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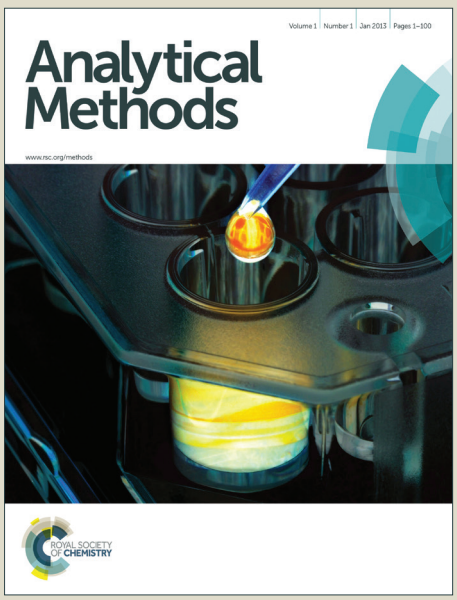

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A sensitive electrochemical method for the determination of 5-hydroxytryptophan in rats' brain tissue based on carbon nanosheets modified electrode

Yahong Chen, Gongke Li*, Yufei Hu*

School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China

* Corresponding author: Gongke Li, Yufei Hu

Tel. : +86-20-84110922

Fax : +86-20-84115107

E-mail : cesgkl@mail.sysu.edu.cn

huyufei@mail.sysu.edu.cn

Abstract

In this paper, a novel electrochemical method was proposed for the determination of 5-hydroxytryptophan (5-HTP) based on carbon nanosheets (CNSs) modified electrode. The CNSs was prepared by one-step flowing electrolysis with a home-made device. The surface morphology and composition of CNSs were characterized by scanning electron microscopy, transmission electron microscopy, X-ray photoelectron spectroscopy and fourier transform infrared. The cyclic voltammetry method was used to study the electrochemical behavior of 5-HTP on CNSs modified electrode. Results suggested that the modified electrode exhibited high electrocatalytic activity and the anodic peak current of 5-HTP was significantly enhanced on the modified electrode. The differential pulse voltammetry method was developed for the determination 5-HTP at the modified electrode. In the optimum experimental conditions, the linear calibration range varied over 5×10^{-8} mol/L to 1×10^{-6} mol/L with detection limit of 3×10^{-8} mol/L. The proposed method was further successfully applied for the determination of 5-HTP in rats' brain tissue sample with the recovery ranged from 90.3 to 98.0%.

Keywords: Electrochemical determination; 5-hydroxytryptophan; carboxyl-riched; carbon nanosheets; rats' brain tissue.

1. Introduction

Serotonin (5-HT) was a monoamine neurotransmitter hypothesized to be involved in several pathological conditions, such as schizophrenia, autism, migraine, and carcinoid syndrome [1, 2]. As the immediate precursor of 5-HT, 5-hydroxytryptophan (5-HTP) has clinical significance for various mental disorders. It has been proved that 5-HTP can easily cross the blood–brain barrier, bypass the rate-limiting step in 5-HT synthesis [3] and then lead to the increase of 5-HT level in brain [4]. The determination of 5-HTP is important for monitoring the level of 5-HT. The past research has also shown that 5-HTP can elevate the levels of some significant hormone in living body, such as serum corticosterone and prolactin [5]. Hence, it is unique important for the sensitive determination of 5-HTP *in vivo/vitro* in biological fluid.

There are several methods for the determination of 5-HTP, such as high performance-liquid chromatography [6, 7], ultraviolet–visible spectrophotometry [8], capillary electrophoresis [9-11]. Among these methods, electrochemical analysis method shows unique advantages such as fast response, low cost, and excellent selectivity, which make it preferable and attractive for the detection of 5-HTP [12-14]. So, it is necessary to develop a new and sensitive electrochemical method for the determination of 5-HTP.

With sizes or features ranging from 1 to 100 nm in one or more dimensions, nanomaterials have been applied in electrochemical detection in recent years [15-18]. Carbon nanomaterials, such as fullerenes [19-21], carbon nanotubes [22-25] and graphene [26-28], have been widely applied to modify electrode surface because of large surface area/volume ratio, good

conductivity and excellent electrocatalytic activity. The hydrophobicity of pristine carbon nanomaterials limits their application due to their strong π-π stacking and vander Waal's interaction [29-31]. The presence of carboxyl groups on carbon nanomaterials can increase their dispersity in aqueous solutions [32-35] and effectively enhance the electrocatalytic activity [36, 37]. Various functionalized methods for carbon nanomaterials have been developed to introduce carboxyl groups [20, 38]. Most of these techniques are based on the use of strong oxidizing agent which may leads to the loss of electronic conductivity by destroying the sp^2-bonded hexagonal configuration [39, 40].

In this work, our study focused on the novel carbon nanosheets (CNSs). They are carboxyl-riched nanomaterials prepared by one-step flowing electrolysis without any other organic reagent.. The well-dispersed CNSs can be deposited on the surface of electrode to form CNSs films. Then a highly sensitive electrochemical method based on the CNSs modified electrode was developed for detection of 5-HTP. The electrochemical properties and electrooxidation activity of 5-HTP on the modified electrode were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The results indicated this modified electrode showed good electrochemical performance to 5-HTP with wide linear range, low detection limit and good stability. The proposed method was further successfully applied for the determination of 5-HTP in rats' brain tissue sample.

2. Experimental

2.1 Reagents

5-HTP was purchased from Sigma-Aldrich Chemical Corporation Industrial (Milwaukee, USA) and the other agents such as L-proline, glycine, L-histidine, D-fructose, L-tryptophan,

dopamine hydrochloride were obtained from Aladdin Industrial Corporation (Shanghai, China). Epinephrine hydrochloride was purchased from J&K Chemical Ltd. (Shanghai, China). Citric acid was obtained from Fuchen Reagent Plant (Tianjin, China). Disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium chloride and perchloric acid were purchased from Guangzhou Chemical Reagent Plant (Guangzhou, China). Doubly distilled water was used throughout. All other reagents were analytical grade and used without further purification.

2.2 Instrumentation

Electrochemical measurements such as CV and DPV were carried out on a CHI832 electrochemical workstation (Shanghai Chenhua Instruments Co., China). A conventional three-electrode system consisted of a bare or modified glassy carbon electrode (GCE, Φ = 3 mm) as the working electrode, a platinum wire as the counter electrode (CE) and a saturated calomel electrode (SCE) as the reference electrode (RE) was employed. For all electrochemical measurement, the volume of the solution was limited to 20 mL.

An S-4300 SEM (HITACHI, Japan) and a Tecnai G2 F30 S-TWIN (FEI, Netherlands) were used to investigate the surface morphology of the modified electrode. The groups studies of CNSs were carried out using an ESCALAB 250 X-ray photoelectron spectrometer and a NICOLET AVATAR 330 Fourier transform infrared (FT-IR) spectrometer. UP-250 ultrasonic cell breaking machine was used for sample preparation.

2.3 Preparation of CNSs and CNSs modified electrode

The CNSs were prepared with a home-made flowing electrolytic device. In briefly, the pure water was driven by liquid chromatography pump to the electrolytic device from bottom

at a flow rate of 1.0 mL/min. The electrolysis current and electrolysis voltage were fixed as 160 mA and 220 V for 5 h. Then, 12 mL pure water was used to rinse out the electrolytic products from the top at the speed of 1.0 mL/min. The rinsing water was collected and the CNSs suspension was obtained after centrifuged for about 10 min at speed of 10000 rpm.

The GCE was polished carefully with 0.05 μm alumina and rinsed ultrasonically with redistilled water to obtain a mirror-like surface. Then 5 μL of the CNSs solution was dropped onto the surface of the freshly polished GCE and dried under an infrared lamp to get the CNSs modified electrode. The CNSs modified electrode was scanned with differential pulse voltammetry from 0.0 V to 0.8 V for 5 cycles in phosphate buffer solutions (PBS, 0.2 mol/L, pH=6.0). This procedure made the modified electrode stable in the electrochemical measurements.

2.4 Standard solutions and Sample pretreatment

The standard stock solution of 5-HTP (1×10^{-2} mol/L) was dissolved at 0.1 mol/L HCl, and stored at −20 ˚C to reduce possible oxidation. The working standard solution was freshly prepared by appropriate dilution of the stock standard solution with redistilled water.

Healthy rats collected from the Center for Disease Control and Prevention of Guangdong province were decapitated rapidly. Whole brain tissues were removed immediately and washed with physiological saline (4 ˚C) to remove traces of blood. Then, the brain tissues were frozen and stored at −20 ˚C. The frozen brain samples were weighed and homogenized in 0.4 mol/L perchloric acid (4 ˚C) containing 0.5 mM ethylenediaminetetraacetic acid disodium salt (10 mL per mg of sample) using a tissue crusher. The homogenized tissue was disruption by an ultrasonic procedure for 5 min in an ice-bath and centrifuged at 10,000 rpm

at 4 °C for 15 min. The supernatant was then collected and used for electrochemical measurements. The experimental were approved by the Animal Ethics Committee of Sun Yat-sen University, and all the procedures were performed in accordance with the Regulations for the Administration of Affairs Concerning Experimental Animals.

3. Results and discussion

3.1 Characterizations of CNSs

The surface of CNSs modified electrode was observed by scanning electron microscopy (SEM) in **Fig. 1a**. It revealed the few-layer planar sheet-like morphology of the CNSs. Transmission electron microscopy (TEM) image of CNSs modified electrode was shown in **Fig. 1b**. It can be seen that CNSs films exhibited layer structure and good attachment to the GCE, which are essential to ensure stability of the modified electrode. The elemental distribution of CNSs was examined by X-ray photoelectron spectroscopy (XPS) and the result showed that the atomic ratios of C and O were about 63.7% and 36.3%, respectively. The C 1s spectrum of XPS (**Fig. 2A**) consisted of four peaks located at 284.8, 286.0, 287.2 and 289.1 eV, corresponding to the C-C, C-O, C=O and -COOH bond. The Fourier transform infrared (FT-IR) of CNSs was also investigated (**Fig. 2B**). The band observed at 3396 cm^{-1} corresponding to the stretching vibration of O-H bonds while that at 1715 cm^{-1} was indicative of C=O stretching. It showed that the strong absorption band at 1433 cm^{-1} and 1249 cm^{-1} could be attributed to the deformation vibration of O-H bonds and the stretching vibration of C-O bonds, respectively. The results of XPS and FT-IR showed that abundant carboxyl groups existed on the CNSs' surface. The carboxyl groups could be beneficial to the dispersion stability of CNSs.

3.2 Electrochemical behavior of 5-HTP on CNSs modified electrode

The electrochemical behaviors of 5-HTP on the bare GCE and CNSs modified electrode were investigated by CV in **Fig. 3A**. In this figure, a weak oxidation peak of 5-HTP was observed at 0.42 V on bare GCE (curve a), while an increased peak occurred at 0.43 V (curve b). In comparison, the anodic peak current of 5-HTP on the CNSs modified electrode was about 29 times larger than that on the bare GCE, indicating the CNSs film can significantly catalyze the 5-HTP oxidation process and accelerate the electron transfer. This may be attributed to the special nanostructure of CNSs, especially the existence of abundant carboxyl groups on the surface, which has a large specific surface area and countless active sites.

In order to investigate further the electrochemical behavior of 5-HTP on the modified electrode, the effect of scan rates ranging from 30 to 300 mV/s was studied (**Fig. 3B**). The anodic peak currents of 5-HTP increased linearly with the square root of the scan rate. It was indicated that the electrochemical oxidation of 5-HTP on the CNSs modified electrode was a diffusion controlled process.

3.3 Optimization of determination conditions

3.3.1 Effect of the pH

The effect of pH on the current response of 5-HTP at the CNSs modified GCE was investigated in the pH range of 4.0 - 9.0. **Fig. 4A** shows that the pH of the supporting electrolyte has a significant influence on the 5-HTP oxidation on the CNSs modified GCE. Seen from **Fig. 4B**, the potentials of peak shifted negatively with the increasing pH values of the solution. The relationship of *Ep* with pH could be described by the following linear regression equation: $Ep = -0.04914\ pH + 0.6928$ ($R = 0.9979$). It was indicated that the

electrochemical oxidative reactions involved the loss of the protons. A slope of 0.0491 V is closed to the Nernst equation value of 0.0591 V and indicates that an equal number of electrons and protons are involved in the electrode reactions. In **Fig. 4C**, the oxidation peak current of 5-HTP reached a maximum value at a pH value of 6.0, that might because the proton-transfer and electron-transfer of the 5-HTP oxidation reached an equilibrium at pH 6.0. Therefore, pH 6.0 was chosen for the subsequent analytical experiments.

3.3.2 Effect of the amount of CNSs

The amount of the CNSs on the modified electrode was also optimized. As shown in **Fig. 5**, the maximum peak current of the 5-HTP was appeared when the volume of CNSs suspension deposited on the surface of GCE was 5 μL. Further increase of the CNSs led to the decrease of peak current. It may be explained that the thicker film of CNSs could block the electron transfer and the mass transfer process of analytes. Consequently, 5 μL was selected as the optimum volume of CNSs suspension for the modified electrode.

3.3.3 Effect of the accumulation conditions

It is stated that accumulation can improve the amount of 5-HTP absorbed on the electrode surface. Therefore, the effect of accumulation times and the stirring rates during accumulating were also investigated. The oxidation peak currents of 5-HTP at different accumulation times were shown in **Fig. 6A**. It can be concluded that the peak current increased slightly with the accumulation time further extending beyond 3 min. This phenomenon may be caused by the saturated adsorption of 5-HTP at the electrode surface. In addition, the influence of the stirring rates during accumulating on the oxidation peak current of 5-HTP was also tested in **Fig. 6B**. The oxidation peak current of 5-HTP increased with the

stirring rates and reached a maximum value at a stirring rates value of 400 rpm. Both sensitivity and work efficiency considered, the optimal accumulation time of 3 min and the optimal stirring rates of 400 rpm during accumulating were employed in further experiments.

3.4 Differential pulse voltammetry method

Under the optimal conditions, a sensitive electrochemical method based on the CNSs modified electrode was proposed for the determination of 5-HTP. As shown in **Fig. 7**, the oxidation peak currents are proportional to the concentration of 5-HTP in a wider range of 5×10^{-8} to 1×10^{-6} mol/L and the linear equation is I_p (nA) = 1449 c (μmol/L) – 20.23 (R = 0.9956). The detection limit was estimated to be 3×10^{-8} mol/L (S/N=3). These results were comparable and even better than those obtained for the other modified electrodes (**Table 1**). The proposed method showed wider linear range and even higher sensitivity indicating that the CNSs modified GCE was suitable for the determination of 5-HTP.

Reproducibility is one of the most important properties of the electrode. The fabrication reproducibility was estimated by five modified electrodes prepared under the same conditions. The RSD was 2.8% for the determination of 5-HTP, suggesting the good reproducibility of the CNSs modified GCE.

3.5 Real sample analysis

In order to apply the proposed method to the determination of 5-HTP in a practical sample, the influence of some possibly coexisting foreign inorganic ions and organic compounds was examined. The interferences studied were conducted by analyzing a solution of 1×10^{-6} mol/L 5-HTP, which varying amounts of possible interference were added. The tolerated limit of each foreign species was taken as a relative error not greater than ±5%. The

tolerable ratio for foreign species was 1000 for NaCl, L-proline, glycine, citric acid, L-histidine, lactic acid and D-fructose, 10 for L-tryptophan, 4 for epinephrine, 1 for dopamine.

The developed method was applied for the determination of 5-HTP in rats' brain tissue. All samples were diluted 50 times with 0.2 mol/L PBS pH 6.0. To ascertain the correctness of the results, the samples were spiked with certain amounts of 5-HTP and then detected. The results indicated that the recovery ranged from 90.3 to 98.0% with relative standard deviation (RSD) ranged from 9.0 to 10% in **Table 2** and the obtained recoveries were satisfactory.

4. Conclusion

In this study, a novel CNSs modified electrode was prepared and applied for the determination of 5-HTP. The CNSs was synthetized by a home-made flowing electrolysis device. The characterizations of the CNSs were investigated by XPS and FT-IR which indicated the presence of abundant carboxyl groups at the surface. The CNSs modified electrode showed efficient electrocatalytic oxidation of 5-HTP. A sensitive electrochemical method based on the CNSs modified electrode was proposed for the determination of 5-HTP. The developed method demonstrated many desirable properties including high sensitivity, low detection limit and wide linear range. Moreover, it was applied for the determination of 5-HTP in rats' brain tissue sample with satisfactory results.

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Figure Captions:

Fig.1 SEM (a) and TEM (b) images of CNSs modified electrode

Fig. 2 XPS spectra (A) of CNPs in C 1s region and FT-IR image (B) of CNSs

Fig.3 (A) CVs of 5-HTP on bare GCE (a) and CNSs modified GCE (b) at scan rate 0.1 V/s. (B) CVs of 5-HTP on CNSs modified electrode with different scan rate from 0.03 (a) to 0.3 V/s (g). Inset is the dependency of peak current with respect to the square root of the scan rate

5-HTP, 1×10^{-6} mol/L; accumulation time: 3 min; amounts of CNSs: 5 μL; supporting electrolyte: 0.2 M phosphate buffer solution (pH 6.0)

Fig.4 (A) DPVs of 5-HTP on CNSs modified electrode with different pH. The dependency of (B) peak potentials and (C) peak currents of 5-HTP on CNSs modified electrode with different pH

5-HTP, 1×10^{-6} mol/L; accumulation time: 3 min; stirring rates: 400 rpm; amounts of CNSs: 5 μL; supporting electrolyte: 0.2 M phosphate buffer solution

Fig.5 The peak currents of 5-HTP on CNSs modified electrodes which were covered with different amounts of CNSs

5-HTP, 1×10^{-6} mol/L; accumulation time: 3 min; stirring rates: 400 rpm; supporting electrolyte: 0.2 mol/L phosphate buffer solution (pH 6.0)

Fig.6 DPVs of 5-HTP on CNSs modified electrode with different accumulation times (A) and different stirring rates (B). The inset is the dependency of peak current with respect to the accumulation times or stirring rates

5-HTP, 1×10^{-6} mol/L; amounts of CNSs: 5 μL; supporting electrolyte: 0.2 mol/L phosphate buffer solution (pH 6.0)

Fig.7 DPVs of different 5-HTP concentrations from 5×10^{-8} (a) to 1×10^{-6} mol/L (f) on CNSs modified electrode. Inset is the dependency of peak current with respect to the concentration of 5-HTP

Accumulation time: 3 min; stirring rates: 400 rpm; amounts of CNSs: 5 μL; supporting electrolyte: 0.2 mol/L phosphate buffer solution (pH 6.0)

Table

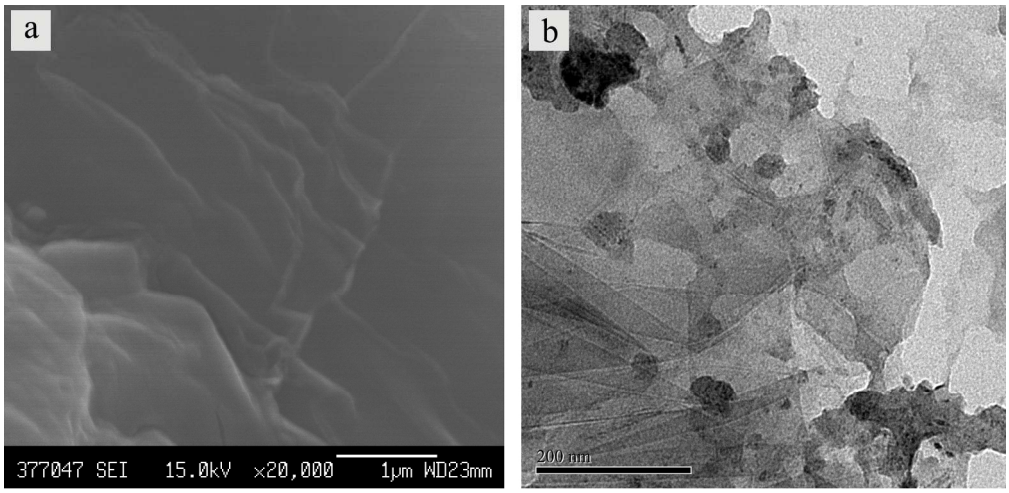
Table 1 Comparison of the efficiency of some electrochemical methods in the determination of 5-HTP

Method	Modified electrode	Linear range(μM)	Detection Limit(μM)	Reference
Linear scan voltammetry	ITO/npSG/CoHCF[a] electrode	10 -1000	2.1	[41]
Differential pulse voltammetry	gold modified pencil graphite electrode	10–60	/	[42]
Amperometric detection	Ru^{II}terpyridine-doped composite electrode	1.0- 40	0.05	[43]
Differential pulse voltammetry	CNSs modified GCE	0.05-1.0	0.03	This work

[a] indium tin oxide/ nanoscale pores sol–gel/ cobalt hexacyanoferrate

Table 2 Analytical results of rats' brain tissue sample

Sample	Added(μM)	Found(μM)	Recovery (%)	RSD (%) (n = 3)
Rats' brain tissue	0	0.084	/	8.9
	0.05	0.128	90.3	9.5
	0.10	0.181	98.0	9.0
	0.20	0.269	93.2	10.0



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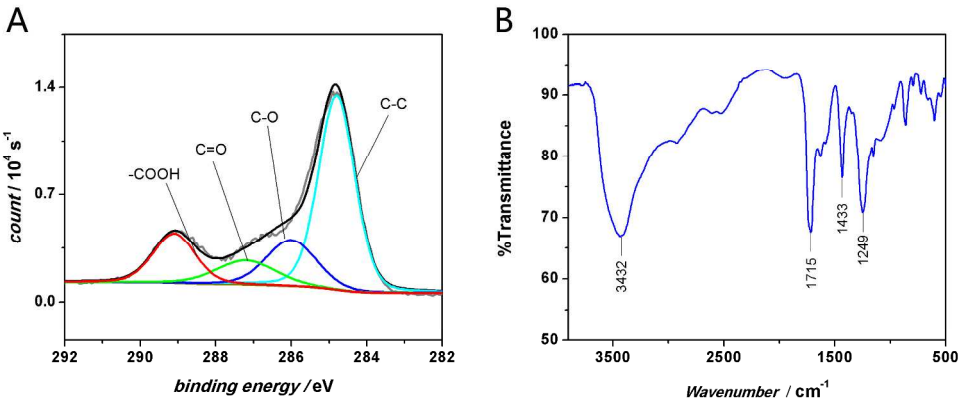

Fig.2
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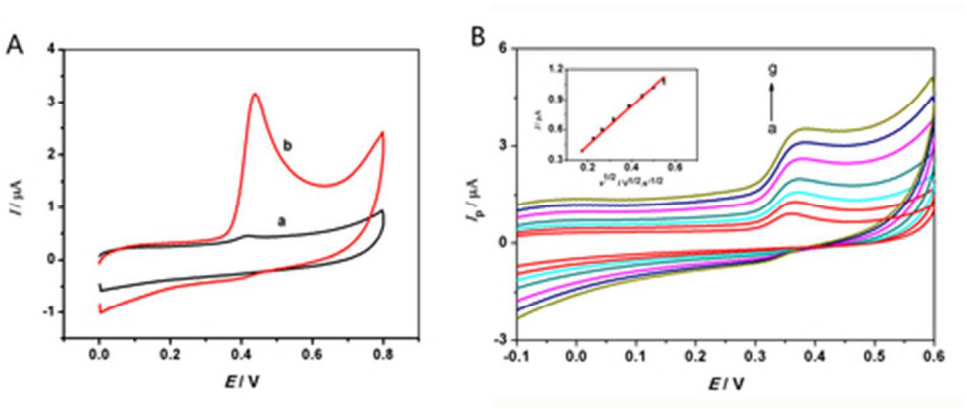

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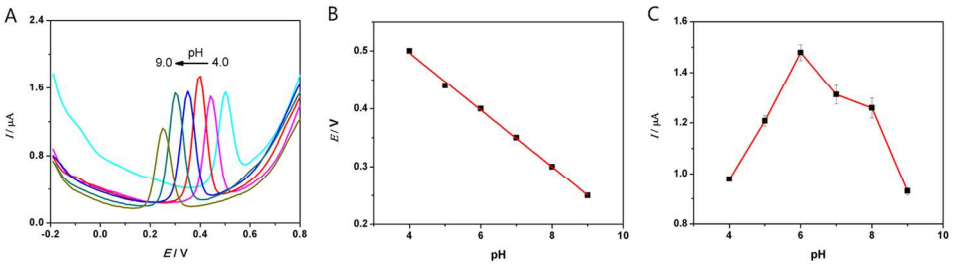

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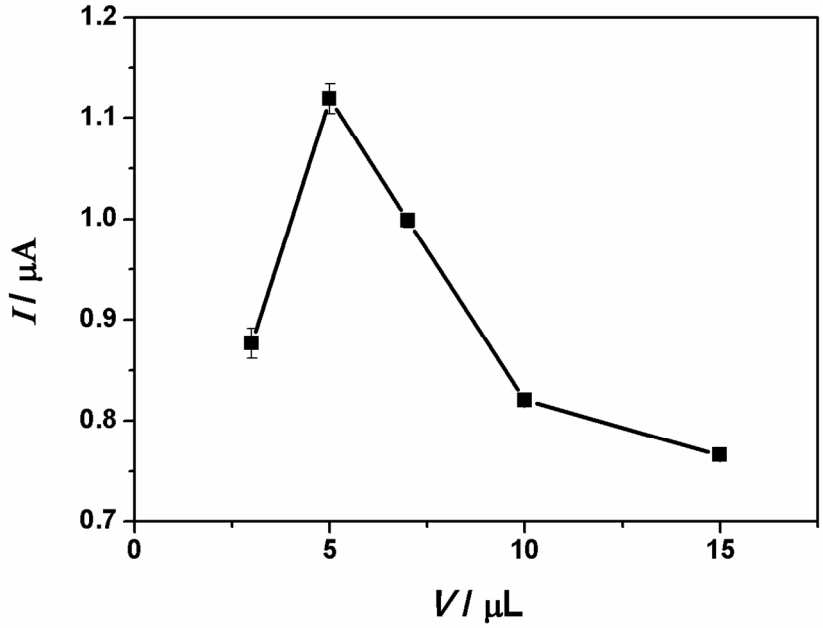

Fig.5
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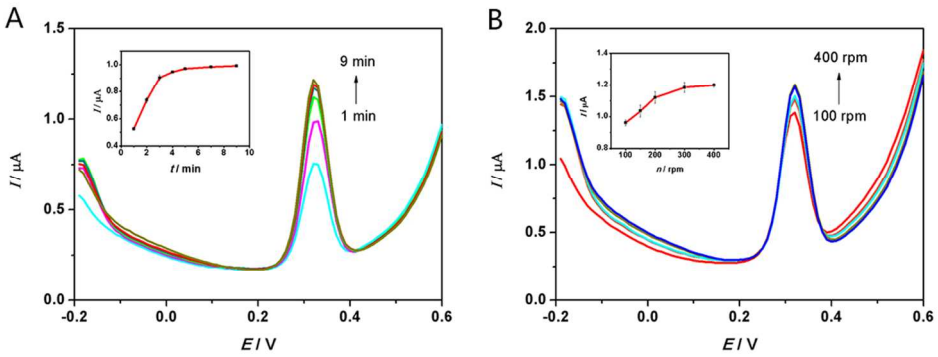

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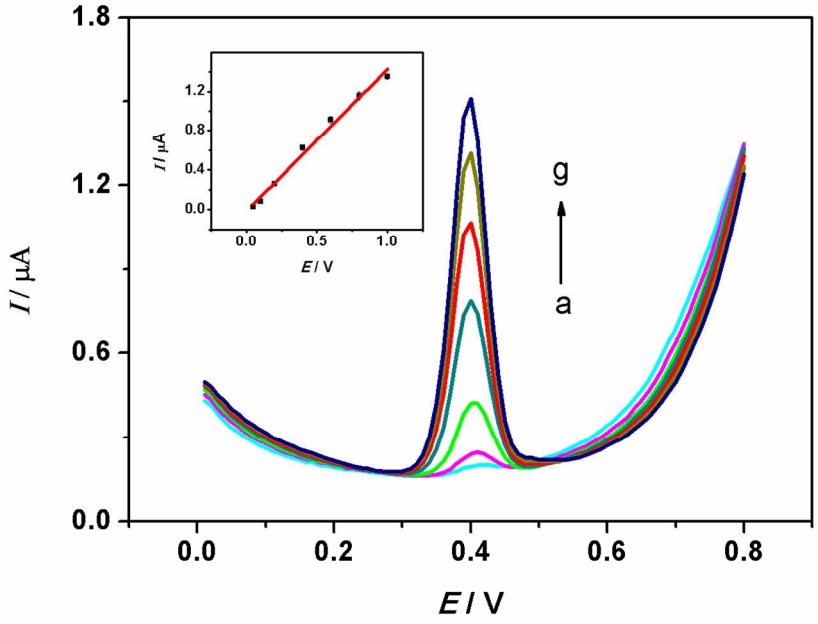

Fig.7
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