Anionic Polymer-Modified Electrodes Based on a Macrocyclic Nickel(II) Complex for Selective Determination of Dopamine in the Presence of Ascorbic Acid

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Selectivity, in addition to sensitivity, is of great interest in the electrochemical determination of compounds in biological samples. Dopamine (DA), a catecholamine neurotransmitter, exists in the brain, blood plasma, and urine, and is involved in diseases such as Parkinson's.[1,2] In biological samples, DA occurs with relatively higher concentrations of ascorbic acid (AA). When DA is oxidized to DA quinone, AA is oxidized at a similar potential to DA at ordinary solid electrodes, which decreases the electrodes' selectivity for DA.

To increase the selectivity for DA, electrodes can be modified with materials that can separate DA and AA electrochemically. Most of these materials are cationic or anionic polymers,[3-6] because cationic DA and anionic AA at the physiological pH of 7.4 can be separated by the exclusion effect of polymer films of the same charge. In addition to ionic polymers, self-assembled monolayers[7] and carbon nanotubes[8] also increase the selectivity for DA.

Anionic polymer-modified electrodes are useful for increasing the selectivity toward cationic DA over anionic AA. The desirable anionic polymer material should be well suited to biological samples. The macrocyclic Ni(II) complex is readily electropolymerized by oxidation or cycling the potential, and the stable polymer film has a catalytic effect on substances such as AA.[9] To obtain an anionic polymer-modified electrode, the macrocyclic Ni(II) complex can be used with an anionic polymer or a macrocyclic Ni(II) complex with an introduced carboxylic acid group. The anionic macrocyclic Ni(II) complex also readily forms a negatively charged electropolymerized film on an electrode surface.

Polyurethane (PU) containing γ-benzyl L-glutamate segments (PUBLG) is a new polymer,[10] which is a segmented PU. Segmented PUs are used in devices that contact blood, such as catheters and artificial hearts, because of their good mechanical properties and tolerance of blood.[11,12] A hydrophobic PUBLG film can be converted into an anionic hydrophilic PU film on introducing carboxylate groups by hydrolysis. We applied an anionic PU film to the selective determination of DA for the first time.

This study examined the selectivity of anionic polymer-modified electrodes for DA over AA and their suitability for use with human urine. Their sensitivity and reproducibility were evaluated using amperometry with flow injection. These anionic polymers were compared with Nafion, a perfluorosulfonated ion-exchange material.

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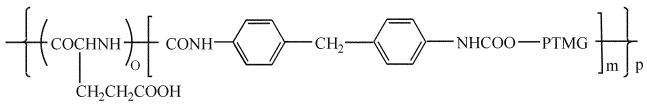
Experimental Section

Two macrocyclic Ni(II) complexes were synthesized: one with and one without a carboxylic acid group. 2,4,9,11-Tetramethyl-1,5,8,12-(benzo)tetraazacyclotetradecinatonickel (II) (Ni(II) complex, NC) was synthesized, as described elsewhere,[9] and we synthesized 2,4,9,11-tetramethyl-1,5,8,12-(benzoic acid)tetraazacyclotetradecinatonickel(II) (carboxylated Ni(II) complex, NC-C) for the first time. The carboxylated Ni(II) complex was characterized using IR (Perkin Elmer Spectrum GX) and ^{1}H and ^{13}C NMR (Bruker Avance 400 MHz) spectra.

A Ni(II) complex-modified electrode was prepared by electropolymerization of the Ni(II) complex on a glassy carbon (GC, 3-mm dia.) surface (GC/NC). The $Ag|Ag^{+}$ (0.01 M $AgNO_3$ in acetonitrile) reference and Pt auxiliary electrodes had already been dipped in a red Ni(II) complex solution under a nitrogen atmosphere. The potential was cycled once between +1.8 and -2.0 V at a scan rate of 0.2 V s^{-1} and 25 °C, using a 0.50-mM Ni(II) complex solution containing 50 mM tetraethylammonium perchlorate in acetonitrile. The GC surface coated with a polymer film of the Ni(II) complex was washed with acetonitrile and water and then used to make measurements. The Ni(II) complex was also electropolymerized on an indium tin oxide-coated glass plate in the same way as on GC to evaluate the hydrophilicity of the modified electrodes by measuring the water contact angle of the films using a Contact Anglemeter (ERMA G-1). An electrode modified with carboxylated Ni(II) complex was prepared in a similar way to the GC/NC. The carboxylated Ni(II) complex was electropolymerized on a GC surface while the potential was cycled 10 times between +1.8 and -2.0 V (*vs.* $Ag|Ag^{+}$) at a scan rate of 50 mVs^{-1} in a 0.50-mM carboxylated Ni(II) complex solution (GC/NC-C). The morphology of the NC-C film using atomic force microscopy differed from that of the NC film.

PU with γ-benzyl L-glutamate segments was synthesized by reacting the PU prepolymer with γ-benzyl L-glutamate oligomer.[10] The PU prepolymer was prepared by reacting

poly(tetramethylene glycol) (PTMG) with 4,4'-diphenylmethane diisocyanate. PUBLG was produced as a precipitate, and the 20% (w/v) solution was diluted with dimethylformamide as necessary. One percent (v/v) PUBLG was drop-coated on a GC/NC surface and was then hydrolyzed to introduce carboxylic acid groups into the PU polymer. Hydrolysis proceeded in a 1 : 3 (v/v) mixture of 4.0 M NaOH and methanol for 7 min at room temperature (GC/NC/PU-C). The structure of PU-C is as follows:



The carboxylate groups introduced into the PUBLG film were identified in the IR spectrum; a new peak, due to the carboxylic acid group, appeared at 1,710 cm^{-1} after hydrolysis, indicating the cleavage of benzyl ester groups in the γ-benzyl L-glutamate segments of PUBLG.

Nafion was used to obtain a Nafion-coated Ni(II) complex-modified electrode (GC/NC/Nafion) as a 0.05% (v/v) solution. DA solutions were prepared with 0.1 M phosphate buffer before use and adjusted to pH 7.4 with 0.2 M KH_2PO_4 and 0.2 M K_2HPO_4. A urine sample from a normal adult was used for the measurements after it had been filtered through a 0.45-μm membrane filter and diluted 100-fold with 0.1 M phosphate buffer.

All of the electrochemical measurements were made using a three-electrode system with a BAS 100W electrochemical analyzer. Cyclic voltammograms were obtained using modified GC, a saturated calomel electrode (SCE), and Pt wire as the working, reference, and auxiliary electrodes, respectively. Amperometry with flow injection was performed using a thin-layer flow cell (BAS) that consisted of dual GC working, Ag|AgCl (3 M NaCl) reference, and stainless steel auxiliary electrodes.

Results and Discussion

A bare GC surface was modified with the electropolymerized film of a Ni(II) complex for oxidizing DA sensitively and selectively. The polymer film forms on the GC surface from radical cations and dications while cycling the potential.[13] Cyclic voltammograms (CVs) of DA and AA were obtained using the GC/NC electrode and are shown in Figure 1. The GC/NC electrode has well-defined redox peaks of DA after modification. The difference between the anodic (E_{pa}) and cathodic (E_{pc}) peak potentials represented by $n(E_{pa}$-$E_{pc})$ was 0.12 V for the GC/NC electrode and 0.36 V for the bare GC electrode. The difference decreased significantly after modification, although the reaction was not Nernstian. The oxidation potential shifted negatively by 0.17 V relative to the bare electrode, and the current roughly doubled. The oxidation peak overlapped that of AA, however, even at the GC/NC electrode, as at a bare GC electrode. Therefore, the GC/NC electrode had an electrocatalytic effect on the oxidation of DA, but its selectivity for DA over AA needed to be improved.

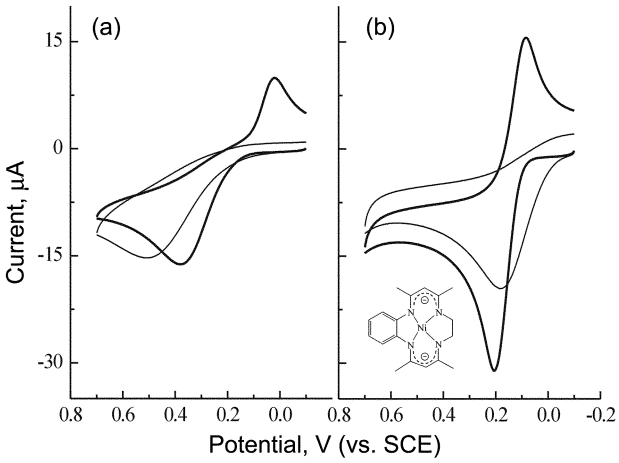


Figure 1. Cyclic voltammograms of 1.0 mM AA (thin line) and 1.0 mM DA (thick line) in 0.1 M phosphate buffer (pH 7.4) using (a) bare GC and (b) GC/NC electrodes at a scan rate of 0.1 V s^{-1} and 25 °C.

To increase the selectivity for cationic DA over anionic AA, the GC/NC was further modified with anionic PU or Nafion. Otherwise, GC was modified with a carboxylated Ni(II) complex that was electropolymerized by cycling the potential in a similar way to GC/NC. CVs of DA and AA using the anionic polymer-modified electrodes are shown in Figure 2. The GC/NC-C, GC/NC/PU-C, and GC/NC/Nafion were all highly selective for DA because they barely responded to AA. Therefore, the anionic polymer-modified electrodes improved the selectivity for DA over AA owing to the anionic exclusion effect of the negatively charged polymer film. The oxidation potential of DA shifted positively for GC/NC/PU-C and GC/NC/Nafion as compared to that for GC/NC-C. This might have been because of the difference between the single and double modifications.

The anionic polymer-modified electrodes had different sensitivities for DA. The oxidation current increased at GC/NC-C and decreased at GC/NC/Nafion compared with GC/NC, and was similar at GC/NC/PU-C. The higher sensitivity of GC/NC-C than GC/NC could have been because of the opposite charge effect of the anionic NC-C film for cationic DA. Its higher sensitivity relative to the doubly modified electrodes, GC/NC/PU-C and GC/NC/Nafion, might have been because of the difference between the single and double modifications. The different sensitivities of GC/NC/PU-C and GC/NC/Nafion can be explained by the hydrophilic nature of the PU-C and Nafion films. The hydrophilicity of the films can be evaluated using the water wettability, by measuring the water contact angle. NC, NC/PU-C, and NC/Nafion films had water contact angles of 60.7 ± 0.3, 45.2 ± 1.0, and 73.5 ± 0.5°, respectively. The PU-C film coating further increased the wettability of the NC film, whereas the Nafion film decreased it. Therefore, sensitivity for DA can be increased by modifying an anionic NC-C polymer as a single modification or a highly hydrophilic PU-C film as a double modification.

The anionic polymer-modified electrodes were used in the

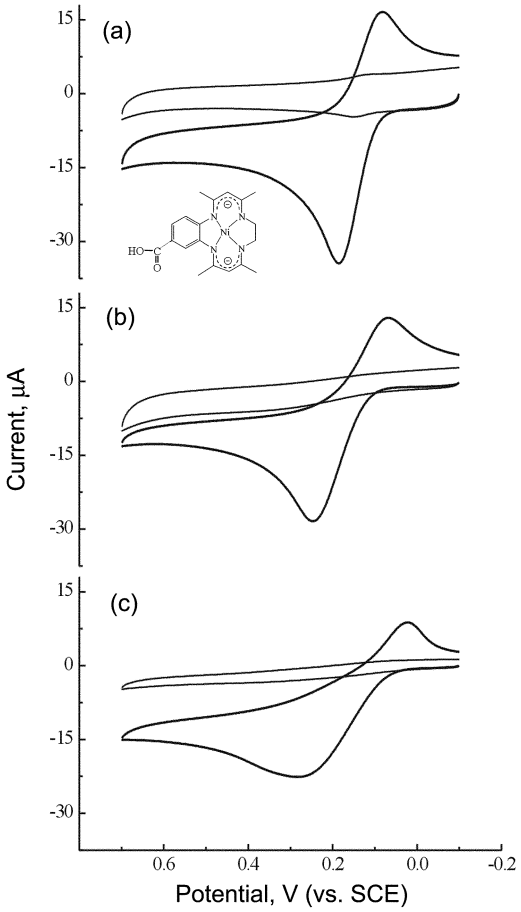


Figure 2. Cyclic voltammograms of 1.0 mM AA (thin line) and 1.0 mM DA (thick line) in 0.1 M phosphate buffer (pH 7.4) using (a) GC/NC-C, (b) GC/NC/PU-C, and (c) GC/NC/Nafion electrodes at a scan rate of 0.1 V s^{-1} and 25 °C.

selective determination of DA in an excess of AA by amperometry with flow injection. The selectivity was examined using the recovery of DA, and the results are listed in Table 1. Anionic AA did not affect the recovery of DA for any of the anionic polymer-modified electrodes, even at 100- and 1000-fold concentrations. Therefore, the anionic polymer-modified electrodes based on the macrocyclic Ni(II) complex are highly selective for DA, even at high AA concentrations.

These electrodes were applied to a human urine sample for the selective determination of DA by amperometric oxidation with flow injection. DA was added to the urine sample and the amperometric responses are shown in Figure 3. The

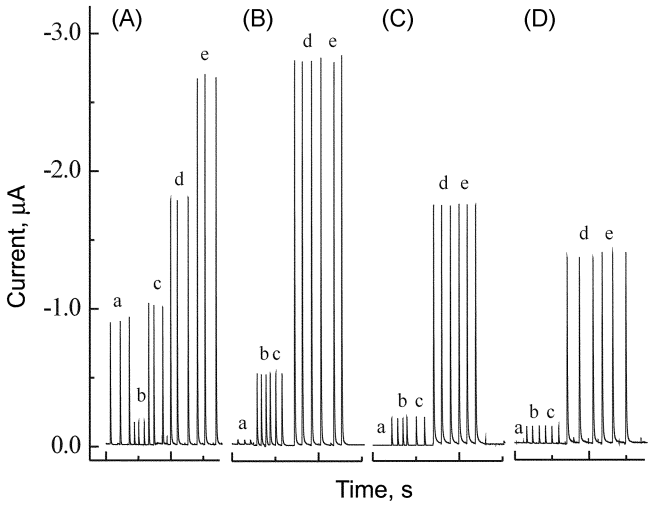


Figure 3. Amperometric responses to consecutive injections of (a) a urine sample, (b) 0.40 μM DA, (c) urine + 0.40 μM DA, (d) 4.00 μM DA, and (e) urine + 4.00 μM DA using (A) GC/NC, (B) GC/NC-C, (C) GC/NC/PU-C, and (D) GC/NC/Nafion electrodes. The other conditions are the same as in Table 1.

results were compared with those for a GC/NC electrode in sensitivity and selectivity. Among the anionic polymer-modified electrodes, sensitivity for DA was highest for GC/NC-C, as in Figure 2, and was even higher than for the GC/NC. In the doubly modified electrodes, GC/NC/PU-C was more sensitive than GC/NC/Nafion. Therefore, the GC/NC-C electrode is still the most sensitive to DA in a urine sample, and highly hydrophilic PU-C is a better anionic polymer for increasing the sensitivity.

The current with GC/NC-C was three to four times higher for 0.40 μM DA than with either GC/NC/PU-C or GC/NC/Nafion, and GC/NC/PU-C had twice the current of GC/NC/Nafion. With a 10-fold higher DA concentration, the current increased about six-fold for GC/NC-C, while it increased about 10-fold for GC/NC/PU-C and GC/NC/Nafion. This means that the DA calibration curve using GC/NC-C with high sensitivity has two linear ranges, as seen with a previously reported anionic polymer-modified electrode.[5] The 0.40- and 4.00-μM DA concentrations may be within different linear ranges on the calibration curve. This might be caused by the saturation effect of DA within the negatively charged NC-C film.

The selectivity of the anionic polymer-modified electrodes for DA was examined using DA recovery in human urine

Table 1. Recovery of DA in an excess of AA using anionic polymer-modified electrodes

Modified electrodes	[DA] added (μM)	[DA] found (μM)		Recovery ± S.D.[d] (%)	
		40 μM AA	0.40 mM AA	40 μM AA	0.40 mM AA
GC/NC-C[a]	0.40	0.40	0.41	100.5 ± 0.1	102.2 ± 0.3
GC/NC/PU-C[b]	0.40	0.41	0.41	101.5 ± 0.1	101.4 ± 0.1
GC/NC/Nafion[c]	0.40	0.40	0.40	100.4 ± 0.1	101.0 ± 0.4

[a]GC modified with a carboxylated Ni(II) complex. [b]Anionic PU coated on GC modified with Ni(II) complex. [c]Anionic Nafion coated on GC modified with Ni(II) complex. [d]The mean of three injections using amperometric detection with flow injection. The applied potential was +0.50 V (*vs.* Ag|AgCl); the carrier solution was 0.1 M phosphate buffer, pH 7.4; the flow rate was 0.7 mL min^{-1} at 25 °C.

Table 2. Recovery of DA in a human urine sample using anionic polymer-modified electrodes

Modified electrodes	[DA] added (μM)	[DA] found (μM)	Recovery ± S.D.[a] (%)
GC/NC-C	0.40	0.38	95.1 ± 1.2
	4.00	3.98	99.6 ± 0.8
GC/NC/PU-C	0.40	0.42	104.5 ± 0.8
	4.00	4.00	100.3 ± 0.1
GC/NC/Nafion	0.40	0.40	100.5 ± 3.0
	4.00	4.11	102.8 ± 0.5

[a]Recovery = $[i_{(DA+urine)}-i_{(urine)}] \times 100/i_{DA}$ and the mean of three injections using amperometric detection with flow injection. The other conditions are the same as in Table 1.

from Figure 3, and the result is given in Table 2. The recovery for two DA concentrations was reliable for all of the anionic polymer-modified electrodes. Their reliability with the urine sample was higher than that of a similar Ni(II) complex-modified electrode.[14] Moreover, these electrodes have improved recovery relative to another anionic polymer-modified electrode[5] and a metal dispersed-sol-gel composite electrode.[15] Therefore, the anionic polymer-modified electrodes based on a macrocyclic Ni(II) complex are highly selective for DA, even in a urine matrix.

When the reproducibility of the modified electrodes was examined for undiluted urine using amperometry, the relative standard deviation for 12 injections was 9.5, 1.9, and 4.7% for the GC/NC-C, GC/NC/PU-C, and GC/NC/Nafion electrodes, respectively. Furthermore, GC/NC/PU-C had a very stable baseline in the amperometric response, whereas GC/NC/Nafion was much less stable than GC/NC-C. Therefore, PU-C is very suitable for biological samples because the GC/NC/PU-C electrode gives the most reproducible results for an undiluted urine sample.

Consequently, anionic polymer-modified electrodes, which are based on a macrocyclic Ni(II) complex, have a high selectivity for the determination of DA in the presence of AA and have reliable recovery even in a urine matrix. The GC/NC-C electrode had the highest sensitivity for DA, and the GC/NC/PU-C electrode was more reproducible than the GC/NC-C or GC/NC/Nafion electrodes. Since PU-C is a highly hydrophilic anionic polymer, the GC/NC/PU-C electrode can be used in the selective, reproducible determination of DA in biological samples.

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