-methylthiophene and two cycles for polypyrrole layer formation. The thicknesses of the poly(3-methylthiophene) and polypyrrole layers of the typical electrodes were estimated to be about 47 and 3 nm, respectively, from the relations reported for the polymer layer thickness and the amount of charge used for polymerization, 2 nm/(mC cm−2) for poly(3-methylthiophene)21 and 0.1 µm/(24 mC cm−2) for polypyrrole.22

Results and Discussion

Voltammetric characteristics of AA and DA at bilayer-coated electrodes

Figure 1 compares cyclic voltammograms for DA, AA and 3,4-dihydroxyphenylacetic acid (DOPAC) obtained at the untreated and the poly(3-methylthiophene)/polypyrrole bilayer coated carbon fiber electrodes at a potential sweep rate of 10 mV/s. These sigmoidal voltammograms indicate the characteristics of quasi-steady state at the microelectrodes. Three distinct differences can be observed in the voltammograms for these species obtained with the bilayer-coated electrodes.
in comparison with those of the untreated ones: (1) a steeper wave-slope, (2) a shift in the half-wave potential to less positive potential, and (3) an increase in background charging current. The inverse slope values of a plot of $\log\left(I/(I_i-I)\right)$ vs. $E$, and the half-wave potentials, $E_{1/2}$, for four species are listed in Table 1, where $I$ is the limiting (plateau) current. These data clearly indicate that the increase in reversibility of the oxidation of these compounds is achieved with the bilayer electrodes. As reported earlier, the electrochemical reversibility of DA is higher than DOPAC at bare carbon fiber electrodes subjected to "mild electrochemical pretreatment". However, the order is reversed at the bilayer-coated electrodes. The shift in $E_{1/2}$ is remarkably large for AA and DOPAC, that are anionic, whereas that for DOPA, that is neutral, is intermediate. These observations are quite similar to those reported by Ewing and co-workers for polypyrrole coated glassy carbon electrodes. Polypyrrole is oxidized at about $-0.2$ V vs. SCE and contains positively charged fixed sites. Doping of AA as counter anions in the polypyrrole layer may be ruled out from the finding that no current for AA oxidation was observed in a PBS solution at the bilayer-coated electrodes, which were transferred from a solution containing 0.1 M AA after repeated cyclic voltammetric measurements. The large increase in the electrochemical reversibility of AA may be ascribed to the presence of positively charged sites in the electronic conductive polypyrrole layer.

It is noted that the limiting current values at the bilayer-coated electrodes are higher by 6 to 20% than those at untreated electrodes for the four species studied (Table 1). This may result, to some extent, from an increase in the geometric electrode surface area due to the bilayer coating. As estimated in the experimental section, however, the bilayer is not thick enough to explain an increase in the current of up to 20%. There might be other contributions from increased electrode surface roughness and possible adsorption of the reactants. Though the background current became much larger at the bilayer-coated electrodes than at the untreated one, this is due to the capacitive current characteristic of electrodes coated with conductive polymers.

Although the data for DOPAC and DOPA are listed here, we will confine our discussion to DA and AA.

The effect of the thickness of the coated polymer layers on the limiting current values for DA and AA in quasi-steady state voltammograms was studied (Fig. 2). In this experiment, the thickness of the polymer layers was controlled by varying the number of the potential sweep cycles used for polymerization; the bilayer was coated using either a procedure of two cycles for 3-methylthiophene polymerization followed by different numbers of cycles for the formation of polypyrrole layer, or a procedure of varied number of cycles for poly(3-methylthiophene) layer formation followed by two cycles for polypyrrole layer.

Figure 2A shows that the thickness of either polypyrrole layer or poly(3-methylthiophene) layer has little significant influence on the limiting currents for AA. This finding suggests that AA is oxidized at the surface of the electronic conductive polypyrrole layer. On the other hand, though the limiting current of DA was not influenced by the polypyrrole layer thickness, it varied with the thickness of the inner poly(3-methylthiophene) layer as shown in Fig. 2B. The limiting current of DA increases with the thickness of the poly(3-methylthiophene) layer when the layer is thin, while the current decreases when the layer becomes thicker. The reason is

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**Table 1** Variation in voltammetric parameters for oxidation of ascorbic acid (AA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3,4-dihydroxyphenyl alanine (DOPA) and dopamine (DA) at the poly(3-methylthiophene)/polypyrrole-bilayer-coated and untreated carbon fiber electrodes

<table>
<thead>
<tr>
<th>Electroactive species</th>
<th>Electrode</th>
<th>$I_i/\mu A$</th>
<th>$E_{1/2}$</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>C</td>
<td>0.089</td>
<td>-50</td>
<td>33.6</td>
</tr>
<tr>
<td>AA</td>
<td>Ut</td>
<td>0.073</td>
<td>310</td>
<td>125.5</td>
</tr>
<tr>
<td>DOPAC</td>
<td>C</td>
<td>0.087</td>
<td>95</td>
<td>33.4</td>
</tr>
<tr>
<td>DOPAC</td>
<td>Ut</td>
<td>0.075</td>
<td>330</td>
<td>135.6</td>
</tr>
<tr>
<td>DOPA</td>
<td>C</td>
<td>0.090</td>
<td>125</td>
<td>33.9</td>
</tr>
<tr>
<td>DOPA</td>
<td>Ut</td>
<td>0.085</td>
<td>265</td>
<td>118.7</td>
</tr>
<tr>
<td>DA</td>
<td>C</td>
<td>0.095</td>
<td>125</td>
<td>32.0</td>
</tr>
<tr>
<td>DA</td>
<td>Ut</td>
<td>0.080</td>
<td>185</td>
<td>92.0</td>
</tr>
</tbody>
</table>

a. Concentrations: 0.1 mmol/dm$^3$ in PBS solution, pH 7.4.
b. Obtained from potential sweep voltammograms at $v=10$ mV/s, background current being subtracted.
c. Limiting (plateau) current values.
d. Half-wave potential vs. SCE.
e. Obtained from plots of $E$ vs. $\log[I/(I_i-I)]$.

Electrodes: C, coated; Ut, untreated.
for this behavior is not clear at present. We usually employed two potential cycles for polymerization of both layers in order to avoid any complications which may result from thicker layers.

**Simultaneous determination of AA and DA**

A cyclic voltammogram recorded with the bilayer coated electrodes for 100 µM DA in the presence of 1.0 mM AA are shown in Fig. 3A. The wave for AA is well separated from that of DA (ca. 200 mV) and the wave heights were found to be proportional to their concentrations. These facts indicate that simultaneous determination of DA and AA is possible. Similar arguments also apply to the combination of DOPAC and AA (Fig. 3B).

DPV is usually used to achieve the higher degree of resolution of waves and the increased sensitivity. Figure 4A shows a voltammogram by DPV obtained at a bilayer coated electrode in a PBS solution containing both 20.0 µM DA and 1.0 mM AA. For comparison, voltammograms recorded by DPV at the electrodes coated with a single layer of either polypyrrole or poly(3-methylthiophene) in the same solution are shown in Fig. 4B. At the electrode coated with only polypyrrole layer, the peak for the oxidation of AA clearly appeared near +0.05 V, but the wave for the DA oxidation was only a shoulder; the latter cannot be used for analytical purposes. At the poly(3-methylthiophene) coated electrode, only a single broad peak was observed. In contrast, at the bilayer coated electrode, two peaks for the oxidation of AA and DA were resolved as expected from the cyclic voltammograms in Fig 3A. The peak potentials for these waves were separated by ca. 160 mV.

Though the DPV waves for AA and DA were not completely resolved, simultaneous determination of DA and AA was possible from the background current corrected peak current heights for DA and AA. Linear dependence of peak heights for DA was found on the DA concentration in the range from 3 to 200 µM (slope: 2.84 nA/µM; correlation factor: 0.995) in the presence of 0.5 mM AA (A) and on the AA concentration in the presence of 20 µM DA (B). In B, solid circles are for AA and open circles are for DA.

\[
\Delta I_p = 2\pi F k c D f(DAt/\alpha^2) \tanh(nF\Delta E/4RT) \\
\Delta I_p = 2\pi F c D f(DAt/\alpha^2) \tanh(nF\Delta E/4RT) \\
\]
where \( n \) is the number of electron transferred, \( a \) the radius of the electrode, \( l \) the length of the electrode, \( \Delta E \) the pulse height, \( \Delta t \) the pulse width, and other symbols have their usual significance. Introducing \( n=2 \), \( D=6.0 \times 10^{-6} \text{ cm}^2/\text{s} \) and the values employed in the experiment into Eq. (1) yields a slope value: \( \Delta I_0/\Delta c=1.27 \text{ nA/µM} \). This result indicates that the sensitivity of the bilayer coated electrodes for DA experimentally obtained is higher than that expected from the theory. In our previous work with mildly pretreated bare carbon fiber electrodes, a result similar to this was obtained in the presence of large excess of AA, while in the absence of AA, the DPV peak current for DA oxidation obeyed Eq. (1). The enhanced DPV peak current values for DA are ascribed to a homogeneous catalytic regeneration of DA through the reduction of the oxidized DA orthoquinone by AA which is present in large excess to DA. Contributions from some increase in the electrode surface area due to bilayer coating and adsorption of DA to the polypyrrole layer surface may also be possible. On the other hand, the lower sensitivity to AA is ascribed to the quasi-reversibility of AA oxidation.

**Stability and response time of bilayer coated electrodes**

In order to develop electrochemical sensors, stability and response time are very important factors. We examined the stability of the bilayer coated carbon fiber electrodes in PBS solutions by cyclic voltammetry. Figure 6A shows background cyclic voltammograms in the base solution for ten runs in a sequence with 1 min waiting periods between runs. The wave observed in the first scan at potentials more positive than 0.2 V gradually diminished and disappeared in the subsequent scans. This wave is due to the capacitive current commonly observed at polypyrrole-coated electrodes. Figure 6 also shows equivalent voltammograms in the presence of 0.1 mM AA and 0.1 mM DA. Except for the first scan, the voltammograms were almost the same for successive runs, indicating that the bilayer electrodes were stable in the time-course we studied. It was found that these electrodes were not deteriorated during the repeated cyclic voltammetric measurements for at least one hour. This result is different from that reported by Ewing and co-workers for polypyrrole-coated glassy carbon electrodes, where they observed a gradual decrease in current with repeated runs. Though the role of the poly(3-methylthiophene) layer in the bilayer coated electrode has not been clarified yet, the poly(3-methylthiophene) may serve to stabilize the electrode.

Recently, cyclic voltammetry with fast sweep rates, e.g., 300 V/s, has been used for in vivo measurements, and electrodes with short response times are desired for this purpose. The differential capacitance of the bilayer-coated electrodes presented in this work was estimated to be ca. 0.25 µF (300 µF/cm²) from the cyclic voltammograms shown in Fig. 1. This high value of the capacitance is ascribed to the capacitive nature inherent to conductive-polymer-coated electrodes. If one assumes an uncompensated resistance of 1 MΩ as a worst case, then the time constant of these electrodes is 0.25 s. In the usual conditions as shown in this paper, however, the response time is much shorter and there would be no serious problem in DPV.

In conclusion, we have shown that the poly(3-methylthiophene)/polypyrrole bilayer coated carbon fiber electrodes can be used to determine simultaneously DA and AA in concentrations higher than 3 µM and 50 µM, respectively.

**References**

5. F. G. Gonon, C. M. Fombarlet, M. J. Buda and J. F.

(Received January 12, 1994)
(Received February 25, 1994)