Short Communication

Fabrication of NiO/MWNTs modified Screen Printed Electrodes for the determination of norepinephrine in the biological fluids

Ji Ma[1],[#], Ying Xiong[2],[#], Yuting Wang[4], Ding Hong[4], Meng Zhao[3],[*]

[1] Department of Endocrinogy, Xiangyang Central Hospital, Affiliated Hospital of Hubei University of Arts and Science, Xiangyang 441053, China.
[2] Department of Pharmacy, Wuhan No.5 Hospital, Wuhan 430050, China.
[3] Department of psychology, Wuhan Wudong Hospital (Wuhan Second Mental Hospital), Wuhan 430084, China.
[4] Department of Pharmacy, Wuhan University, Wuhan 430072, China.
[#]These authors equally contributed to this work.
[*]E-mail: 13969397@qq.com

Depression was a most common human disorder disease because of insufficient neurotransmitter supply such as norepinephrine (NE). Hence, the accuracy measurement for NE levels in biological fluids including cerebrospinal fluid, brain tissue or blood sample was essential and important to diagnosis some psychiatric and neurological disorders diseases. However, a novel layered-designed composite sensor of NiO/MWNTs/SPE was fabricated and developed to detect brain NE level in depression mice model. The developed SPE exhibited good electrocatalytic activity response to the oxidation of NE during the brain tissue sample detection. The oxidative peak current was linearly related with the NE concentration from the range of 0.75 μM to 30.0 μM, measured by square wave voltammetry (SWV) under the optimal conditions. The detection limit for NE was calculated as 0.05μM (S/N). The newly method presented excellent selectivity and sensitivity for the NE detection in real brain samples with good linear equation compared to the HPLC method, which was benefit for the fast diagnosis of neurological disorders diseases. In conclusion, this experiment demonstrated that the developed sensor was attractive for NE detection in the clinical future diagnosis of mental disease.

1. INTRODUCTION

Norepinephrine (NE), acted as one of the important neurotransmitters in the central nervous systems, played the critical roles in the physiological processes such as anxiety, Parkinson's disease and depression [1,2]. The depression was involved in different symptoms including sadness, despair or

even suicidal inclination due to the neuropathological function changes and the decline of the monoamine neurotransmitters [3,4]. However, many researches supported that the pathological process of depression was associated with the volume loss and neurochemical alterations of NE [5]. Clinical reports [6] also confirmed that the monoamine neurotransmitter NE of patients in the central nervous monoaminergic systems was obviously decreased. Therefore, it was essential to develop a simple, fast, accurate and sensitive method to quantitatively measure NE concentration in biological system, which could provide the important information for the diagnosis, treatment and prognosis of depression.

Several studies had been published about the analytical methods of NE including the high-performance liquid chromatography (HPLC) [7], spectrophotometry [8] and the electrochemistry with various sensors [9,10]. Above traditional methods were rather complicated, expensive and time-consuming, which was required the pretreatment of the derivatization, deproteinization, extraction or purification. Electrochemical analytical method presented had been payed much attention owing to the advantages of low cost, high sensitivity and no sample pretreatment [11]. Screen-printed electrode (SPE) was previously reported to be the inexpensive instrumentation, which was widely applied for the fabrication of disposable sensor to detect the different compounds [12]. Whereas, the SPE sensor used to determinate NE in the depression model had not been published before. Therefore, the purpose of this work was to provide a simple, inexpensive and reliable method for the exact detection of NE based on SPE sensor. Meanwhile, HPLC was also used as the gold method to verify the established SPE sensor technology.

In the earlier studies, it was reported the miniaturized electrochemical sensors based the multi-walled carbon nanotubes (MWNTs), ZnO and Au et al. metal nanoparticles exhibited rapid response, good sensitivity and the low limit of detection. MWNTs could enhance the redox signal because of the excellent electrochemical catalytic properties including the excellent thermal conductivity, nice mechanical strength and chemical stability [13]. Different sensors modified with NiO, metal oxides nanoparticles, were also appealing good properties for electrochemical sensing of dopamine (DA) and 5-hydroxytryptamine (5-HT) by other scholar, which showed low background current and sensitive detect limitation [14]. Hence, according above researches, MWNTs and NiO nanoparticles were aptly chosen as the substrate for the modification in our study.

In this work, we described the application of MWNTs and NiO modified the disposable SPE sensor for the detection of brain NE for the first time. Although various methods were suggested in the previous researches for determination of NE in biological samples, it was still a challenging question to monitor the concentration NE in the tissues of depression model. Moreover, the developed SPE sensor provided the excellent sensing with good selectivity, wide linear ranges, low detection limits and anti-interfering ability in the presence of uric acid (UA) and ascorbic acid (AA) for the long-time application in depression mice model. This novel method was great promising in the diagnosis, treatment and prognosis of depression.

2. REAGENTS AND METHODS

2.1 Reagents and apparatus

NE standards were employed from Sigma. Nano-materials of MWNTs (-OH, 3.06 wt%) were purchased Chengdu Organic Chemicals Co. (Sichuan, China) and . Furthermore, $NiCl_2$ was obtained from Shanghai Kerui.

The stock solution of NE (1mM) was prepared with the doubled distilled water, which then stored at -20°C. The supporting electrolyte solution (0.05 M, pH 7.5) was obtained with adding a certain amount of Tris and HCl. All other chemicals and solvents in this experiment were of analytical reagent (AR) graded that was used without further purification.

Based on the previous report [15], the screen-printed electrodes (SPE) were obtained to be instead of glass carbon electrode, which acted as the three electrode system. EC 570 electrochemical workstation from Gaoss Union Technology (Wuhan, China) was employed to perform all voltammogram experiments with the developed SPE. Moreover, SEM instrument (Quanta 200, FEI Coropration, Holland) and Atomic Force Microscope (AFM, Japan, Shimadzu) were also used to identify the characterization of newly fabricated SPE sensors.

2.2 Preparation of NiO/MWNTs SPE

The disposable NiO/MWNTs SPE sensor was fabricated as following steps [8]. First step, the MWNTs solution with 2mg/mL concentration was dropped on the carbon working electrode to obtain MWNTs SPE, which then needed to be air-dried at room temperature for 30 min. After that, Ni particles were desposited on the surface of MWNTs SPE to prepare Ni/MWNTs SPE. Then, the Ni particles were oxidized to NiO particles through the cyclic voltammogram (CV) with the scan range from -0.8 V to 1.5 V at 100mV/s over 12 cycles in supporting Tris-HCl (0.05mol/L, pH 7.5) buffer to obtain NiO/MWNTs SPE [14]. However, MWNTs SPE and NiO/MWNTs SPE were investigated through approaches for the comparison of superior performance.

2.3 Electrochemical analytical procedure

The electrochemical experiments in this work were all carried out at room temperature (25°C±1) in this work. NE stock solution was dispersed in supporting buffer solution (Tris-HCl, 0.05mol/L, pH 7.5) and the total volume was made to 2 mL. Voltammograms were then recorded under the optimized parameters with CV and square wave voltammogram (SWV), respectively. The scan potential range of CV was from -0.2 V to 0.8 V. And the optimized SWV parameters were shown as following: initial potential (E): -0.2 V, final potential (E): 0.8 V, square wave amplitude: 20 mV, potential step: 6 mV and square wave frequency (f): 15 Hz. Furthermore, all potential were recorded versus Ag/ AgCl electrode of SPE.

After that, NE levels in the depression mice were detected by the developed method. The brain tissues were homogenized under the ice condition and then centrifuged to obtain the supernatant. All

brain tissues samples were diluted with 1.0 mL Tris-HCl to obtain 2.0 mL test sample for the direct determination with no other pretreatment. The proposed electrochemical method with SWV and the HPLC analysis approach were both employed to detect NE content in the brain tissues of the depressed mice. The detail analytical of HPLC was carried out as published with a little modification [7, 16,17]. HPLC system was consisting a LC-10Avp plus HPLC module (Shimadzu, Japan). And Agilent C18 column was provided to perform the analysis for the chromatographic separation, which the parameter was 250·4.6 mm and DI. 5 μm. Moreover, the acetate buffer and UV-grade acetonitrile (pH 3.5) were mixed with 1:9 (v/v) to prepare the mobile phase. The fluorescence detector was employed for the chromatograms analysis, and the excitation wavelength was 254 nm.

2.4 Animal treatment and real sample administration

All animals and experimental procedures complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No.85-23, revised 1996). Meanwhile, the experiment was already approved by the Animal Care Committee at Huazhong University of Science and Technology. Male Kunming mice, weighted (25±3) g, were employed from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. All mice were kept in standard conditions with free access to food and drinking water for 1 week. Then, twenty mice were randomly divided into two groups: control (C) group and model (M) group. As reported, mice in M group were exposed to the chronic unpredictable mild stress (CUMS) to establish the depression model [18,19]. The detail CUMS procedure were administered with a little changes containing the 24 h food deprivation, 24 h water deprivation, a soiled cage (100 mL water in sawdust bedding), 1-min tail pinch (1 cm from the end of the tail), 5-min cold swimming (at 4 °C), two periods (7 and 17 h) of 45∘ cage tilt, two periods (12 h to 12 h) of continuous overnight illumination and dark in day. One unpredictable stressor was randomly given every day 4 weeks. The brain samples of mice in C group and M group were obtained at the end of the experiment for the subsequent NE detection.

3. RESULTS AND DISCUSSION

3.1 Characterization of modified screen-printed electrode (SPE) by SEM and AFM analysis

The surface morphology of modified SPEs was evaluated by SEM and AFM as exhibited in Figure 1. SEM and AFM images revealed the MWNTs SPE and NiO/MWNTs SPE had completely different morphologies. As previous reported [15], MWNTs could provide the excellent electrocatalytic activities owing to the high surface area, rapid response and low resistance for the sensor. Meanwhile, Fu et al reported MWCNTs presented significant oxidation catalysis [20]. SEM (Figure 1A) and AFM (Figure 1C) results confirmed the surface of MWNTs SPE presented many the electron binding sites with the tubular structure and the relative smooth and homogeneous film. However, it could be found that NiO was desposited to cover onto the surface of MWCNTs. The

surface structure and composition of NiO/MWNTs SPE was large, rugged and rough as exhibited in SEM and AFM images (Figure 1B and Figure 1D). According to the previous study [21], NiO could decrease Ohmic resistance of the electrolyte, and then reduce transfer resistance of the electrolyte interface, which thus was proposed. NiO/MWNTs SPE was subsequently fabricated to increase the binging sites of the template molecules, which was beneficial to increase the mass transfer and magnify the electrochemical signal.
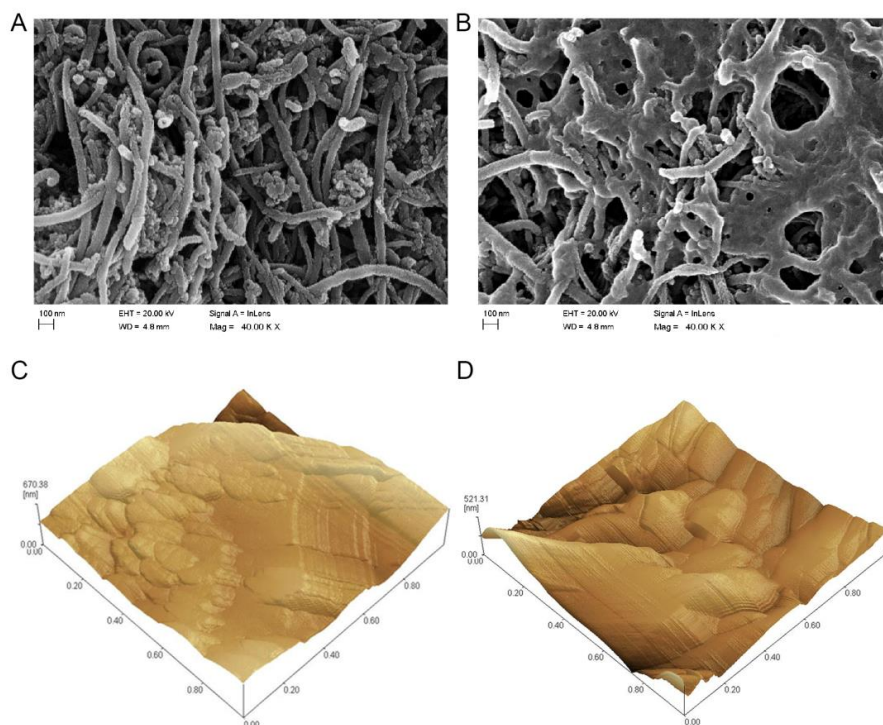

Figure 1. The morphology characterization of MWNTs/SEM SPE (A, C) and NiO/MWNTs SPE (B, D) with SEM images and AFM images

3.2 Electrochemical behavior of NE at NiO/MWNTs SPE

The target molecule NE responds to MWNTs SPE and NiO/MWNTs SPE were obtained by CV and SWV methods. As shown in Figure 2A, CV signal of NE at MWNTs SPE was a poor irreversible redox, and an interference peak appeared at about 0.1 V that resulted in the current intensity was not satisfactory. Contrarily, CV current sensitivity was observed to notably improve several folds when NiO/MWNTs composite was used to modify SPE.

However, Figure 2 B displayed SWV behavior of NE at different sensor. SWV peaks were observed at about 0.2 V to 0.3 V for 10 μM NE. Interestingly, SWV detection sensitivity at NiO/MWNTs SPE was increased approximately five-times than MWNTs SPE. This might be attributed to the aligned NiO/MWNTs composite which provided a high-surface-area material to accelerate the electron transfer rate from binding site to the electrode [14,22]. Hence, the electro-conductivity was obviously enhanced to obtain the maximum current response.
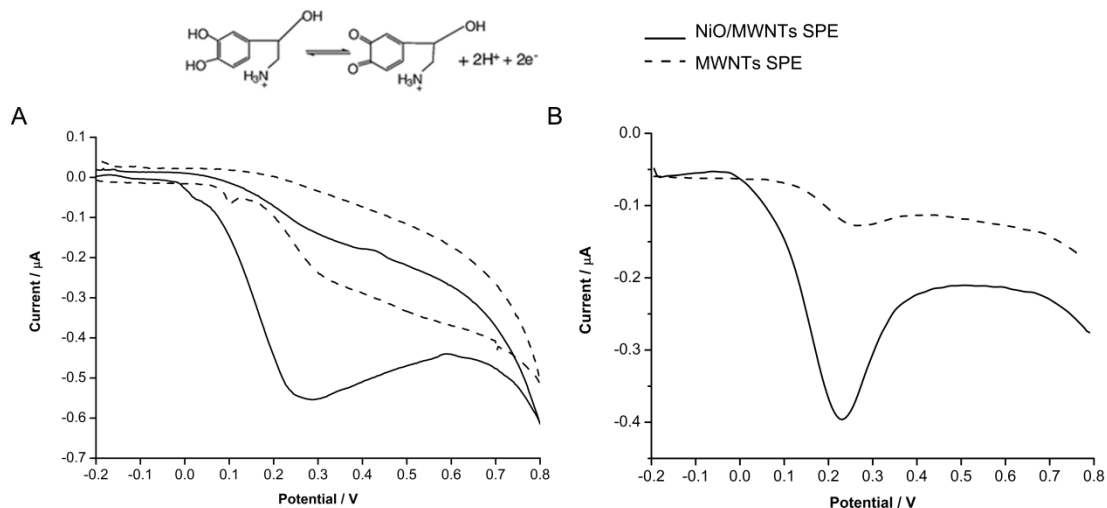
Figure 2. CV curves (A) and SWV curves (B) of NE at MWNTs SPE and NiO/MWNTs SPE. The parameters were as following, scan rate of CV: 100mV/s, frequency: 15Hz, solution: 0.05 M Tri-HCl (7.5) containing 20 µM NE. Insert: NE oxidation reaction.

3.3 The influence of electrochemical condition

The solution pH had a significant influence on the electrochemical performance of NiO/MWNTs SPE. Therefore, choice of pH was investigated from 6.5 to 8.5 by SWV measurement. The maximum peak current response was observed at pH 7.5 as shown in Figure 3A.

The influence of the accumulation time on NE oxidation was also examined by SWV, which peak current response increased fast with the increasing accumulation time, but it nearly remained stable after 8 min (Figure 3B). Therefore, the accumulation time of 8 min was selected as the optimized analytical condition for the subsequent experiment.
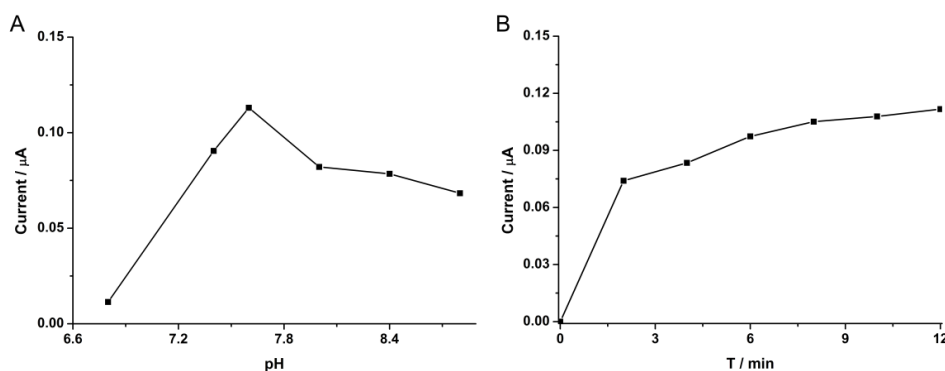

Figure 3. The influence of pH and accumulation time on the electrochemical current responses of 10 µM NE in 0.05 M Tris-HCl buffer at NiO/MWNTs SPE. The peak current response of NE to pH (A) and accumulation time (B).

3.4 Calibration curve

Under the optimum experiment condition, the sensitivity, working curves and detection limit of NE at NiO/MWNTs SPE were investigated by SWV with optical parameters as mentioned in method. The calibration curves of NE in the 0.05 M Tris-HCl (pH 7.5) were obtained as shown in Figure 4, which the calibration equation was observed to be linear with the peak currents in the range of 0.75 μM to 30.0 μM: Ip (μA) = 0.00814c (μM)-0.01407 (R=0.9989). Moreover, various concentrations of NE in the real brain samples were also examined by SWV, which the NE peak current increased linearly with the increasing concentrations. The relationship between the peak current and NE concentration presented a good linear result over the range from 1μM to 30 μM. The linear regression equation was found: Ip (μA) = 0.00855c (μM)-0.01747 (R=0.9979). Moreover, the detection limit of developed method in the real samples was estimated to be as low as 0.05μM (signal/ noise = 3).
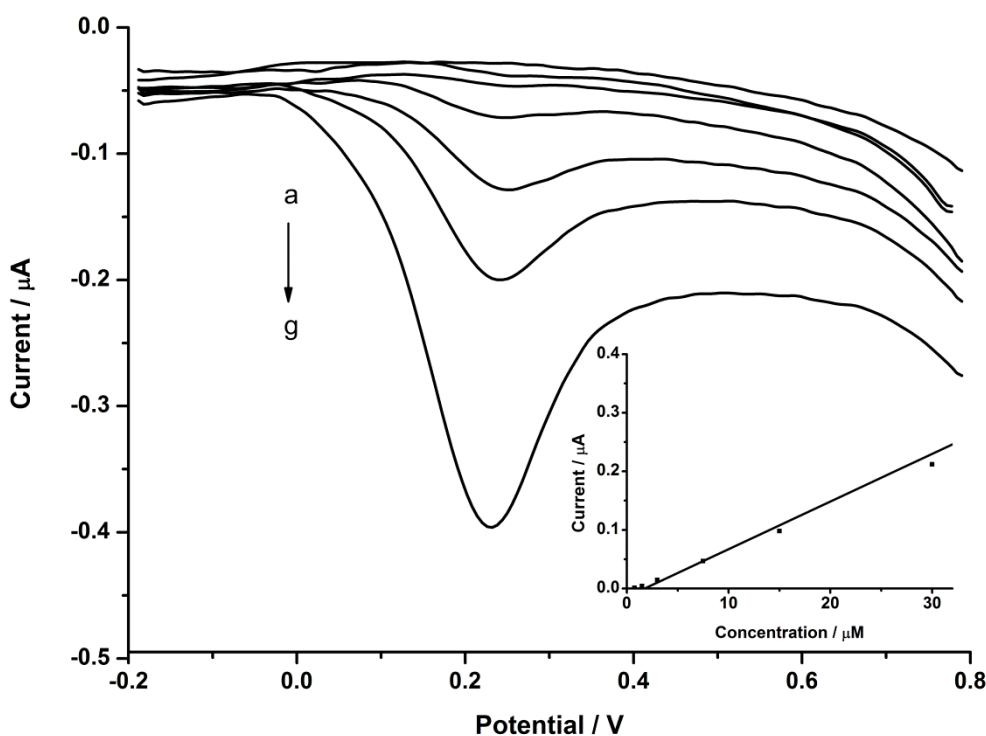

Figure 4. Square wave voltammograms for increasing concentrations of NE at MWNT-ZnONPs SPE under optimum conditions; NE concentration (1-7): 0 mol/L, 7.5×10^{-7}mol/L, 1.5×10^{-6} mol/L, 3×10^{-6} mol/L,7.5×10^{-6} mol/L, 1.5×10^{-5} mol/L, 3×10^{-5} mol/L

3.5 Interferences, reproducibility and stability

The selectivity of modified sensor was tested such as ions and possible interfering biomolecules at the same condition for the NE detection. 100-fold concentrations of K^+, Mg^{2+}, Ca^{2+}, Na^+ and 20-fold concentrations ascorbic acid （AA）, uric acid (UA), glucose and 5-HT were investigated in the presence of 10 μM NE. It was found that the fabricated SPE presented excellent

selectivity for NE determination with no interference. Given the fact that AA and UA were the main biological interference, which could interfere SWV detection for NE, the extra study was carried out to investigate the effect of 50-fold concentrations AA and UA. A far and separate alone peak for UA were observed, and no peaks were obtained for AA, suggesting that above two species do not interfere with the simultaneous detection of NE. Thus, the NiO/MWNTs SPE exhibited the satisfactory selectivity for the detection of NE in real samples.

To investigate the intra-assay reproducibility and inter-assay repeatability of NiO/MWNTs SPE, the NE (10 μM) SWV curves were also recorded. The intra-assay relative standard deviation (RSD) in 5 successive measurements was obtained to be 3.4%, and inter-assay RSD of ten prepared sensors detecting in same conditions was 4.2% respectively. Above results revealed NiO/MWNTs SPE presented an acceptable repeatability. Moreover, the stability of modified SPE at room temperature for 4 weeks was examined, and the SWV responses remained to be 96.8% compared with the initial value, which the good stability was mainly attributed to the NiO/MWNTs composites film.

3.6 Real brain sample analysis in depression mice

The practicability of newly developed sensor for the real sample analysis was evaluated with the standard addition method in depression mice model. The depression mice model was successfully constructed by 4 weeks of CUMS, which was also confirmed through the behaviors experiments such as the open field test and tail suspension test [18,19]. Then, different concentrations of NE were added to the mice brain tissues. Then, the oxidation peak currents were detected with modified SPE by SWV method. However, HPLC detection results of NE were showed in Figure 5A as comparison. It was observed that a separated main peak of NE appeared and the retention time was about 4 min (as shown in Figure 5A). HPLC calibration equation ($Y=181.21X+8091.15$, $R=0.9986$) was also obtained to examine the NE concentrations in mice brain tissues. The brain NE in M group was lower than in S group. While, the experiment was carried out to measure NE content in depression mice with the developed sensor. Curve a was SWV response of NE in spiked standard sample of brain tissue, which the oxidation peak at about 0.24 V was observed. Thus, the prepared sensor could be well applied to exam NE in brain biological samples (curve b in S group and curve c in M group). The fact revealed that the RSD of NE measurement was less than 5% between two methods of fabricated SPE and HPLC. Brain NE content of depression mice in M group was decreased compared to the normal mice, which was the same to other literatures [5, 22]. Hence, the developed sensors were reliable and well used for NE detection in mice biological samples.
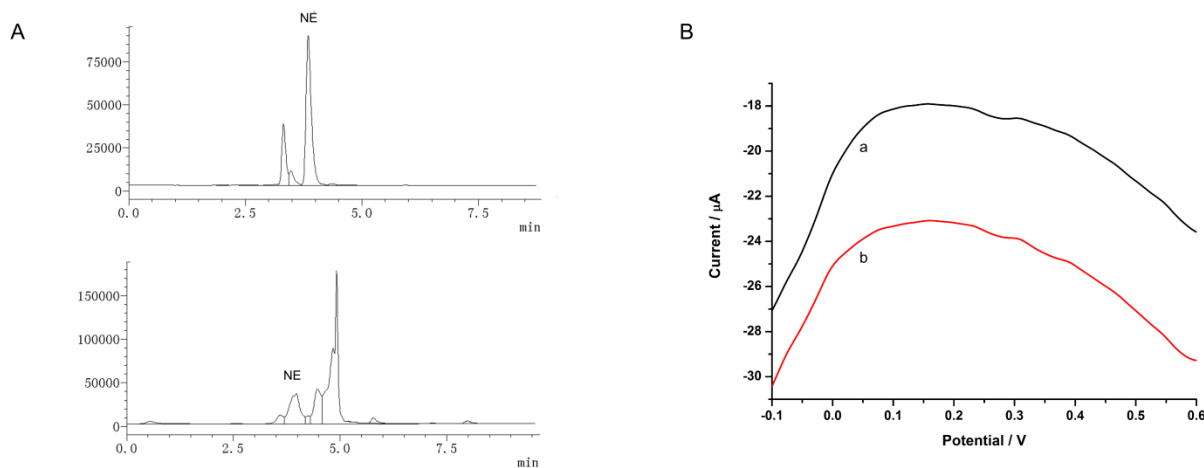
Figure 5. The chromatogram of a standard sample spiked with 5 µM NE and the chromatogram a brain tissue sample (A); SWV curves of NE in brain tissue samples of S group (curve a) and M group (curve b).

4. CONCLUSION

In present paper, a novel NiO/MWNTs SPE for detection NE in depression mice brain tissues was developed and shared. The introduction of NiO nano-particles and MWNTs promoted the electrons transfer of biological molecules, which resulted in the increase of electrochemical signals and presented the high sensitivity. It was firstly reported that NiO/MWNTs SPE was conveniently applied for the brain NE detection in the depression mice, which displayed a good linear relationship between the peak current and NE concentration. Meanwhile, a newly simple method was confirmed to be reliable compared with HPLC during the determination of real brain sample. To our best knowledge, the modified SPE was fewly used to monitor brain neurotransmitter NE, and the proposed sensor provided excellently selective and sensitive approach for NE in biological fluids, benefiting for the fast diagnosis of neurological disorders diseases. Therefore, it seemed that the developed sensor was attractive for the application in the clinical future monitoring and desiease diagnosis.

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