Talanta 80 (2009) 1007–1011

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Amperometric detection of dopamine based on tyrosinase–SWNTs–Ppy composite electrode

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ARTICLE INFO

Article history:
Received 31 July 2009
Accepted 24 August 2009
Available online 29 August 2009

Keywords:
3-Dimensional composite electrode
Tyrosinase
Chemically polymerized polypyrrole
Single wall carbon nanotube
Dopamine sensor
Ascorbic acid

ABSTRACT

A novel 3-dimensional single wall carbon nanotubes (SWNTs)–polypyrrole (Ppy) composite was prepared as an electrode by chemically polymerizing polypyrrole onto SWNTs using a $LiClO_4$ oxidant. This composite electrode was characterized by scanning electron microscopy (SEM) and cyclic voltammetry with 1 mM $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$. The SWNTs were thickly coated with chemically polymerized polypyrrole and the composite had many surface pores and crevices which could enhance mass transfer. The SWNTs–Ppy composite electrode showed a large specific surface area (30 m^2/g) and a good reproducible current response, at about 100 times the peak current of a glassy carbon electrode (GCE). The diffusion coefficient was calculated to be 4.81×10^{-6} cm^2/s. As a biosensor application, tyrosinase was immobilized on the functionalized SWNTs and tyrosinase–SWNTs–Ppy composite was prepared in the same manner. This tyrosinase–SWNT–Ppy composite electrode was used for amperometric detection of dopamine in the presence of ascorbic acid and showed high sensitivity (467 mA/M cm^2) and lower detection limit (5 μM) compared to previous reports.

1. Introduction

Recently, the combination of nanoscale materials and biomolecules has been of great interest in the field of biotechnology, especially in bioelectronics [1–4]. Among several currently available nanoscale materials such as carbon nanotubes (CNTs), nanoporous silica, and gold nanodots, CNTs have drawn the highest interest since their discovery by Iijima in 1991 because of their extraordinary physical properties [5]. CNTs have been used as nanoscale conductors, catalyst carriers, and electrode materials [6–8]. In electrochemistry, CNTs have been widely used as a modified material in the preparation of electrodes because CNTs have advantages such as a large surface area, a good electrical conductivity, and easy surface functionalization that can enhance the performance of the electrodes. In previous studies, carbon nanotube modified electrodes (CNT-MEs) have been prepared by (1) filling a bare electrode with a mixture of the CNTs and mineral oil, (2) embedding or mixing carbon nanotubes into a graphite or a glassy carbon electrode and (3) coating directly onto the electrode surface [9–11]. However, the previous CNT-MEs have made a limited use of the advantages of CNTs, the large specific surface area and the good electrical conductivity. In the case of the CNT-MEs constructed by embedding or coating of the substrate electrode, which have a small, specific surface area, the large surface area of CNTs could not be used to the full effect. Also, chemical functionalization, such as an acidic treatment for the immobilization of proteins, can affect defeat sites, which are the broken parts of the bond network, and consequently affect the electrical properties of the CNT-MEs. In this paper, a single walled carbon nanotubes (SWNTs)–polypyrrole (Ppy) composite electrode was prepared to make the best use of the large surface area and the good electrical conductivity of CNTs. Although substrate such as glassy carbon electrodes or metal which can play a role as a current collector in the composite electrodes have been required in previous methods, a SWNTs–Ppy composite can be applied to the electrode itself without any substrate. For the electrode preparation, the conducting polymer polypyrrole was electrically polymerized as a thin film on the surface of the substrate electrode in general. However in this research, polypyrrole was chemically polymerized using $LiClO_4$ as an oxidant that played the role of a cross-linker for connecting the SWNTs and forming the thick 3-dimensional SWNT–Ppy composite, not an electrically deposited thin film.

Tyrosinase (E.C.1.14.18.1) is a multifunctional copper containing oxidoreductase widely distributed in nature. Tyrosinase has two catalytic activities in the presence of oxygen; hydroxylation of monophenols by a cresolase activity and oxidation of diphenols to quinine by a catecholase activity. Tyrosinase has a broad substrate specificity for various phenolic compounds such as phenol, catechol, cresol, dopamine and ephinephirine, which can be

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doi:10.1016/j.talanta.2009.08.032

applied for the determination of phenolic compounds in food, environment and clinical diagnosis [12–14]. Dopamine, which can be a substrate of tyrosinase, is one of the most important neurotransmitter which affects the brain function. Deficiency of dopamine in the brain tissue causes Parkinson's disease, a neurological disorder which many elderly people have suffered from. Development of a tool for dopamine measurement in tissues can make a great contribution to diagnose Parkinson's disease. An electrochemical method has been widely used as a direct measurement for dopamine and has several advantages such as a small dimension, an easy operation, a fast response, and a high accuracy. However, the major problem of the electrochemical detection of dopamine in a real tissue is the co-existence of many interfering compounds. Among the interfering compounds, ascorbic acid is particularly important because both ascorbic acid and dopamine can be oxidized at similar potentials and these results in an overlap of the voltammetric response [15–17]. Therefore it is important to develop a method for the selective determination of dopamine with a high sensitivity and a low detection limit. For the biosensor application, tyrosinase was immobilized on the functionalized SWNTs and the prepared tyrosinase–SWNTs–Ppy composite was tested for determination of dopamine in the presence of ascorbic acid.

The goals of this study were (1) the characterization and the calibration of a 3-dimensional SWNTs–Ppy composite as an electrode and (2) the applications of the tyrosinase-immobilized SWNTs–Ppy composite to detect dopamine selectively in the presence of ascorbic acid.

2. Material and methods

2.1. Materials

SWNTs were purchased from IlJin NanoTech (Seoul, Korea). Tyrosinase (E.C. 1.14.18.1), monomer pyrrole, $LiClO_4$, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ and all other reagents were obtained from Sigma–Aldrich at the highest grade available and were used without a further purification.

2.2. Apparatus

Electrochemical measurements were performed with an AUTOLAB potentiostat at ambient temperature with a conventional 3-electrode system: working electrode, the SWNT–Ppy composite; counter electrode, coiled Pt wire; reference electrode, Ag/AgCl. Scanning electron microscope images were photographed out using a JEOL JSM-5210 to characterize the SWNT–Ppy composite electrode.

2.3. Tyrosinase immobilization

Before immobilization, 1 mg SWNTs were functionalized by 1.5 ml 1-pyrenebutyric acid (0.1 M in dimethylformaldehyde) for non-covalently introducing carboxyl groups on the surface, shaking at 200 rpm for 1 h. After washing the functionalized SWNTs with methanol and distilled water, respectively, tyrosinase was covalently attached onto the functionalized SWNTs by 100 mM EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide), in 50 mM phosphate buffer (pH 6.5),with an activation time of 30 min [18].

2.4. Preparation of 3D SWNTs–Ppy composite electrodes

The SWNTs–Ppy composite was prepared by chemical polymerization of monomer pyrrole by mixing the monomer pyrrole and 0.1 M $LiClO_4$ in a phosphate buffer (50 mM, pH 6.5) in a 2:1 volume ratio. SWNTs with/without tyrosinase were added rapidly to the mixture, which was then cast in an electrode holder and hardened overnight at the ambient temperature.

3. Results and discussion

3.1. Characterization of the composite electrode

3.1.1. SEM of the SWNTs–Ppy composite

Fig. 1 shows the SEM images of the SWNTs–Ppy composite electrode. As shown in Fig. 1(a), the SWNT was thickly coated by chemically polymerized polypyrrole. The SWNTs–Ppy composite had many pores on the surface which could prevent mass

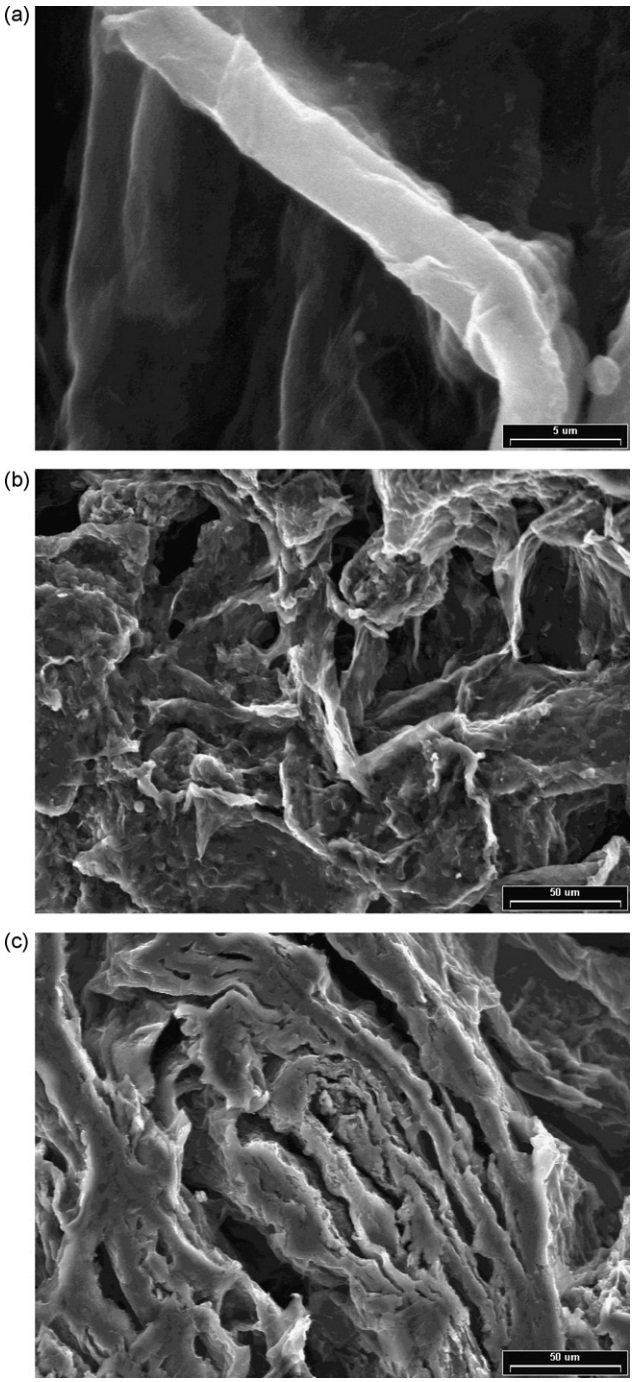

Fig. 1. SEM image of SWNT–Ppy composite (a) Ppy coated SWNT, (b) surface of SWNT–Ppy composite and (c) cross-section image of SWNT–Ppy composite.

transfer limitation as shown in Fig. 1(b). Fig. 1(c) reveals that the SWNTs–Ppy composite electrode had several layers and many crevices in the cross-section, which could also reduce mass transfer limitation. The composite surface was non-uniform and had some partially excessive polymerized surface. To prevent the excessive polymerization, the ratio of the oxidant $LiClO_4$ and monomer pyrrole was optimized. As a result, the excessive polymerization was minimized by using a 2:1 ratio mixture of the reactants (v/v, monomer pyrrole to 0.1 M $LiClO_4$) and 10 μl of the mixture was enough to produce 1 mg SWNT.

3.1.2. Cyclic voltammogram of the SWNTs–Ppy composite

Fig. 2 shows the cyclic voltammograms for a glassy carbon electrode(GCE) and the SWNTs–Ppy composite in 0.1 M $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ solution in 10% H_2SO_4 (v/v%) with a scanning rate of 20 mV/s. From Fig. 2, the current response of the SWNTs–Ppy composite electrode was about 100 times larger than that of the GCE under the same testing conditions. As shown in Table 1, compared to other papers previously reported, the current response of the SWNTs–Ppy composite electrode was higher although the system was in low $K_3Fe(CN)_6$ concentration and with a slow scan rate. The difference between the GCE and SWNTs–Ppy composite also indicated that the SWNTs–Ppy composite had a larger capacitance than the GCE. This enhanced that the current response could be attributed to the much larger specific surface area of the composite (30 m^2/g, from BET result, data not shown) effectively increasing the electron transfer and consequently increasing the current response. The large specific surface area of the SWNTs–Ppy composite provides a high probability of contacts between electrons and the electrode surface and causes the increase in the current response, consequently. The larger current response of SWNTs–Ppy composite electrode was also the result of the increased numbers of defeat sites on the electrode surface.

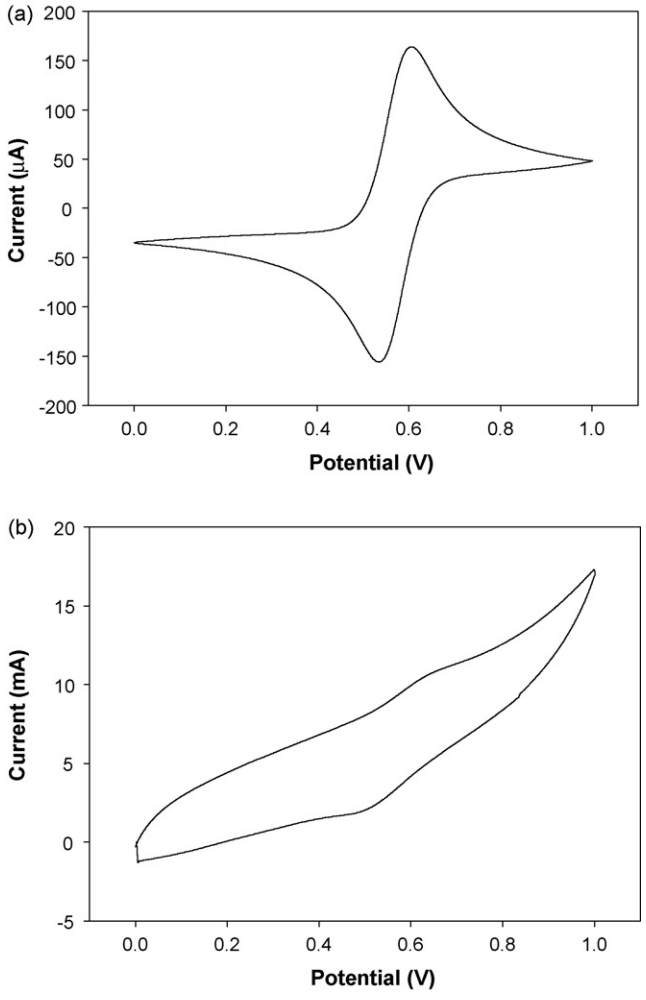

Fig. 2. Cyclic voltammogram of (a) glassy carbon electrode and (b) SWNT–Ppy composite with 0.1 M $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ solution in 10% H_2SO_4 (v/v%) with a scanning rate 20 mV/s.

Table 1
Current responses for the different carbon based composite.

Electrode	I_p (A)	Ref.
Carbon–castor oil polyurethane composite	5.0×10^{-5} [a]	[23]
Carbon nanotube–epoxy composite	1.6×10^{-3} [b]	[24]
Graphite–silicon rubber composite	7.9×10^{-6} [c]	[25]
Graphite–poly(methylmethacrylate) composite	2.2×10^{-5} [d]	[26]
SWNT–Ppy composite	1.0×10^{-2} [e]	This study

[a] 5 mM $K_3Fe(CN)_6$, scan rate 100 mV/s.
[b] 10 mM $K_3Fe(CN)_6$, scan rate 100 mV/s.
[c] 5 mM $K_3Fe(CN)_6$, scan rate 50 mV/s.
[d] 5 mM $K_3Fe(CN)_6$, scan rate 200 mV/s.
[e] 1 mM $K_3Fe(CN)_6$, scan rate 20 mV/s.

The SWNTs–Ppy composite electrode, which showed the large current response and had the high effective surface area, may contribute to develop a biosensor system with a high sensitivity. Recently, amperometric enzyme-based biosensors have been developed for chemical and biomedical analysis [19–21]. However, the sensitivity of these biosensors is low without a mediator because the active sites of the enzymes are deeply buried and the electron transfer efficiency is not good in the absence of mediator. This SWNTs–Ppy composite with a high current response can be a potential candidate for the development of mediator-less enzyme-based biosensors. This SWNTs–Ppy composite can also provide a large surface, which is for a large amount of enzyme loading and for frequent contacts with electrons generated from the enzyme catalysis, and additionally enhance the current response.

In this study, a 3-dimensional SWNTs–Ppy composite electrode was prepared without a substrate electrode as a current collector. It was unknown whether the prepared 3-dimensional composite electrode could exhibit accuracy without a current collector, so the accuracy of the prepared composite as an electrode was also examined by the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ pair solution. As shown in Fig. 2, in the case of the GCE, the oxidation peak voltage and the reduction peak voltage were 603 and 535 mV, respectively. In the case of the SWNTs–Ppy composite, the oxidation peak voltage and the reduction peak voltage were 600 and 469 mV, respectively. The difference between the oxidation peak voltage and the reduction peak voltage was small, because the system was reversible. The high regression coefficient of the relationship between the oxidation peak current and the square root of the scanning rate also indicated the accuracy of the prepared SWNTs–Ppy composite electrode. Fig. 3 indicates that the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ system with the prepared SWNTs–Ppy composite electrode was a reversible system. In fact, the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ was a reversible oxidation–reduction pair. Consequently, the prepared SWNTs–Ppy composite electrode-incorporated system appeared reliable.

3.1.3. Diffusion coefficient

In the composite electrode, the accessibility of the reactant to the inner part of the electrode has been one of the most important factors. Although the SWNTs–Ppy composite electrode had enough pores and crevices detectable in SEM images to decrease mass transfer limitation, the question remains whether the reactant can actually access the SWNT through the thickly polymerized

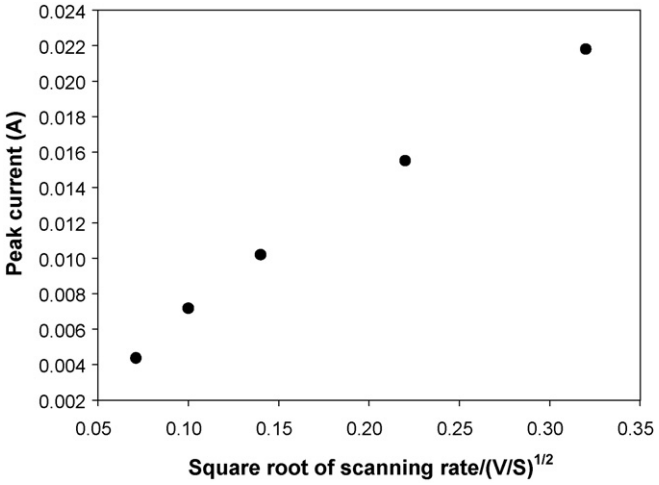

Fig. 3. Relationship between the square root of scanning rates and the peak currents of the SWNT–Ppy composite in the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ solution.

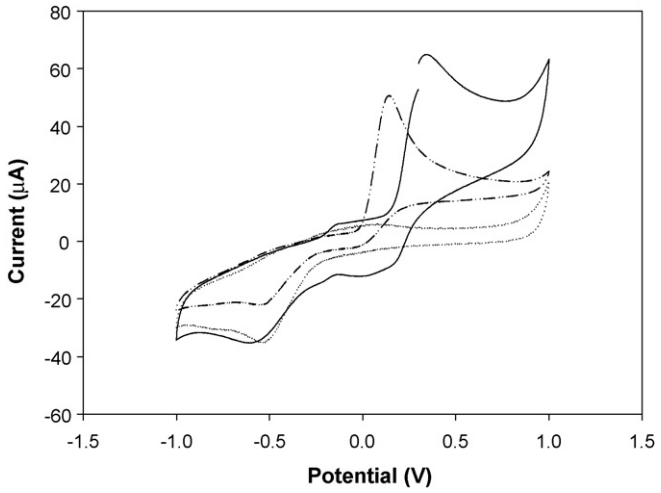

Fig. 4. Cyclic voltammogram of 1 mM dopamine (—), 1 mM ascorbic acid (— - - —), and phosphate buffer (- - - -) using 20 unit free tyrosinase and glassy carbon electrode, scanning rate 50 mV/s.

polypyrrole layer. To validate the accessibility of the reactants to the SWNTs–Ppy composite electrode, the diffusion coefficient was determined from cyclic voltammetry at different scanning rates operating with the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ solution. The relationship between the oxidation peak current and the square root of the scanning rate was studied as shown in Fig. 3, which yielded a linear relationship with a high regression coefficient ($R^2 = 0.996$). The slope is proportional to the diffusion coefficient of $Fe(CN)_6$ within the SWNT–Ppy composite and the diffusion coefficient was calculated from the following equation, shown in Table 2 [22].

$$I_p = 2.69 \times 10^5 \times n^{3/2} \times S \times C \times V^{1/2} \times D^{1/2} \quad (1)$$

where I_p, peak current (A); n, charge transfer number ($n = 1$); S, surface area of electrode (cm^2); D, diffusion coefficient (cm^2/s); V, scanning rate (V/s).

Based on the diffusion coefficient, we believe that this 3-dimensional composite electrode will solve the problem of mass transfer limitation in enzyme-based biosensors.

3.2. Application for dopamine sensor in the presence of ascorbic acid

3.2.1. Determination of working potential for dopamine detection

Fig. 4 shows the cyclic voltammograms of the 200 mM phosphate buffer (pH 6.5), 1 mM dopamine and 1 mM ascorbic acid with a glassy carbon electrode and 20 unit free tyrosinase at the scan rate 50 mV/s. In the absence of dopamine, there is no peak in the cyclic voltammogram. In the presence of 1 mM dopamine, the cyclic voltammogram had a definite oxidation peak at 346 mV, which was due to the oxidation of the dopamine by the enzyme catalysis on the electrode surface. As shown in Fig. 4, ascorbic acid is also oxidized at 143 mV. Therefore, the potential of 346 mV was determined as a working potential for the amperometric measurement of dopamine.

Table 2
Diffusion coefficients for the different electrodes.

Electrode	Diffusion coefficient (cm^2/s)	Ref.
SWNT–epoxy composite	6.32×10^{-6} [a]	[24]
Polycarbonate membrane	4×10^{-6} [b]	[27]
SWNT–Ppy composite	4.81×10^{-6} [c]	This study

[a] 10 mM $K_3Fe(CN)_6$.
[b] 1 mM $K_3Fe(CN)_6$.
[c] 1 mM $K_3Fe(CN)_6$.

3.2.2. Amperometric response of the tyrosinase–SWNTs–Ppy composite electrode for dopamine detection in the presence of ascorbic acid

Dopamine was detected by the tyrosinase–SWNTs–Ppy composite electrode in 50 mM phosphate buffer (pH 6.5) at a working potential of 346 mV (vs. Ag/AgCl) under constant stirring. The amperometric responses to the addition of various concentration of dopamine are indicated in Fig. 5. A well-defined oxidation current was observed in proportion to the concentration of dopamine. A major problem of the electrochemical detection of dopamine in real tissues is the co-existence of many interfering compounds. Among the interfering compounds, ascorbic acid is particularly important because both ascorbic acid and dopamine can be oxidized at similar potentials and this results in an overlap of the voltammetric responses. As shown in Fig. 5, only ascorbic acid solution with no dopamine did not show any amperometric response although the ascorbic acid concentration (40 μM) was 8 times higher than the initial concentration of dopamine (5 μM). The selective determination of dopamine in the presence of ascorbic acid is feasible

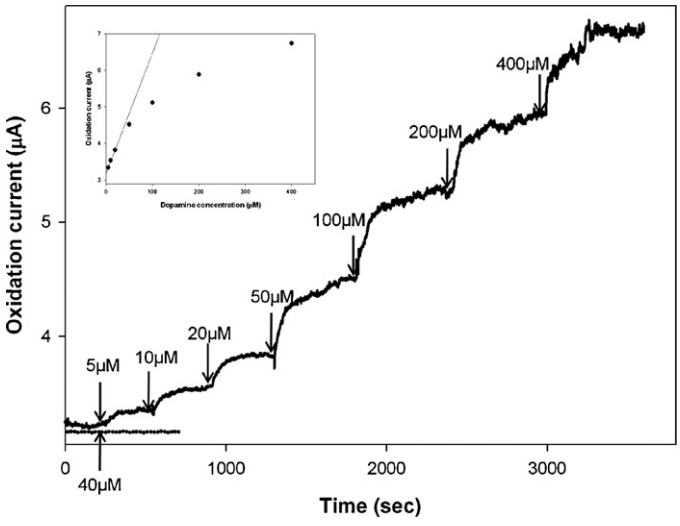

Fig. 5. Amperometric response of the tyrosinase-immobilized carbon nanoparticle polypyrrole composite electrode (- - - - -) ascorbic acid (—) dopamine in an air-saturated blank buffer (50 mM phosphate buffer, pH 6.5) under stirring. Inset: calibration plots of the electrode to dopamine.

Table 3
Performances of the tyrosinase-immobilized dopamine sensors.

Sensitivity (mA/M cm^2)	Linear range (μM)	R^2[a]	Detection limit (μM)	Response time (s)	Reference
1.66	120–360	0.995	39.6	438	[28]
12	5–23	0.998	0.52	<20	[29]
10.6	50–250	0.972	25		[30]
68.6	5–120	0.999		<10	[31]
467	5–50	0.998	5	<5	This study

[a] R represented as correlation coefficients of the linear ranges.

using the tyrosinase–SWNT–Ppy composite electrode. This electrode also shows a rapid response within 5 s when dopamine was added to the system. Inset of Fig. 5 shows the dependence of the steady state current on the concentration of dopamine. Linearity was observed within the range 5–50 μM and the sensitivity corresponding to the linear range for dopamine was 467 mA/M cm^2. The characteristics of the tyrosinase-immobilized electrode for dopamine detection was summarized in Table 3, where the tyrosinase–SWNTs–Ppy composite electrode shows the highest sensitivity and a low detection limit compared with the previously reported tyrosinase-immobilized biosensor for dopamine detection. The tyrosinase–SWNTs–Ppy composite electrode showed a good reproducibility. The relative standard deviation estimated by 5 measurements of 20 μM dopamine at 346 mV (vs. Ag/AgCl) was about 2.5%.

4. Conclusions

The 3-dimensional SWNTs–Ppy composite was prepared with chemically polymerized polypyrrole using $LiClO_4$ as an oxidant without any substrate such as GCE or metal plate. The prepared SWNTs–Ppy composite electrode showed a much larger current response than the GCE, due to the large specific surface area that increased the frequency of contacts between the electrode surface and electrons and enhanced the electron transfer rate. This system has a great potential for applications in the amperometric enzyme-based biosensor for high sensitivity because the amount of enzyme loading could be increased considering the large specific surface area of the SWNTs–Ppy composite leading to a large current response. Considering the diffusion coefficient determined with the $[Fe(CN)_6]^{-3}/[Fe(CN)_6]^{-4}$ solution, mass transfer could not be a problem in the amperometric enzyme-based biosensors. The tyrosinase–SWNTs–Ppy composite was developed for the determination of dopamine. This composite electrode showed a high sensitivity and a low detection limit and could make a contribution to the diagnosis of neurological disorders.

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