Determination of Dopamine Using Screen-printed Carbon Electrode Modified by PEDOT-MWCNTs double-layer Composites

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The poly(3,4-ethylenedioxythiophene) (PEDOT)-MWCNTs composites with double-layer structure ((MWCNTs/PEDOT)$_2$-PEDOT) were prepared on the commercially available screen-printed carbon electrode (SPCE) by a simple solution coating approach. Due to the unique double-layer structure and the good synergistic effect of PEDOT and MWCNTs, the (MWCNTs/PEDOT)$_2$-PEDOT modified SPCE ((MWCNTs/PEDOT)$_2$-PEDOT/SPCE) demonstrated excellent electrocatalytic properties for the oxidation of dopamine (DA). Under the optimal experimental conditions, the oxidation peak currents of DA increased linearly with two concentration intervals over the range of 0.02 - 0.95 μM and 0.0.95 - 15 μM, respectively. And the detection limit (S/N=3) was 0.01 μM. Furthermore, the proposed sensor was applied to the electrochemical determination of DA in Human serum samples, and the recoveries for DA were from 98.3% to 101.5% with relative standard deviation (RSD) between 2.7% and 3.8%. It showed great application prospects in pharmaceutical analysis.

1. INTRODUCTION

Dopamine (4-(2-Aminoethyl) benzene-1, 2-diol, DA) is an important biomolecule in human metabolism process, conveys cognitive, behavioral and motor functions. And it is associated with several neurological disorders, such as Parkinson's disease [1], schizophrenia [2], Tourette's syndrome and addiction [3]. Studies have shown that the abnormal level of DA is the cause of neurological

disorders [4]. Therefore, it is necessary to develop an accurate and sensitive DA detection method for the diagnosis of neurological diseases.

Many traditional assay methods have been developed for the determination of DA, such as high-performance liquid chromatography (HPLC) [5], capillary electrophoresis (CE) [6], and HPLC-mass spectrometry (HPLC-MS) [7]. Compared with these methods, electrochemical detection method has many advantages, such as high sensitivity, simple operation, low cost, rapid detection and feasibility of miniaturization [8, 9], so it has been paid much attention.

In recent years, screen printed electrode (SPCE) has become a research hotspot in the field of electrochemical analysis due to its low power consumption, fast response, simple operation, low reagent consumption, high repeatability and easy miniaturization [10, 11]. SPCEs has been used for portable electrochemical sensors and in situ analysis [12]. In order to fabricate efficient electrochemical sensors, various materials, such as conductive polymers, metal nanoparticles, selective membranes and carbon nanomaterials have been used as electrode modifiers. At the same time, many composite materials with novel and unique structures have been designed and developed for the fabrication of electrochemical sensors based on SPCEs [13].

Conductive polymers have been widely studied in sensing applications because of their easyprocessing, high conductivity and biocompatibility [14, 15]. Poly (3,4- ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT:PSS) is a promising conductive polymer, which can be used to modify SPCEs due to its softness, electronic/ionic conductivity, and printability [14, 16]. Multi-walled Carbon Nanotubes (MWCNTs) have unique structures and properties, such as good electrical conductivity, high electrocatalytic effect and strong adsorptive ability [17]. Recently, the combination of carbon nanomaterials and conductive polymers has become a new research hotspot of electrochemical sensing platform due to the excellent performance and synergistic effect of both inorganic and organic components[18, 19]. Studies revealed that the multi-layer composites have excellent biocompatibility, remarkable conductivity, high operational stability and large active surface area. Furthermore, the multi-layer composites with double-layer structure can obtain better mechanical flexibility, electrochemical stability and coplanar integration capability [20].

Herein, we report the fabrication of PEDOT-MWCNTs multi-layer composites modified SPCE for the determination of DA. The modified SPCEs of different PEDOT-MWCNTs layer structure composites were prepared by a simple solution coating method. The PEDOT-MWCNTs double-layer structure composites modified SPCE ((MWCNTs/PEDOT)$_2$-PEDOT/SPCE) showed good electrochemical performance and it can be used to detect DA in human serum samples.

2. EXPERMENT

2.1. Apparatus and reagents

Electrochemical measurements were performed on a CHI 830 electrochemical analyzer equipped with data processing software (Shanghai Chenhua Instrument Co., Ltd, Shanghai, China). Commercially available integrated three-electrode screen-printed carbon electrode (SPCE) (Metrohm DropSens, Spain) with a carbon working electrode (4 mm diameter), a carbon auxiliary electrode and a silver reference

electrode was used to carry out the experiments. The pH measurements were performed using a pHS-3C digital pH meter (INESA Scientific Instrument Co., Ltd, Shanghai, China).

All reagents were analytical grade and used as received. Dopamine was obtained from Sigma-aldrich (Shanghai, China). Acetic acid, boric acid, phosphoric acid, potassium ferricyanide and potassium ferrocyanide were obtained from Sinopharm Chemical Reagent (Shanghai, China). PEDOT (1% in PSS, w/w) were acquired from Cool chemistry (Beijing, China). Multi-walled carbon nanotubes (MWCNTs) (>97% purity, <2 μm length and 10 - 20 nm internal diameter) were purchased from Nanotech Port (Shenzhen, China). The MWCNTs were purified according to the following process conditions：stirring for 10 h in 3 M HNO_3 at 60 °C then filtered and rinsed to neutral pH value with water, followed by drying in a vacuum oven [21].

All solutions were prepared with ultrapure water (Millipore, USA).

2.2. Fabrication of bare/modified SPCEs

Before the modification of SPCE, it should be ultrasonically cleaned in absolute ethanol and ultrapure water for 8 minutes, respectively [22]. After that, the fabrication of multi-layer composites modified SPCE was performed, as shown in Scheme 1A. Firstly, a self-adhesive polyethylene terephthalate (PET) label paper was used to make a ring with an inner diameter of 4 mm and an outer diameter of 6 mm, and the ring was attached to the SPCE to protect the carbon auxiliary electrode and the silver reference electrode from the contamination of the modified material, as shown in Scheme 1A (a) [23]. Secondly, 20 μL PEDOT:PSS (0.04% in water, v/w) was dropped onto the carbon working electrode of SPCE, and the PEDOT modified SPCE (PEDOT/SPCE) was prepared after standing for 2 h at 20 ℃, as shown in Scheme 1A (b). Thirdly, 10 μL MWCNTs (0.2% in water, w/w) was dropped onto the working electrode of the PEDOT/SPCE, and the MWCNTs modified PEDOT/SPCE (MWCNTs/PEDOT/SPCE) was prepared after standing for 1 h at 20 ℃, as shown in Scheme 1A (c). Fourthly, 10 μL PEDOT:PSS (0.02% in water, v/w) was dropped onto the working electrode of MWCNTs/PEDOT/SPCE, and the PEDOT-MWCNTs single-layer structure composites modified SPCE ((MWCNTs/PEDOT)$_1$-PEDOT/SPCE) was prepared after standing for 2 h at 20℃ , as shown in Scheme 1A (d). Fifthly, as shown in Scheme 1A (e - f), repeated the third and fourth steps above to prepare the PEDOT-MWCNTs double-layer structure composites modified SPCE ((MWCNTs/PEDOT)$_2$-PEDOT/SPCE). Similarly, the PEDOT-MWCNTs triple-layer structure composite modified SPCE ((MWCNTs/PEDOT)$_3$-PEDOT/SPCE was prepared. Finally, the PET protection ring was carefully removed to expose the carbon auxiliary electrode and the silver reference electrode of the modified SPCE, as shown in Scheme 1A (g).

As illustrated in Scheme 1B, in order to test the performance of the modified SPCEs of different PEDOT-MWCNTs layer structure composites, the SPCEs were immersed in the test solution, which was thoroughly purged with nitrogen. Then, the electrochemical performance test or the DA detection test can be carried out by connecting with the electrochemical analyzer.
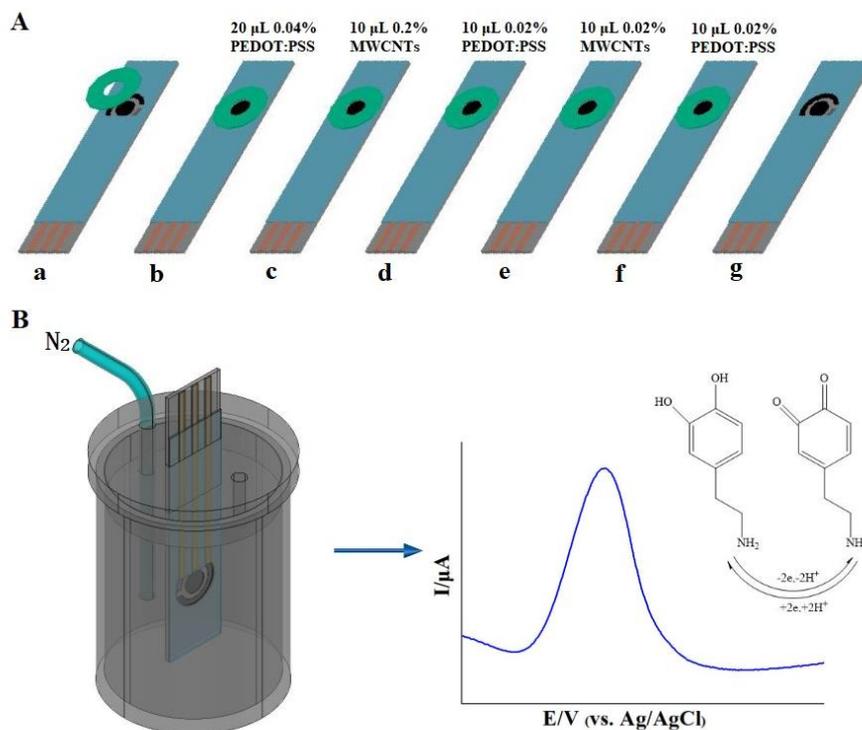
Scheme 1. (A) Schematic representation of fabrication of multi-layer composites modified SPCE (the (MWCNTs/PEDOT)$_2$-PEDOT/SPCE). (B) The detection device and the oxidation-reduction mechanism for DA at the modified SPCE.

2.3. Electrochemical measurements

Stock solution of DA was prepared in Britton-Robinson buffer solution (BRBS). Different concentrations of DA solutions were prepared by diluting the stock solution with buffer solution.

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were carried out for the electrochemical detection of DA in BRBS. The test solutions were thoroughly purged with nitrogen before experiments. All electrochemical measurements were performed at room temperature.

2.4 Sample preparation

Human blood obtained from healthy volunteers in Henan University of Science and Technology First Affiliated Hospital was centrifuged at 3500 rpm for 30 min to separate serum. Then, the human serum sample was stored at 4 °C when not in use. The serum sample was diluted 10 times with BRBS (pH 5.0) without any pretreatment before measurement. The concentration of DA in human serum was determined by standard addition method. A known amount of DA was added to the human serum sample and measured by LSV.

Int. J. Electrochem. Sci., 16 (**2021**) Article ID: 210612

5

3. RESULTS and DISCUSSION

3.1 Electrochemical characterization of the bare SPCE and the modified SPCEs

The redox couple of ferricyanide/ferrocyanide ($[Fe(CN)_6]^{3-}/Fe(CN)_6]^{4-}$) provides a classical electroactive system, which could be be used to characterize the electrochemical performance of the working electrode [22]. In order to explore the electrochemical performance of the bare SPCE and the modified SPCEs, the external redox probe $[Fe(CN)_6]^{3-}/Fe(CN)6]^{4-}$ had been studied by cyclic voltammetry (CV). Fig. 1A shown the cyclic voltammograms obtained at the bare SPCE and the modified SPCEs in 0.1 M KCl containing $1.0×10^{-4}$ M $K_3Fe(CN)_6$ and $1.0×10^{-4}$ M $K_4Fe(CN)_6$ at a scan rate of 50mV·s^{-1}. The peak current （the anode peak current (Ipa) or the cathode peak current (Ipc)） obviously increased when PEDOT (curve b) or different PEDOT-MWCNTs layer composites (curve c - e) were modified on the bare SPCE (curve a) , which indicated that PEDOT or different PEDOT-MWCNTs layer composites increased electrochemical active sites. However, through the comparison of the CV curves of the modified SPCEs (curve b - e), it can be found that the peak current increases most at the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE. This should be attributed to the double-layer structure of conductive PEDOT and MWCNTs, which effectively accelerates electron transfer rate, resulting in a best synergistic effect.

Fig. 1B shows the linear fifitting curves of current response at the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE at different scan rates in 0.1 M KCl containing $1.0×10^{-4}$ M $K_3Fe(CN)_6$ and $1.0×10^{-4}$ M $K_4Fe(CN)_6$. The peak currents increased with the increase of potential scan rates and were linearly correlated with the square root of the scan rates in the range of 10 - 100 mV·s^{-1}. The linear equations were Ipa (μA) = 101.253 μA $v^{1/2}$ (V·s^{-1})$^{1/2}$ - 1.836 μA (R^2 = 0.994) and Ipc (μA) = -85.659 μA $v^{1/2}$ (V·s^{-1})$^{1/2}$ + 0.028 μA (R^2 = 0.995), respectively. These results indicate that the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE shows a diffusion-controlled electron transfer behavior [24].
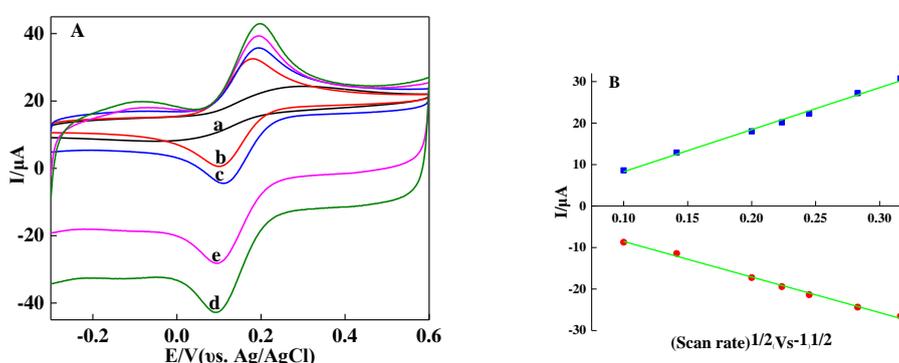

Figure 1. (A) CV curves obtained at the bare SPCE (a), the PEDOT/SPCE (b), the (PEDOT-MWCNTs)$_1$-PEDOT/SPCE (c), the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE (d) and the (PEDOT-MWCNTs)$_3$-PEDOT/SPCE (e) in 0.1 M KCl containing $1.0×10^{-4}$ M $K_3Fe(CN)_6$ and $1.0×10^{-4}$ M $K_4Fe(CN)_6$ at a scan rate of 50mV·s^{-1}. (B) The plots of the peak current at the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE against different scan rates (10 - 100mV·s^{-1}) (I: Ipa, II: Ipc).

In order to further evaluate the effect of the layer number of PEDOT-MWCNTs composites on the performance of modified SPCEs, different-layer composites modified SPCEs were characterized by CV in BRBS with pH 5.0 containing 2 μM DA, as shown in Figure 2A. The results demonstrated that the PEDOT-MWCNTs double-layer composites modified SPCE ((PEDOT-MWCNTs)$_2$-PEDOT/SPCE) showed the best performance for the detection of DA, which was consistent with the detection of $[Fe(CN)_6]^{3-}/Fe(CN)_6]^{4-}$. Fig. 2B shows the linear fitting curves of the current response of the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE at different scan rates in BRBS (pH 5.0) containing 2 μM DA. The peak currents were linearly correlated with the square root of the scan rates in the range of 10 - 100 mV·s^{-1}. And the linear equations respectively were Ipa (μA) = 90.668 μA $\nu^{1/2}$ (V·s^{-1})$^{1/2}$ - 4.931 μA (R^2 = 0.995) and Ipc (μA) = -71.373 μA $\nu^{1/2}$ (V·s^{-1})$^{1/2}$ + 3.359 μA (R^2 = 0.995). These results indicate that the reaction of DA at the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE is a diffusion-controlled process [24].

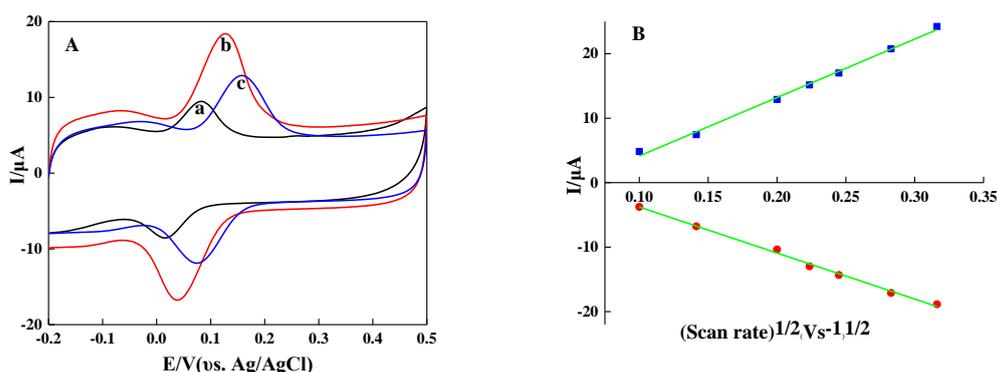**Figure 2.** (A) CV curves obtained at the (PEDOT-MWCNTs)$_1$-PEDOT/SPCE (a), the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE (b) and (PEDOT-MWCNTs)$_3$-PEDOT/SPCE (c) in BRBS (pH 5.0) containing 2 μM DA, scan rate: 50 mV·s^{-1}. (B) The plots of the peak current at the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE against different scan rates (10 - 100mV·s^{-1}) (I: Ipa, II: Ipc).

3.2 Influence of supporting electrolyte solution pH

The pH value of supporting electrolyte solution is very important for detection of DA. BRBS is a kind of supporting electrolyte solution which is often used to determine DA at the electrochemical sensors. BRBS can be used below pH 6.0, but it can not be used above pH 6.0 because of the interaction between borate and *o*-dihydroxy moiety of catecholamines. So, the oxidation potentials and current responses of DA at (PEDOT-MWCNTs)$_2$-PEDOT/SPCE were investigated in BRBS with different pH value. Fig. 3A shown the effect of pH on the peak potentials of DA. With the increase of pH, the anode peak potential (Epa) and the cathode peak potential (Epc) shifted negatively, indicating that protons participated in the electrode reaction process of DA [25]. As shown in Fig. 3B, when the pH value increased from 4.0 to 6.0, the peak current increased firstly and then decreased. And the maximum of Ipa was observed at pH 5.0, while Ipc reached the maximum at pH 4.5. Considering that Ipa at pH 5.0 was higher than Ipc at pH 4.5, Ipa was selected for the determination of DA, and the BRBS with pH 5.0

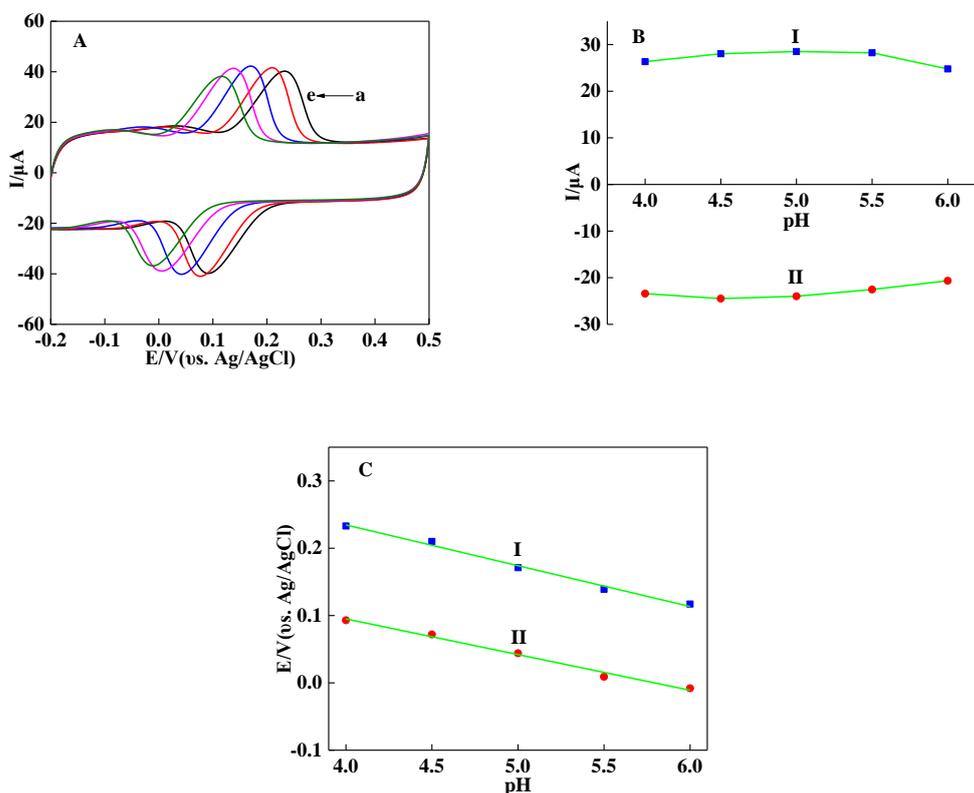was selected as an optimumsupporting electrolyte solution in the next experiments to achieve higher sensitivity.

Figure 3. (A) CV curves obtained at (PEDOT-MWCNTs)₂-PEDOT/SPCE in BRBS with different pH (a→e: pH values of 4.0-6.0). (B) Influence of pH value on the current response of DA (I: Ipa, II: Ipc). (C) The plots of peak potential of DA versus pH values (I: Epa, II: Epc).10 μM DA, Scan rate: 50 mV·s⁻¹.

Besides, as shown in Fig. 3C, the Epa and Epc were linearly correlated with pH values.The linear equations were Epa (V) = 0.477 - 0.061 pH (R^2 = 0.991) and Epc (V) = 0.307 - 0.053 pH (R^2 = 0.990), respectively. And the slope (dEp/dpH) values (Epa: -61mV/pH, Epc: -53 mV/pH) are close to the anticipated Nernstian value of -59 mV/pH. All these results demonstrates that the proton takes part in the oxidation reaction process of DA directly, and the oxidation-reduction of DA involves the electrons transfer process of the same number of protons and electrons [26,27]. The possible reaction process of DA at (PEDOT-MWCNTs)₂-PEDOT/SPCE was described as Scheme 1B.

3.3 Analytical performance of the sensors

Fig. 4A and Fig. 4B shown the LSV responses of (PEDOT-MWCNTs)₂-PEDOT/SPCE sensor toward the successive additions of DA with varying concentrations in BRBS (pH 5.0). As shown in Fig. 4A, when the concentration of DA was 0.01 μM, there was a definite LSV response at (PEDOT-MWCNTs)₂-PEDOT/SPCE. However, when the concentration of DA was lower than 0.01 μM, there

Int. J. Electrochem. Sci., 16 (**2021**) Article ID: 210612

8

was no recognizable LSV response. According to these, it can be determined that the detection limit of DA is 0.01 µM. As shown in Fig. 4C and Fig. 4D, the peak current and the concentration of DA showed good linear relationships over two concentration intervals, which range from 0.02 - 0.95 µM (Fig. 4C) and 0.95 - 15 µM (Fig. 4D). The linear equations were I (µA) = 2.663c + 0.568, (R^2=0.995), and I (µA) = 3.309c + 0.434 (R^2=0.997), respectively.
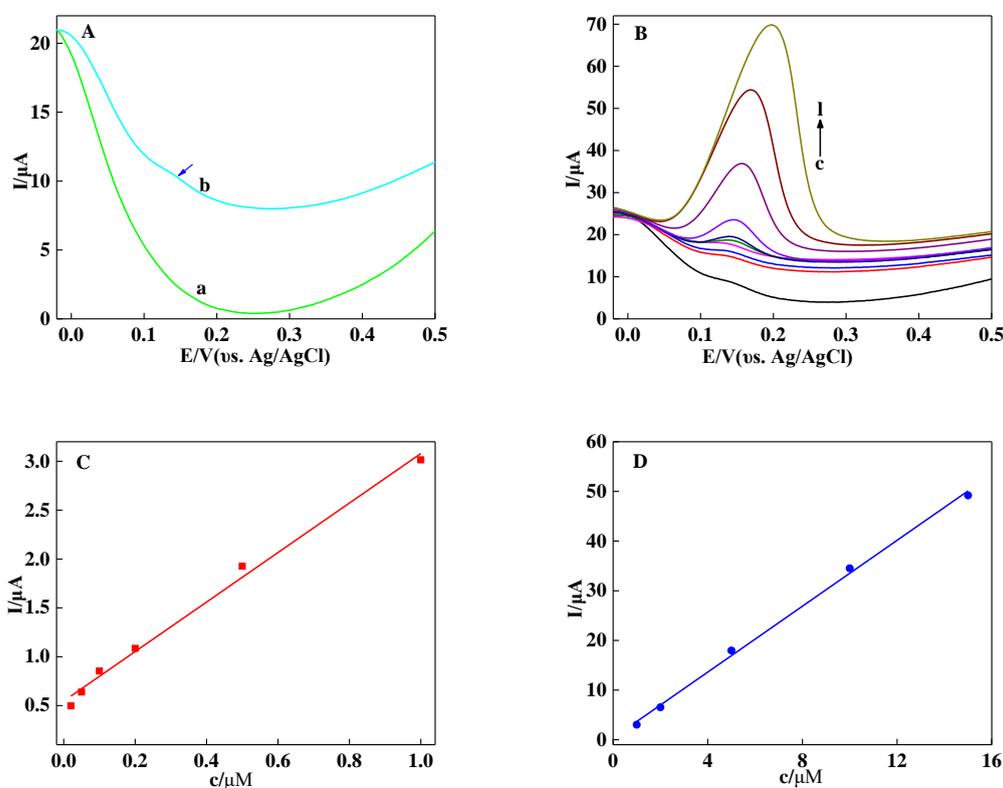

Figure 4. (A) LSV curves at (PEDOT-MWCNTs)$_2$-PEDOT/SPCE in blank BRBS (pH 5.0) (a) and in BRBS (pH 5.0) containing 0.01 µM DA (b). (B) LSV curves at (PEDOT-MWCNTs)$_2$-PEDOT/SPCE in BRBS with pH 5.0 containing different concentrations of DA (c→l : 0.02 - 15 µM) . (C) The linear relationship of peak current and the concentration of DA (0.02 - 0.95 µM). (D) The linear relationship of peak current and the concentration of DA (0.95 - 15 µM). Scan rate: 50 mV·s^{-1}.

3.4 Comparison between the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE and other modified electrodes

The comparison between the analytical performance of the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE and previously reported modified electrodes for the electrochemical determination of DA is given in Table 1. The data shows that the (PEDOT-MWCNTs)$_2$-PEDOT/SPCE has considerable or even superior analytical performance, especially for the determination of low concentration DA, which may be related to the synergistic effect of PEDOT-MWCNTs composites with double-layer structure.

Table 1. Comparison with other modified electrodes for the determination of DA.

Electrode	Target Molecule	Linear range (μM)	LOD (μM)	Ref.
AuNPs/Trp-GR/GCE	DA	0.5 - 411	0.056	[28]
Py-PBA/GCE	DA	0.05 - 10	0.033	[29]
GQD/SPE	DA	0.1 - 1000	0.05	[30]
Porphyrin-clay/CPE	DA	0.4 - 10.3	0.1	[31]
N-rGO/GCE	DA	1.0 - 60.0	0.1	[32]
Ni-MOF/GCE	DA	0.2 - 100	0.06	[33]
HNGA/GCE	DA	0.6 - 75	0.22	[34]
(PEDOT-MWCNTs)2-PEDOT/SPCE	DA	0.02 - 0.95 0.95 - 15	0.01	This work

3.5 Preliminary analysis of real samples

Human serum sample was selected as the real sample to investigate the reliability of the proposed method using the standard addition method. Under the optimized conditions, each sample was measured five times, and the average value of the measurement results was taken. As shown in Table 2, DA could be detected in human serum samples with the appropriate recovery. The quantitative recoveries for DA were from 98.3% to 101.5% with relative standard deviation (RSD) between 2.7% and 3.8%. These results indicated the applicability and reliability of the proposed method.

Table 2. Analytical results for DA in human serum samples (n=5).

Method	Added/(μM)	Determined/(μM)	Recovery/%	RSD/%
	2.00	2.03	101.5	3.8
LSV	4.00	3.93	98.3	2.7
	8.00	7.95	99.4	3.1

4. CONCLUSION

In this paper, the PEDOT-MWCNTs double-layer structure compositesmodified SPCE ((MWCNTs/PEDOT)$_2$-PEDOT/SPCE) was prepared for the sensitive determination of DA. The synergistic effect of PEDOT and MWCNTs results in good analytical performance of the (MWCNTs/PEDOT)$_2$-PEDOT/SPCE. The proposed sensor was also utilized for the serum sample analysis, and further studies prove that the (MWCNTs/PEDOT)$_2$-SPCE/SPCE is confirmed to be reliable and applicative in real sample analysis.

Int. J. Electrochem. Sci., 16 **(2021)** Article ID: 210612

10

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Int. J. Electrochem. Sci., 16 **(2021)** Article ID: 210612

11

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