Magnetism-assisted modification of screen printed electrode with magnetic multi-walled carbon nanotubes for electrochemical determination of dopamine

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Abbreviations

DA: Dopamine; AA: Ascorbic acid; UA: Uric acid; CNTs: Carbon nanotubes; CNT-

N: Carbon nanotube nanoweb; MWCNTs: Multi-walled carbon nanotubes; mMWCNTs: Magnetic multi-walled carbon nanotubes; MNPs: Magnetic nanoparticles; GCE: Glass carbon electrode; CPE: Carbon paste electrode; MCPE: Modified carbon paste electrode; SPE: Screen printed electrode; m/SPE: Magnetic nanoparticles modified electrode; mMWCNTs/SPE: Magnetic multi-walled carbon nanotubes modified electrodes; PBS: Phosphate buffered saline; HPLC: High performance liquid chromatography; CV: Cyclic voltammetry; SWV: Square wave voltammetric; TEM: Transmission electron microscope; EDS: Energy dispersive spectrometry; VSM: Vibrating sample magnetometer; LOD: Limit of detection.

ABSTRACT:

A simple and sensitive dopamine (DA) electrochemical sensor was fabricated based on magnetism-assisted modification of screen printed electrode (SPE) with magnetic multi-walled carbon nanotubes (mMWCNTs). The mMWCNTs modified

electrodes (mMWCNTs/SPE) combines the advantages of SPE and the simultaneous contribution of magnetic nanoparticles (MNPs) and MWCNTs, increasing sensitivity and selectivity of DA detection. The linearity was found between 5 μ M to 180 μ M, with the limit of detection (LOD) of 0.43 μ M. In the mean time, this modified electrode exhibited excellent selectivity for DA detection with almost no interference from ascorbic acid (AA), which co-exists with DA in many bio-samples and causes common interference. Finally, this novel electrode has been applied to determine DA concentration in spiked human blood serum and satisfactory recovery was found in the range of 97.43 - 102.94% with the RSDs of less than 2.27%. This work developed a sensitive and reliable electrochemical analytical method based on mMWCNTs/SPE, which exhibits great potential for diagnosis of the diseases related to DA.

Keyword: Magnetic multi-walled carbon nanotubes; Screen printed electrode; Electrochemistry; Dopamine

1. Introduction

Dopamine (DA) is a catecholamine neurotransmitter that plays pivotal roles in the functioning of renal and hormonal systems [1]. Abnormal alterations of DA level might cause several neurologic diseases such as Parkinsonism, schizophrenia, senile dementia and HIV infection [2, 3]. Various analytical methods have been reported to analyze DA in biofluids, including high performance liquid chromatography (HPLC)

[4], spectrophotometry [5], ion-exchange chromatography [6], fluorescence [7] and capillary electrophoresis [8]. However, these methods exhibited certain disadvantages such as dependence on expensive and sophisticated instrumentation, complicated operation procedures and long analysis time. Therefore, it is important to develop rapid, simple, accurate, sensitive and cost-effective method for quantitative determination of DA.

Recently, electrochemical approaches have attracted considerable interests for detection of DA due to the high sensitivity, ease to operate, fast response and low cost [9]. But a major problem comes from the interference of ascorbic acid (AA) which possesses close oxidation potential to DA on a traditional electrode and has much higher concentration in serum samples [10]. In order to overcome this difficulty, various modified electrodes have been explored such as metal complexes [11], metal nanoparticles [12, 13], conducting polymers [14] and carbon nanomaterials [15, 16]. Considering the different pK_a of these two compounds (8.87 for DA and 4.10 for AA) [17], modifying the electrode with negatively charged material is a good choice to detect DA from AA.

Carbon nanotubes (CNTs) have been widely applied for the development of chemical sensor, gas adsorbents, catalyst supports due to their excellent electrical conductivity, high surface area, remarkable mechanical strength and good chemical stability [18]. Electrodes modified with CNTs exhibited significantly enhanced electron transfer efficiency owing to the high electronic conductivity and the topologic defects of the CNTs [19]. The carboxylated CNTs can be deprotonated at pH 7.0 and thus are negatively charged, whereas DA was dissociated as cationic and AA as anionic. As a result, carboxylated CNTs modified electrode can effectively

repel negatively charged AA and thus only respond to positively charged DA. Therefore, carboxylated CNTs modified electrode can exhibit high selectivity for determination of DA in the presence of AA [20]. However, there exist many problems when using carboxylated CNTs to modify all the electrodes. For example, in the straightforward manual modified carbon paste electrode (MCPE) preparation process involving the grind of the modifier (carboxylated CNTs or CNT-based materials), the graphite powder and the binder (paraffin oil or silicone oil), it is difficult to distribute the modifier homogenously in the MCPE, and the refreshment process is time consuming [21]. On the other hand, casting method usually used for the preparation of glass carbon electrode (GCE) [22] and screen printed electrode (SPE) modification [23] presents the disadvantages of tedious operating procedure including electrode pretreatment, electrode modification and electrode refreshment, the difficulty in controlling the thickness and homogeneity of modifier films, and the weak physical stability of the modified electrodes [24]. Recently, Fe_3O_4 magnetic nanoparticles (MNPs) have exhibited promising potential for application in magnetic separation, target drug delivery, sensing and imaging for the superparamagnetism property [25-28]. They have also been widely used to modify electrodes to increase the electrochemical response due to their large surface area to volume ratio, excellent catalytic activity and high electron transfer rate [29]. N-doped carbon nanotubes functionalized Fe_3O_4 nanoparticles was synthesized to modify GCE to discriminate AA, DA and uric acid (UA) [30], and multi-walled carbon nanotubes decorated with magnetic core-shell $\text{Fe}_3\text{O}_4@\text{SiO}_2$ was used to prepare a MCPE for detection of UA [31]. However, both examples used only the high electrocatalytic activity of CNTs and MNPs, making no use of the superparamagnetism property of MNPs which can

bring easiness for preparation of the modified electrodes. The modification is simply and conveniently carried out by magnetically immobilizing modifier onto the surface of electrode with the external magnetic field, resulting in a stable modifier film on the electrode surface. The electrode is modified by only one step, which greatly simplifies the preparation of electrode to avoid the possible instability and modifier activity loss resulted from multiple modifications. Moreover, the MNPs-based materials modified electrode can be used straightforward for sample analysis, avoiding the long time of drying necessary for the other modified electrodes. In the mean time, among the three most frequently used electrodes, superior to the “beaker”-type reaction cells of bulky electrodes (GCE and CPE), SPE can achieve the miniaturization and diversification of biosensors for on-line determination of a droplet analyte [32]. Therefore, a novel modified SPE combing all the advantages of CNTs and MNPs will afford a promising solution for identification of DA in biofluids.

In this work, magnetic multi-walled carbon nanotubes (mMWCNTs) synthesized by a modified polyol-medium solvothermal method were immobilized on the surface of SPE to fabricate a novel electrode (mMWCNTs/SPE) for the detection of DA. The electrochemical behaviors of DA on different electrodes were investigated by cyclic voltammetry (CV), and the electrochemical determination of DA was carried out on mMWCNTs/SPE by square wave voltammetric (SWV). Additionally, the reproducibility, repeatability and selectivity of the modified electrode were investigated by SWV. Finally, mMWCNTs/SPE was used for determination of DA in spiked human blood serum successfully.

2. Experimental

2.1 Materials

Ferrous chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), trisodium citrate, sodium acetate and poly (ethylene glycol) of average mol. wt 2000 were supplied by Chengdu Tianhua Chemical Technology Co., Ltd. (Chengdu, China). MWCNTs with 20 - 40 nm in diameter were purchased from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). Dopamine hydrochloride and ascorbic acid were purchased from Sigma-Aldrich. SPEs were obtained in Shanghai Molybdenum Magnesium Electronic Technology Co., Ltd. (Shanghai, China). Phosphate buffered saline (PBS) (0.1 M pH 7.0) was freshly prepared. Other chemicals and solvents were all of analytical reagent and used as received.

2.2 Apparatus

Cyclic voltammetry (CV) and square wave voltammetry (SWV) measurements were carried out on EC 550 electrochemical workstation (Wuhan Gaoss Union Instruments Cot., China). Carbon electrode, saturated Ag/AgCl and the mMWCNTs modified carbon electrode were used as the counter electrode, reference electrode and working electrode of SPEs, respectively. The morphology of the carboxylated MWCNTs and mMWCNTs was explored by transmission electron microscope (TEM) (Hitachi HT7700, Japan). Chemical composition of mMWCNTs was confirmed by energy dispersive spectrometry (EDS) (BRUKER QUANTAX400, Germany). Magnetic properties of MNPs and mMWCNTs were measured on a vibrating sample magnetometer (VSM) (Lakeshore 7307, USA). All measurements were carried out at the room temperature.

2.3 Material synthesis

The mMWCNTs were synthesized by a modified polyol-medium solvothermal method [33] as shown in Fig. 1A. Briefly, the pristine MWCNTs (0.40 g) were dispersed in 50 mL of concentrated nitric acid at 60 °C with magnetic stirring for 7 h to obtain the carboxylated MWCNTs. The carboxylated MWCNTs were rinsed several times with distilled water until the pH value reached 7.0 and then dried by a vacuum freeze dryer. The dried carboxylated MWCNTs (0.15 g) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.81 g) were dispersed in 40 mL of ethylene glycol solution by ultrasonication for 2 h, and trisodium citrate (0.15 g), sodium acetate (3.60 g) and poly (ethylene glycol) of average mol. wt 2000 (1.0 g) were then added to be magnetic stirreing for 1 h to form a stable solution. Afterwards, the mixture was sealed in an autoclave and kept at 200 °C for 10 h. The autoclave was then cooled down to room temperature naturally, and the gained mMWCNTs were washed with anhydrous ethanol and distilled water thoroughly. The same procedure was employed to synthesize MNPs without adding carboxylated MWCNTs. The MNPs and mMWCNTs were dried by vacuum freeze dryer and stored for further use.

2.4 Electrode preparation

A magnet was firstly fixed to the bottom of the SPE reaction cell. The mMWCNTs (0.006 g) were dispersed in 2 mL of 0.1M PBS (pH 7.0) to be vigorously shaken to obtain a homogeneous solution, and 10 μL of which was dropped on the SPE to form the mMWCNTs modified electrode (mMWCNTs/SPE). In addition, the MNPs modified electrode (m/SPE) was prepared by the same procedure by dropping MNPs solution on SPE. The modification process for electrodes was depicted in Fig.

1B. On the other hand, we also immobilized mMWCNTs onto the SPE through casting method. Briefly, 10 μL of homogeneous solution of mMWCNTs was cast on the SPE surface and then left to dry in air, yielding the modified SPE with non-magnetic immobilized way which was denoted as * mMWCNTs/SPE.

Fig. 1. (A) Synthetic route of mMWCNTs. (B) magnetism-assisted modification of SPE with mMWCNTs and electrochemical response of DA on mMWCNTs/SPE.

3. Results and discussion

3.1 TEM characterization of the carboxylated MWCNTs and mMWCNTs

The morphologies and microstructures of the carboxylated MWCNTs and mMWCNTs were observed by TEM. As shown in Fig. 2A, the carboxylated MWCNTs exhibited tubular structure and their walls were relatively smooth, indicating the structure of MWNTs has not been largely impaired upon acid treatment. The diameter and length of the carboxylated MWCNTs were about 25 - 35 nm and several micrometers, respectively. In the mean time, Fig. 2B showed that the

carboxylated MWCNTs were covered with spherical MNPs with an average diameter of about 180 nm, confirming the successful modification of MNPs on the surface of the carboxylated MWCNTs.

Fig. 2. TEM images of (A) the carboxylated MWCNTs and (B) mMWCNTs.

3.2 EDS and VSM characterization of the nanocomposites

The EDS spectrum was recorded to analyze the chemical composition of mMWCNTs. Fig. 3A revealed that the weight ratio for C, O and Fe in mMWCNTs was 45.52, 21.72 and 32.76%, respectively. Magnetic properties of MNPs and mMWCNTs were analyzed by VSM at room temperature. The magnetization curves for both materials showed no coercivity and remanence as in Fig. 3B, indicating that they had no hysteresis and thus possessed superparamagnetism property. Compared to the saturation magnetization of MNPs ($155.30 \text{ emu g}^{-1}$), the value of mMWCNTs (56.62 emu g^{-1}) was much lower, suggesting a tight binding pattern between MNPs and MWCNTs. According to saturation magnetization values, the content of MNPs in mMWCNTs was calculated to be 36.46%, which was in agreement with the weight ratio of Fe obtained by EDS. Therefore, this magnetic nanocomposite could be used to modify SPE by simply fixating an external magnet under the reaction cell.

Fig. 3. (A) EDS spectrum of mMWNTs. (B) Magnetization curves of MNPs (a) and mMWNTs (b).

3.3 Electrochemical characterization of the modified electrodes

The electrochemical behavior of different modified electrodes was characterized by CV in the presence of $K_3[Fe(CN)_6]$ as a redox probe. In the CV experiment, the potential range was set from -0.2 to 0.6 V at a scan rate of 50 mV s^{-1} . As shown in Fig. 4, a pair of well-defined redox peaks was observed on different electrodes. Compared to the bare SPE (curve a), m/SPE (curve b) produced lower redox peak currents which was probably due to the aggregation of MNPs that diminished the electrochemical response of the electrode [34]. On the other hand, mMWNTs/SPE (curve c) generated much higher redox peak currents, indicating an improved electrochemical activity toward $[Fe(CN)_6]^{3-/4-}$. This improvement could be explained by the increased electron transfer rate at the electrode/electrolyte surface, which was attributed to the excellent electrical conductivity of MWCNTs. To investigate the role of the magnet, the redox peak currents of $[Fe(CN)_6]^{3-/4-}$ on mMWNTs modified electrodes prepared by magnetic and non-magnetic immobilization were compared. It was found that there was no significant difference between the two modified SPEs,

i.e. mMWCNTs/SPE (curve c) and *mMWCNTs/SPE (curve d), indicating that the magnet merely holds the mMWCNTs onto electrodes without other electrochemical roles. In addition, the background currents were significantly increased on mMWCNTs/SPE and *mMWCNTs/SPE, which was probably resulted from the enlarged SPE effective surface area brought by the large surface and nanograde size of mMWCNTs [35]. To verify this assumption, the effective surface areas (A) of the different electrodes were estimated by the $K_3[Fe(CN)_6]$ redox system. For a reversible process, the A value could be calculated according to the following Randles-Sevcik formula [36]:

$$(1)$$

where I_{pa} refers to the oxidation peak current, n the electron transfer number, C_0 the molar concentration of $K_3[Fe(CN)_6]$, D the diffusion coefficient for $K_3[Fe(CN)_6]$, and ν the scan rate. Under the optimized conditions ($n = 1$, $\nu = 0.5 \text{ V s}^{-1}$, $C_0 = 5 \times 10^{-6} \text{ mol cm}^{-3}$, $D = 7.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) in this work, the A values of bare SPE, m/SPE, mMWCNTs/SPE and *mMWCNTs/SPE were 0.029, 0.026, 0.081 and 0.080 cm^2 , respectively. The effective electrode area of mMWCNTs/SPE and *mMWCNTs/SPE was nearly 2 times greater than that of bare SPE, suggesting that high surface effect of mMWCNTs enhance electrochemical response towards the electroactive molecules.

Fig. 4. CV of 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with 0.1 M KCl on bare SPE (a), m/SPE (b), mMWCNTs/SPE (c) and *mMWCNTs/SPE (d) at a scan rate of 50 mV s^{-1} .

3.4 Electrochemical behavior of DA on mMWCNTs/SPE

Fig. 5 showed the CV response to 0.1 M PBS (pH 7.0) with or without $100 \mu\text{M}$ DA on the modified and unmodified electrodes with a scan rate of 50 mV s^{-1} . It was observed that there was no redox peaks in the blank PBS (pH 7.0) on bare SPE (curve a1), m/SPE (curve b1) and mMWCNTs/SPE (curve c1). When DA was added to the PBS, the CV obtained on the bare SPE (curve a) displayed a pair of broad and asymmetric redox peaks with a weak peak current. The anodic (E_{pa}) and cathodic (E_{pc}) peak potentials were 0.23 and -0.11 V with a peak separation potential (ΔE_{p}) of 0.34 V. For DA, m/SPE (curve b) did not lead to significant changes in current compared with bare SPE (curve a), which was probably due to the aggregation of MNPs that diminished the electrochemical response of the electrode [34]. But the redox process become less irreversible as the ΔE_{p} reduced to 0.25 V ($E_{\text{pa}} = 0.18 \text{ V}$ and $E_{\text{pc}} = -0.07 \text{ V}$). For mMWCNTs/SPE (curve c), the appearance of a pair well-defined

redox peaks with $\Delta E_p = 0.17$ V ($E_{pa} = 0.13$ V and $E_{pc} = -0.04$ V) showed that the reversibility of DA redox process changed better [37]. It was clear that the anodic peak potentials of DA on m/SPE (curve b) and mMWCNTs/SPE (curve c) had a negative direction shift, and the cathodic ones shifted to positive direction compared to the bare SPE (curve a), indicating that MNPs and mMWCNTs could promote electron transfer [12, 38]. Moreover, the anodic and cathodic peak current signals of DA oxidation on mMWCNTs/SPE (curve c) were substantially enhanced in comparison with bare SPE (curve a) and m/SPE (curve b). The best performance of DA oxidation on mMWCNTs/SPE might be due to the following reasons: Firstly, the carboxylated MWCNTs functionalized with rich carboxyl groups are deprotonated at pH 7.0 and thus are negatively charged [20]. The positively charged DA can effectively bind to negatively charged carboxyl groups of mMWCNTs by electrostatic interactions. Secondly, the easy enrichment of DA to the modified SPE by π - π stacking to MWCNTs, will enhance the electrochemical response [39]. Thirdly, the edge plane defects and the large edge plane to basal plane ratio of MWCNTs are believed to be able to ease the electron transfer kinetics [40, 41]. Fourthly, MNPs can also accelerate the electron transfer rate (increase the sensitivity in detection) [30]. To sum up, mMWCNTs/SPE exhibited excellent electrochemical response to DA due to the simultaneous contribution of MNPs and MWCNTs, suggesting it is a more suitable method for DA determination.

Fig. 5. CV of 0.1M PBS (pH 7.0) in the absence (a1, b1, c1) and presence of 100 μM DA (a, b, d) on bare SPE (black line), m/SPE (red line) and mMWCNTs/SPE (blue line) at a scan rate of 50 mV s⁻¹.

3.5 Effect of scan rate

The effect of scan rate on the electrochemical response of DA on mMWCNTs/SPE was tested in 100 μM DA in 0.1 M PBS (pH 7.0) (Fig. 6A). As shown in Fig. 6B, the redox peak currents increased linearly with the increment of scan rate in the range from 20 to 200 mV s⁻¹. The linear regression equations for the anodic and cathodic peak currents of DA were: $I_{pa} (\mu A) = -0.49 v (mV s^{-1}) - 30.17$ ($r^2 = 0.9920$) and $I_{pc} (\mu A) = 0.48 v (mV s^{-1}) + 25.48$ ($r^2 = 0.9911$), respectively. The good linear relationship demonstrated that the oxidation of DA was a typical adsorption-controlled process on mMWCNTs/SPE [42]. Notably, the slope of the equation for the oxidation peak was larger than that of the reduction peak, indicating that the electrode modified by mMWCNTs possessed a higher electrocatalytic activity toward the oxidation process than to the reduction process [43]. In addition, the surface

concentration (Γ) of DA on the surface of mMWCNTs/SPE could be estimated by using the following equation [24]:

$$(2)$$

where I_p is the peak current, F the Faraday, R the universal gas constant, and T the Kelvin temperature. In the experiment, A was 0.089 cm^2 as obtained above, and the electron transfer number involved in DA oxidation is 2 [44]. Based on the slopes of the anodic and cathodic peak currents vs. scan rate, the average surface concentration of DA was calculated to be approximately $1.44 \times 10^{-6} \text{ mol cm}^{-2}$, which is higher than the reported value of $8.385 \times 10^{-10} \text{ mol m}^{-2}$ [45], suggesting the enlarged surface area of mMWCNTs/SPE could offer more adsorption sites for DA.

Fig. 6. (A) CV of mMWCNTs/SPE in 0.1 M PBS (pH 7.0) containing 100 μM DA at different scan rates (a - j: 20, 40, 60, 80, 100, 120, 140, 160, 180, and 200 mV s⁻¹). (B) The linear relationship of anodic (I_{pa}) and cathodic (I_{pc}) peak currents against scan rate.

3.6 Effect of pH

The buffer's pH value is one important factor which plays crucial role in the electrochemical reaction. The influence of pH values on the electrochemical response

of DA on mMWCNTs/SPE was studied in 0.1 M PBS containing 100 μ M DA with SWV method. It was clear that the anodic peaks varied when the pH values ranging from 3.0 to 8.0 (Fig. 7A), and the highest anodic peak was obtained at pH 7.0 (Fig. 7B). In order to achieve higher sensitivity and simulate the biological environment, pH 7.0 was chosen as the supporting electrolyte in this work.

In Fig. 7B, the anodic peak potential shifted negatively with the increment of pH value in the range from 3.0 to 8.0, indicating that protons participated in the electrode reaction [46]. The linear regression equation could be expressed as E_{pa} (V) = 0.46358 – 0.05547 pH ($r^2 = 0.9941$). Based on the following Nernst equation [47]:

$$(3)$$

where n and m refer to the number of electrons and protons, a and b are coefficients of oxidant and reductant involved in the reaction, respectively. The slope value of 55.47 mV/pH matched closely to the Nernst equation theoretical slope value (59.1 mV/pH), suggesting that DA oxidation was a two-proton and a two-electron transferring electrochemical reaction ($n = m = 2$) [48]. The redox mechanism of DA was illustrated in Fig. 1B, where the oxidation product of DA was the corresponding quinine [49].

Fig. 7. (A) SWV of mMWCNTs/SPE in 0.1 M PBS at different pH values (a - f: 3.0,

4.0, 5.0, 6.0, 7.0, and 8.0) in the presence of 100 μM DA; the inset shows the plot of anodic peak current (I_{pa}) against pH of PBS from 3.0 to 8.0. (B) Calibration plots of anodic peak current against pH and the linear relationship of anodic peak potential with pH.

3.7 Electrochemical determination of DA

The electrochemical determination of DA was carried out on mMWCNTs/SPE by SWV with the above optimized conditions. The anodic peak currents exhibited a linear response with increasing DA concentrations as shown in Fig. 8. The linear regression equation was expressed as $I_{\text{pa}} (\mu\text{A}) = -0.35 C_{\text{DA}} (\mu\text{M}) - 3.39$ ($r^2 = 0.9980$) in the range from 5 to 180 μM . The limit of detection (LOD) was 0.43 μM as calculated from the signal-to-noise ratio of 3 ($S/N = 3$). Our linear range is not applicable for common concentration of DA in both normal human body as low as between 0.01 - 1 μM in human brain [10] and between 0.5 - 25 nM in the cerebrospinal fluid [50], nor it is applicable for those abnormal DA related diseases as far as we know. However, abnormally high concentration of DA can cause damage to some nerve cells. For example, 20-50 μM of DA can decrease neuronal NMB cell growth, induce apparent differentiated cell morphology and increase ^3H -dopamine uptake. Even higher concentration (100 - 300 μM) of DA is neurotoxic and induces apoptosis [51]. Therefore, our linear range is useful for judgment of some nerve cell damage relating to abnormality of DA. The analytical performances of the present modified electrode and previously reported CNT-based modified electrodes were summarized in Table 1. The LOD obtained was higher than that of β -CD-MWNTs/PANI and β -CD-MWCNTs/Plu-AuNPs modified GCE, the reason of which might

be due to the formation of inclusion complexes between β -CD and DA, and the high conductivity and electrocatalytic effect of PANI and Plu-AuNPs [45, 52]. However, compared to the electrode modified with the other materials i.e. α -CD/CNT, (SWCNTs/CPB)₃, MWCNT/ β -CD, CNT/PEDOT and HCNTs/GCE, our mMWCNTs modified electrode showed a lower LOD and a broader linear range, which could be attributed to the simultaneous contribution of MNPs and MWCNTs. Furthermore, the mMWCNTs modified electrode in this work can be prepared quickly and simply with the aid of an external magnet. The modifier film is much more stable and is refreshed easier than those manual and casting modified electrodes. Therefore, mMWCNTs modified SPE could serve as a potential platform for DA at trace concentration in various bio-samples.

Fig. 8. (A) SWV of mMWCNTs/SPE in 0.1M PBS (pH 7.0) containing various concentrations of DA (a - j: 0, 5, 10, 20, 40, 60, 80, 100, 140, and 180 μ M). (B) The linear relationship of anodic peak current (I_{pa}) with concentration of DA.

3.8 Interference of AA

It is known that AA co-exists with DA in extracellular fluid of the central nervous system and serum samples with much higher concentration than the latter. In

the mean time, AA possesses the oxidation potential close to DA which will cause overlapping voltammetric response on a traditional electrode. In a control test, AA (curve a) was detected at 0.1V and the mixture of DA and AA (curve c) exhibited overlapping signal on bare SPE (Fig. 9A). Interestingly, no apparent signal was observed for AA (curve a) when mMWCNTs/SPE was used (Fig. 9B). Compared to DA (curve b), the anodic peak current of the mixture of DA and AA (curve c) had no notable changes. Moreover, the I_{pa} for 20 μ M DA (curve b) on mMWCNTs/SPE was 1.5 times higher than that on the bare SPE. The reason for the clearance of the AA interference could be explained as following, i.e. the carboxylated MWCNTs were predominantly deprotonated at pH 7.0, whereas DA was dissociated as cationic and AA as anionic. As a result, mMWCNTs modified SPE effectively repelled negatively charged AA and thus only respond to positively charged DA. Therefore, the modified electrode in this work could be used to well discriminate DA from AA in complex samples.

Fig. 9. SWV of (A) bare SPE and (B) mMWCNTs/SPE in 0.1M PBS (pH 7.0) containing 600 μ M AA (a), 20 μ M DA (b) and 20 μ M DA and 600 μ M AA (c).

3.9 Analysis of DA in human blood serum

In order to evaluate the practical application of proposed sensor, concentration of DA in spiked human blood serum was determined by SWV. In the blank control test, no responses were observed in the 20 times diluted human serum. The recovery and RSD values of the spiked samples were presented in Table 2, and each of the concentrations was repeated for 5 times ($n = 5$). The recoveries were calculated to be in the range of 97.43 - 102.94% and the RSDs of all concentrations were less than 2.27%, which is in agreement with the data reported by some other labs doing the same test [3, 9]. The satisfactory results suggested that this electrochemical analytical method based on mMWCNTs modified SPE was precise and reliable for the analysis of DA in real samples.

3.10 Reproducibility and repeatability of mMWCNTs/SPE

To evaluate the reproducibility of DA response on mMWCNTs/SPE, five mMWCNTs modified electrodes were tested for the detection of 80 μ M DA in 0.1 M PBS (pH 7.0) by SWV. Relative standard deviation (RSD) (2.37%) of five electrodes demonstrated that mMWCNTs/SPE prepared in this work had excellent reproducibility. The repeatability of mMWCNTs/SPE was also examined under the same experimental conditions. The RSD of eight consecutive measurements was 1.82%, revealing an appreciable repeatability of this modified electrode. These results confirmed that mMWCNTs modified SPE possesses good reproducibility and repeatability for DA determination.

4. Conclusions

The mMWCNTs were synthesized by a modified polyol-medium solvothermal

method and characterized by TEM, EDS and VSM. The mMWCNTs not only inherent catalytic ability of the nanomaterials, but also possess the combined advantages of the large surface area and superparamagnetism of MNPs and the high conductivity, fast electron transfer rate and more electroactive interaction sites of MWCNTs. The modified electrodes were characterized by CV. The mMWCNTs modified electrode had higher electrocatalytic activity toward DA oxidation than bare SPE, and it could uniquely respond to DA when AA is presented because of the different electrostatic interactions of DA and AA on mMWCNTs/SPE. Additionally, satisfactory recovery of DA in spiked human serum was achieved in the range of 97.43 - 102.94% with the RSDs of less than 2.27%. Consequently, mMWCNTs/SPE can be used as a sensor for judgment of some nerve cell damage relating to abnormality of DA, and mMWCNTs could act as excellent modifier to modify different electrodes for detection of various electroactive species.

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Tables

Table 1

Comparison of analytical performances of mMWCNTs/SPE with previously reported CNTs modified electrodes for the determination of DA.

Modified electrodes	LOD	Linear range	Technique	Reference
	(μM)	(μM)		
α -CD/CNT/PGE	1.0	2-1000	DPV	[53]
β -CD-MWNTs/PANI/GCE	0.012	0.1-1000	CV	[45]
(SWCNTs/CPB) ₃ /GCE	0.6	4-120	DPV	[54]
MWCNT/ β -CD/GCE	6.7	10-80	CV	[55]
CNT/PEDOT/GCE	10	10-330	DPV	[56]

β -CD-MWCNTs/Plu-AuNPs/ GCE	0.019	1-56	Amperometry	[52]
HCNTs/GCE	0.8	2.5-105	DPV	[57]
mMWCNTs/SPE	0.43	5-180	SWV	This work

CD - cyclodextrin, CNT - carbon nanotube, PGE - pyrolytic graphite electrode, MWCNTs - multiwalled carbon nanotubes, PANI - polyaniline, GCE - glassy carbon electrode, SWCNTs -single-wall carbon nanotubes, CPB - charged cetylpyridinium bromide, PEDOT - poly(3,4-ethylendioxythiophene), Plu- poly(luminol), AuNPs - gold nanoparticles, HCNTs -helical carbon nanotubes, SPE - screen printed electrode.

Table 2

Determination results of DA in spiked human blood serum at mMWCNTs/SPE by SWV (n = 5).

Sample	DA added (μ M)	DA found (μ M)	Recovery (%)	RSD (%)
1	-	< LOD	-	-
2	40	39.78	99.45	2.27
3	80	82.35	102.94	1.77
4	120	116.92	97.43	1.04