Electrochemical Analysis of Nicotine Based on Multi-Walled Carbon Nanotube/Graphene Composite

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In this article, multi-walled-carbon nanotube/graphene hybrid (MWCNT-G) was prepared by directly adding graphene powder to the surface functionalized multi-walled carbon nanotubes (MWCNT) dispersion. The morphology of the MWCNT-G was observed using SEM and TEM. Observation found the MWCNT-G represented a network morphology, which is beneficial for the electrochemical reaction. Therefore, the MWCNY-G was used for commercial electrode surface modification. The electrochemical performance of the modified electrode was tested by electrochemical determination of nicotine.

1. INTRODUCTION

Nicotine is one of the pyridine derivative alkaloid with high toxicity, which commonly present in the tobacco product such as cigarette and cigar. Nicotine has three different structural forms at different pH conditions and their percentages changes during the pH change. Studies showed the nicotine is associated with many diseases such as lung cancer. The major route for nicotine intake is smoking. Tobacco smoke could be potentially harmful to active smoker as well as passive smoker. The lethal dose of the nicotine for children and adult is 10 mg and 60 mg, respectively [1]. However, recent

report also indicated the nicotine had a potential for Alzheimer's disease treatment [2]. Despite the medical potential usage, many governments have published rules against the use of nicotine or banned smoking in public area. In order to help the quite smoking treatment, many anti-smoking products were developed and contain certain amount of nicotine.

Especially for tobacco products, nicotine is the major alkaloid (about 95% in total) in the cigarette or cigar. Depending on the brand, the content of the nicotine varies from 1 to 30 mg/g [3]. Due to smoking is worldwide behavior and large of evidences proved the harmful to human health, development of an accurate, efficiency, sensitive and selective analytical approach is essential. So far, many analytical methods were developed for nicotine determination such as GC-MS [4-6], capillary electrophoresis with MS [7], HPLC [8, 9], fluorometry [10], chemiluminiscence [11] and electrochemical approach. However, many of these methods suffer some practical problems such as long time sample purification process, complicated operation process, high cost instrument and narrow detection range. Among them, electrochemical approach has been to found satisfy many of the current analytical requirements due to its simplicity, excellent stability, high selectivity and low cost. However, electro-oxidation of nicotine at bare electrode requires very high potential. Therefore, the determination of nicotine is limited by the usable potential range of the electrode material. Moreover, the high potential determination could overlap with many other substances. Electrode surface modification is an alternative way to enhance the electrochemical performance of the electrode. For nicotine detection, several attempts were carried out for commercial electrode surface modification. For example, Xiao and co-workers demonstrated an electroreduced carboxylated graphene modified glassy carbon electrode (GCE) for nicotine analysis [12]. Shehata and co-workers demonstrated a Nano-TiO$_2$ modified carbon paste sensor for nicotine analysis [13]. Fekry and co-workers demonstrated a novel electrochemical nicotine sensor based on Ce NPs with anionic surfactant [14].

Electrode modifier is the key for the performance of the fabricated electrochemical sensor. So far, many materials were applied for electrode surface modification such as semiconductors [15, 16], noble metals [17-19] and carbon based materials [20-27]. Among them, carbon based material showed promising properties towards electrocatalysis. For example, Govindhan and co-workers demonstrated a multi-function electrochemical sensor based on the carbon nanotube and successfully applied for phenolic pollutants detection [28]. Arduini et al. [29] reported an electrochemical sensor based on screen-printed electrodes modified with a carbon black-Au NPs composite and successfully applied for determination of glucose, hydrogen peroxide and hydroquinone. Moreover, Huang and co-workers fabricated an Ag NPs coated graphene composite as an electrochemical sensor for avian influenza virus H7 analysis [30]. In this contribution, we proposed a novel carbon based composite, which contains both multi-walled carbon nanotube (MWCNT) and graphene. The composition preparation was achieved by dispersing graphene powder into the surface modified MWCNT. The prepared MWCNT-G composite was then applied for GCE surface modification and then uses for determination of nicotine.

2. EXPERIMENTS

2.1. Materials

Nicotine standard sample and tobaccos samples were supplied by the tobacco industry technology research and development center of Henan. Multi-walled carbon nanotubes (MWNT, average diameter: 40 nm) was purchased from XFNANO Co. Ltd. All other chemical reagents were analytical grade and used as received without further purification. Milli-Q water was used through the experiments.

2.2. Acidification of MWCNT

MWCNT surface modification was carried out using strong acids. Specifically, 10 mg MWCNT was added in to 20 mL acid mixture contains 8 M sulfuric acid and 8 M nitric acid (1:3). The dispersion was sonicated in a both sonicater for 10 h at room temperature. After acidification, NaOH was gradually added into the dispersion for neutralization. The surface functionalized MWCNT was collected using a filtration method and washed by water. Dry sample was collected by drying the MWCNT in an oven overnight.

2.3. Preparation of MWCNT-G composite

MWCNT-G composite was prepared according to following procedure. Typically, 10 mg surface acidified MWCNT was firstly dispersed into 24 mL water using 2 h sonication. After a homogenous black solution was formed, 2 mg graphene powder was added into MWCNT dispersion followed with further 5 h intense sonication. Due to the π-π supramolecular interactions, MWCNT-G composite dispersion was formed [24, 31]. The morphology of as-synthesized composite was observed using a ZEISS, SUPRA 55 field emission scanning electron microscopy (FESEM) and a transmission electron microscopy (TEM, JEOL JEM-100CX). A Fourier transform infrared spectrometer (FT-IR, Thermo-Nicolet Nexus 670) was used to characterize the MWCNT-G composite.

2.4. Electrochemical determination of nicotine

MWCNT-G composite was used for electrode surface modification. Specially, 6 μL of MWCNT-G composite dispersion (0.5 mg/mL) was dropped on the GCE surface and dried at a fume hood. Then, 5 μL of Nafion (1 wt% in ethanol) was dropped onto the MWCNT-G/GCE and dried at room temperature. All electrochemical characterizations and determinations were carried out at a CHI430A electrochemical working station. Three electrode system was applied for all experiments, which contains an Ag/AgCl reference electrode, a Pt wire auxiliary electrode and MWCNT-G/GCE working electrode. Electrochemical impedance spectroscopy (EIS) was used for characterizing the electrode resistance performance. 5 mM $[Fe(CN)_6]^{3-/4-}$ was used as probe, 0.1 M KCl was used as supporting electrolyte. Frequency range was set as 10^1 to 10^5 Hz and the amplitude was set as 5 mV.

Electrochemical determination of nicotine was performed using CV method at 0.1 M PBS (pH = 5) at scan range between —0.7 to —1.5 V using scan rate of 50 mV/s. The DPV measurements were carried out at scan range between —0.7-—1.5 V. The modulation time was set as 0.05s with a time interval of 0.2s and a step potential of 0.6 mV/s.

3. RESULTS AND DISCUSSION

Surface morphology of MWCNT-G composite was firstly investigated by scanning electron microscopy (Figure 1A). It can be seen that the graphene sheets showed a typical crumpled structure with lots of bundles of carbon nanotubes. The insertion of MWCNT between graphene sheets could prevent the restacking effect of the graphene, which is a current main drawback using graphene as electrocatalyst material. The restacking effect of the graphene could reduce the surface area of the electrode modifier and hinder its electrochemical activities [32]. The microstructure of MWCNT-G composite was then investigated using TEM. As shown in Figure 1B, the MWCNT showed an even distribution on the graphene sheets surface due to the interaction of π-conjugated aromatic domain on the basal plane of the graphene [33]. The attachment of MWCNT on the graphene surface results steric barriers between adjacent graphene sheets, further confirmed the successful prevention of aggregation of the graphene sheets.

FT-IR spectroscopy of graphene and MWCNT-G composite are shown in Figure 1C. In the spectrum of graphene, the peak located at 1622 cm^{-1} can be assigned to the C=C skeletal vibrations of unoxidized graphitic domains. In the spectrum of MWCNT-G composite, a broad peak at 3400 cm^{-1} can be observed, corresponding to the oxygen-containing groups on the acidified MWCNT, suggesting the treatment with strong acid could effectively modify the MWCNT surface and enhance its dispersibility.
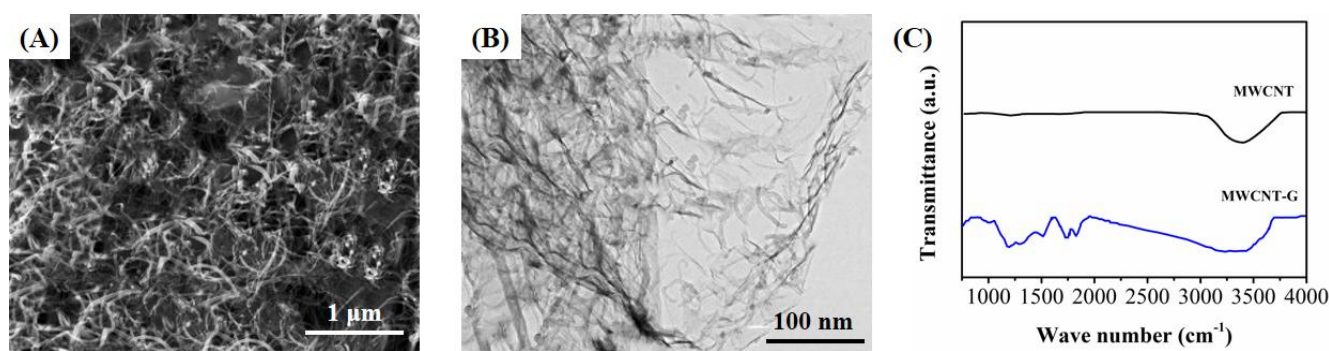

Figure 1. (A) SEM and (B) TEM images of MWCNT-G composite. (C) FTIR spectra of graphene and MWCNT-G composite.

The electrochemical performance changes of GCE before and after surface modification were investigated using electrochemical impedance spectroscopy (EIS). Figure 2 shows the EIS curves of the bare GCE, MWCNT/GCE and MWNT-G/GCE in the presence of 5 mM [Fe(CN)$_6$]$^{3-/4-}$ solution.

As shown in the figure, the EIS curves can be divided into two parts, including a semicircular and a straight line. The semicircular implied the electron transfer limited process status of the electrode. The diameter is equivalent to the electron transfer resistance value. The straight line implied diffusion process status of the electrode. As shown in the figure, the bare GCE shows a relatively bigger semicircular with a straight line. After modification of MWCNT, the MWCNT/GCE shows an increasing of semicircular compared with the bare GCE, suggesting immobilization of MWCNT on the GCE hindered the charge transfer. This phenomena can be ascribed to the presence of the surface oxygen containing groups on the MWCNT surface, which limited the electron transference of ferri/ferro cyanide [34]. In contrast, a much smaller semicircular was observed on the MWCNT-G composite modified GCE, demonstrating very low-electron-transfer resistance to the redox-probe dissolved in the electrolyte solution. Therefore, the addition of graphene sheets could highly enhance the electrochemical performance of the electrode.
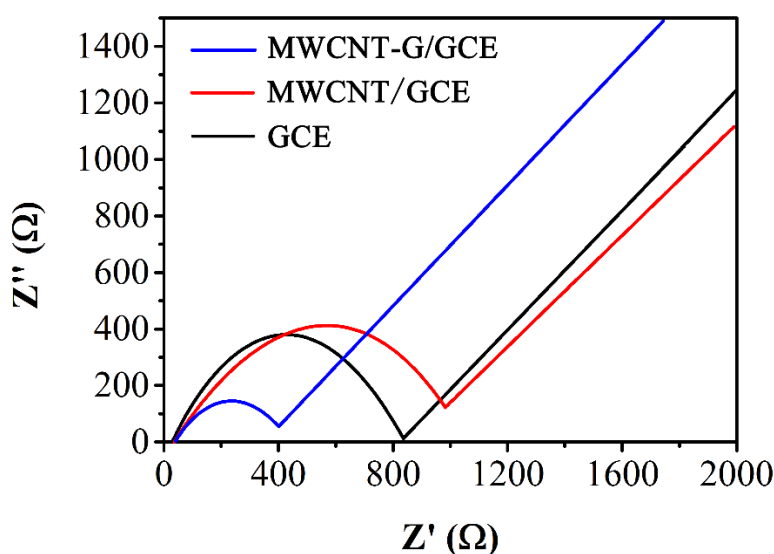

Figure 2. Nyquist diagrams of bare GCE, MWCNT/GCE and MWCNT-G/GCE in 5 mM $K_4[Fe(CN)_6]$ + 0.1 M KCl.

Cyclic voltammograms (CVs) of 100 μM nicotine in PBS (pH 5) at different electrodes were investigated and shown in Figure 3. At scan range, bare GCE, MWCNT/GCE and MWCNT-G/GCE all showed irreversible response towards reduction of nicotine without oxidation peaks. Significant difference can be observed in the CV profiles using different electrode. Although the MWCNT/GCE showed a higher electron transfer resistance compared with the bare GCE, the nicotine reduction response at MWCNT/GCE showed a clear enhancement compared with that of the bare GCE due to the larger surface area of MWCNT, which could effectively absorbed the nicotine molecules and result a high signal. Moreover, a much higher current response was observed at MWNCT-G/GCE with a positive shift of the reduction potential (—1.07 V). No peak was observed when the absence of nicotine, suggesting the peak appeared at —1.07 V indeed related to the reduction of nicotine.
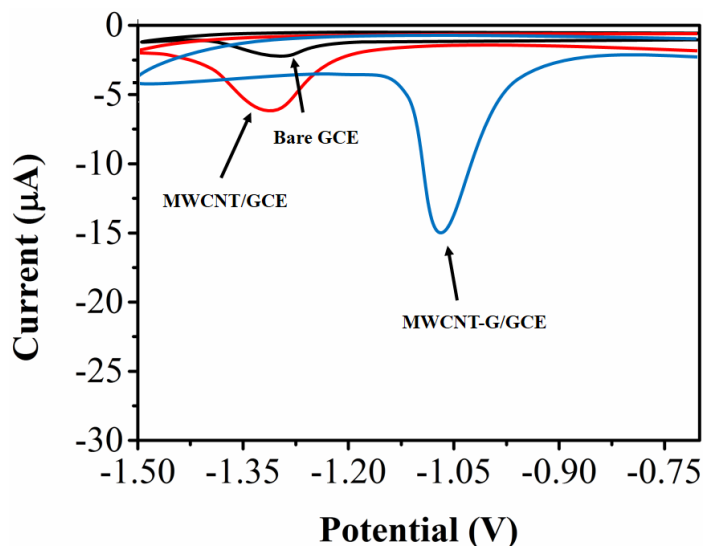
Figure 3. Cyclic voltammograms of bare GCE, MWCNT/GCE and MWCNT-G/GCE toward 100 μM nicotine in PBS (pH = 4) with scan rate of 50 mV/s.

The influence of the electrolyte was investigated. The response of nicotine reduction was recorded at 0.1 M PBS, $Na_2C_2O_4$, H_2SO_4, HAc-NaAc and the reduction values were presented in Figure 4A. It can be seen that the PBS showed the highest signal response towards reduction of nicotine. Therefore, PBS was chosen for this study. The influence of pH value was then investigated as well. As shown in the Figure 4B, the peak current showed an elevation when the pH value increased from 3 to 5 and then decreased further increasing. Therefore, the pH value of 5 was chosen in further study. The relationship between amount of modifier and current response was also studied. Figure 4C shows the effect of the current response with the amount of MWCT-G composite modification. When the amount of the MWCNT-G dispersion increased from 2-6 μL, the nicotine reduction current enhanced greatly. With further increase of the modifier to 10 μL, the response conversely showed a decline due to the thinker layer of MWCNT-G composite, which hampered the electrochemical reaction process.
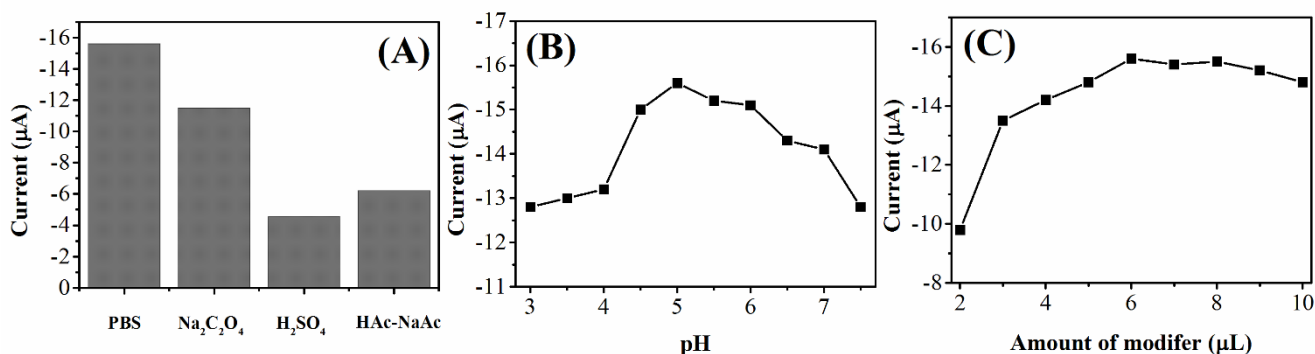

Figure 4. Effect of (A) electrolyte types (B) PBS pH values and (C) amount of MWCNT-G composite on electrochemical reduction of nicotine.

Under optimum conditions, the analytical performance of MWCNT-G/GCE was studied using DPV method. Figure 5 shows the differential pulse voltammetry (DPV) curves of the MWCNT-G/GCE towards different concentrations of nicotine. It can be seen that the current was proportional to the concentration of nicotine from 2 to 600 μM. This detection linear range is wider than previous studies. For example, the detection linear range of nicotine at pencil graphite electrode is between 7.6 to 107.5 μM [35]. The detection linear range of nicotine at boron-doped diamond electrode is between 5 to 500 μM [36]. The detection linear range of nicotine at MWCNT modified electrode is between 31 to 220 μM [37]. The detection limit of MWCNT-G/GCE could estimate to be 0.52 μM, which is lower than other existing studies [38-40].
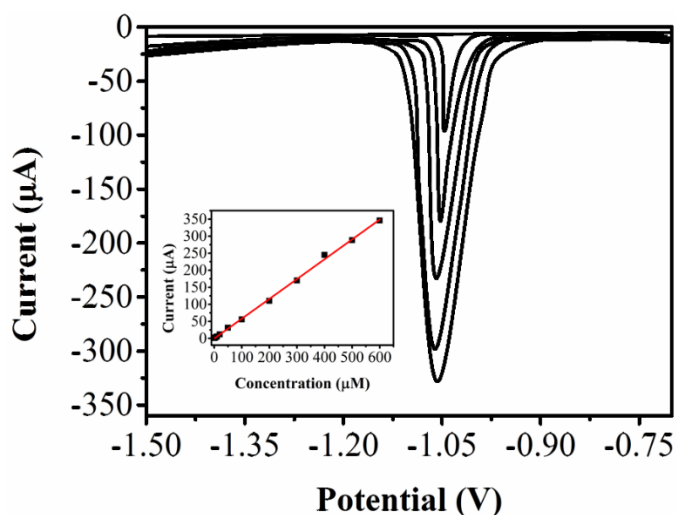

Figure 5. DPV curves of MWCNT-G/GCE towards different concentrations of nicotine in PBS (pH = 5). Inset: plots of reductive peak current vs concentration.

The reproducibility of the MWCNT-G/GCE was tested by determination of 100 μM nicotine using ten freshly prepared electrodes. The relative standard deviation (RSD) obtained was 2.21%, suggesting the excellent reproducibility of the proposed nicotine electrochemical sensor. The stability test of the MWCNT-G/GCE was tested by five times determination of 100 μM nicotine. The responses showed that the proposed MWCNT-G/GCE decreased about 4.18% current response. To evaluate the long-term storage stability, the MWCNT-G/GCE was tested by storing in fridge for one month. The current responses showed the MWCNT-G/GCE still remains 98.1% of their original activity.

In order to evaluate the applicability of the proposed MWCNT-G/GCE for nicotine determination in real samples, two brands of cigarette samples were analyzed. Spike and recovery procedure was adopted for testing the validation of the proposed method. As shown in Table 1, the proposed nicotine electrochemical sensor showed an excellent performance towards nicotine detection in the samples of cigarettes, suggesting our proposed method is an effective analytical tool for nicotine detection in the real samples.

Table 1. Determination of nicotine content in two brands of cigarettes using MWCNT-G/GCE.

Sample	Addition (μM)	Found (μM)	Recovery (%)
1	0	20.16	—
	10	30.25	100.29
	20	40.23	100.17
	30	49.87	99.42
2	0	5.55	—
	20	24.45	95.69
	50	55.11	99.21
	100	104.99	99.47

4. CONCLUSION

In conclusion, we proposed a novel electrochemical nicotine sensor based on MWCNT-G composite. A simple approach was used for dispersing graphene into surface functionalized MWCNT dispersion. The MWCNT-G composite showed an excellent enhancement of electrochemical performance for GCE. The MWCNT-G modified GCE was successfully applied for electrochemical determination of nicotine. A wide detection range with a low detection limit was achieved in this study. Moreover, the MWCNT-G/GCE also showed excellent reproducibility, stability and long-term storage stability.

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