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RESEARCH PAPER

Selective Determination of Dopamine with an Amperometric Biosensor Using Electrochemically Pretreated and Activated Carbon/Tyrosinase/Nafion®-Modified Glassy Carbon Electrode

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Abstract Dopamine, the most important neurotransmitter in the human brain, controls various functions. Dopamine deficiency causes fatal neurological disorders such as Parkinson's disease. Even though various types of electrochemical sensors have been studied to measure dopamine levels, they often have poor selectivity for dopamine due to co-existence of interfering substances (*e.g.* ascorbic acid). Herein, we aimed to develop a highly sensitive dopamine detection method in the co-existence of ascorbic acid, a major interfering substance in real sample by designing an electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE as an amperometric dopamine biosensor. To maximize the biosensor performance, pH, volume of Nafion®, and scan rate were optimized. This electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE could detect as low as 50 μM of dopamine with a wide linear range (50 ~ 1,000 μM) within a few seconds. In addition, it had a sensitivity of 103 mAM/cm^2, which was higher than all previously reported tyrosinase-based dopamine biosensors. In addition, interference effect caused by 4 mM of ascorbic acid was negligible in the co-existence of 1 mM of dopamine. Consequently, this electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE might be applicable as amperometric biosensor for selective detection of dopamine in real samples with interfering substances.

Keywords: dopamine, selectivity, biosensors, tyrosinase

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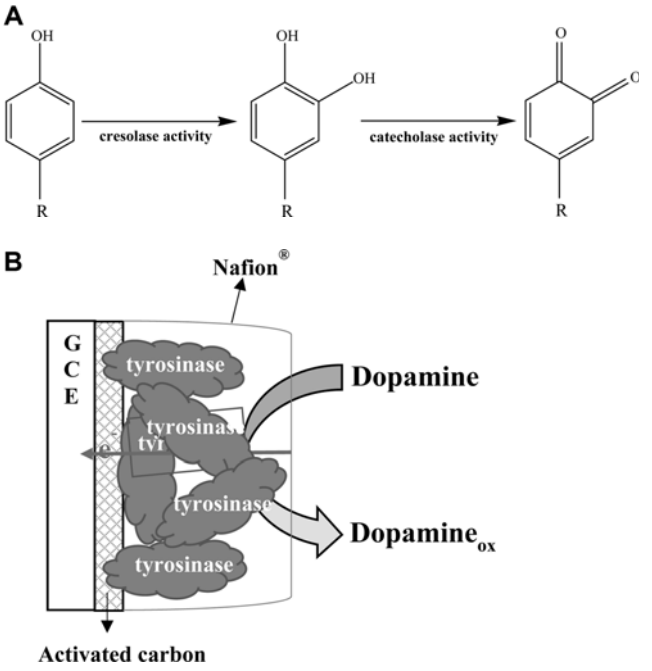
1. Introduction

Tyrosinase (E.C. 1.14.18.1), a copper-containing oxidoreductase, is widely distributed in nature. It is a bifunctional enzyme (hydroxylation of monophenols to *o*-diphenols by its cresolase activity and sequentially oxidation of *o*-diphenols to *o*-quinones by its catecholase activity) as shown in Scheme 1 [1,2]. Tyrosinase has broad spectrum of substrate, including various phenolic compounds. Therefore, tyrosinase has been widely used for determining phenolic compounds in food [3], environment [4], and clinical diagnosis [2].

Dopamine, a substrate for tyrosinase, is the most important neurotransmitter in the human brain. It controls various functions such as locomotor activity, cognition, emotion, positive reinforcement, food intake, and endocrine regulation [5]. Dopamine imbalance is often fatal for humans. For example, dopamine deficiency in human brain can cause Parkinson's disease, a neurological disorder that many elderly people suffer [6]. Hence, it is very important to develop a method for measuring dopamine levels for the purpose of diagnosing neurological disorders such as Parkinson's disease. Various types of electrochemical



Scheme 1. (A) Tyrosinase is a biofunctional enzyme. Its cresolase activity catalyzes *o*-hydroxylation of monophenols to diphenols. Sequentially, its catecholase activity oxidizes diphenols to quinones. (B) Illustration of sensing mechanism of the electrochemically pretreated and activated carbon/Nafion®-modified GCE.

sensors have been studied and commonly used to directly measure dopamine levels. Even if one electrochemical sensor in small-dimension is highly accurate and easy to operate with fast response, it is often limited by poor selectivity for dopamine due to the co-existence of many interfering compounds contained in real samples.

Among various interfering compounds, ascorbic acid is particularly crucial because both ascorbic acid and dopamine might be simultaneously oxidized at a similar potential range, thereby overlapping the response [7]. Accordingly, tyrosinase-based biosensor might be an attractive strategy to achieve highly selective dopamine measurement because tyrosinase is only specific for dopamine whereas ascorbic acid is not a substrate of tyrosinase [8].

Recently, the combination of carbon materials and biomolecules has been researched in the fields of bioelectronics, especially as biosensors and biofuel cells [9]. Among several carbon materials including activated carbon, carbon nanotube, and graphene, we are interested in activated carbon because it is inexpensive and chemically inert. In addition, it offers large specific surface area which may enhance dopamine response [10,11]. In addition, previous studies have reported that electrochemical pretreatment can improve the electrochemical properties of carbon materials (*e.g.*, a better electron transfer between soluble analyte and carbon-based electrode) [12,13]. For integration of carbon material and biomolecules, Nafion®, a negatively charged perfluorinated sulfonate polymer, has been used in biosensor fabrication because it has good electrical conductivity [14,15]. In addition, it can block anionic ascorbic acid to access the electrode [12].

Herein, we aimed to develop a biosensor for highly sensitive dopamine detection in the presence of ascorbic acid by using tyrosinase/activated carbon/Nafion®-modified glassy carbon electrode as amperometric biosensor.

2. Materials and Methods

2.1. Materials

Activated carbon and platinum wire were purchased from Hankook Bay Chemical Co. Ltd., (Seoul, Korea) and Dongsun Science Co. Ltd., (Ansan, Korea), respectively. Ag/AgCl and glassy carbon electrode (GCE) were obtained from WanA Tech. (Seoul, Korea). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the highest grade available and used without further purification.

2.2. Preparation of activated carbon/tyrosinase/Nafion®-modified GCE

At first, a bare GCE was polished with aluminum slurry and ultrasonically cleaned with distilled water to remove adsorbed impurities on the GCE. Activated carbon (10 mg) was dispersed in 1 mL of dimethylformamide and sonicated for 30 min to obtain stably dispersed and activated carbon. To make larger specific surface area that might enhance dopamine response, 8 μL of activated carbon (10 mg/mL) was dropped into bare GCE and dried at ambient temperature. After adsorbing activated carbon on GCE, 500 unit of tyrosinase was used to cover the GCE and allowed to dry at ambient temperature. To block anionic ascorbic acid to access the electrode, 4 μL of Nafion® was coated with the activated carbon/tyrosinase-modified GCE. The prepared activated carbon/tyrosinase/Nafion®-modified GCE was then electrochemically pretreated at 2.0 V for 300 sec in phosphate buffer (50 mM, pH 7.0) to improve its electrochemical properties [12,13]. The electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE was used in further experiment as working electrode and stored in dry condition at 4°C when not in use. In order to confirm that the fabrication was reproducible, we checked oxidation peak current of dopamine (1 mM in 50 mM phosphate buffer, pH 7.0) using the biosensor as working electrode.

2.3. Electrochemical measurement

Cyclic voltammetries and amperometric measurements were

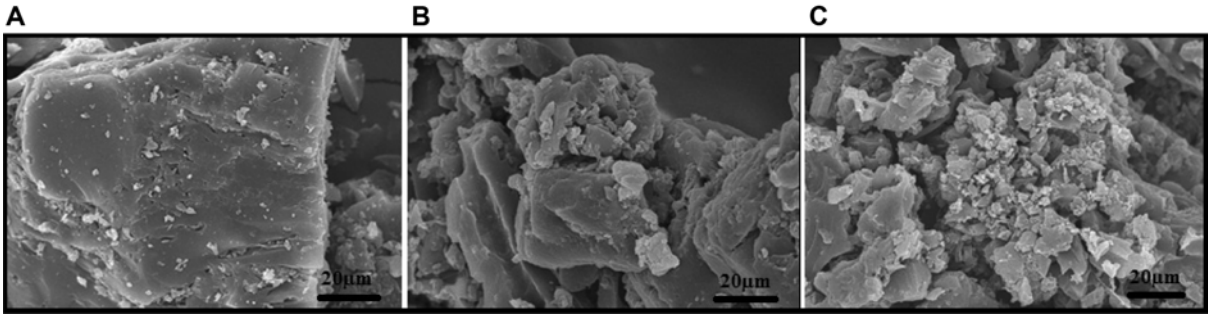


Fig. 1. SEM image of activate carbon (A) and tyrosinase/activated carbon/Nafion composite before (B) and after (C) electrochemical pretreatment.

carried out using an AUTOLAB potentiostat (PGSTAT302N, Metrohm, Netherlands) with commercial software NOVA [2]. The electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE, Ag/AgCl electrode, and coiled Pt wire were used as working electrode, reference electrode, and counter electrode, respectively. All measurements were carried out at 25°C in phosphate buffer solution with different pH.

2.4. Morphological characterization

The surface morphologies of activated carbon and activated carbon/tyrosinase/Nafion® before and after electrochemical pretreatment were analyzed by scanning electron microscope (SEM, Hitachi-S4700, Tokyo, Japan) at 10 kV. A specimen coated with activated carbon/tyrosinase/Nafion® was prepared on separate GCE. The upper portion was analyzed after cutting and fixing with an adhesive tape holder [16].

3. Results and Discussion

3.1. Surface morphology

To observe the changes in surface morphology of tyrosinase/activated carbon/Nafion® before and after the electrochemical pretreatment, the surface of the working electrode was examined by SEM. SEM images revealed that the surface of activated carbon after the electrochemical pretreatment was rougher compared to that before the pretreatment, resulting in larger specific surface. Surface roughness-driven large surface area might enhance the performance of the biosensor. It has been reported that surface roughness is good for reducing the interference from chemicals that are usually present in biological samples such as ascorbic acid [17].

3.2. Electrochemical oxidation of dopamine

For amperometric detection of tyrosinase-driven dopamine oxidation, cyclic voltammogram of dopamine was performed. Results are shown in Fig. 2. Neither the bare GCE (x in Fig. 2) nor the tyrosinase-adsorbed GCE (■ in Fig. 2) exhibited definite oxidation or reduction peak (x in Fig. 2). However, the activated carbon/tyrosinase/Nafion®-modified GCE (● in Fig. 2) oxidized dopamine at 332 mV and then reversibly reduced it at 260 mV with peak separation ΔE_p of 72 mV. Such small ΔE_p value indicated that the electron abstracted from dopamine by tyrosinase was rapidly transferred to the electrode surface [12]. Some reports have shown activity of carbon materials toward dopamine detection [18]. Therefore, we investigated whether the activated carbon/Nafion®-modified GCE could affect dopamine response. As a result, without tyrosinase, the activated carbon/Nafion®-modified GCE (▼ in Fig. 2) did not have electrochemical response to 1 mM of dopamine. The electrochemical pretreatment enhanced the current response with the highest oxidation and reduction peaks (▲ in Fig. 2). Accordingly, the electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE was

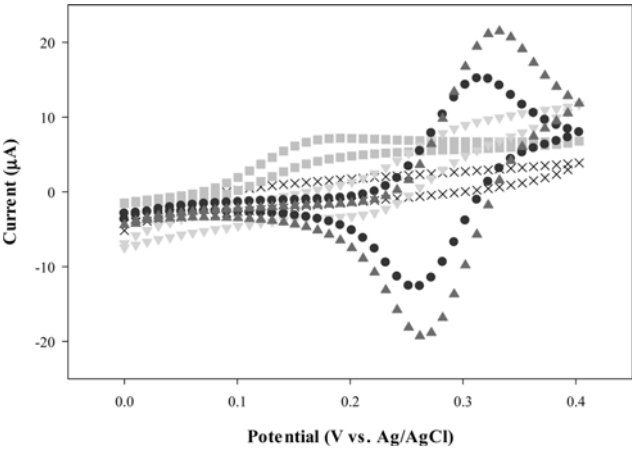


Fig. 2. Cyclic voltammogram of dopamine (1 mM in 50 mM phosphate buffer, pH 7.0) with bare GCE (x), tyrosinase adsorbed GCE (■), activated carbon/Nafion®-modified GCE (▼), and tyrosinase/activated carbon/Nafion®-modified GCE before (●) and after (▲) electrochemical pretreatment. Coiled Pt wire and Ag/AgCl electrode were used as counter electrode and reference electrode, respectively. Scan rate: 10 mM/sec.

used as the working electrode for further experiments. For selective determination of dopamine, cyclic voltammogram of ascorbic acid was also investigated using the electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE as working electrode. As a result, ascorbic acid was not oxidized at 330 mV, while dopamine was oxidized at 330 mV (Supplementary Fig. S1), leading to selective determination of dopamine without interference of ascorbic acid. This result was consistent with result of previous reports [2].

3.3. Effect of pH and amount of Nafion® on current response

Herein, the effect of pH and Nafion® on anodic current response was studied to optimize the performance of the amperometric dopamine sensor. The effect of pH on the anodic current response was investigated using pH ranging from 4 to 8. The optimum pH was determined to be at pH 7 as shown in Fig. 3A. Given that the optimum pH of tyrosinase was also found to be at pH 7, the optimum pH of the biosensor was absolutely dependent on tyrosinase activity. Similar to pH, temperature might influence the performance of the biosensor. Considering temperature that a real sample will be tested, dopamine detection was performed at 25°C which was not far from the optimum temperature of tyrosinase [2].

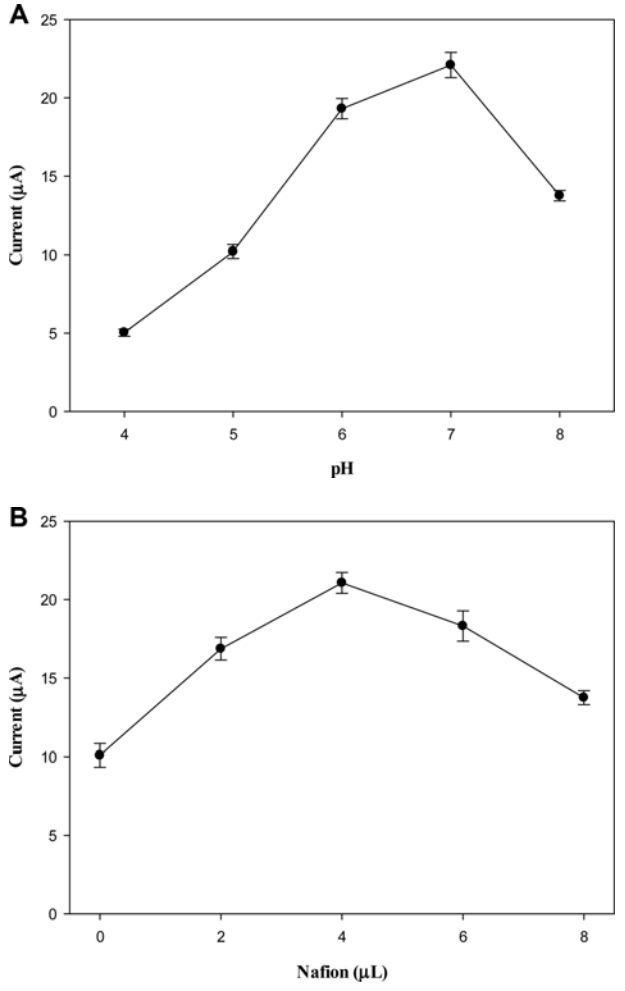


Fig. 3. Effect of pH (A) and Nafion volume (B) on current response of electrochemically pretreated and tyrosinase/activated carbon/Nafion-mediated GCE.

Nafion®, a perfluorinated sulfonate polymer, is the most widely used proton-exchanger with adsorption capability due to its large surface area [19]. The amount of Nafion® used in the biosensor fabrication might change its electrical properties, thereby affecting the anodic current response. Accordingly, the relationship between the anodic peak current and the amount of Nafion® was examined in this study. Results are shown in Fig. 3B. The anodic current response was gradually increased with the increasing amount of Nafion®. However, it began to decrease when the volume of Nafion® exceeded 4 μL. The thick Nafion® coating might have retard electron transfer, resulting in higher electrical resistance and lower current response.

3.4. Amperometric detection of dopamine

Cyclic voltammogram was performed using the electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE as working electrode under different scan rates. As shown in Fig. 4A, both anodic and cathodic peak currents were linearly dependent on the scan rate, a characteristic of surface control process.

In addition, cyclic voltammogram was performed at various concentrations of dopamine at a scan rate of 10 mV/sec. Given that anodic peak currents were increased at higher dopamine concentrations (Fig. 4B), the electron transfer from dopamine oxidation might have been controlled by diffusion [20]. The anodic peak current had a linear relationship with dopamine concentration within a range from 50 μM to 1.0 mM. The correlation coefficient was 0.991 (Fig. 4B). Regression equation indicated that the detection limit and the sensitivity of activated carbon/tyrosinase/Nafion®-modified GCE were 50 μM and 103 mAM/cm^2, respectively. The performances of various types of tyrosinase-based amperometric biosensors for dopamine detection were compared. Results are shown in Fig. 5 and Table 1. Similar to our activated carbon/ tyrosinase/Nafion®-modified GCE, MWNT-Nafion-tyrosinase-modified GCE fabricated by Tsai *et al*. has been used as an amperometric biosensor. Although the MWNT-Nafion-tyrosinase-modified GCE exhibited the lowest detection limit for dopamine (as low as 0.52 μM), its sensitivity was 8.6-fold lower than our activated carbon/ tyrosinase/Nafion®-modified GCE. In addition, it showed very narrow linear range (5 ~ 23 μM) [21]. Tembe *et al.* have constructed dopamine biosensor

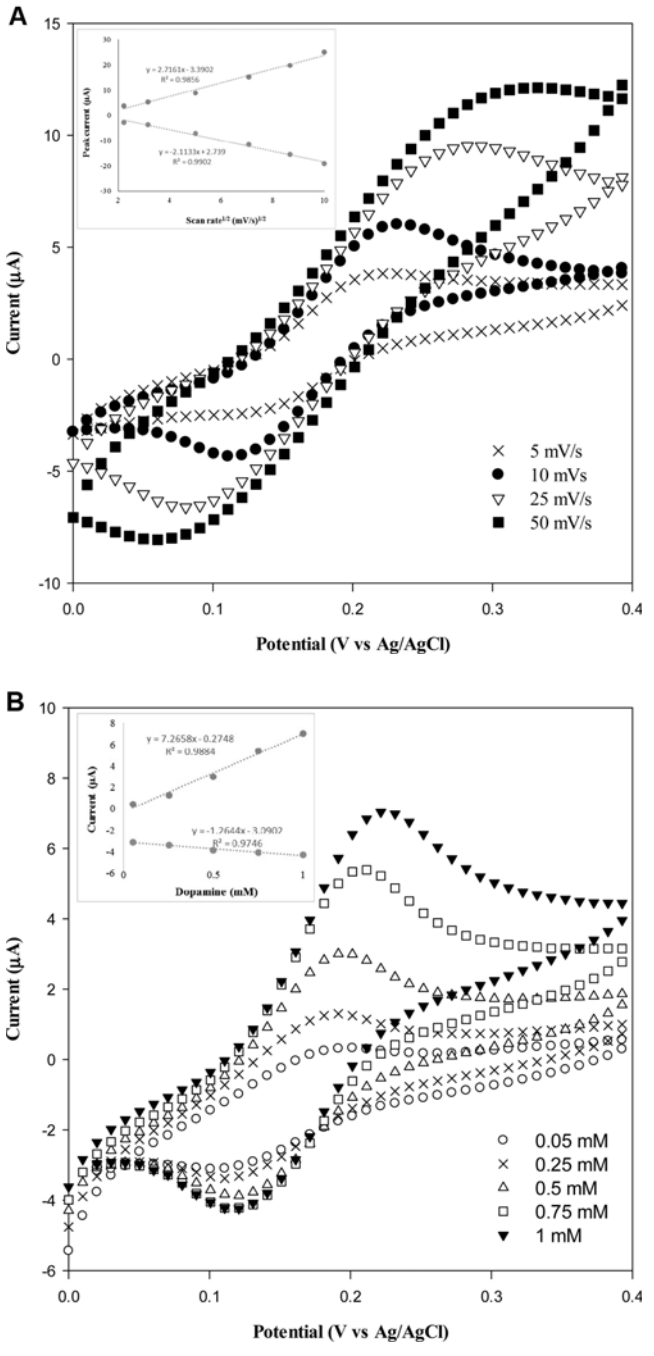


Fig. 4. Amperometric response of tyrosinase/activated carbon/Nafion-modified GCE to scan rate (A) and dopamine concentration (B).

using plant tyrosinase from *Amorphophallus companulatus* by immobilizing them onto egg shell membrane with glutaraldehyde as cross-linking reagent. Although the biosensor was stable under wide pH range (pH 5.0 ~ 6.5) at different temperatures (20 ~ 45°C), its sensitivity and detection limit were only 10.6 mAM/cm^2 and 25 μM, respectively [22]. Zhou *et al.* have developed a novel method for immobilizing tyrosinase onto a boron-doped

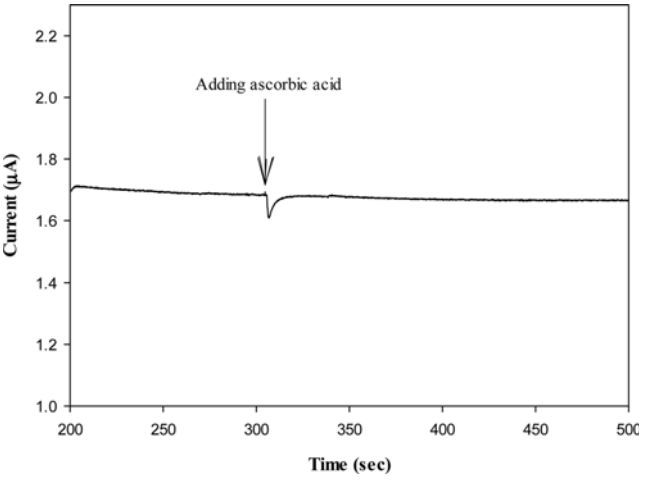


Fig. 5. Interference study using ascorbic acid (4 mM) to monitor dopamine (1 mM) detection using the biosensor.

diamond electrode and then designed an amperometric biosensor. The biosensor was applicable for selective detection of dopamine in the presence of ascorbic acid. However, its sensitivity (68.6 mAM/cm^2) and detection limit (1.4 μM) were under linear range of 5 ~ 120 μM [23]. Pérez *et al*. have developed an amperometric biosensor based on tyrosinase entrapped in polyacrylamide microgels for detecting phenolic compounds. Although the biosensor was active for various phenolic compounds, its sensitivity (1.66 mAM/cm^2) and detection limit (1.66 μM) for dopamine were not excellent [24]. Compared to these tyrosinase-based dopamine biosensors reported previously, the electrochemically pretreated and activated carbon/ tyrosinase/ Nafion®-modified GCE exhibited the highest sensitivity (103 mAM/cm^2, see Table 1) developed in this study had a wide linear range (50 ~ 1,000 μM), although its detection limit (50 μM) was not excellent. Additionally, our activated carbon/tyrosinase/Nafion®-modified GCE showed good reproducibility. The relative standard deviation estimated with five measurements using 500 μM of dopamine was about 1%.

3.5. Interference study and stability

The most common substance that will have interference with dopamine detection is ascorbic acid (50 ~-70 μM at physiological level [16]). It is electroactive in the blood. Therefore, we investigated the interference effect of ascorbic acid on dopamine detection in this study. As a result, the interference effect of ascorbic acid (4 mM) was negligible in the co-existence of 1 mM of dopamine as shown in Fig. 5. Consequently, the electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE is applicable for highly sensitive dopamine detection as an amperometric biosensor.

Table 1. Performances of different tyrosinase-based amperometric dopamine sensors

Type	Linear range (μM)	Detection limit (μM)	Sensitivity (mAM/cm^2)	Stability	References
MWNT-Nafion-tyrosinase nanocomposite	5 ~ 23	0.52	12		[21]
Tyrosinase-immobilized eggshell membrane	50 ~ 250	25	10.6	Shelf-life > 6 month at 4°C	[22]
Tyrosinase immobilized on boron-doped diamond electrode	5 ~ 120	1.3	68.6	After 1 month, 90% of initial activity at 4°C	[23]
Tyrosinase entrapped in polyacrylamide gel	120 ~ 360	39.6	1.66	Useful life time was 27 days	[24]
Tyrosinase immobilized onto ferrocene encapsulated Pd-linked ormosil	50 ~ 1,000	50		After 3 month, 95% of initial activity at 4°C	[25]
Activated carbon/tyrosinase/ Nafion®-modified GCE	50 ~ 1,000	50	103	80. 9% of initial activity after 15 days	This study

Stability is a basic requirement of biosensors. Therefore, the stability of the electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE was investigated in this study. When the anodic current response of 1 mM of dopamine solution was measured, the electrochemically pretreated and activated carbon/ tyrosinase/Nafion®-modified GCE retained 80. 9% of its initial activity after 15 days.

4. Conclusion

Herein, an electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE was developed as an amperometric biosensor for selective detection of dopamine. Compared to previously reported tyrosine-based dopamine biosensors, this electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE showed the highest sensitivity and a wide linear range for the detection of dopamine. This might be due to its high enzyme loading (500 unit) with a large surface area resulting from the use of activated carbon on GCE. In addition, the interference effect of ascorbic acid, a major interfering substance for dopamine detection in real samples, was found to be negligible. Consequently, our electrochemically pretreated and activated carbon/tyrosinase/Nafion®-modified GCE might be applicable as an amperometric biosensor for selective detection of dopamine in real samples.

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