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Sensitive Electrochemical Determination of Indole-3-Acetic Acid Based on Multiwalled Carbon Nanotubes and Sucrose-Derived Carbon Composites

Limin Lu[1],*Yongfang Yu*[1], *Hui Zhou*[2], *Ping Li*[1], *Yingqiong Peng*[1,*], *Wenmin Wang*[1], *Haohua He*[1,*]

[1]Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Ministry of Education, College of Software, Jiangxi Agricultural University, Nanchang 330045, PR China
[2]Key Laboratory of Organo-Pharmaceutical Chemistry of Jiangxi Province, Gannan Normal University, Ganzhou 341000, China

[*]E-mail: hhhua64@163.com (H. He), jneyq_pyq@163.com

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In this paper, a novel composite electrode consisting of multiwalled carbon nanotubes and sucrose-derived carbon (MWCNTs/SDC) was successfully developed for the electrochemical detection of indole-3-acetic acid (IAA). The obtained composites were characterized by scanning electron microscopy (SEM), and the surface morphology results indicated that the MWCNTs/SDC provided a higher specific surface area as it formed an open network structure than the pure MWCNTs. The electrochemical behaviors of IAA on different electrodes were investigated by cyclic voltammetry (CV) and differential pulse voltammograms (DPV). The results demonstrated that the oxidation peak currents of IAA obtained at MWCNTs/SDC/GCE were much higher than those at SDC/GCE and MWCNTs/GCE, which can be ascribed to the high conductivity and the large specific surface area of the composite. At MWCNTs/SDC/GCE, the response current exhibited a linear range from 0.1 μM to 30 μM. The detection limit was 0.0296 μM (S/N = 3). In addition, the proposed sensor exhibited high selectivity, good stability and reproducibility.

Keywords: Indole-3-acetic acid; Sucrose-derived carbon; Multi-walled carbon nanotubes; Electrochemical detection

1. INTRODUCTION

Indole-3-acetic acid (IAA), which is released by the terminal bud of a shoot, acts as a true chemical messenger regulating some important biological processes of plants, such as division, elongation and differentiation of cells [1]. And the effects of dynamic change of IAA level in agriculture on the environment and human health have attracted a widespread concern [2]. However, it is hard to detect IAA because of its very low concentration in plant. Also, the phytohormone is easily to be decomposed by heat, light and oxygen. Thus, searching for a fast, precise, sensitive and convenient measurement method for IAA detection is significant. So far, many techniques have been utilized for the determination of IAA in biological samples, such as immunoassay [3], capillary electrophoresis (CE) [4], liquid chromatography [5], chemiluminescence [6] and electrochemical methods [7]. Among these methods, electrochemical method shows several advantages of high sensitivity, rapid response, low cost and simple instrumentation. However, IAA exhibits slow electron transfer on bare glass carbon electrodes (GCEs) [8]. Thus, the development of new functional materials is very necessary for fabricating sensitive electrochemical sensors for its detection.

In recent years, multiwalled carbon nanotubes (MWCNTs) have been extensively used because of their low cost, good mechanical strength, high electrical conductivity, and their chemical stability [9-11]. In particular, the electronic properties of MWCNTs demonstrate that they are beneficial to promote electron-transfer reactions and can be used as electrode modified materials in electrochemical reactions. To further enhance their performance, broaden their properties and expand their applications, different approaches have been used to modify the walls of MWCNTs. Up to now, various nano- composites combining MWCNTs with inorganic and organic materials have been reported. For instance, in the study of Zhao et al [12], by combining the advantages of carbon nanotubes and Ag nanoparticles, MWCNTs/Ag nanoparticles, the nanohybrids were used for the electrochemical detection of hydrogen peroxide with good sensitivity. The report of Zheng et al. proved that carbon fiber microelectrodes modified with polyvanillin/MWCNTs composite showed a sensitive response towards the electrochemical oxidation of nitrite [13]. Zhang et al. used graphene oxide as a surfactant to disperse MWCNTs and synthesized graphene oxide/MWCNTs film. This nanocomposite exhibited excellent electrocatalytic activity for the oxidation of Azithromycin [14]. These MWCNTs-based hybrid materials showed enhanced sensitivity and great potential application in electronics, catalysis, and sensors, etc.

In the present work, a composite electrode combining of MWCNTs and SDC with a highly accessible surface area and a high electrical conductivity was prepared. Then, the MWCNTs/SDC modified GCE used for constructing a low-cost and high-efficiency sensor for detecting IAA. MWCNTs/SDC/GCE exhibited excellent enhancement effect on the electrochemical reactions of IAA compared with bare GCE, SDC/GCE and MWCNTs/GCE, which was due to the large accessible surface area and good electrical conductivity. Consequently, a sensitive voltammetric method for the detection of IAA was established by using MWCNTs/SDC/GCE.

2. EXPERIMENTAL

2.1 Chemicals and reagents

MWCNTs (purity > 95%) were purchased from Shenzhen Nanotech Port Co., Ltd. Sucrose was obtained from Aladdin Reagent Co., Ltd. IAA was purchased from Energy Chemical Co., Ltd. IAA stock solution (0.1 M) prepared with absolute ethyl alcohol was stored at 278–281 K. Phosphate buffer were prepared from stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, which were obtained from Sinopharm chemical reagent Co. Ltd. All other reagents were of analytical grade, and double distilled water was used throughout the experiment.

2.2 Apparatus

Scanning electron microscopy (SEM) analysis was obtained using a Hitachi S-3000 N scanning electron microscope. The cyclic voltammetric measurements were carried out on a CHI660D electrochemical workstation (Shanghai, China). A three-electrode cell (5 mL) was used with the modified glassy carbon electrode (GCE, Φ = 3 mm) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode and a platinum foil electrode as the counter electrode. All potentials were measured and reported versus the SCE and all experiments were carried out at room temperature. The differential pulse voltammetry (DPV) was carried out with the parameters of an increment potential of 0.004 V, a pulse amplitude of 0.05 V, a pulse width of 0.2 s, a sample width of 0.02 s, a pulse period of 0.5 s and a quiet time of 20 s.

2.3. Preparation of the MWCNTs/SDC nanocomposites

The sucrose suspension (0.1 M) was prepared by adding 34 mg sucrose powder into 1 mL deionized water. Then, 1 mg MWCNTs were added into the as-prepared sucrose solution and stable sucrose/MWCNTs suspensions (1 mg mL^{-1}) were obtained by sonicating for 1 h. Subsequently, H_2SO_4 (95–97%) was dropped into the above suspensions, and a black bread-like composite materials of MWCNTs/SDC were formed immediately. Finally, the resultant composite was centrifuged and washed with doubly distilled water until the pH value was close to 7, and then the black solid powder was redispersed in water for characterization and further use. For comparison, SDC was prepared under the same conditions without adding the MWCNTs.

2.4. Preparation of the modified electrodes

The modified electrodes were prepared by a simple casting method. Prior to modification, the GCEs were polished with chamois leather containing 0.05 μm Al_2O_3 slurry, rinsed thoroughly with double distilled water, then washed successively with double distilled water, anhydrous ethanol and acetone in an ultrasonic bath, and dried under N_2 before use. 5 μL of the MWCNTs/SDC suspension was dropped on the surface of GCE and then dried in air. The resulting modified electrode was named as MWCNTs/SDC/GCE. For comparison, MWCNTs/GCE was prepared by casting 5 μL of the MWCNTs suspension on the surface of GCE and then dried in air. SDC/GCE was prepared by casting 5 μL of the SDC suspension on the surface of GCE and then dried in air.

3. RESULTS AND DISCUSSION

3.1 Surface morphologies of the films

SEM was employed to characterize SDC (A), MWCNTs (B) and MWCNTs/SDC (C) films. As can be seen from Fig. 1A, it was obvious that the SDC sheets exhibited a typical wrinkled surface morphology. For MWCNTs, as shown in Fig. 1B, it clearly demonstrated that the bundles were interlocked together and formed a netlike nanostructure, which was attributed to the strong π–π electronic interactions and lack of attached oxygenated groups on the surface of MWCNTs [15]. In contrast, the MWCNTs/SDC formed a porous network structure that remained unchanged even (Fig. 1C). When an acid treatment is used to sucrose, the MWCNTs serve as a framework to bind the SDC *via* strong π–π stacking and van der Waals forces between the neighboring surfaces. Thus, the SDCs were distributed through the entangled MWCNT framework to provide a high specific surface area as it forms an open network structure [16].

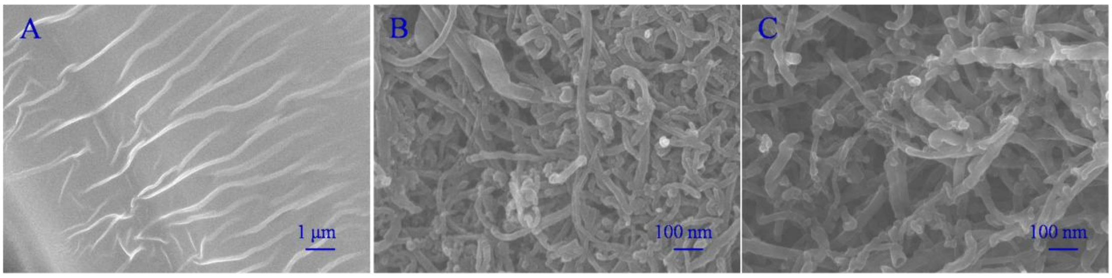

Figure 1. SEM images of SDC (A), MWCNTs (B) and MWCNTs/SDC (C).

3.2 Electrochemical behaviors of IAA at various electrodes

The electrochemical behaviors of IAA on bare GCE (a), SDC/GCE (b), MWCNTs/GCE (c) and MWCNTs/SDC/GCE (d) were investigated by cyclic voltammetric (CV) in 0.1 M phosphate buffer (pH 6.5) containing 5 μM IAA (Fig. 2).

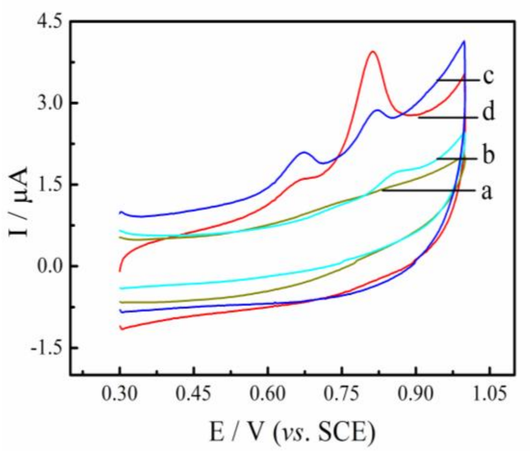

Figure 2. Cyclic voltammograms of 5 μM IAA in 0.1 M phosphate buffer (pH 6.5) at bare GCE (a), SDC/GCE (b), MWCNTs/GCE (c) and MWCNTs/SDC/GCE (d) at the scan rate of 50 mV s^{-1}.

The broaden voltammetric profile with low background current on bare GCE reveals sluggish electrochemical kinetics on this surface. Compared with bare GCE, SDC/GCE (b) was more sensitive to the electrochemical oxidation of IAA. At MWCNTs/GCE (c), the oxidation peak currents increased, which was due to the strong interfacial accumulation abilities and excellent conductivity of MWCNTs [17,18]. An obvious enhancement was observed in the oxidation peak currents at MWCNTs/SDC/GCE (d), where the excellent conductivity of MWCNTs enhanced the charge electron transfer between MWCNTs/SDC/GCE and IAA solution, and the large specific surface area of MWCNTs/SDC can increase the loading amount of IAA.

3.3 Optimization of the experimental conditions

3.3.1. Effect of the accumulation time

The influence of accumulation time was studied in 0.1 M phosphate buffer (pH 6.5) containing 5 μM IAA on MWCNTs/SDC/GCE. As shown in Fig. 3, the oxidation peak currents of IAA increased gradually with time increasing before 20 s. With further increase of the accumulation time, the oxidation peak currents changed slightly, indicating that 20 s was sufficient to reach the IAA saturation on MWCNTs/SDC/GCE. Thus, 20 s was chosen to be the optimum accumulation time for analytical determination.

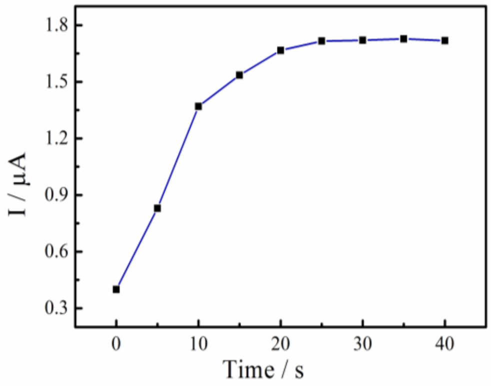

Figure 3. Influence of the accumulation times of 10 μM IAA on the oxidation peak currents at MWCNTs/SDC/GCE in 0.1 M phosphate buffer (pH 6.5).

3.3.2. Effect of scan rate on the peak currents of IAA

The influence of scan rates on the oxidation peak of 6 μM IAA at MWCNTs/SDC/GCE was also investigated by CV. As shown in Fig. 4, the redox peak currents clearly increased with increasing potential scan rate. Moreover, the oxidation peak currents (I_{pa}) was proportional to the scan rate v (Fig. 4), and the liner regression equations were expressed as I_{pa} (μA) = 0.04087v (mV s^{-1}) + 2.43605 (R^2 = 0.9944). It indicated that the electrochemical behavior of IAA at MWCNTs/SDC/GCE was an adsorption-controlled electrode process. The adsorption of IAA at the surface of the

MWCNTs/SDC/GCE can be ascribed to the π–π interaction between the IAA and the MWCNTs/SDC [8,19].

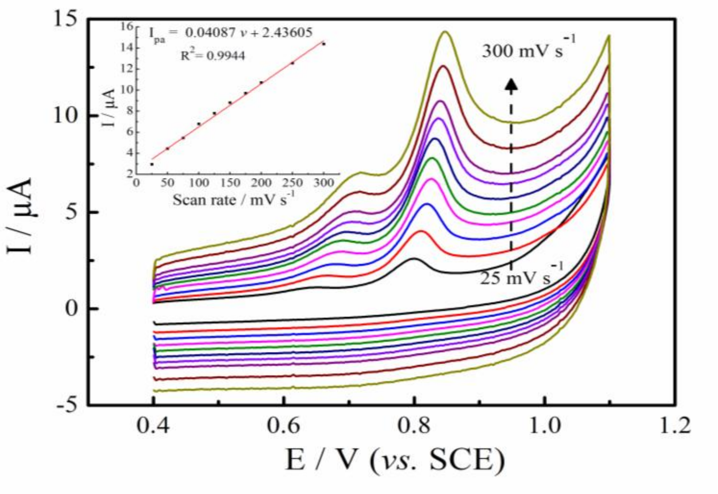

Figure 4. CVs of 6 μM IAA on MWCNTs/SDC/GCE at different scan rates in 0.1 M phosphate buffer (pH 6.5) (from the inner to the outer are 25, 50, 75, 100, 150, 175, 200, 250 and 300 mV s^{-1}). Insert: the plot of the peak currents versus scan rate.

3.4 Electrochemical determination of IAA

Differential pulse voltammetry (DPV) method was selected for determination trace amounts of IAA in 0.1 M phosphate buffer (pH 6.5).

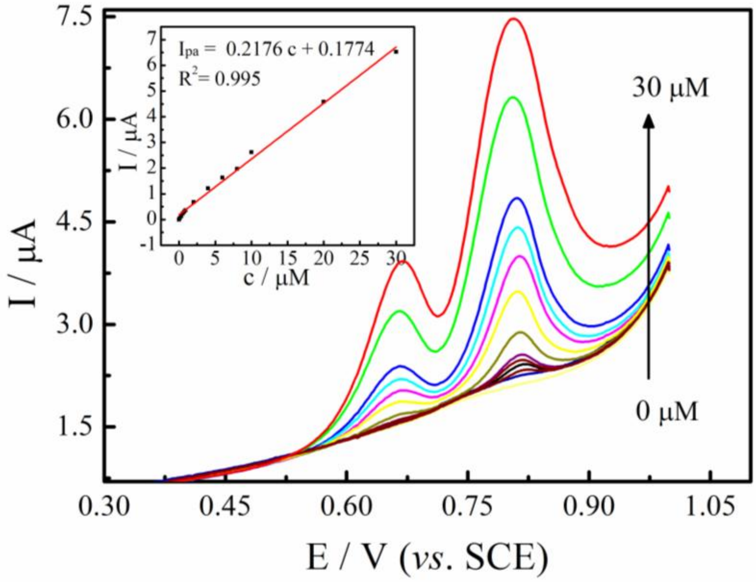

Figure 5. DPV of MWCNTs/SDC/GCE in different concentrations of IAA solutions (0, 0.1, 0.3, 0.5, 0.7, 0.9, 2, 4, 6, 8, 10, 20 and 30 μM). Insert: plot of the oxidation peak current against the concentration of IAA.

Fig. 5 shows DPV responses of different IAA concentrations at MWCNTs/SDC/GCE, it can be seen that the oxidation peak currents increased linearly with the increase in the IAA concentration from 0.1 μM to 30 μM (Inset of Fig. 5) and the corresponding linear function was $I_{pa} = 0.2176\ c + 0.1774$ with a correlation coefficient of 0.995. The value of the detection limit of IAA was found to be 0.0296 μM based on S/N=3. The MWCNTs/SDC/GCE provided a reasonable linear range for IAA detection and featured a lower detection limit compared to some previous reports [8, 19-23] (Table 1), indicating that MWCNTs/SDC nanocomposite was ideal sensing materials for the fabrication of electrochemical sensors for IAA.

Table 1. Comparison of the proposed sensor for determination of IAA with others.

Type of electrode	Linear range (μM)	Detection limit (μM)	Refs.
[a]PST-rGO	0.1–7.0	0.05	[8]
GCE	1.8–660	2.7	[20]
Graphite-polyurethane/GCE	1.3–94.7	0.15	[21]
Boron-doped diamond	5-50	1.22	[22]
[b]MWNT/MPS/Pt	1–20	0.1	[23]
Nano Au/MWNTs/chitosant	5–200	8.33	[24]
MWCNTs/SDC/GCE	0.1–30	0.0296	This work

[a] PST–rGO: Poly(safranine T)–reduced graphene oxide (rGO);

[b] MWNT/MPS: Multiwalled carbon nanotubes (MWNTs) modified (3-mercaptopropyl)trimethoxysilane (MPS) microelectrode.

3.5 Reproducibility, stability and selectivity of MWCNTs/SDC/GCE

To investigate the reproducibility of the MWCNTs/SDC/GCE, five modified electrodes were prepared independently, which showed a satisfactory RSD value of 3.46% for the detection of 1 μM IAA, indicating the good reproducibility. Also, in order to test the repeatability of the modified electrode, 1 μM IAA was determined successively 15 times using the same electrode in Fig. 6A. The value of RSD was 2.18%, which indicated the MWCNTs/SDC/GCE had a good repeatability. The stability of the modified electrode has been investigated by measuring the current response to 1 μM IAA in pH 6.5 phosphate buffer every few days. The test results showed that there was no significant change of the current responses after being stored for three weeks in the air at room temperature, indicating that the MWCNTs/SDC/GCE was quite stable.

Potential interference in the determination of IAA was studied. Under the optimized conditions, the oxidation peak current of 1 μM IAA was individually measured in the presence of different concentrations of interferents. The results were shown in Fig. 6B. It was found that 100-fold of K^+, Na^+, Al^{3+}, Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, 20-fold of glucose, folic acid, salicylic acid and ascorbic acid did not interfere with the detection, with the peak current changes less than ±5%.

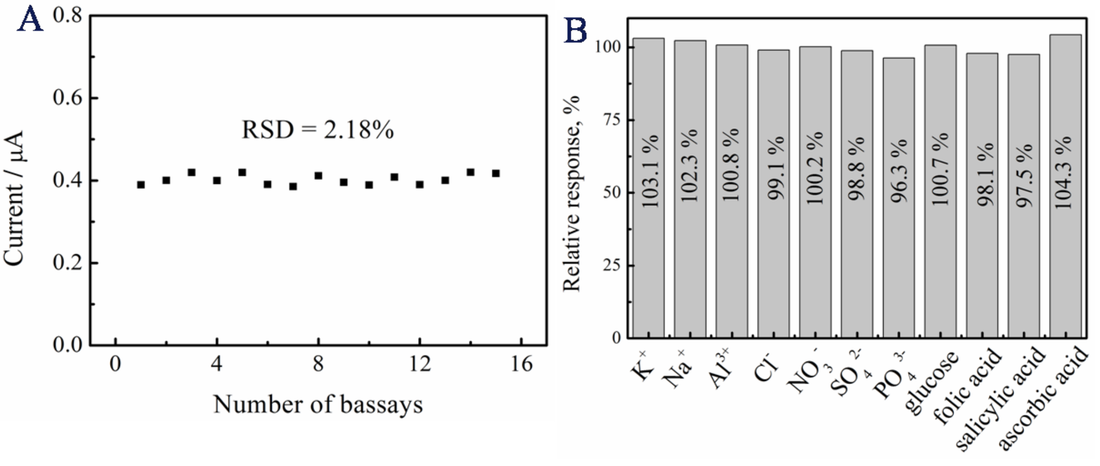

Figure 6. (A) The repeatability of the current response of the MWCNTs/SDC/GCE in 0.1 M phosphate buffer (pH 6.5) containing 1 µM IAA for 15 successive assays; (B) Interference experiments for the detection of IAA at MWCNTs/SDC/GCE in 0.1 M phosphate buffer (pH 6.5) containing 1 µM IAA in the presence of different concentrations of interferents.

4. CONCLUSIONS

In this work, a new modified electrode was successfully fabricated for the sensitive determination of IAA based on MWCNTs/SDC/GCE composite. The composite exhibited excellent conductivity and provided a large specific surface area to increase the loading amount of IAA, thus, the modified electrode demonstrated enhanced electrocatalytic activity toward IAA oxidation with a low detection limit. Owing to the excellent selectivity, high stability and good reproducibility, the designed IAA biosensor provides a good platform in the sensitive detection of real samples.

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