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Bioelectrochemistry 72 (2008) 87–93
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

Electropolymerization of negatively charged Ni(II) complex for the selective determination of dopamine in the presence of ascorbic acid

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Received 7 June 2007; received in revised form 9 November 2007; accepted 18 November 2007
Available online 23 November 2007

Abstract

Electrodes for the dopamine (DA) determination in biological samples have been developed with improved selectivity and sensitivity in an excess of ascorbic acid (AA). Negatively charged Ni(II) complex was synthesized and electropolymerized on the glassy carbon electrode to impart the surface with anionic characteristics that could act both as a catalyst and as a discriminating layer against AA based on the electrostatic interaction. Thus prepared electrodes enabled selective determination of DA even in a large excess of AA by differential pulse voltammetry at physiological pH. Linear response was found down to 1.0×10^{-7} M with 5.0×10^{-9} M of LOD (Limit of Detection). In a flow injection analysis performed in an amperometric mode, the detection limit was lowered by two orders of magnitude down to 1.0×10^{-9} M with a linear range of 1.0×10^{-9} to 1.0×10^{-6} M. The relative standard deviation was found to be 3.36% from 25 independent measurements for 1.0×10^{-5} M of DA. Stable oxidation current of DA was observed even after 30 days storage in air. The recoveries of DA in the 100-fold diluted human urine samples were 97.7% for 4 measurements. The rate constant for the DA oxidation was 1.3×10^{-3} cm s^{-1} from hydrodynamic experiments using a rotating disk electrode.

Keywords: Dopamine; Negative charged Ni(II) macrocyclic complex; Ascorbic acid; Differential pulse voltammetry; RDE experiments

1. Introduction

Dopamine is a biogenic catecholamine formed by decarboxylation of 3,4-dihydroxyphenylalanine. It is a precursor to epinephrine and norepinephrine in a biosynthetic pathway. One of the important functions of DA is as a neurotransmitter in the central and peripheral nervous systems. It also functions as a biological messenger. Insufficient DA level due to the loss of DA-producing cells may lead to disease called Pakinson's disease, in which a person loses the ability to execute smooth and controlled movements [1–3].

Many attempts, therefore, have been made to develop an efficient and selective way of determining DA level in biological samples. Earlier attempts by fluorometric methods required a large volume of samples, but still lacking selectivity and sensitivity [4]. Later, methods based on chromatography combined with spectrometry as a detection method have been developed. For example, mass spectrometry has been combined with GC [5], HPLC [6,7], and capillary electrophoresis [8]. Although these methods are highly specific and sensitive, they require sophisticated and expensive instrumentation, and are time-consuming. As an alternative to mass spectrometry, electrochemical detection methods have also been introduced. Originally developed by Kissinger [9], electrochemical methods are widely used in liquid chromatography and capillary electrophoresis [10–14] for the reason that they offer a simple, rapid, and very sensitive way of detecting DA.

Certainly the best way is to directly determine DA without any separation steps of samples. Main obstacle to this goal in

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doi:10.1016/j.bioelechem.2007.11.007

electrochemical detection is the existence of ascorbic acid (AA) in large amount. AA hinders DA detection as the oxidation potential of AA is close to that of DA at most solid electrodes. Therefore, one should block the effect of AA and other interfering species in DA analysis or devise a method to simultaneously detect both DA and AA at different potentials by modifying electrode surfaces. Surface modification by surfactants [15], self-assembled monolayers [16,17], and polymers [18–20] shifts oxidation potentials of DA and AA so that each peak can be separately detected by voltammetry. Other methods such as enzyme-based techniques [21], electrochemical pretreatment [22] were also reported.

A polymer modification of the electrode surface may be the most commonly practiced way to separate or eliminate the oxidation peak of AA from DA, which includes electropolymerization of sulfosalicylic acid [23], eugenol [24], acetylaniline [25], pyrrole [26], *N*,*N*-dimethylaniline [27], and neutral red [28]. Nafion [29–31], zeolite [32,33], nontronit clays [34], ultrafine TiO_2 [35], and hydrophilic polyurethane film [36] coated on an electrode surface also made possible effective elimination of AA. However, these methods need complicated electrode preparation process and suffer from low detection limit.

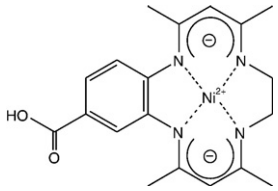
We have long been studying the electrocatalytic properties of nickel tetraaza macrocyclic complexes on the DA and AA oxidation. A polymeric film of symmetric metal tetraaza macrocycles are known to exhibit electrocatalytic activity for substrates such as O_2 and NO_3^- [37,38]. In the meantime, asymmetric 1,5,8,12-tetraaza-2,4,9,11-tetramethyl-cyclotetradecinatonickel(II) complex synthesized in our group [39] was found to be an effective electrocatalyst for DA and AA oxidation when formed a polymer film on glassy carbon electrodes. But since oxidation potentials of DA and AA are very close, it is not possible to determine DA in the presence of AA. One way to solve this problem was to form a second layer over the nickel complex layer to block the entrance of AA to the electrode. Negatively charged polyurethane or Nafion films not only provided selectivity toward DA by effectively removing AA interference due to the electrostatic repulsion at neutral pH, but also offered additional advantages such as better stability and reproducibility over unmodified electrodes [40].

In this paper, we report further improvement of our electrodes in which negatively charged nickel macrocyclic monomer could be electropolymerized to impart negative charges to the produced polymeric film. Thus prepared modified electrodes showed both electrocatalytic activity toward DA oxidation and screening effect against AA at physiological pH.

2. Experimental

2.1. Synthesis and reagents

An asymmetric, negatively charged Ni(II) macrocyclic complex [1,5,8,12-(benzoic acid)tetraaza-2,4,9,11-tetramethyl-cyclotetradecinatonickel(II)] (Scheme 1) was synthesized in our group. 0.04 mol of 2,4-pentandione was added to 50.0 mL of methanolic solution containing 0.02 mol of nickel(II) acetate tetrahydrate under reflux for 30 min with stirring. To this hot



Scheme 1. Structure of negatively charged Ni(II) macrocyclic complex.

(60 °C) mixture was added ethylenediamine (0.02 mol) and 1,2-phenylenediamine (0.02 mol) under reflux for 2 h in nitrogen gas. The solution was then refluxed for 24 h until precipitation of the product was complete. The carboxyl group dissociates in neutral pH to take negative charge. The product was characterized by IR (Spectrun GX, Perkin Elmer, USA), GC-MS (HP 5973, USA) and NMR (Bruker, Avance Digital 400, Germany) measurements. IR (KBr disc, cm^{-1}): ν(C═C), 1486; ν(C═N), 1531; $\nu(C_6H_6)$, 749; ν(C═O), 1687; ν(C–O), 1293; EIMS: 396 *m*/*z*, and ^{1}H NMR ($CDCl_3$): 2.097, 2.402, 2.454(s) (methyl); 5.112, 5.166(s) (methine); 3.401(s) (ethylene); 7.917 (s) (carboxylic acid); 7.188–7.488(m) (aromatic).

Tetraethylammonium perchlorate (TEAP) was synthesized and followed by recrystallization according to the literature [41]. Acetonitrile (Merck, Darmstadt, Germany) was distilled and checked by cyclic voltammetry prior to use. Dopamine (Sigma, St. Louis. USA), ascorbic acid (Sigma, St. Louis. USA) and other reagents were used without further purification and of guaranteed grade quality. DA solutions were prepared with phosphate buffer of pH 7.4 using KH_2PO_4 and K_2HPO_4. All solutions were prepared with deionized water purified by Milli-Q water system (Millipore).

2.2. Preparation of modified electrodes

The laboratory-built GC disk electrodes (diameter 3.0 mm, Tokai Carbon, Japan) were consecutively polished with aqueous alumina slurries of 1.0, 0.3, and 0.05 μm (Buehler Inc, Lake Bluff, ILL, USA) until mirror finish. Electrodes were thoroughly rinsed with water in each polishing step. The polished electrodes were then subject to sonication in acetonitrile and water, respectively, and dried at room temperature. Polymer-modified electrodes were prepared by cycling the potential (10 cycles) between 1.8 and −2.0 V vs $Ag|Ag^+$ at 50 mV s^{-1} in acetonitrile solution containing 0.5 mM nickel complex and 0.05 M TEAP as supporting electrolyte. The morphology of GC surfaces was analyzed by Field Emission Scanning Electron Microscope (FESEM, Hitachi, S-4300, Japan). Hereafter, the polymer-modified electrodes will be referred as GC/NC-C electrodes. The wettability change of the electrode surface was monitored by measuring the water contact angle using a Contact Anglemeter (Model G-1, ERMA Inc., Tokyo, Japan).

2.3. Electrochemical measurement

Electrochemical measurements were performed at 25 °C with an electrochemical analyzer (BAS 100B/W, Bioanalytical

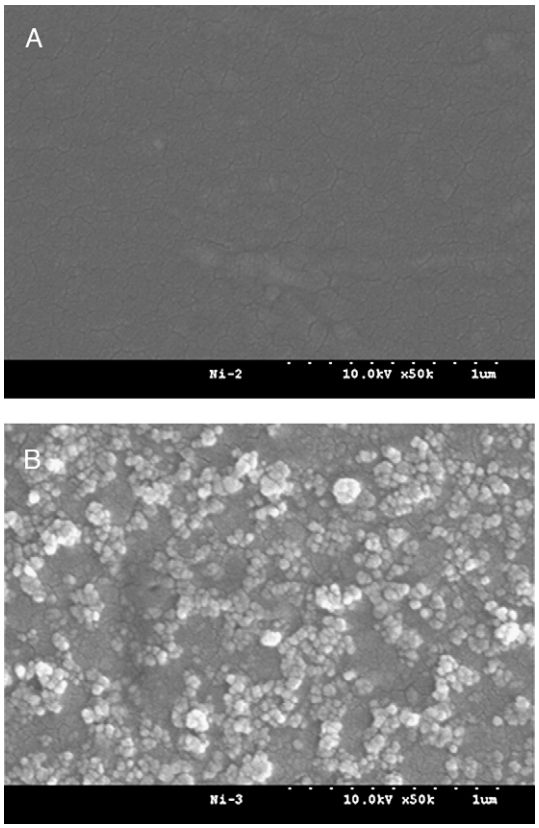


Fig. 1. SEM images. SEM images of a glassy carbon surface before (A) and after (B) electropolymerization of negatively charged nickel complex.

Systems, USA). GC, SCE, and Pt wire were used as working, reference, and auxiliary electrodes, respectively. All solutions of pH 7.4 were deaerated in a nitrogen atmosphere before measurements. RDE experiments were carried out using a RDE system (EG & G PARC 616 RDE, USA).

3. Results and discussion

3.1. Electrocatalytic oxidation of DA

Fig. 1 shows the FESEM images of the GC surface before (a) and after electropolymerization (b). As expected, the bare GC did not show any feature. But uniformly distributed small particles of ca. 50 nm in diameter were observed upon electropolymerization, indicating that polymerization starts after nucleation.

Fig. 2 shows cyclic voltammograms of 1.0 mM dopamine at a bare GC (a) and a GC/NC-C electrode (b). It clearly shows that the polymerized film reduces the oxidation overvoltage of DA by ca. 200 mV, shifting E_p from +400 mV to +190 mV. Peak current was doubled upon polymerization. This result indicates that polymer film of negatively charged Ni(II) complex exhibits catalytic activity for the DA oxidation. A similar effect was reported by Joshua et al. [42] in which the carbon paste electrode modified with iron tetrasulfophthalocyanine complex provided electrocatalytic activity for DA.

3.2. Elimination of AA interference

The AA oxidation at a bare GC and a GC/NC-C electrode was examined (Fig. 3). AA is irreversibly oxidized at a bare GC with E_p at ca. 500 mV (curve a). However, no redox peaks are observed at a GC/NC-C (curve b). This is because ascorbic acid has pK_a of 4.2 and thus is effectively repulsed by the similarly charged polymeric film. Since our monomer has benzoic acid moiety fused into the tetraaza macrocyclic ring, the –COOH group may have the similar pK_a value to that of benzoic acid, which is 4.2. At pH 7.4, almost all –COOH dissociate to become $–COO^-$ and convert the surface more hydrophilic as a result. This surface has strong affinity toward dopamine for the easy electron transfer. If the carboxylate group is introduced on the electrode surface, the contact angle on the film would decrease due to the increased wettability. We obtained 52.0±

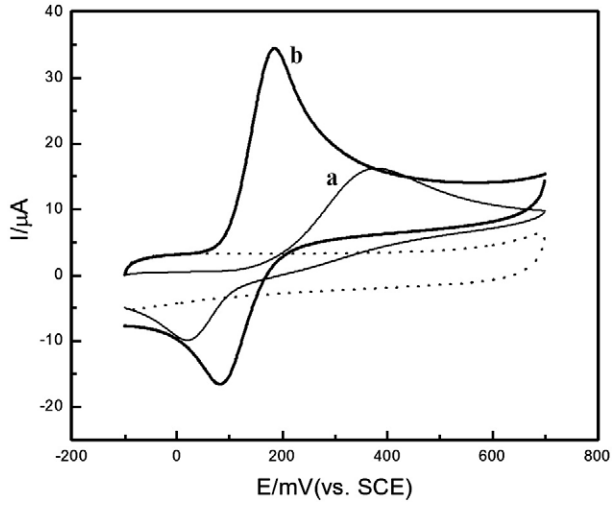


Fig. 2. Cyclic voltammograms of dopamine. Cyclic voltammograms of 1.0 mM DA at a bare GC (a) and at a GC modified with negatively charged Ni(II) complex (b) in pH 7.4 buffer. Scan rate: 100 mV s^{-1}. Dot line is a base line.

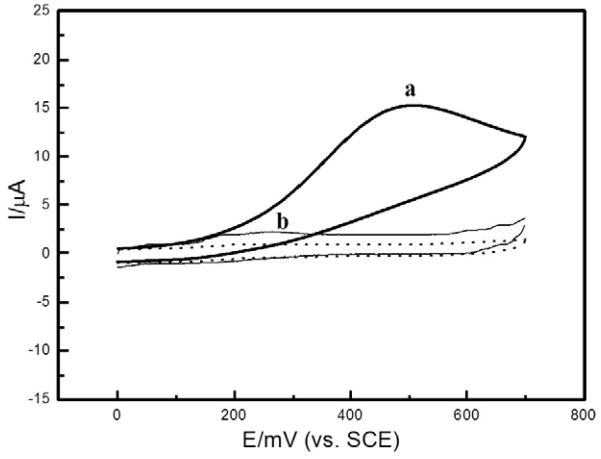


Fig. 3. Cyclic voltammograms of ascorbic acid. Cyclic voltammograms of 1.0 mM AA at a bare GC (a) and at a GC modified with negatively charged Ni(II) complex (b) in pH 7.4 buffer. Scan rate: 100 mV s^{-1}. Dot line is a base line.

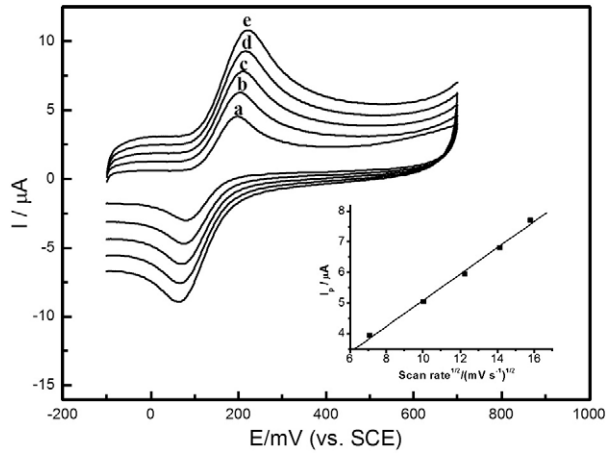


Fig. 4. Cyclic voltammograms of DA in the presence of AA. Cyclic voltammograms for the oxidation of DA (0.2 mM) in the presence of 0.5 mM AA at the GC/NC-C electrode in pH 7.4 buffer with scan rates of 50 (a), 100 (b), 150 (c), 200 (d), and 250 mV s^{-1}. Inset: plot of scan rate dependency of peak currents.

1.9° on our surface for three independent measurements. As a control, we prepared the surface in the same way with the same nickel macrocyclic complex but without –COOH group. The contact angle was 60.7±0.3°. This clearly indicates that carboxyl group plays a crucial role in discriminating DA from AA. We believe that this may be the simpler and easier way to prepare modified electrodes to detect DA in the presence of AA.

Fig. 4 shows the scan rate dependence of the oxidation current of DA (0.2mM) on the scan rate in the presence of 0.5 mM AA. Observed redox peaks are due only to DA when compared to Figs. 2 and 3. AA does not affect DA measurement although it is present at large amount. However, an AA oxidation peak was observed when a modified electrode by electropolymerized film of Ni(II) tetraaza macrocyclic compound without –COOH group was used (data not shown). DA oxidation current is linearly proportional to the square root of scan rate, indicating that DA oxidation process is diffusion-controlled (Inset). The diffusion coefficient of DA was calculated using the equation,

$$I_p = 2.69 \times 10^5 \left[\mathrm{A\,s\,mol^{-1}V^{-1/2}}\right] A_r D_o^{1/2} n^{3/2} v^{1/2} C_o \qquad (1)$$

where n, A_r (cm^2), D_o (cm^2 s^{-1}), C_o (mol cm^{-3}), and v (V s^{-1}) have their usual meanings. I_p (A) is the peak current. By calculating the slope and putting the necessary values to the equation, we obtained D_o value of 6.3×10^{-6} cm^2 s^{-1}. This value agrees well with the reported ones [43].

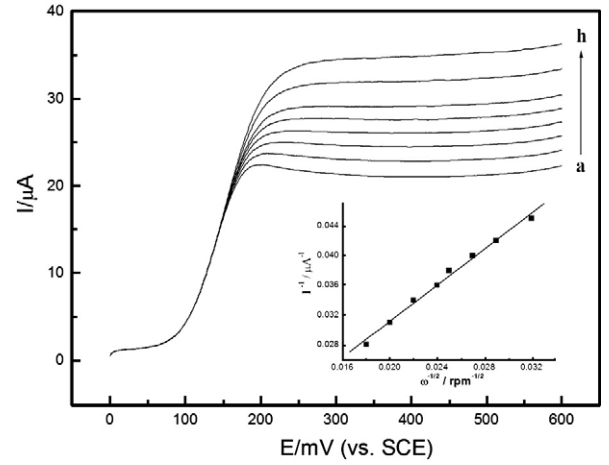


Fig. 5. RDE experiments. Linear scan voltammetry of at a GC/NC-C electrode for the DA (0.2 mM) oxidation as a function of rotation speed. Curves (a) to (h) correspond, respectively, to 1000, 1200, 1400, 1600, 1800, 2000, 2500, and 3000 rpm. Inset: Koutecty-Levich plot of I_{lim}^{-1} vs $\omega^{-1/2}$. Current was read at +0.4 V.

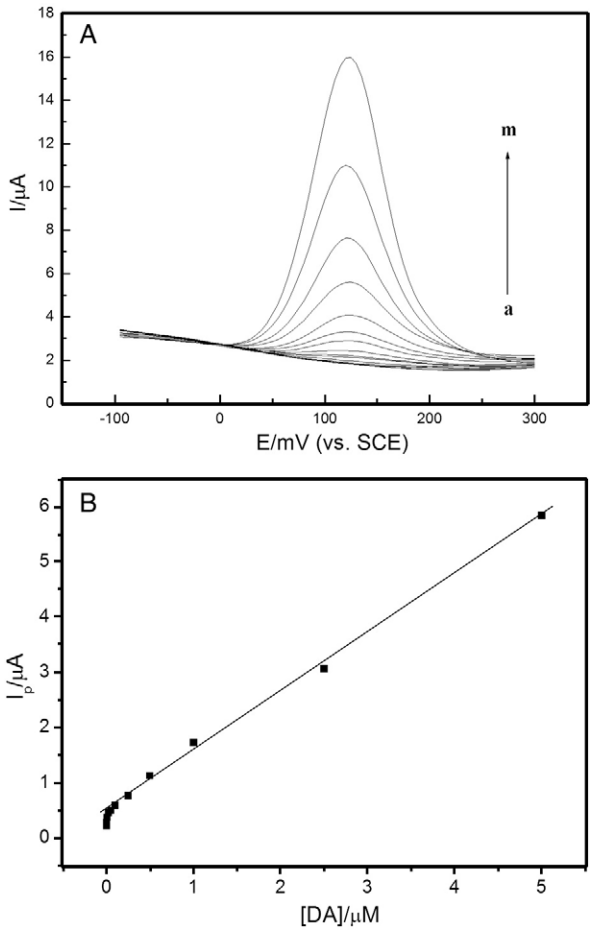


Fig. 6. Differential pulse voltammograms of DA. Panel A: Differential pulse voltammograms of DA oxidation at GC/NC-C electrodes. Curves (a) to (m) correspond, respectively, to 0, 5.0×10^{-9}, 1.0×10^{-8}, 5.0×10^{-8}, 1.0×10^{-7}, 1.0×10^{-6}, 2.5×10^{-6}, 5.0×10^{-6}, 1.0×10^{-5}, 2.5×10^{-5}, 5.0×10^{-5}, 1.0×10^{-4}, and 2.0×10^{-4} M DA concentration. Panel B: Plot of I_p vs [DA] obtained from panel A.

3.3. Kinetic study of dopamine oxidation

The kinetics of DA oxidation was investigaged at a GC/NC-C electrode using a rotating disk electrode (RDE) (Fig. 5). The limiting current, I_{lim}, is a function of Levich current I_{lev}, representing the mass-transfer and the kinetic current I_k, corresponding to the electron cross-exchange between DA and

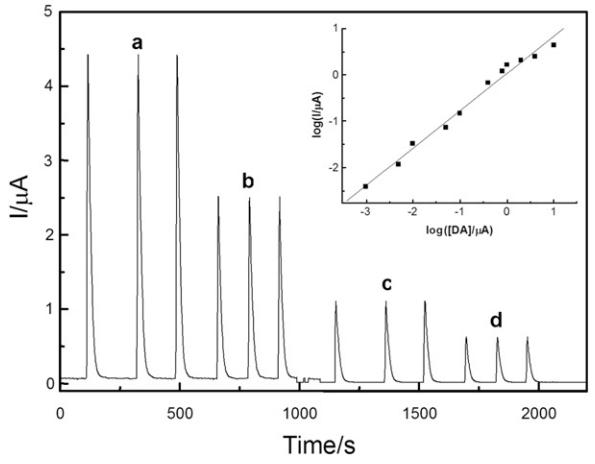


Fig. 7. Flow injection analysis. Flow injection analysis of DA in an amperometric mode at a GC/NC-C electrode. E_{appl}=+0.5 V. Flow rate=0.7 mL min^{-1}. Inset: Plot of log I_p vs log [DA]. Three injections were made for each DA concentration: (a) 10, (b) 4.0, (c) 0.8, (d) 0.4 μM.

negatively charged polymerized Ni(II) complex. I_{lim} is expressed by the Koutecky–Levich equation [44],

$$\frac{1}{I_{lim}} = \frac{1}{I_{lev}} + \frac{1}{I_k} = \frac{1}{0.62nFAD^{2/3}\omega^{1/2}\nu^{-1/6}C_o} + \frac{1}{I_k} \quad (2)$$

D, ν, and C_o are diffusion constant of DA, kinematic viscosity of electrolyte, and bulk concentration of DA, respectively, and other symbols have their usual meanings. By plotting I_{lim}^{-1} vs $\omega^{-1/2}$, I_k can be obtained from an y-intercept. I_k is expressed by

$$I_k = nFA\,kC_o \quad (3)$$

where k is the electron transfer rate constant for the DA oxidation. The value of k was found to be 1.3×10^{-3} cm s^{-1}.

3.4. Calibration curves for dopamine in the presence of ascorbic acid

A differential pulse voltammetry (DPV) has been employed to construct the calibration curve for DA in the presence of AA using GC/NC-C (Fig. 6A). DA concentration was varied from 5 nM to 0.2 mM while AA concentration was fixed at 0.05mM. Voltammetric response was stable over a given concentration range. A calibration curve (Fig. 6B) from DPV shows that the oxidation current linearly responded to the DA concentration range of 0.1 μM to 10 μM with r-factor of 0.999. The slope in this range was 1.05 μA/μM DA. Although the constant slope was not obtained at lower concentrations of DA, it was possible to detect DA by this electrode. The detection limit at signal-to-noise ratio of 3 was calculated to be 5 nM. This value is much lower than 0.2 μM by Protiva et al. [45] who used GC electrodes modified with *N*,*N*-dimethylaniline by electropolymerization.

The GC/NC-C electrode was used as a detector in the amperometric determination of DA with flow injection (Fig. 7). +0.5 V was applied to ensure the complete oxidation of DA. 0.1 M phosphate buffer of pH 7.4 was used as a carrier solution. DA samples of 50 μL were continuously injected at flow rate of 0.7 mL min^{-1}. The linear range was obtained between 1.0×10^{-9} and 1.0×10^{-5} M (r=0.995) with 1.0×10^{-9} M of LOD and 1.62 A M^{-1} of sensitivity. Inset is the plot of peak current vs DA concentration.

Table 1
Effect of interfering species in the determination of dopamine by DPV

Additive	[additive]/[DA][a]	Recovery of DA (%) (±s.d.%)
Urea	100	98.8 (±0.8)[b]
Aspartic acid	100	100.1 (±0.5)
Glutamine	100	101.3 (±0.1)
Xantine	100	101.4 (±0.3)
Glucose	100	99.1 (±0.7)
Guanosine	100	99.5 (±0.4)
Ascorbic acid	1000	110.5 (±0.7)
Uric acid	1000	109.8 (±0.5)
Acetaminophen	100	103.4 (±0.3)
Guanine	100	100.9 (±0.6)
Oxalic acid	100	100.8 (±0.2)
Cysteine	100	98.1 (±0.5)

[a] DA concentration was fixed at 0.4 μM.
[b] Mean value taken after three independent measurements.

3.5. Reproducibility and stability

Reproducibility of GC/NC-C electrodes in the measurements was evaluated by the current reduction for the successive use of the electrodes in a solution containing 10 μM DA and 0.1 mM AA. The relative standard deviation of less than 3.4% was resulted for 25 successive experiments. The long-term stability was tested by cyclic voltammetry. When stored in air, GC/NC-C electrodes did not show any significant performance reduction. They retained 98.9, 97.5, and 95.2% of activity measured for the freshly prepared electrode after 10, 20, and 30 days, respectively. This level of reproducibility and stability could be good enough to apply to the real samples.

3.6. Effect of interfering species on the DA determination

To apply to an in vivo system, interference from various compounds has been investigated by DPV using GC/NC-C electrodes. DA concentration was fixed at 0.40 μM. Although the interfering species were present at a 100 or 1000-fold higher concentration than DA, any significant interference was not observed. Almost 100% DA was recovered (Table 1). However, it was not possible to eliminate the interference from serotonin and (nor)epinephrine because of their structural similarity with DA.

Table 2
Recovery of dopamine in a spiked human urine samples determined by DPV

Number	[DA] added (μM)	[DA] found (μM)	Recovery % (±s.d.%)
1	4.00	3.90	97.5
2	4.00	3.92	98.0
3	4.00	3.91	97.8
4	4.00	3.89	97.3
Mean	4.00	3.90	97.7 (±3.3)

The recovery of DA in a spiked human urine sample was measured at the same electrode by DPV. The sample was 100-fold diluted. The results are listed in Table 2. For the four independent measurements, the recovery was 97.7%. This indicates that GC/NC-C electrodes have high selectivity for DA and can be used in a biological matrix where the large amount of ascorbic acid and uric acid are present.

In a review by Selvaraju et al. [46], the recovery of DA in real sample was 100.8% at polymer film of phenosafranine formed by electropolymerization.

4. Conclusions

In this work, we have shown that polymeric film of negatively charged Ni(II) macrocyclic compound formed on an electrode surface can act both as an electrocatalyst for the DA oxidation and as a discriminating layer for DA against AA and other interfering species. For this purpose, we synthesized 1,5,8,12-(benzoic acid)tetraaza-2,4,9,11-tetramethyl-cyclotetradecinatonickel(II) compound in which –COOH group dissociates to $–COO^-$ and thus imparts negative charge to the molecule at physiological pH. AA is effectively excluded by the polymeric layer based on the electrostatic repulsion. This can make it simpler to construct a selective electrode for DA. Thus prepared GC/NC-C electrode gave a linear response over the concentration range of 0.1 μM and 10 μM determined by DPV. GC/NC-C electrodes have also been proven to be stable and give reproducible results. Average 97.7% DA was recovered for the 100-fold diluted human urine samples. Diffusion coefficient of DA and the rate constant for DA oxidation were determined.

Acknowledgement

This work was supported by Korea Research Foundation (Grant No. KRF-2006-005-C00009).

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