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Development of an Electrochemical Sensor for Determination of Ligustrazine Based on Nitrogen-Doped Multi-Walled Carbon Nanotube

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In this work, β-cyclodextrin and nitrogen-doped multi-walled carbon nanotube (NCNT) were directly incorporated to graphite powder, and an electrochemical treatment was implemented for the fabrication of a new sensor. The characteristics of the as-prepared electrode were studied using cyclic voltammetry (CV) measurement. Due to the synergic effects of β-cyclodextrin and NCNT, the response of our developed sensor for ligustrazine was more significant than that of other electrodes. Our developed sensor exhibited excellent performance in the quantitative determination of ligustrazine in Ligusticum wallichii.

Keywords: Ligustrazine; β-cyclodextrin; Nitrogen-doped multi-walled carbon nanotube; Electrochemical determination; Ligusticum wallichii

1. INTRODUCTION

It has been widely accepted that cardiovascular disease poses great risk to human health. One of the most common causes of cardiac-cerebral vascular disease is thromboembolism. Hence thrombus prevention has been a great concern in the therapeutic field of cardiovascular disease. As a typical conventional Chinese medicine, Ligusticum wallichii can be used for blood-activating and stasis-dissolving. And Ligustrazine (chemical name, 2,3,5,6-tetramethyl pyrazine, abbreviated as TMPZ) is one of the main active ingredients in Ligusticum wallichii [1]. TMPZ has gained extensive use in the treatment of proliferative vitreoretinopathy [2], atherosclerosis [3], reperfusion injury [4], expansion of

coronary artery [5], anticoagulation, antiplatelet aggregation [6], and against myocardial ischemia [7], as shown in recent reports. Therefore it is of great necessity to clinically determine TMPZ, considering the aforementioned applications. Currently several strategies have been reported for the TMPZ detection, including capillary electrophoresis and HPLC [8, 9]. However, the aforementioned strategies are not suitable for fast on-site monitoring, due to their requirement of time-consuming specimen pretreatment, highly professional operators, despite their desirable precision and sensitivity. However, an electrochemical technique is relatively cost-effective, time-saving, simple in operation, rapis in response, and can provide real-time in-situ determination.

As we know, carbon nanotubes (CNTs) possesses extensive range of usages, remarkable characteristics and distinct structure, and has become increasingly appealing in diverse research fields in the last decade [10, 11]. In addition, CNT-based sensors have gained extensive application in the determination of molecules including NADH [12], uric acid [13], homocysteine [14], ascorbic acid [15], dopamine [16], nitric oxide [17], H_2O_2 [18], glucose [19], hesperidin [20], DNA [17], and TNT [21], due to their excellent electron transfer kinetics, low limit of detection (LOD), along with desirable sensitivity. The analytical behaviors of the above sensors are affected by the biocompatibility, solubility, and electronic features of CNTs. Normally, these characteristics are significantly influenced by the surface structure, including the number of functional groups and defective sites present on the ends and side walls of CNTs [17, 22, 23]. To ensure the favorable features including excellent catalytic behavior, solubility, and sensing ability, some techniques have been developed such as physical adsorption, covalent bonding, etc. [24]. For instance, the conversion of the defective sites on the CNTs surface into carboxylic functional groups occur during the acid treatment [24]. The resultant CNTs are excellent in electrocatalytic activity, wettability, and dispersion for the ascorbic acid and homocysteine oxidation at a low potential. Under alkaline solution, the redox-mediation of the oxygen reduction could occur in the presence of the oxygen-containing groups on the tube surface [25]. Furthermore, the modification of the CNTs could be achieved using several organic compounds having redox-mediation features (such as orthoquinone [17] and toluidin blue O [26]) via π–π electronic or hydrophobic interactions. The combination of a redox mediator and CNTs contributes to adequate electrocatalysis, thus facilitating the CNTs functionalization for electrochemical utilities. Several metal particles could be encapsulated in the interior of the nanotubes or decorate the walls of the nanotubes [27] to improve the catalytically activity [28].

An effective strategy has been proposed for regulating the electronic and structural features of the nanotubes, where substantial defective sites could be formed on the surfaces of the nanotube after the CNTs are doped with N [29-31]. For instance, Pt-based nanoparticles could be immobilized in the absence of premodification by the direct use of the NCNTs ascribed to involvement of nitrogen, thus facilitating the electrocatalysts preparation [32, 33]. Therefore, this report investigated the features of TMPZ based on the NCNTs using a sensitive and facile electroanalytical strategy, with a linear range of 2.0×10^{-7} to 1.0×10^{-5} mol L^{-1}. And the LOD was calculated as 1×10^{-7} mol L^{-1}. Our developed strategy provided repeatable and accurate detection of the TMPZ content in Ligusticum wallichii.

2. EXPERIMENTS

2.1. Chemicals

Chemical vapor deposition was carried out at 650 °C for the synthesis of NCNTs (nitrogen accounting for 3−5%), and the precursor was pyridine. Then sequential reflux was implemented on the above NCNTs in NaOH (6 M) and HCl (6 M) aqueous solution in turn for 4 at 110 °C, leading to the removal of the Al_2O_3 support and metal catalysts, respectively. This was followed by thorough washing of the as-prepared NCNTs using distilled water till the pH 7 (the filtrate), along with drying overnight at 70 °C before following experiments. Multi-walled carbon nanotubes (MWCNTs, diameter range, 10−20 nm; purity, ca. 95%) were present in CNTs that were commercially available in Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). TMPZ was of analytical grade, and was commercially available in Aladdin Co. Ltd. (Shanghai, China). Methanol was used as the source material for 1 mM standard stock solution of TMPZ, which was then stored below 4 °C. β-cyclodextrin (β-CD) and $ZrOCl_2$ nanoparticle were commercially available in Aladdin Co. Ltd. (Shanghai, China).

2.2. Instrument

An electrochemical analyzer (CHI832B, CHI Instrument) controlled by a computer was used for the electrochemical assays, consisting of a traditional triple-electrode configuration. Herein the working, reference, and counter electrodes were modified glassy carbon electrode (GCE), saturated calomel electrode, and a platinum wire. For the amperometric experiment, the convective transport was achieved using a magnetic stirrer. Rotating ring-disk electrode (RRDE) voltammetry was performed using a speed controller (HP-1A, Jiangsu, China) and CHI 900C electrochemical workstation (Shanghai, China), equipped with a modified glassy carbon ring-disk electrode (glassy carbon core diameter, 5 mm; outer diameter, 9 mm) and a Pt ring (+0.5 V for polarization) under oxygen condition.

2.3. Electrode fabrication

After successive polishing with alumina powder (1.0 μm and 0.3 μm), the GCE was completely rinsed using double-distilled water. Then the GCE was successively sonicated in acetone, nitric acid, and double-distilled water (1:1), followed by rising using double-distilled water, and drying at ambient temperature. MWCNT modified GCE or NCNT modified GCE were obtained after dropping 10 μL of MWCNT or NCNT suspension (2 mg/mL) onto the as-prepared GCE surface (or dropping 5 μL of MWCNT or NCNT suspension (2 mg/mL) onto the GCE disk section for RRDE), along with drying in vacuum condition. On the other hand, certain amount of β-CD and NCNT was obtained after accurate weighing, and then introduced to graphite powder. This was followed by thorough mixing of the above mixed solution in a quartz mortar. Then the as-prepared mixed solution was further mixed with paraffin, followed grinding for 0.5 h to obtain a homogeneous paste. This was followed by tightly packing the as-prepared paste into one side of a Teflon tube by hand, and inserting a copper wire to the

above tube on the other end. Finally the terminal product was obtained as β-CD-NCNT modified GCE.

2.4. Real sample preparation

Ligusticum wallichii was initially ground using a mortar, followed by accurate weighing to obtain three portions (1 g for each). After separate dispersion of these portions into 10 mL of methanol solution (80%), ultrasonic wave was applied for 0.5 h. This was followed by the mixture centrifugation at 3000 rpm for 10 minutes. And the supernatant was separately reserved. We repeated the extraction for one, two and three times, for the first, second and last portions, respectively. Eventually the supernatant of these portions was mixed, followed by volatilization until a volume of 10 mL under a water bath before following experiments.

3. RESULTS AND DISCUSSION

The peak currents of TMPZ are proportional to the scan rates in the range of 100–1000 mV/s, which indicates that the electrode reaction of TMPZ is controlled by the adsorption process [34]. The electrochemical features of the test electrodes were compared using CV measurement, and the probe was 1 mM $Fe(CN)_6^{3-/4-}$ that contained 0.1 M KCl. The original GCE exhibited a weak electrochemical response (Fig. 1A). Nevertheless, the GCE after the MWCNT addition showed no current response towards $Fe(CN)_6^{3-/4-}$. Then a pair of significant redox peaks was observed after doping. Compared to the original GCE, the NCNT modified GCE showed larger peak current and smaller peak-to-peak potential separation (ΔE_P), suggesting effective surface area and the charge transfer rate were both improved in the presence of NCNT. Hence it was essential to electrochemically pre-treat the β-CD-NCNT modified GCE. Fig. 1B showed much higher charge current and peak currents obtained at the β-CD-NCNT modified GCE, even compared with the sum of that obtained at the β-CD modified GCE and β-CD-MWCNT modified GCE.

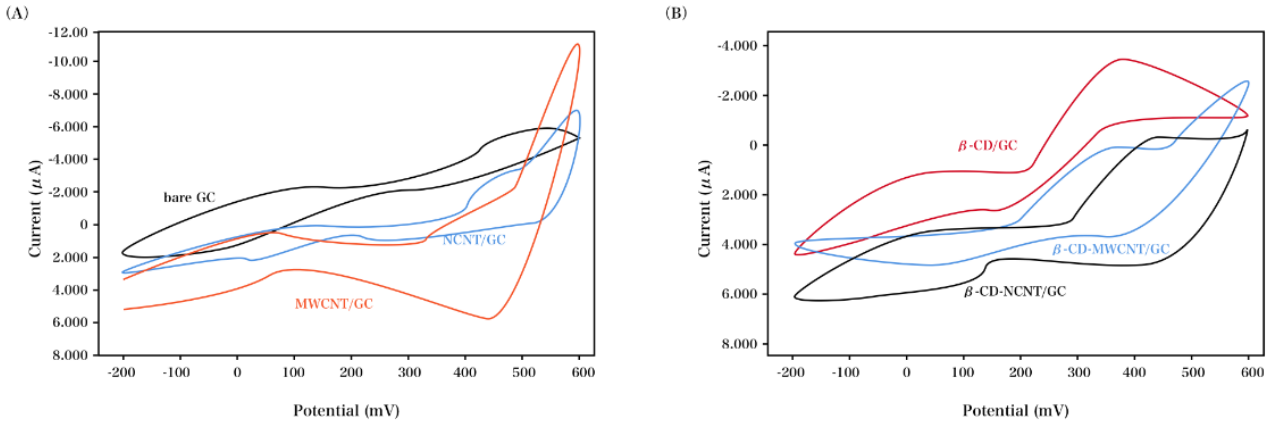

Figure 1. CVs of 1 mM $[Fe(CN)_6]^{3-}$ that contained 0.1 M KCl on varying electrodes as follows: (A) the original GCE, MWCNT modified GCE, NCNT modified GCE and (B) β-CD modified GCE, β-CD-MWCNT modified GCE, β-CD-NCNT modified GCE at a scan rate of 0.1 V/s.

As shown in the N 1s spectra of XPS (Fig. 2), three well-defined peaks were shown for the NCNT at ca. 404.9 eV, 401.5 eV, and 398.9 eV. The distribution of the peaks varied in different reports, since the nitrogen atoms possessed diverse chemical states, and the CN reference materials were absent. The quantum-chemical calculations on the N1s binding energies confirmed the peak distribution [35]. The molecular form of nitrogen was indicated by the N 1s peak at 404.9 eV [36], since it can be seen that the corresponding characteristic observed in X-ray absorption spectra at the NK-edge of CN_x nanotubes evolved to a fine structure ordinarily recorded in the vibrational fine structure of the $\pi*$ resonance of N_2 gas [37].

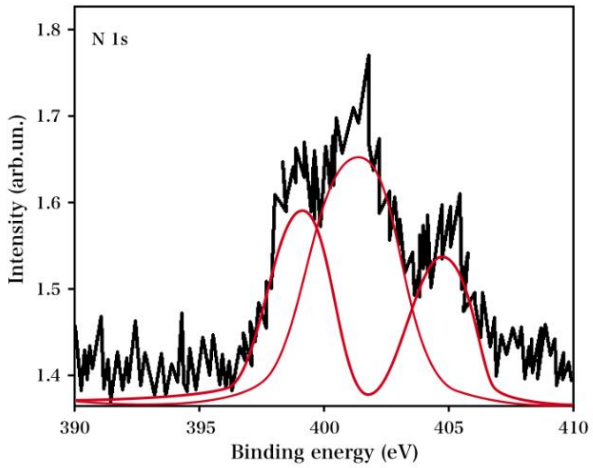

Figure 2. N 1s spectrum of NCNT in XPS.

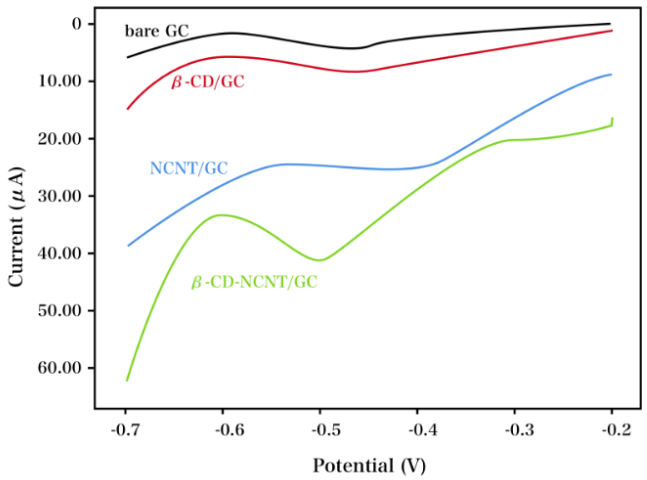

Figure 3. SWV profiles of 3 μM TMPZ on the original GCE, β-CD modified GCE, NCNT modified GCE, β-CD-NCNT modified GCE in a blank solution.

Square wave voltammetry (SWV) measurement was performed to investigate the electrochemical features of TMPZ in pH 1.4supporting electrolyte (sulfuric acid) using varying electrodes. Compared with the original GCE, the NCNT modified GCE and β-CD modified GCE

exhibited enhanced response towards TMPZ (Fig. 3). In specific, the response towards TMPZ obtained at the GCE after simultaneous addition of NCNT and β-CD along with an electrochemical treatment, was more significant compared with the sum of that obtained at the NCNT modified GCE and β-CD modified GCE. It is worth noting that when the concentration of TMPZ is low, the cathodic peak increases in an unpredictable manner [38]. This increase was ascribed to the synergic effects of NCNT and β-CD.

Voltammetric strategies including differential pulse voltammetry (DPV), SWV, linear sweep voltammetry (LSV), and CV measurements were performed and compared in their response sensitivity towards TMPZ. Through comparison, the optimal response towards TMPZ was observed using SWV, and determined as the analytical technique for TMPZ. Overlap voltammograms obtained using the β-CD-NCNT modified GCE with varying concentrations of TMPZ were displayed in Fig. 4A. As indicated in Fig. 4B, the concentration was found linearly related with the current with the gradual concentration increase (linear range, 0.2 to 10 μM). And the LOD was calculated as 0.1 μM.

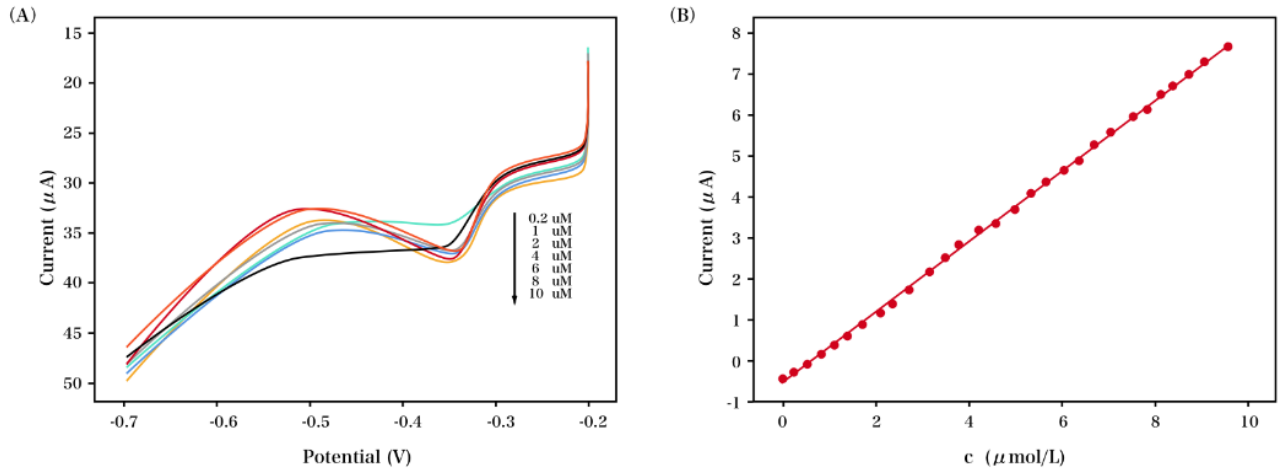

Figure 4. (A) SWV profiles of TMPZ on β-CD-NCNT modified GCE with varying concentrations. (B) The ipa vs. TMPZ concentration plot.

The effect of several potential co-present agents in the TMPZ detection was investigated. Under the same test condition, we assessed a certain amount of TMPZ containing other species using SWV. 10-fold inorganic ions (including SO_4^{2-}, CO^{3-}, Co^{2+}, Cu^{2+}, Mg^{2+}, Zn^{2+}), 5-fold glucose, dopamine, ascorbic acid, and uric acid produced no interfering effects, when the peak current response error was controlled no more than ca. 5%. Therefore, this method can be used for the rapid determination of TMPZ [39]. Investigations were carried out to obtain the optimum conditions for TMPZ detection. The pH of the working solution significantly alters the electrochemical sensitivity of TMPZ. We observed that the peak current of TMPZ increases with increasing pH until pH 7.0, and decreases when the pH is over 7.0. Therefore, we chose pH 7.0, the most sensitive pH, as the optimum pH in this work. The adsorptive behavior of TMPZ at the electrode surface was also examined [40, 41]. The peak intensity of TMPZ increases in line with the adsorption time, reaching its maximum at about 30 s.

We also studied the stability and repeatability of the β-CD-NCNT modified GCE, with the relative standard deviation (RSD) obtained as 2.4% after eight consecutive measurements. The response currents recorded after the electrode preparation was repeated for five times were rather similar, and the RSD was 2.7%. The above results suggested the remarkable repeatability of the as-prepared β-CD-NCNT modified GCE towards the TMPZ detection. The proposed electrode was stored at ambient temperature in dry atmosphere for 1 month, with measurements performed every two days. Within 1 month, no obvious variation in the response was observed, suggesting the desirable stability of the β-CD-NCNT modified GCE, and the potential to be employed for TMPZ detection. To allow for realistic comparison to previous reports, the characteristics of different electrochemical sensors for TMPZ are summarized in Table 1.

The feasibility and the reliability of our developed strategy were investigated using Ligusticum wallichii (Chinese herbal medicine) as the real specimen. According to the determination of TMPZ content in three specimen portions, the specimen two showed higher content than the specimen one, and the same content with the specimen three, suggesting that three times of extraction was enough, and we determined specimen two as the test specimen. Table 2 displayed the results of the recovery test using the standard addition method. 17.54 mg/g was obtained as the TMPZ content in this real specimen. Our developed strategy showed desirable specificity and reliability, as shown in the RSD and recovery results.

Table 1. Comparison of the major characteristics of electrochemical sensors used for the detection of TMPZ.

Electrode	Linear detection range	Detection limit	Reference
Glassy carbon electrode	1 - 100 μM	0.7 μM	[34]
Carbon electrode	0.5 - 10 μM	0.25μM	[42]
β-CD-NCNT modified GCE	0.2 - 10 μM	0.1 μM	This work

Table 2. Results of TMPZ detection in Ligusticum wallichii (n = 4)

Found	Addition	Total found	Recovery (%)	R.S.D (%)
3.132 μM	2 μM	5.127 μM	99.90	2.54
2.619 μM	2 μM	4.633 μM	100.30	3.61
4.228 μM	2 μM	6.301 μM	101.17	1.85
4.341 μM	2 μM	6.288 μM	99.16	3.22

4. CONCLUSIONS

This study proposed the fabrication of a new electrochemical TMPZ sensor based on the β-CD-NCNT modified GCE for the sensitive voltammetric detection of TMPZ. The sensitive TMPZ detection on the β-CD-NCNT modified GCE was achieved by the investigation on the TMPZ reaction mechanism and on the analytical techniques. The detection strategy used was characteristic of

convenience, as well as desirable stability, sensitivity, and accuracy. Our developed sensor could be successfully used to quantatively analyze TMPZ in real specimens.

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