*Int. J. Electrochem. Sci.*, 11 **(2016)** 2360 - 2376

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

# Simultaneous Electrochemical Determination of Dopamine and Serotonin in Rat Cerebrospinal Fluid Using Screen-Printed Electrode Modified with MWNTs-$SiO_2$-chitosan Composites

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*Received:* 18 November 2015 / *Accepted:* 15 December 2015 / *Published:* 1 February 2016

Dopamine (DA) and serotonin (5-HT) are directly and simultaneously determined in cerebrospinal fluid (CSF) by using MWNTs-$SiO_2$-chitosan composites modified screen-printed electrode (SPE). Owing to the high catalytic activity of composites, the modified electrode is found to exhibit a remarkably enhanced electro-activity toward the sensing of DA and 5-HT. Under optimum experimental conditions, the MWNTs-$SiO_2$-chitosan composites modified SPE could be used for the determination of DA and 5-HT in wide concentration ranges of 1 μM~20 μM and 0.1 μM~2 μM, respectively, whereas the detection limits have been found to be 0.2 μM and 0.01 μM, respectively. Furthermore, the proposed sensor is successfully used for the detection of DA and 5-HT in rat CSF samples. The results display rapid response, expanded linear response range and excellent repeatability.

**Keywords:** dopamine; serotonin; screen printed electrode; rat cerebrospinal fluid; simultaneous determination

## 1. INTRODUCTION

Dopamine (DA) and serotonin (5-HT) are important monoamine neurotransmitters and involved in a variety of central nervous system functions. For example, DA has been linked to many disorders [1, 2], and 5-HT plays a variety of physiological functions and pathological states[3]. The relative levels of DA and 5-HT have been involved in many diseases and response to drug treatments[4, 5]. Considering the coexistence of DA and 5-HT, simultaneous measurement is

particularly important. However, according to clinical evidence, DA and 5-HT influence each other in their respective releasing[6]. Hence, it is extremely useful to develop a method that can separate and simultaneously determine DA and 5-HT effectively in biological samples for diagnosis and treatment [7]. In particular, the recognition and sensing of DA and 5-HT in cerebrospinal fluid (CSF) has been of great interest. The researches have reported CSF is a major medium in the dynamics and metabolism of monoaminergic system in human, [8]. The major monoamine neurotransmitters concentrations in CSF are commonly used as indices of activity[9, 10].

Numerous techniques like High Performance Liquid Chromatography (HPLC) [11], capillary electrophoresis[12], fluorescence[13], mass spectrometry[14], Liquid chromatography electrospray ionisation tandem mass spectrometry (LC-ESI-MS-MS) [15] have been used to simultaneously determine the concentration of DA and 5-HT. However, the mentioned techniques are expensive, time-consuming and inappropriate for the analysis of large number of samples in clinic. Electrochemical techniques provide a faster and convenient method for the determination of drugs. Because DA and 5-HT are readily oxidized, electrochemical methods have been explored for their analyses in biological matrices and pharmaceuticals [16-24]. Each technique, however, generally suffers from some disadvantages, such as poor selectivity, complex sample preparation procedures and long analysis time. For example, DA and 5-HT have similar oxidation potentials, so it is difficult to distinguish them by using many traditional electrodes [25]. Therefore, the aim of this study is to investigate the electrochemical behaviors of DA and 5-HT using the disposable screen-printed electrode (SPE) and to develop a sensitive method for determination of these two monoamine neurotransmitter in CSF with simple pretreatments. To our knowledge, the research is few about the simultaneous determination of DA and 5-HT in CSF samples using SPE.

Because of various advantages including simple fabrication, low cost, small size, disposability and easily mass production, SPE has attracted much more interest and challenged the conventional three-electrode system[26, 27]. The small working area of the SPE surface is well adapted to the analysis of small volumes of CSF sample. However, added samples as a droplet on the SPE, a high-sensitivity working surface is inevitable required for electrochemical analysis. Moreover, SPE should offer remarkably high separation efficiencies for the analysis of DA and 5-HT mixtures. In this respect, we developed the sensitive and selective MWNTs-$SiO_2$-chitosan composites modified SPE, which could provide direct, simpler analysis of DA and 5-HT in CSF sample than the earlier methods.

Multi-walled carbon nanotubes (MWNTs), nanomaterials, are often used to fabricate matrices for sensors. It is an exciting approach because nanomaterials have a unique structure and high surface-to-volume ratio [28]. Besides the nano-size effects common to other nano materials, MWNTs also exhibit the characters of excellent electronic semi-conductivity and conductivity, especially high specific surface area [29-31]. In previous work, MWNTs was discovered to be a highly active electrocatalyst during the determination of DA and/or 5-HT [32-34].

Recently, composite materials, based on integration of carbon nanotubes and some other materials to promote properties of the individual components with synergistic effect, have gained growing interest[35, 36]. As a new kind of inorganic materials, $SiO_2$ is particularly attractive to the progress of electrochemical biosensor. Under mild conditions, $SiO_2$ can be prepared and present chemical inertness, negligible swelling in aqueous and non-aqueous solution, tunable porosity, high

thermal stability. Most important of all is the ability to form films [37]. According to the studies, sensors based on $SiO_2$ were fabricated [37-39]. However, neurotransmitter sensor based on $SiO_2$ has never been reported.

On the other hand, incorporation of organic polymers, especially those with amino or amide groups, such as chitosan, allows the formation of molecular hybrids often stabilized by strong hydrogen bonding. Chitosan is a polysaccharide with the N-deacetylated derivative of chitin, which contains a high content of hydroxyl and amino groups along its chains. It has been widely used for the preparation of biosensors due to their nontoxic behavior, good mechanical strength, excellent film forming ability, high permeability, and cost-effectiveness[40-42]. Ashutosh Tiwari et al. [43] reported a chitosan-SiO2-multiwall carbon nanotubes sensor to study the immobilization of creatine amidinohydrolase (CAH).

In this work, we describe the fabrication of a novel matrix based on MWNTs-$SiO_2$-chitosan nanocomposites modified SPE. It was applied for simultaneous detection of DA and 5-HT in rat cerebrospinal fluid using electrochemical techniques. And our goal in the future work is to develop a portable device to provide a direct answer for applications in diagnostics and point-of-care testing with only a simple sample introduction. This new method might be a promising new technology for rapid detection in clinical.

## 2. EXPERIMENTAL

### *2.1 Reagents and instruments*

DA, 5-HT, AA and UA were purchased from Sigma. The MWNTs（purity > 90 %）was employed from Chengdu Organic Chemicals Co., Lit, Chinese Academy Sciences (Sichuan, China). SiO2 (15~20 nm, 99.9 %) was purchased from Aladdin Chemistry Co., Ltd (Shanghai). Chitosan (CHIT, MW ~ 1×106; 75 ~ 80 % deacetylation) was purchased from Sigma. The stock solutions of DA（5 mM）and 5-HT (1 mM) were attained through dissolving in doubled distilled water, and then, stored at -20℃. Tris-HCl buffer solutions (0.05 M, pH 7.5) were performed as supporting electrolyte. Other chemicals and solvents used were all of analytical grade.

The electrochemical system was produced using polymeric commercial inks: ED427 (silver ink, Acheson, American), Electrodag 423SS (carbon ink, Acheson, Japan), CNC-01 (silver/silver chloride ink, CamNano, China), and IN-15M (insulator ink, JUJO, Japan).

Screen-printed electrodes (SPE) were produced with the semi-automatic screen printer (Samwo, China) using polyester screens with appropriate stencil designs mounted at 45° to the printer stroke. RD-GGJ-200/I UV curing machine (Baoding rongda electronic equipment, Heibei, China) was used for drying insulation layer. The EC 570 electrochemical workstation (Gaoss Union Technology, Wuhan, China) was used to record the voltammograms by SPE. FE-SEM instrument (Quanta 200, FEI Coropration, Holland) and XRD instrument (X'Pert Pro, PANalytical) were employed for the characterization of modified SPE.

*2.2 Fabrication of MWNTs-SiO2-chitosan SPE*

The electrodes were composed of Ag lead, Ag/AgCl reference electrode, carbon working and counter electrodes, which were prepared using the screen-printed technique (Figure 1A). The method was as follows: (a) Ag lead used Ag ink; (b) Ag/AgCl reference electrode printed with Ag/AgCl ink; (c) counter electrode was prepared by graphite ink; (d) working electrode was prepared as follows: A stock solution of MWNTs-$SiO_2$ in 1 mL aqueous solution of 1 % CTAB was produced by dispersing 2 mg MWNTs and 2 mg $mL^{-1}$ $SiO_2$ with ultrasonication for 2 h, then the MWNTs-$SiO_2$ suspension solution was dropped on the carbon working electrodes, air-dried for 30 min at room temperature. Chitosan solution (0.5%) was obtained by dissolving chitosan in 1 % acetic acid solution with magnetic stirring for about 2 h. After that, MWNTs-$SiO_2$-chitosan SPE was prepared by coating the working electrode surface with 1 μL chitosan solution. (e) Finally, insulation layer was used the UV light-cured ink and dried in UV curing machine. Then MWNTs-$SiO_2$-chitosan SPEs were stored in the desiccator before use. Besides, MWNTs SPE and MWNTs-$SiO_2$ SPE were prepared to compare during the further investigation.

*2.3 Analytical procedure*

Appropriate amount of DA and 5-HT was mixed to add to the model systems and real samples containing 0.05 mol $L^{-1}$ Tris-HCl (pH 7.5) buffer solution. The cyclic voltammetric curves (CV) were recorded over the potential window from -0.2 to 0.8 V versus the Ag/AgCl. Scan rate: 100 mV $s^{-1}$. Square wave voltammogram (SWV) was recorded over the potential window from -0.1 to 0.6 V versus the Ag/AgCl. The frequency was 25 Hz, scan increment were 6 mV and pulse amplitude were 25 mV. Differential pulse voltammogram (DPV) was scanned in potential interval from -0.1 to 0.6 V vs. Ag/AgCl, PH= 0.025 V; SH= 0.005 V; PW= 0.05 s; ST= 0.1 s. All electrochemical experiments were carried out at room temperature.

*2.4 Analysis of CSF sample*

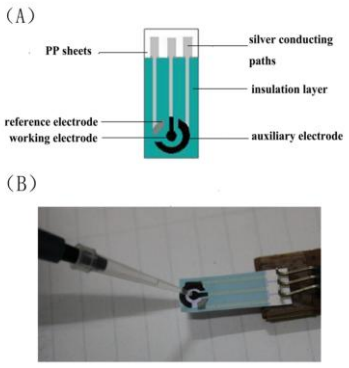


**Figure 1.** (A) Image of the screen-printed electrode (SPE); (B) Analysis of a 20 μl rat CSF drop on the strip.

Sprague-Dawley (SD) rats (200 ± 20 g) were obtained from Laboratory Animal Center of Wuhan University. The certification numbers was No.00018995 (SCXK 2008-0004). The rat CSF was obtained and detected for analysis without any pretreatment. As shown in Figure 1B, the 75 μL CSF was diluted to 150 μL by 0.05 mol $L^{-1}$ Tris-HCl (pH 7.5). Next, the diluted CSF (20 μL) was directly dropped on the surface of SPE. And then, the electrochemical assays were carried to scan immediately by SWV.

## 3. RESULTS AND DISCUSSION

### *3.1 Characterization of SPE*

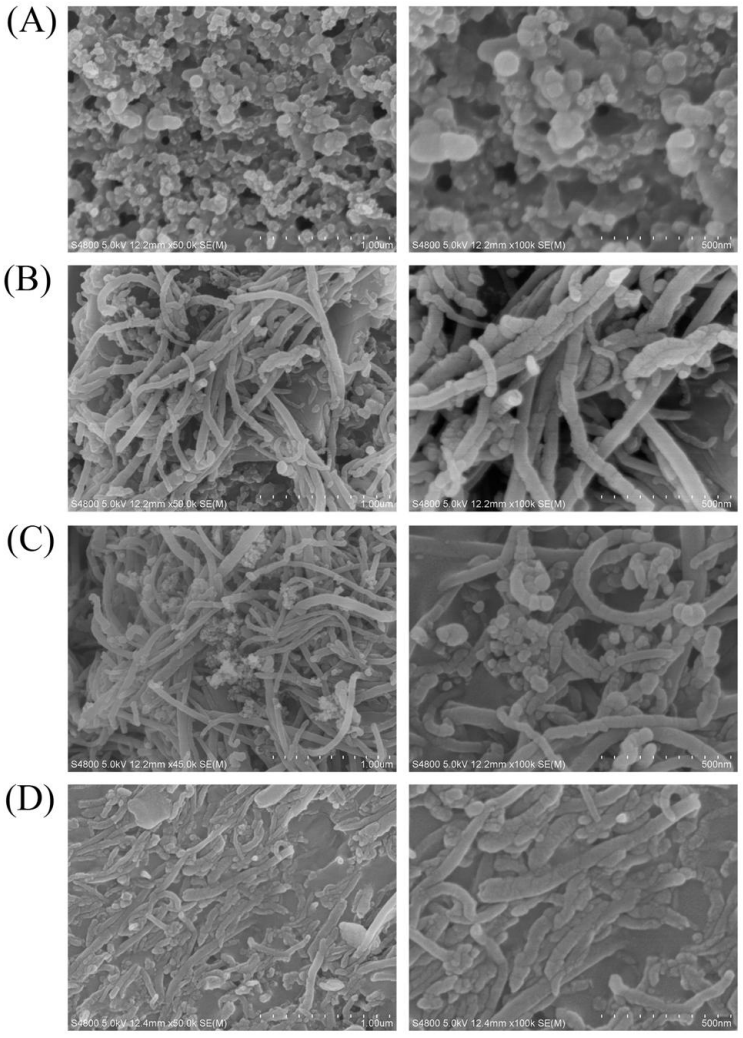


**Figure 2.** SEM images of different SPEs: bare SPE (A), MWNTs modified SPE (B), MWNTs-$SiO_2$ modified SPE (C), MWNTs-$SiO_2$-chitosan modified SPE (D). Scale bar: 1.00 μm (left) and 500 nm (right).

The SEM images of the working electrode from bare and modified SPE are shown in Figure 2 A-D. The surface of bare SPE is shown in Figure 2A, and the high-resolution SEM image shows that

the carbon surface is covered with a great content of graphite particles. As shown in Figure 2B, the SEM images of MWNT-SPE reveal that the MWNTs are well distributed on the surface, which could improve the electron transfer reaction and enhance detection sensitivity because of their remarkable catalytic effect. From Figure 2C, it can be seen the $SiO_2$ particles are dispersed well on the MWNTs substrate. These nanostructured $SiO_2$ functionalized MWNTs can exhibit better transmission performance due to unique electrochemical property. However, in our previous work, we found that the modified composite film would flake off easily during the detection system, so MWNTs-$SiO_2$ modified SPE was not very stable. In order to fully utilize the catalytic property of $SiO_2$ particles and MWNTs, it is extremely important to disperse them efficiently on the SPE. Various researches have indicated that the chitosan, a copolymer, is widely used to act as electrochemical film. In this study, chitosan was used to contribute the MWNTs-$SiO_2$-chitosan (MSC) film. After using chitosan, MWNTs-$SiO_2$ composite was firmer stable. It could be observed that the MSC film are compact, homogeneous and densely packed on the electrode surface (Figure 2D). This film combines the merits of $SiO_2$, MWNTs, and chitosan, then it provides a favorable environment for simultaneously analyzing DA and 5-HT. So the response of target compounds can remarkably increased in the MSC modified SPE, even MSC may has substantial capacitive currents.

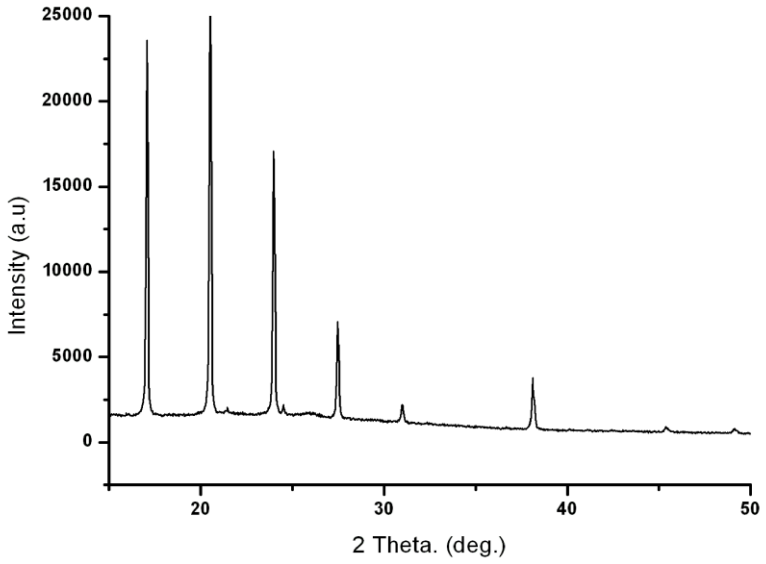


**Figure 3.** XRD patterns of MWNTs-$SiO_2$.

In order to further investigate the characterization of the MWNTs-$SiO_2$-chitosan SPE, the XRD pattern experiment was also employed for examining the composite of MWNTs-$SiO_2$. The diffraction peak at $2\theta = 23.97°$ is a typical peak of pristine MWNTs [44]. And the absence of $SiO_2$ peaks in the XRD pattern (Figure 3), which are located at $2\theta$ of 17.11°, 20.53°, 27.49°, 38.11° respectively, indicate $SiO_2$ nanoparticles densely covered on the surface of MWNTs.

*3.2 Electrochemical behavior of DA and 5-HT at MWNTs-$SiO_2$-chitosan SPE*

The electrochemical behaviors of DA and 5-HT were examined using cyclic voltammetry (CV) in 0.05 M Tris-HCl buffer with pH of 7.5. As shown in Figure 4, DA and 5-HT show sluggish and much smaller CV peak responses at bare SPE (curve bare). Whereas at the MWNTs-$SiO_2$-chitosan SPE, the peak currents increase greatly.

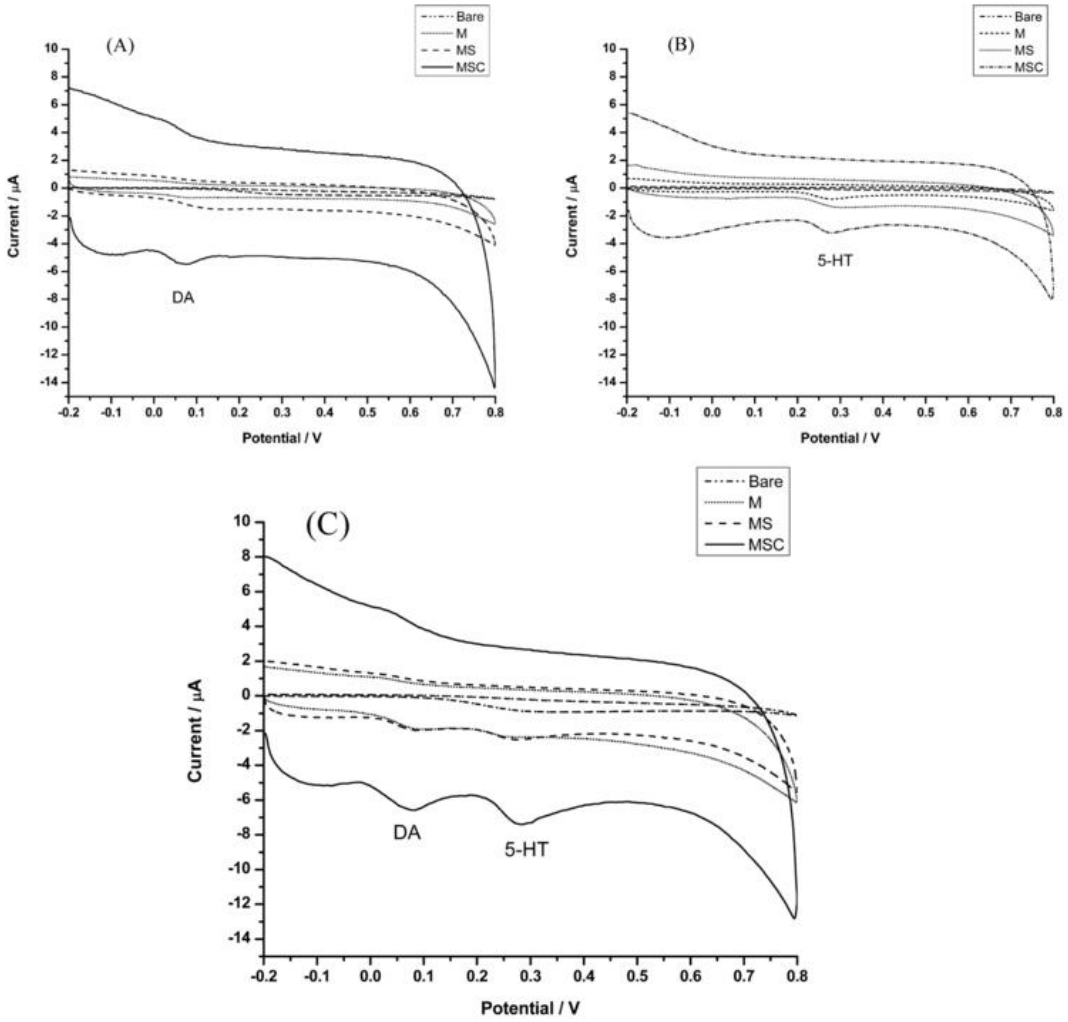


**Figure 4.** Cyclic voltammogram curves of 50 μM DA (A), 5 μM 5-HT (B) , coexisted 50 μM DA and 5 μM 5-HT (C) at bare SPE (curve bare), MWNTs SPE (curve M), MWNTs-$SiO_2$ SPE (curve MS) and MWNTs-$SiO_2$-chitosan SPE (curve MSC) in 0.05 M Tris-HCl buffer, scan rate: 100 mV $s^{-1}$.Potential interval from -0.2 to 0.8 V vs. Ag/AgCl.

The distinguished peaks at 0.081 V for 50 μM DA, and 0.289 V for 5 μM 5-HT (Figure 4C) are observed with large peak separation of 0.208 V. The oxidation peaks of DA and 5-HT were well separated on the surface of the MWNTs SPE (curve M). Besides, the oxidation peak currents were also greatly enhanced, which could be ascribed to the large surface area and excellent electro conductivity of MWNTs. However, the enhancements of DA and 5-HT peak currents are not obvious at MWNTs-$SiO_2$ SPE (curve MS). This phenomenon may because of the MWNTs-$SiO_2$ composite were not stable enough on electrode's surface and were possibly washed off by the electrochemical determination

samples. Thus, we used chitosan to contribute the MWNTs-$SiO_2$-chitosan (MSC) film. After using the chitosan, MWNTs-$SiO_2$ was more stable. Curve MSC demonstrates that MWNTs-$SiO_2$-chitosan SPE could greatly catalyze the electrooxidation of DA and 5-HT and obviously improve the peak shapes. Curve MSC also shows that MSC has substantial capacitive current. This capacitive current didn't facilitate the target compounds sensing. However, the MSC film could make the MWNTs-$SiO_2$ firmer stable, and then combine the merits of all these three materials, such as the catalytic effects of $SiO_2$, the subtle electronic properties of MWNTs, and the ion-exchange characters of chitosan. So the response of DA and 5-HT can remarkably increased in the MSC modified SPE, even MSC may has substantial capacitive currents.

### *3.3 The effect of scan rate*

Different scan rates of CV were used for recording mixed 50 μM DA and 5 μM 5-HT in 0.05 M Tris-HCl (pH 7.5). When the scan rate increased, the peak potentials both shifted to more positive values. The oxidation peak currents of 5-HT was linear with the scan rate in the in the range of 10 to 300 mV $s^{-1}$, which indicate an adsorption controlled process. The anodic and cathodic peak currents of DA at MWNTs-$SiO_2$-chitosan SPE was also proportional to the scan rate over the range of 10 to 300 mV $s^{-1}$, displaying a typical surface adsorption kinetics. The linear equations of DA and 5-HT between oxidation peak currents and scan rates were respectively found as following: Ip = 1.3229 + 0.0289v, R = 0.9925 (DA); Ip = 0.0128 + 0.0169v, R = 0.9959 (5-HT).

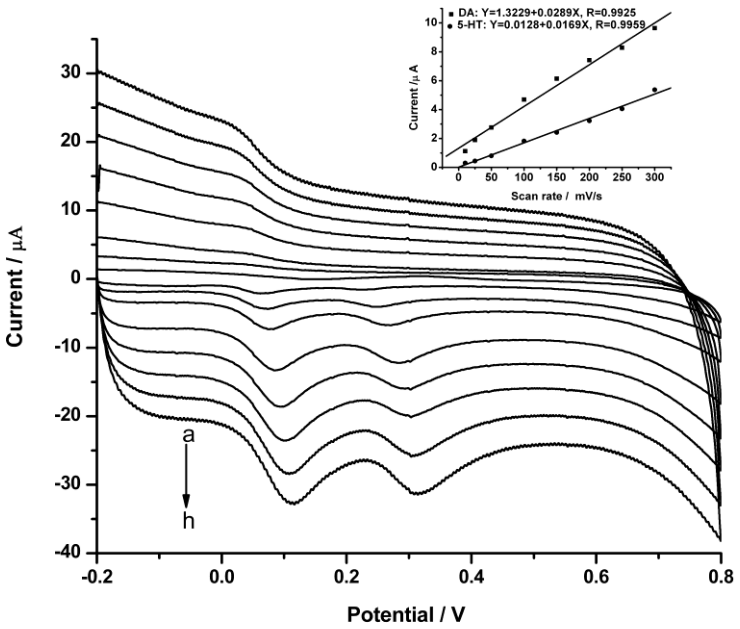


**Figure 5.** CV of 50 μM DA and 5 μM 5-HT in in 0.05 M Tris-HCl (pH 7.5) at MWNTs-$SiO_2$-chitosan SPE at different scan rates: (a) 10 mV $s^{-1}$; (b) 25 mV $s^{-1}$; (c) 50 mV $s^{-1}$; (d) 100 mV $s^{-1}$; (e) mV $s^{-1}$; (f) 200 mV $s^{-1}$; (g) 250 mV $s^{-1}$; and (h) 300 mV $s^{-1}$. (Insets) the relationships of peak currents vs. scan rate and peak potentials vs. logarithm of scan rate.

*3.4 The effect of pH*

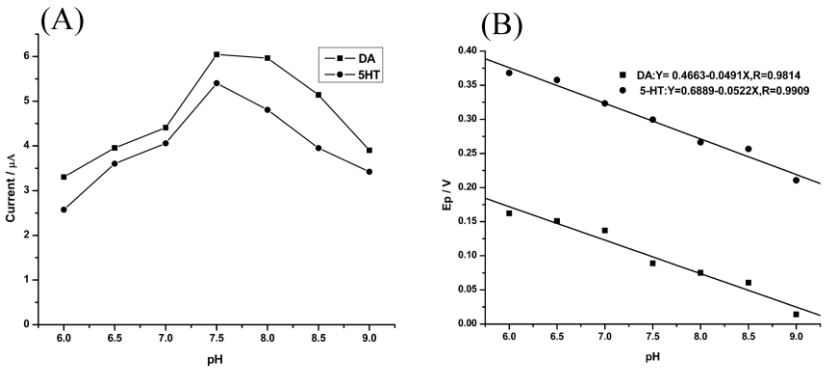


**Figure 6.** Influences of pH on the electrochemical responses of 50 μM DA and 5 μM 5-HT at MWNTs-$SiO_2$-chitosan SPE in 0.05 M Tris-HCl buffer. (A)The currents of DA and 5-HT responses to pH, (B) The relationships of the peak potential of DA and 5-HT against pH (n = 4).

The effect of pH values of supporting electrolyte was examined for DA and 5-HT oxidation at MWNTs-$SiO_2$-chitosan SPE. And finally, the relationship between pH value and the peak potential (Ep) was studied. The peak currents of DA and 5-HT increased with the increase of pH, and reached the maximum at pH 7.5. After that, they oppositely decreased with the increase of solution pH further (Figure 5A). So the pH 7.5 Tris-HCl was gotten as the supporting electrolyte for the experiment. In addition, as shown in Figure 5B, the peak potentials of DA and 5-HT shifted with pH as following: Epa (DA) = 0.4663−0.0491 pH (r = 0.9814), Epa (5-HT) = 0.6889−0.0522pH (r = 0.9909).

*3.5 The effect of MWNTs-$SiO_2$-chitosn amounts and accumulation time*

The modifier volume of MWNTs-$SiO_2$-chitosn was an indispensable factor in electrochemical response to DA and 5-HT. So the relationship between different volumes (from 1.0 to 5.0 μL) of the MWNTs-$SiO_2$ and the peak current was examined. The response of the electrode improved maximum at 3.0 μL. the peak currents reduced (Figure 6A), as the modified composition was more than 2.0 μL. Therefore, 2.0 μL was chosen as the optimized amount of the MWNTs-$SiO_2$ suspension. On the other hand, after coating the surface of MWNTs-$SiO_2$ modified electrode with more than 1.0 μL chitosan solution, the background current became larger, and this may cause the poor sensitivity for DA and 5-HT detection. So, 3.0μL MWNTs-$SiO_2$-chitosn, containing 2.0 μL MWNTs-$SiO_2$ (2 mg $mL^{-1}$) and 1 μL. Chitosan (0.5 %) was selected for the fabrication of the disposable sensor.

Under the different accumulation time, square wave voltammograms (SWV) responses of DA and 5-HT mixture (50 μM DA + 5 μM 5-HT) at MWNTs-$SiO_2$-chitosan SPE were explored, too. The experimental results indicated that, with the time prolonging, both peak currents of 5-HT and DA increased. When the preconcentration time closed to 3 min, the peak currents remained almost stable (Figure 6B). This may caused by saturation of the amount of DA and 5-HT adsorbed on the modified electrode surface. Thus, to compromise between the sensitivity and running time, accumulation step was performed for 3 min for subsequent analysis.

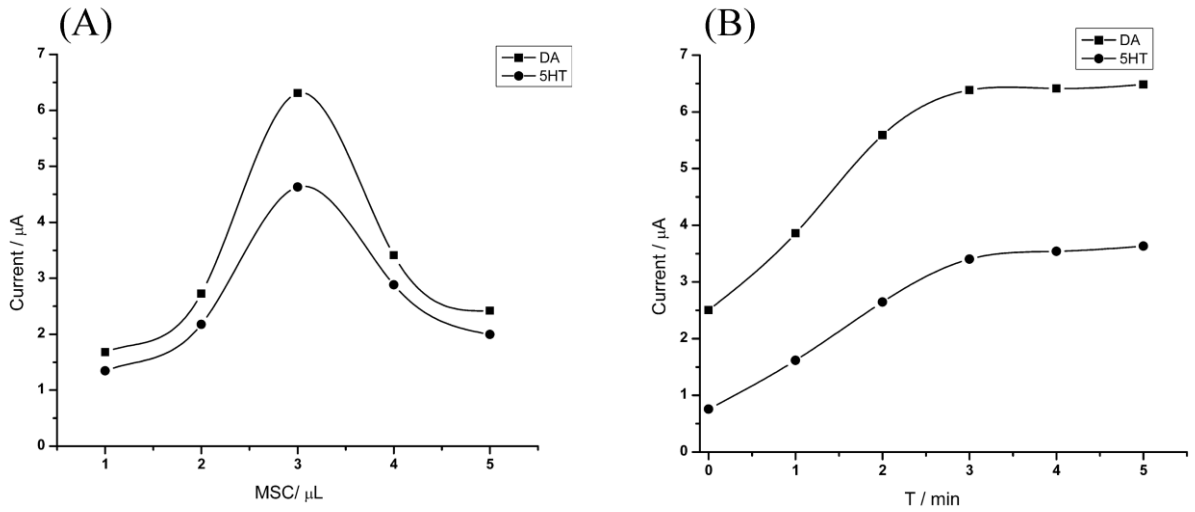


**Figure 7.** Relationship between MWNTs-$SiO_2$-chitosn (MSC) amounts (A) and accumulation time (B) against peak currents of 50 μM DA and 5 μM 5-HT in 0.05 M Tris-HCl buffer (pH 7.5) (n = 6).

*3.6 Calibration curve and limit detection*

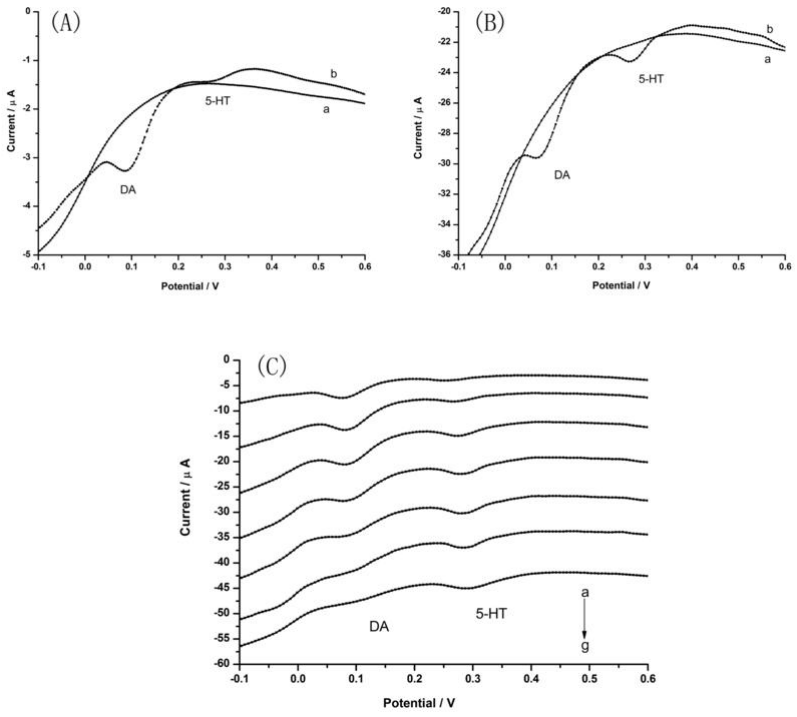


**Figure 8. (**A**)** DPV curves of 0.05 M Tris-HCl buffer (curve a) and 10 μM DA and 1 μM 5-HT (curve b) at MWNTs-$SiO_2$-chitosan SPE. Potential interval from -0.1 to 0.6 V vs. Ag/AgCl, PH= 0.025 V; SH= 0.005 V; PW= 0.05 s; ST= 0.1 s; (B) SWV curves of 0.05 M Tris-HCl buffer (curve a) and 10 μM DA and 1 μM 5-HT (curve b) at the MWNTs-$SiO_2$-chitosan SPE. Potential interval from -0.1 to 0.6 V vs. Ag/AgCl, frequency: 25 Hz; scan increment of 6 mV and pulse amplitude of 25 mV; **(**C**)** SWV curves of 10 μM DA and 1 μM 5-HT in different frequency: 10, 15, 20, 25, 30, 35 and 40 Hz (a→g); scan increment of 6 mV and pulse amplitude of 25 mV.

The properties of DPV and SWV have been compared in order to find a more sensitive method for DA and 5-HT coexist determination. And considering the current of $O_2$ at 0.8V could affect the peaks of target compounds, potential interval was change to -0.1 - 0.6 V vs. Ag/AgCl. The SWV curves (Figure 7B) shows greatly enhanced of both the peak currents of DA and 5-HT compared with the DPV (Figure 7A), which is not sensitive for 5-HT detection. So, when DA and 5-HT coexist, SWV was further employed to learn more about the electro-chemical responses.

An important condition in SWV is the frequency. So the effects of different scan frequency were studied. From Figure 7C, it can be obtained that the DA currents enhance in the range of 10–15 Hz and gradually decrease in the range of 20–40 Hz. However, the peak currents of 5-HT increase with the improving the frequency in the range of 10–30 Hz, and decrease when frequency is exceed 30 Hz. Therefore, considering the specificity of both DA and 5-HT peak currents, 25 Hz was selected for further simultaneous determination. The optimal parameters were step width = 6 mV; amplitude = 25 mV, frequency = 25 Hz, potential interval from -0.1 to 0.6 V vs. Ag/AgCl.

The electrochemical behaviors of DA (or 5-HT) were detected in the presence of 5-HT (or DA) by SWV to study the responses when DA and 5-HT coexist.

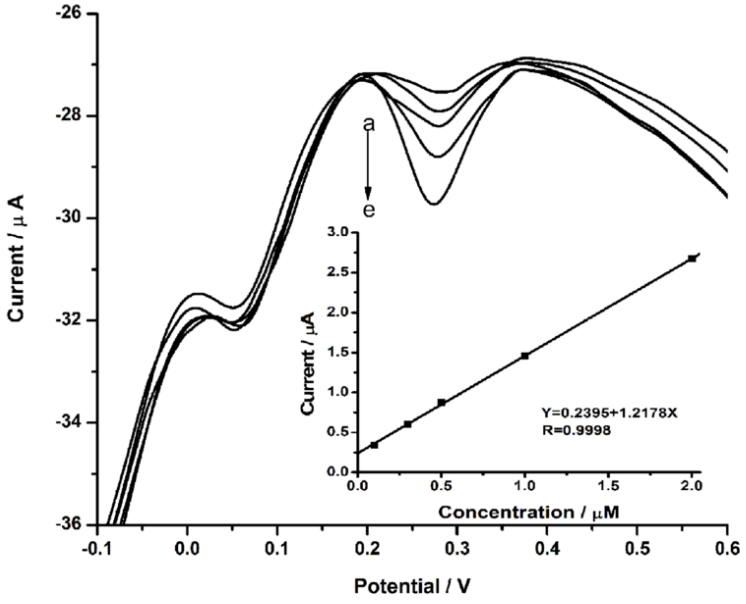


**Figure 9.** SWV of different concentration 5-HT in the presence of 3 μM DA in 0.05 M Tri-HCl (pH 7.5).The 5-HT concentrations from a to e are: 0.1, 0.3, 0.5, 1, and 2 μM, respectively.

Various concentrations of DA (or 5-HT) in the presence of 5-HT (or DA) were examined under optimal condition. As shown in Figure 8 and Figure 9, the peak currents of DA (or 5-HT) almost remained invariant at different concentrations of 5-HT (or DA). In Figure 7, the calibration equation of 5-HT was obtained in the range of 0.1 to 2 μM in the presence of 3 μM DA: Ip (μA) = 1.2178c + 0.2395 (R = 0.9998). The detection limit was found to be 0.01 μM (S/N = 3.0). Figure 8 exhibited the relationship between the peak current and the concentration of DA in the presence of 0.5 μM 5-HT. The calibration curve was performed from 1 to 20 μM DA, with a detection limit of 0.2 μM (S/N =

3.0). The linear regression equation was Ip (μA) = 0.3542c + 0.3186 (R = 0.9973). Good linearity was obtained between peak currents of DA (or 5-HT) and their concentrations in the mixture. Obviously, the proposed method could be applied for the simultaneous determination of DA and 5- HT concentrations.

The reproducibility of the modified electrode was also examined in present study. 10 continuous repetitive SWV were obtained about 5 μM DA and 0.5 μM 5-HT. The relative standard deviation (R.S.D) was about 4.7 % and 3.1 % for DA and 5-HT, respectively, indicating that the exhibited sensor had good reproducibility. Besides, the longtime stability of modified SPE was also evaluated after3 months. The average responses of DA and 5-HT decrease were found to be less than 5 %. The excellent stability may be attributed to the effect of MWNTs-$SiO_2$-chitosan composites film.

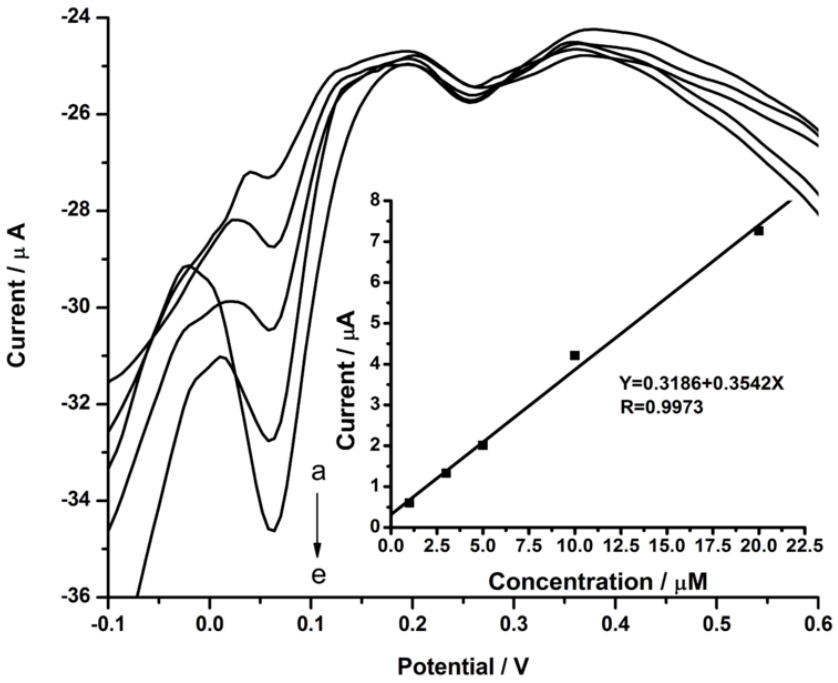


**Figure 10.** SWV of different concentration DA in the presence of 0.5 μM 5-HT in 0.05 M Tri-HCl (pH 7.5).The DA concentrations from a to e are: 1, 3, 5, 10, and 20 μM.

*3.7 Interferences*

The influences of various foreign species on the determination of DA and 5-HT were evaluated. In biological systems, the amount of ascorbic acid (AA) and uric acid (UA) are larger than monoamine neurotransmitters. Thus, the interference of high-concentration AA and UA should be considered during the determination of DA and 5-HT. Figure 10A indicated that the detection of 5 μM DA and 0.5 μM 5-HT in the presence of 100 μM AA was not disturbed. And the Figure 10B explained the effect of different concentration of UA didn't interfere the determination of DA and 5-HT (approximately ± 5 % relative error).

The other main interfering substances were examined, and it was observed that many substances did not obstruct the simultaneous determination of DA and 5-HT such as 200 μM glucose, 200 μM $Ca^{2+}$, 200 μM $Mg^{2+}$, and 200 μM citric acid. The results showed that MWNTs-$SiO_2$-chitosan possessed excellent anti-interference capability for simultaneous determination of DA and 5-HT in biological sample.

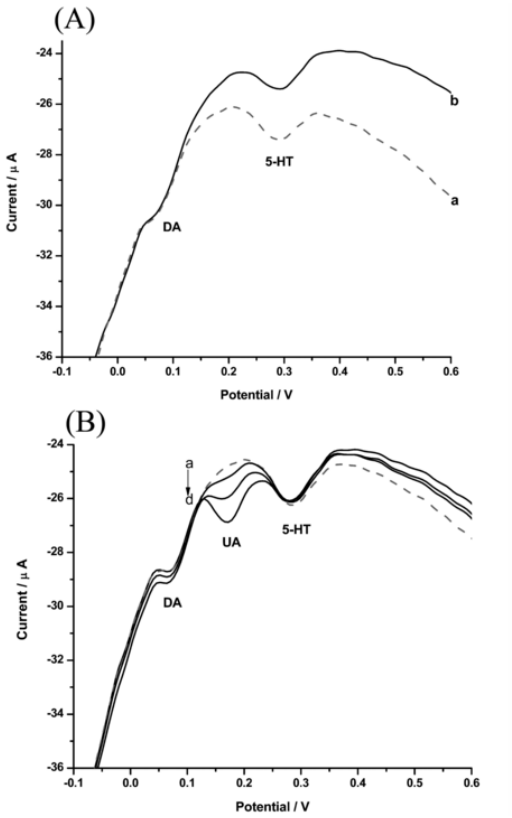


**Figure 11. (**A**)** Effects of 100 μM AA (curve b) on the response to 5μM DA and 0.5μM 5-HT at MWNTs-$SiO_2$-chitosn SPE; (B) different amounts UA on the response to 5μM DA and 0.5μM 5-HT at MWNTs-$SiO_2$-chitosn SPE. The UA concentration (curve a to d): 0, 50, 100, 200 μM, respectively.

*3.8 Detection of DA and 5-HT in rat cerebrospinal fluid*

The MWNTs-$SiO_2$-chitosn SPE were used to the determination of DA and 5-HT in rat cerebrospinal fluid (CSF) using SWV. According to the results in Figure 11, 75 μL original CSF was diluted to 150 μL with the Tri-HCl buffer solution (curve a). Then different volumes of DA and 5-HT were mixed to the diluted CSF samples. The calibration curves of 5-HT were: Ip (μA) = 2.8198c + 0.1218 (R = 0.9959) in the range of 0.1 μM to 1 μM. While, the calibration curve for additional DA was Ip (μA) = 0.1394c + 1.0341 (R = 0.9913) in the range of 1 μM to 20 μM. The recoveries in the range of 92.64 ~ 108.62 % and 91.41 ~ 109.57 % were also gotten for 0.5 μM 5-HT and 10 μM DA, respectively.

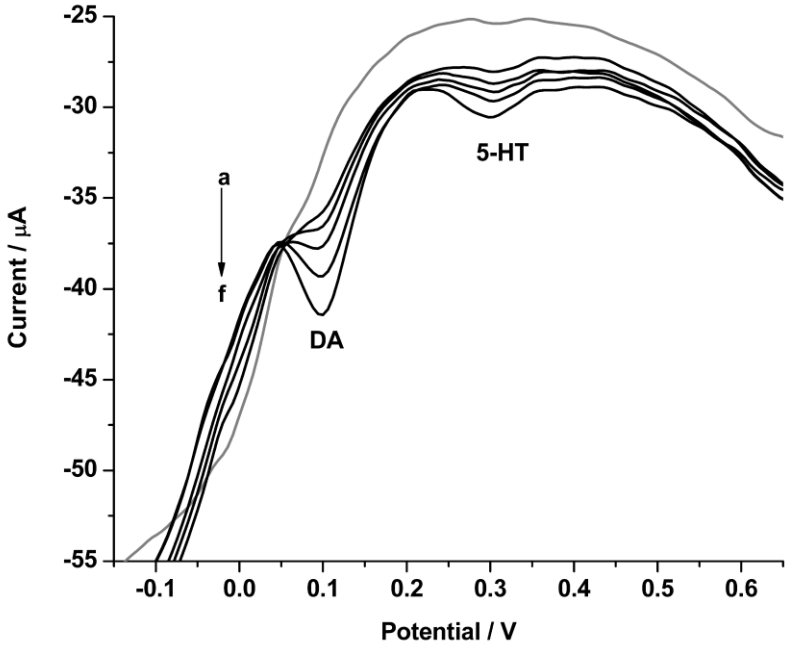


**Figure 12.** SWV of diluted rat cerebrospinal fluid at MWNTs-$SiO_2$-chitosn SPE. The 5-HT concentration from curves a to f: 0, 0.1, 0.2, 0.3, 0.5 and1.0 μM; The added DA concentration curves from curves a to f: 0, 1, 3, 5, 10 and 20 μM.

Besides, the table 1 compared the MWNTs-$SiO_2$-chitosan SPE with other sensors. It could be obtained that the MWNTs-$SiO_2$-chitosan SPE was successfully used to simultaneously determinate DA and 5-HT in CFS. And the linear range was able to apply for actual clinical monitor. As can be seen in the table 1, glassy carbon electrode (GCE) is the most common type of electrode for voltammetric determination of DA and/or 5-HT. In the recent years, multiwall carbon nanotube[16, 33], acetylcholine[18], 5-hydroxytryptophan[19], carbon nanotubes-ionic liquid gel[20], gold nanocluster/overoxidized-polypyrrole composite[21], electrochemically reduced GO-porphyrin [23], mobile crystalline material-41[32], functionalized carbon nanotube/ionic liquid nanocomposite[45], double-layered membrane of reduced graphene oxide/polyaniline nanocomposites and molecularly imprinted polymers embedded with gold nanoparticles[46], Safranine O[47], ferulic acid functionalized electrochemically reduced graphene[48], dopamine-functionalized graphene [49], GS@ Mn3O4/Nafion film [50], molecularly imprinted poly (nicotinamide)/CuO nanoparticles[51], and other materials have been used to modify GCE to increase the selectivity and sensitivity of DA and/or 5-HT. However, the preparation of modified GCE is time-consuming and complicated. Compared with GCE, SPE offers significant advantages including simple fabrication, disposability and portability. By comparison with other electrodes, such as calf-thymus DNA on a carbon fiber electrode [17], edge plane pyrolytic graphite electrode[22], carbon nanofiber[24], PbS nanoparticles Schiff base-modified carbon paste electrode[52], and Poly (pyrrole-3-carboxylic acid)-modified pencil graphite electrode[53], MWNTs-$SiO_2$-chitosn SPE couldn't offer the highest sensitivity. However, it has significant advantages such as fast speed, low cost, and it may provide a direct answer for applications in diagnostics with only a simple sample introduction.

**Table 1.** Comparison of different electrochemical sensors for the determination of DA and/or 5-HT.

| Modified electrode | The linear range | | Detection samples | Reference |
|---|---|---|---|---|
| | DA (μM) | 5-HT (μM) | | |
| MWNT-DHP/GCE | 0.05–5 | 0.02–5 | human blood serum | [33] |
| MWNT-IE/GCE | 0.5–10 | 1.0–15 | pH 7.4 PBS | [16] |
| DNA-PPyox/CFE | 0.01–1.0 | 0.3–10 | human blood serum | [17] |
| ACh/GCE | 0.7–5.0 | 1.0–30 | pH 4.0 PBS | [18] |
| 5-HTP/GCE | 0.5–35 | 5–35 | injections | [19] |
| MWNTs-IL-Gel/GCE | 0.1–12 | 0.02–7.0 | human blood serum | [20] |
| Nano-Au/PPyox/GCE | 0.75–20 | 0.07–2.2 | human blood serum | [21] |
| EPPGE | 0.2–25 | 0.1–100 | laked horse blood | [22] |
| ERGO-P/GCE | 0.1–500 | 0.1–300 | pH 7.4 PBS | [23] |
| CNF electrode | 0.05 -10 | 0.1 -10 | modified Tris buffer | [24] |
| MCM-41-$NH_2$/GCE | 0.2–103 nM | 0.2–94.4 nM | human serum | [32] |
| IL–DC–CNT/GCE | - | 5.0–900 | pH 7.0 PBS | [45] |
| (AuNPs@MIPs) membrane/GCE | - | 0.2-10 | human serum samples | [46] |
| poly(SFO)/ GCE | - | 0.03-10 | spiked blood serum samples | [47] |
| ERGO–FA/GCE | 0.6–1000 | - | pH 6.0 citrate buffer solution | [48] |
| ERGO-DA/GCE | 0.5–100 | - | human serum samples | [49] |
| GS@Mn3O4/Nf/GCE | 1.0-1300 | - | injection solution | [50] |
| MIPs@CuO/GCE | 0.02-2.5 | - | serum samples | [51] |
| PSNSB/CPE | 0.05–120 | - | real and synthetic samples | [52] |
| p(P3CA)/PGE | - | 0.01-1 | human blood serum and urine samples | [53] |
| MWNTs-$SiO_2$-chitosn SPE | 1-20 | 0.1-1 | rat CFS | This work |

## 4. CONCLUSIONS

In this study, a novel MWNTs-$SiO_2$-chitosn modified screen-printed electrode was fabricated. The MWNTs-$SiO_2$-chitosn composite were characterized with different methods such as SEM and XRD. The MWNTs-$SiO_2$-chitosn composite could not only efficiently improve the electron transfer but also enhance the accumulation efficiency. The developed SPE are suitable for simultaneously determining DA and 5-HT in rat cerebrospinal fluid. The oxidation peaks of DA and 5-HT could be perfectly separated with good results. Further, interference materials such as AA, UA had negligible effects on the DA and 5-HT response, demonstrating the excellent selectivity and sensitivity of the proposed biosensor. We believe this approach is attractive for use in field based Portable Electronics.

ACKNOWLEDGEMENTS
This project is supported by the Science Research Programs of the Natural Science Foundation of Hubei Province (2015CFB367), the National Natural Science Foundation of China (21301055), the Natural Science Foundation of Hubei Province of China (WJ2015MB147, WX12B06), and the Science Research Programs of Science Technology Department of Hubei Province of China (2013CFB359).

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