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Liquid chromatography with amperometric detection using functionalized multi-wall carbon nanotube modified electrode for the determination of monoamine neurotransmitters and their metabolites

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Abstract

The fabrication and application of a novel electrochemical detection (ED) method with the functionalized multi-wall carbon nanotubes (MWNTs) chemically modified electrode (CME) for liquid chromatography (LC) were described. The electrochemical behaviors of dopamine (DA) and other monoamine neurotransmitters at the CME were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The results indicated that the CME exhibited efficient electrocatalytic effects on the current responses of monoamine neurotransmitters and their metabolites with high sensitivity, high stability and long-life activity. In LC–ED, DA, norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) had good and stable current responses at the CME. The linear ranges of seven analytes were over four orders of magnitude and the detection limits were $2.5\cdot10^{-10}$ mol/l for DA, 2.5×10^{-10} mol/l for NE, $5.0\cdot10^{-10}$ mol/l for MHPG, $3.0\cdot10^{-10}$ mol/l for DOPAC, $3.5\cdot10^{-10}$ mol/l for 5-HT, $6.0\cdot10^{-10}$ mol/l for 5-HIAA, $1.25\cdot10^{-9}$ mol/l for HVA. The application of this method coupled with microdialysis sampling for the determination of monoamine neurotransmitters and their metabolites in Parkinsonian patients' cerebrospinal fluid was satisfactory.

Keywords: Multi-wall carbon nanotube; Monoamine neurotransmitters

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative diseases in the aged. Although its characteristic pathological finding is the selective cell death of dopamine neurons in subatantia nigra [1], the real pathogeny of PD is not clear to date. However, all experimental studies showed that in PD patients some kinds of monoamine neurotransmitters and their metabolites, such as dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), have abnormal changes compared with those in normal persons [2]. Therefore, in order to realize and control the change regularity of the monoamine neurotransmitters in PD

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patients, it is important to develop the method that can separate and determine these compounds effectively.

Concentrations of monoamine neurotransmitters and their metabolites in cerebrospinal fluid (CSF) have been used extensively as indirect measures of monoamine metabolism in human brain [3]. The CSF surrounding the brain and spinal cord of mammals not only can provide the microclimate to support neuronal function but also may be involved in diffusion neurotransmission [4]. In human, CSF is a major medium in which the dynamics and metabolism of monoaminergic system can be readily accessed and studied. The CSF concentrations of major monoamine neurotransmitters and their metabolites are commonly used as indices of the monoaminergic activity.

A variety of techniques have been applied for the determination of these monoamine neurotransmitters and their metabolites, e.g., spectrophotometry [5], fluorometry [6], ultraviolet–Vis [7], chemiluminescence [8], pseudopolarography [9], flow injection analysis (FIA)-potentiometric sensor [10], voltammetry [11] and amperiometry [12]. Some of these methods have been used in biological matrices and pharmaceuticals to determine the monoamine neurotransmitters. However, because the monoamine neurotransmitters are absence of spectrum group and present with very low concentrations in biological matrix, the applications of fluorometric or other photometric methods for their determination were limited.

Liquid chromatography (LC) is the most commonly employed method for biological analysis because of its small sample volume, high sensitivity, effective separation and small injection volume. Many methods, such as high-performance liquid chromatography (HPLC) with electrochemical [13], fluorometric or ultraviolet [14,15] detection have been used to determine an important number of interesting compounds with a single analysis. Now, electrochemical detection (ED) is gaining more acceptance in HPLC for its more sensitivity and simple operation [16]. In order to improve the sensitivity and selectivity of HPLC–ED, chemically modified electrode (CME) has received rather extensive interest as a HPLC electrochemical detector [17,18]. Considerable work has been done on the ideal modifier of the CME for monoamine analysis.

Recently, carbon nanotubes have come to be one kind of most interesting material since they were discovered in 1991 [19]. Carbon nanotubes were found in two types of structure: the multi-wall carbon nanotubes (MWNTs) and the single-wall carbon nanotubes (SWNTs). Besides the nano-size effects common to other nano materials, carbon nanotubes also show the characters of unique size distribution, novel hollow-tube structure, high specific surface area and excellent electronic semi-conductivity and conductivity. These properties of carbon nanotubes bestow them with a broad range of potential applications such as catalyst [20], biological cell electrodes [21], nanoscale electronic [22] and mechanical systems [23], scanned probe microscope and electron field emission tips [24,25]. In addition, depending on their atomic structures, the subtle electronic properties suggest that carbon nanotubes have the ability to promote electron-transfer reactions when used as an electrode in electrochemical reactions. The MWNTs were first used to fabricate carbon nanotubes electrodes and have been applied to probe bioelectrochemical reactions [21,26] and in the electrocatalysis of oxygen [27]. Their performance has been found to be superior to other carbon electrodes in terms of reaction rates and reversibility.

In this paper, MWNTs were functionalized with nitric acid and the carboxyl groups were introduced to the open ends of the MWNTs. The MWNT solution treated with nitric acid was cast on a glassy carbon electrode to form a MWNT-COOH CME. When the CME was used as an amperometric detector for LC, it showed very stable electrochemical behavior and could be used to catalyze the electrochemical reaction of the monoamine neurotransmitters and their metabolites. Coupled with microdialysis sampling, the application of this method for the simultaneous determination of monoamine neurotransmitters and their metabolites in Parkinsonian patients' cerebrospinal fluid was satisfactory.

2. Experimental

2.1. Regents

MWNTs with diameters of 10–20 nm and lengths of 1–10 μm were obtained from Sun Nanotech (China). DA, norepinephrine (NE), 3-methoxy-4-hy-

droxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5-HT), 5-HIAA, and HVA were analytical grade and purchased from Sigma (USA). All buffer components were of analytical-reagent grade or better. Double-distilled deionized water was used for all solutions. Prior to use, all solutions were degassed with purified nitrogen for 20 min.

2.2. Apparatus

Electrochemical experiments were performed with a CHI832 electrochemical system (CHI, USA). The three-electrode system consisted of a MWNT-COOH CME or a glassy carbon (GC) electrode as working electrode, a saturated calomel electrode as reference electrode and a gold wire electrode as counter electrode.

LC experiments were conducted on a Model 510 pump and a U6K injector (Waters, USA). The injection volume was 20 μl. The column was a Luna 5 μm C_{18} (25 cm×4.6 mm) (Phenomenex, USA) directly attached to a C_{18} precolumn (15×1.0 mm). The detector consisted of a laboratory-made thin-layer cell and a CH1 Potentiostat (Jiangsu Electrochemical Instruments Works, Jiangsu, China). The working electrode was a MWNT-COOH CME or GC electrode. The mobile phase was 0.2 mol/l phosphate buffer (pH 5.0) which was delivered at a constant flow-rate of 1.0 ml/min. All the experiments were performed at room temperature (25 °C) and the pH value was calibrated with a pH meter (Horiba, Japan)

Microdialysis was accomplished by using a CMA 101 microdialysis pump (CMA Microdialysis, Stockholm, Sweden) and a CMA 12 microdialysis probe (dialysis length, 3 mm; diameter, 0.24 mm, BAS, Japan). The probe was perfused with Ringer's solution [28] (147 m*M* Na^+, 4.0 m*M* K^+, 2.2 m*M* Ca^{2+}) at a flow-rate of 1.0 μl/min and the microdialysis device was performed according to the literature [29].

Fourier transform (FT) IR spectra were recorded on a Nexus 670FT IR spectrometer (Nicolet, USA).

2.3. Functionalization of MWNTs with carboxyl groups

The MWNTs functionalized with carboxyl groups were prepared by refluxing MWNTs with HNO_3 for 4–5 h. Then the black MWNT suspension was filtered and the solid was dried under an infrared lamp. After these procedures, the MWNTs were broken and the carboxyl groups were introduced into the cross section. On the FT-IR spectra of the handled MWNTs, the peaks at 1716 and 1575 cm^{-1} were corresponding to ν(C=O, $-COOH^-$) and ν(C=O, $-COO^-$), respectively, which were in accordance with the literature [30,31], and indicated that $-COOH$ and $-COO^-$ were present on the surface of the broken MWNTs. The MWNT-COOH suspension was prepared by dispersing 1 mg of MWNT-COOH solid in 10 ml of *N*,*N*-dimethylformamide (DMF) with ultrasonic agitation.

2.4. Preparation of MWNT-COOH modified electrode

Prior to preparation of the MWNT-COOH CME, the GC electrode surface was polished with 0.3 μm alumina on a polishing micro-cloth and rinsed with deionized water. Subsequently it was ultrasonicated thoroughly with acetone, NaOH solution (50%, w/w), HNO_3 (1:1, v/v) and doubly distilled water. The CME was prepared by dropping the MWNT-COOH suspension (10 μl) on the GC electrode surface and then evaporating the solvent under an infrared lamp.

2.5. Collection and preparation of cerebrospinal fluid samples

Fifteen patients with Parkinsion's disease and six control subjects without neurological disorders in the local hospital participated in the study. The patients were divided into two groups. Eight patients treated with the medicine of L-DOPA were in one group, and the other seven untreated patients were in the other group. After informed consent was obtained from each patient or family members, CSF was collected by lumbar puncture with the patients in the lateral decubitus position in the morning after overnight bed-rest and fasting. An initial 3 ml of the CSF was used for routine examination. The additional 1 ml of the CSF was immediately placed in ice-chilled tubes and stored at −80 °C until analysis.

In order to prevent the interference of large

molecules, such as proteins, in the biological matrix, microdialysis sampling was applied before the CSF was analyzed by HPLC. The CSF in ice-chilled tube was thawed at room temperature. The microdialysis probe was stereotaxically implanted into the tube containing CSF. According to the microdialysis conditions in Section 2.2, samples were then collected continuously at the microdialysis rate of 1.0 μl/min for 25 min and the dialytes were directly analyzed by HPLC–ED.

3. Results and discussion

3.1. Electrochemical behavior of the MWNT-COOH CME

Fig. 1 shows the cyclic voltammograms of the MWNT-COOH CME in 0.2 mol/l phosphate buffer solution (pH 5.0). In the potential range from −0.2 V to +0.6 V, a pair of redox waves were observed with the anodic peak potential at +0.063 V and the cathodic peak potential at +0.0070 V vs. saturated calomel electrode (SCE), respectively, which were related to the redox of the carboxylic group [31]. The peak potentials and peak currents remained very stable after the second cycle. Compared to GC electrode, the background current of the MWNT-COOH CME was apparently large which might be attributed to the increased surface charges. The stability of the MWNT-COOH CME was examined by removing it from the solution after the cyclic voltammetric experiment. The MWNT-COOH CME was rinsed with water and ethanol, then exposed in air for 24 h. When the same cyclic voltammetric experiment was performed with the MWNT-COOH CME again, little changes both in the peak potentials and peak currents were found. These results indicated that the CME was fairly stable.

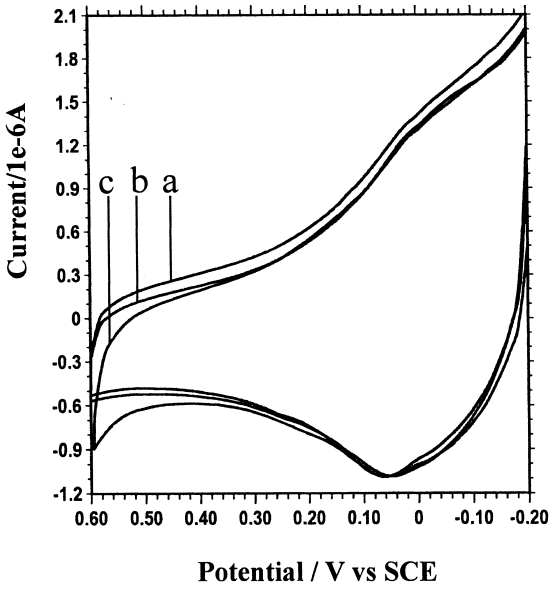

Fig. 1. Cyclic voltammograms of the MWNT-COOH CME in 0.2 mol/l phosphate buffer solution (pH 5.0), 0.1 V s^{-1} scan rate between −0.20 V and 0.60 V. (a) The first, (b) the second and the third cycle, and (c) after 24 h.

3.2. Electrocatalytic oxidation of DA at the MWNT-COOH CME

Fig. 2A shows the cyclic voltammograms of DA at the bare GC electrode and the MWNT-COOH CME in 0.2 mol/l phosphate buffer solution (pH 5.0). With the addition of DA to the buffer solution, a pair of redox peaks was observed at both electrodes. However, compared to the GC electrode, the peak current of DA at the MWNT-COOH CME increased greatly and the reversibility was also improved significantly. This is due to the fact that MWNT-COOH CME could act as a promoter to enhance the electrochemical reaction, increasing the rate of the heterogeneous electron transfer, so the overpotential of DA at the MWNT-COOH CME was lower than that at the bare GC electrode. In addition, depending on the carbon nanotube dimension, the electronic structure and the topological defects present on the tube surface [21], the MWNT-COOH could increase the effective area of the electrode, so the peak current of DA at the CME increased. The differential pulse voltammetry (DPV) responses of DA with different concentrations at the MWNT-COOH CME are shown in Fig. 2B, which indicates that with the growth of DA concentrations, the oxidation currents increased evenly. The DA oxidation currents had a good linear correlation with DA concentrations. Besides the electrochemical behavior of DA, the responses of other monoamine neurotransmitters and their metabolites at the GC electrode and the MWNT-COOH CME were also investigated. The

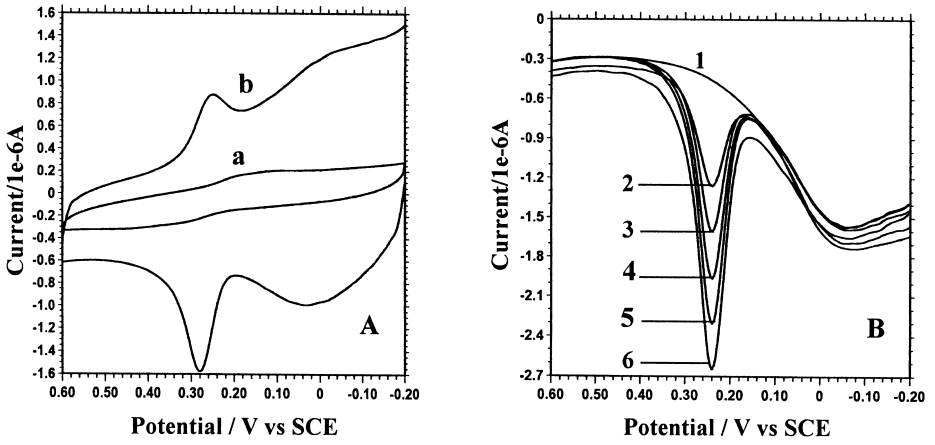

Fig. 2. (A) Cyclic voltammograms of DA at bare GC electrode (a) and the MWNT-COOH CME (b), containing $1.0\cdot10^{-6}$ mol/l DA. (B) Differential pulse voltammograms of DA at the MWNT-COOH CME: (1) 0 mol/l DA; (2) $1.0\cdot10^{-6}$ mol/l DA, (3) $1.5\cdot10^{-6}$ mol/l DA, (4) $2.0\cdot10^{-6}$ mol/l DA; (5) $2.5\cdot10^{-6}$ mol/l DA; (6) $3.0\cdot10^{-6}$ mol/l DA. Electrolyte: 0.20 mol/l phosphate solution (pH 5.0).

oxidation peak currents are summarized in Table 1. It was found that the MWNT-COOH CME had an effective catalysis function and could be used as the electrochemical detector for the monoamine neurotransmitters and their metabolites in HPLC.

3.3. Hydrodynamic voltammetry (HDV)

HDV is a suitable method to select the appropriate potential applied to HPLC–ED. In this study, standard solutions of each of the seven analytes were repetitively injected while the HPLC–ED operating potential was increased from 0.0 to 0.8 V in 0.1 V increments. Fig. 3 shows the hydrodynamic voltammograms of the mixed sample containing $1.0\cdot10^{-6}$ mol/l DA, NE, MHPG, DOAPC, 5-HT, 5-HIAA and HVA on the MWNT-COOH CME. When the applied potential was greater than +0.20 V, the current responses of DA, NE, DOPAC increased, and reached maximum values at +0.40 V. But the current responses of 5-HT, HVA and MHPG were still very low. When the potential increased continuously, the current response of 5-HT, HVA and MHPG increased quickly and reached maximum values at +0.70 V. When the potential increased to greater than +0.70 V, although all the current responses underwent a slight increase, the baseline current also became high and the other substances maybe responded at the electrode. In order to obtain the best selectivity and signal/noise ratio, +0.70 V was chosen as the optimum detection potential.

3.4. Effect of the mobile phase pH

In order to obtain the optimum amperometric responses of the neurotransmitters and their metabolites, it is important to examine the effect of mobile phase pH. Fig. 4 shows the pH effect of mobile phase on the amperometric responses of the monoamine neurotransmitters and their metabolites at the MWNT-COOH CME with HPLC. In consideration

Table 1
The current responses of monoamine neurotransmitters and their metabolites at the MWNT-COOH CME and at the bare GC electrode[a]

Analyte ($1.0\cdot10^{-6}$mol/l)	DA (μA)	NE (μA)	5-HT (μA)	DOPAC (μA)	5-HIAA (μA)	HVA (μA)	MHPG (μA)
At MWNT-COOH CME	0.617	0.684	0.486	0.406	0.299	0.152	0.352
At glassy carbon electrode	0.025	0.029	0.032	0.042	0.029	0.017	0.022

[a] Conditions as in Fig. 2B.

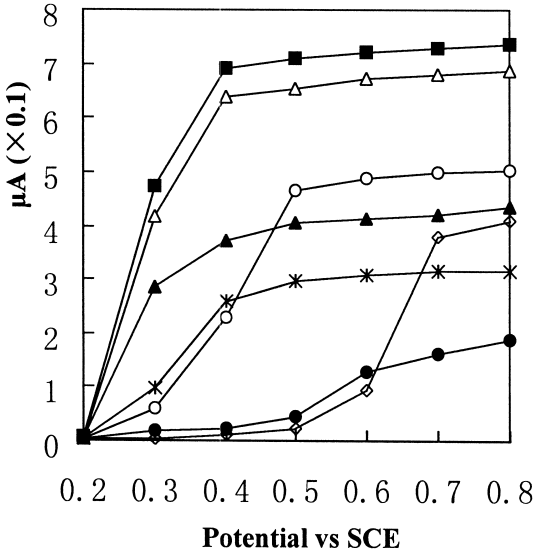

Fig. 3. Hydrodynamic voltammograms of a mixture of $1.0 \cdot 10^{-6}$ mol/l (■) NE, (△) DA, (○) 5-HT, (▲) DOPAC, (◇) MHPG, (*) 5-HIAA, (●) HVA at the MWNT-COOH CME in LC–ED. Column was a Luna 5 μm C_{18} (25 cm×4.6 mm) directly attached to a C_{18} precolumn (15×1.0 mm); injection volume: 20 μl; mobile phase: 0.2 mol/l phosphate solution; pH 6.0; flow-rate: 1.0 ml/min.

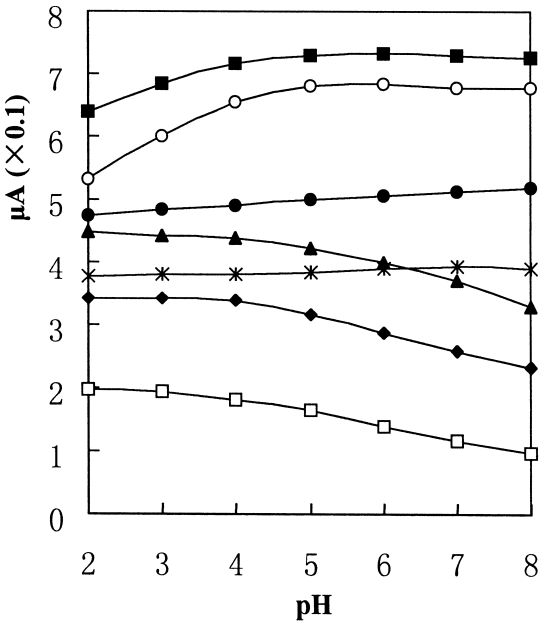

Fig. 4. The pH effect of the mobile phase on the amperometric responses of $1 \cdot 10^{-6}$ mol/l (■) NE, (○) DA, (●) 5-HT, (▲) DOPAC, (*) MHPG, (♦) 5-HIAA, (□) HVA at the MWNT-COOH electrode. Other experimental conditions as in Fig. 3.

of all the monoamine neurotransmitters and their metabolites having good amperometric responses simultaneously, the mobile phase pH was selected as 5.0.

3.5. LC–ED of monoamine neurotransmitters and their metabolites: linearity, detection limits and reproducibility

Fig. 5 shows the current responses of the monoamine neurotransmitters and their metabolites at the GC electrode and at the MWNT-COOH CME in HPLC–ED, respectively. It was found that the current responses of the monoamine neurotransmitters and their metabolites at the MWNT-COOH CME were much larger than those at the GC electrode. The detection limits of these analytes at the CME were investigated and the data are shown in Table 2.

To determine the linearity for DA, NE, MHPG, DOPAC, 5-HT, 5-HIAA and HVA at the MWNT-COOH CME in LC–ED, a series of mixed standard solutions of these analytes ranging from $0.5 \cdot 10^{-9}$ to $5.0 \cdot 10^{-5}$ mol/l were tested. The ranges of the linear relationships were over four orders of magnitude, and all the coefficients were more than 0.995.

The reproducibility of the CME was estimated by eight repetitive injections of a standard solution containing 1.0 μmol/l mixture for the seven analytes under the same conditions every 30 min. The relative standard deviations (RSDs) of the peak currents were found to be 1.2% for DA, 1.3% for NE, 1.6% for MHPG, 1.5% for DOPAC, 1.6% for 5-HT, 1.8% for 5-HIAA, and 1.7% for HVA.

In addition, the long-term stability of the MWNT-COOH CME stored at 4 °C in phosphate-buffered saline was examined by checking its relative activity periodically. No apparent change in the current responses on these analytes was observed over a 1-month period. The results indicated that the MWNT-COOH CME had a good stability and reproducibility when it was used as the HPLC

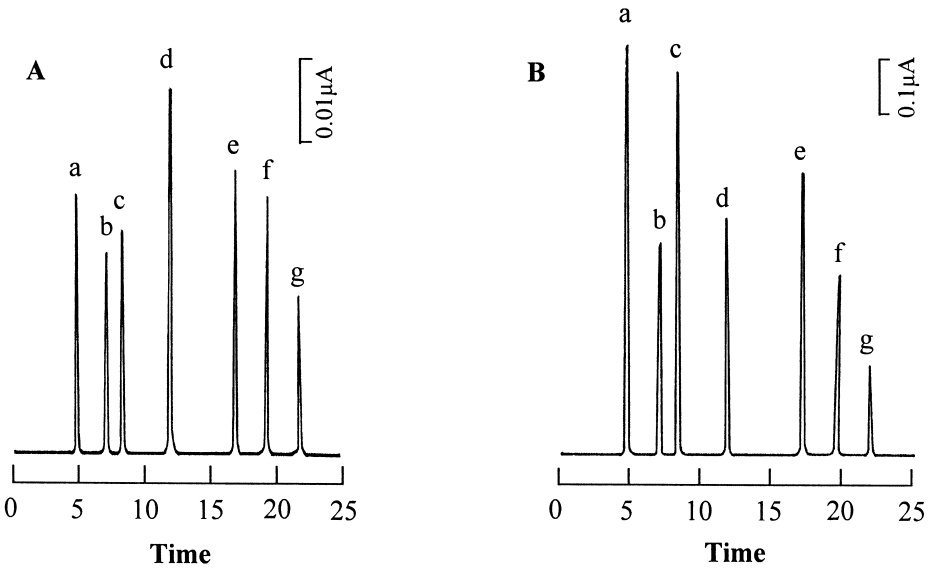

Fig. 5. Chromatograms of $1.0 \cdot 10^{-6}$ mol/l: (a) NE; (b) MHPG; (c) DA; (d) DOPAC; (e) 5-HT; (f) 5-HIAA; (f) HVA at the (A) the bare GC electrode and (B) the MWNT-COOH CME. Applied potential: +0.70 V; other conditions as in Fig. 3.

detector to determine the monoamine neurotransmitters and their metabolites. The analytical data are shown in Table 2.

3.6. Relative recovery of microdialysis sampling experiment

The relative recovery of microdialysis probe for the analyte is equal to the ratio of its concentration in the microdialysate, i.e., the outlet from the probe (C_{out}) to its concentration in the medium surrounding the probe (C_{in}). Therefore the recovery is C_{out}/C_{in}. The relative recovery of a microdialysis probe is affected by the microdialysis rate. At low microdialysis rates, there will be high relative recoveries. However, low microdialysis rates may lead to the pollution of the collected dialysate due to the long collection time. In this paper, several microdialysis rates were investigated for a proper relative recovery. In order to detect the monoamine

Table 2
Analytical data of the seven analytes by LC–ED at the MWNT-COOH CME[a]

Analyte	Regression equation[b]	Correlation coefficient (R^2)	Range (mol/l)	Detection limit (mol/l)[c]	RSD (%)
NE	$y=0.729x+0.002$	0.998	$5.0 \cdot 10^{-10}$–$1.0 \cdot 10^{-5}$	$2.5 \cdot 10^{-10}$	1.3
MHPG	$y=0.382x+0.001$	0.997	$1.0 \cdot 10^{-9}$–$2.0 \cdot 10^{-5}$	$5.0 \cdot 10^{-10}$	1.6
DA	$y=0.680x-0.001$	0.998	$5.0 \cdot 10^{-10}$–$1.0 \cdot 10^{-5}$	$2.5 \cdot 10^{-10}$	1.2
DOPAC	$y=0.421x-0.0014$	0.997	$6.0 \cdot 10^{-10}$–$1.0 \cdot 10^{-5}$	$3.0 \cdot 10^{-10}$	1.5
5-HT	$y=0.501x-0.005$	0.997	$7.0 \cdot 10^{-10}$–$1.0 \cdot 10^{-5}$	$3.5 \cdot 10^{-10}$	1.6
5-HIAA	$y=0.315x+0.008$	0.996	$1.2 \cdot 10^{-9}$–$1.6 \cdot 10^{-5}$	$6.0 \cdot 10^{-10}$	1.8
HVA	$y=0.163x-0.0027$	0.996	$2.5 \cdot 10^{-9}$–$3.0 \cdot 10^{-5}$	$1.25 \cdot 10^{-9}$	1.7

[a] LC–ED conditions as in Fig. 5.
[b] y and x represent the peak current (nA) and the concentration of the analytes (nmol), respectively.
[c] The detection limits of the analytes were investigated using a signal-to-noise ratio of 3 ($S/N=3$).

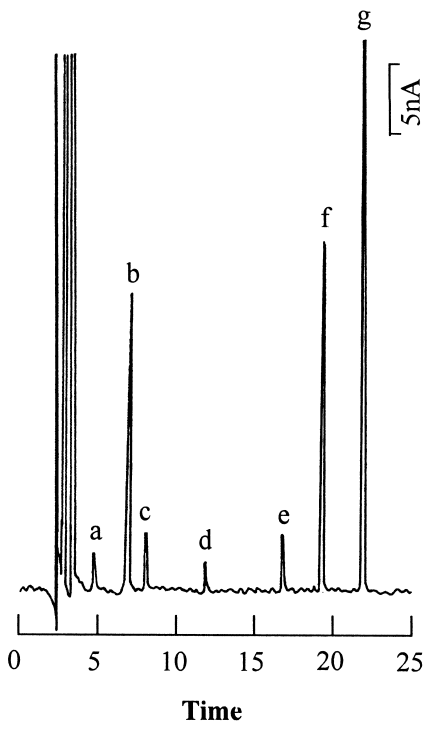

Fig. 6. Chromatograms of the monoamine neurotransmitters and their metabolites: (a) NE; (b) MHPG; (c) DA; (d) DOPAC; (e) 5-HT; (f) 5-HIAA; (g) HVA, in CSF from one control patient by LC–ED coupled with microdialysis sampling. Other conditions as in Fig. 5.

neurotransmitters and their metabolites rapidly and accurately, 1.0 μl/min was selected as optimum microdialysis rate. The microdialysis relative recoveries were found to be 38.8% for DA, 39.6% for NE, 31.2% for MHPG, 38.5% for DOPAC, 37.8% for 5-HT, 29.9% for 5-HIAA and 30.9% for HVA under these conditions.

3.7. Analytical application

To demonstrate the feasibility of the proposed MWNT-COOH CME as the electrochemical detector for HPLC, the determination of monoamine neurotransmitters and their metabolites in human CSF was performed. The original CSF was sampled by microdialysis and injected into the HPLC–ED system directly. Fig. 6 shows the chromatograms of the monoamine neurotransmitters and their metabolites in CSF from one control patient. The average concentrations of monoamine neurotransmitters and their metabolites in CSF samples from the patients and controls are given in Table 3. The detected concentrations of the monoamine neurotransmitters and their metabolites in different CSF samples were within the normal ranges [3,32–37]. All these results suggested that the MWNT-COOH CME was very reliable and sensitive for the determination of the monoamine neurotransmitters and their metabolites in real samples. Further studies are proceeding now in our laboratory.

4. Conclusion

In this paper, the fabrication and application of MWNT-COOH CMEs were studied. Both cyclic voltammetric and liquid chromatographic experiments showed that the MWNT-COOH CME had excellent catalytic activity for the oxidation of

Table 3
The cerebrospinal fluid concentrations of monoamine neurotransmitters and their metabolites in controls and Parkinsonian patients by LC–ED[a]

	Controls [6] (mol/l)	Parkinsonian patients	
		Untreated [8] (mol/l)	Treated [7] (mol/l)
DA	$(4.65\pm1.08)\cdot10^{-9}$	$(2.36\pm0.85)\cdot10^{-9}$	$(16.22\pm7.20)\cdot10^{-8}$
NE	$(2.68\pm1.54)\cdot10^{-9}$	$(1.80\pm0.84)\cdot10^{-9}$	$(1.93\pm1.39)\cdot10^{-9}$
MHPG	$(5.34\pm1.82)\cdot10^{-8}$	$(4.79\pm1.61)\cdot10^{-8}$	$(4.55\pm2.01)\cdot10^{-8}$
DOPAC	$(3.59\pm1.02)\cdot10^{-9}$	$(1.66\pm0.74)\cdot10^{-9}$	$(2.73\pm0.89)\cdot10^{-9}$
5-HT	$(6.41\pm2.03)\cdot10^{-9}$	$(4.60\pm1.29)\cdot10^{-9}$	$(3.28\pm1.18)\cdot10^{-9}$
5-HIAA	$(7.91\pm2.14)\cdot10^{-8}$	$(6.23\pm1.77)\cdot10^{-8}$	$(5.92\pm1.97)\cdot10^{-8}$
HVA	$(23.60\pm9.67)\cdot10^{-8}$	$(12.21\pm6.26)\cdot10^{-8}$	$(29.26\pm11.02)\cdot10^{-8}$

[a] The values shown are calculated from the calibration curves and are mean of $n=3$ in each case. LC–ED conditions as in Fig. 5.

monoamine neurotransmitters and their metabolites. In LC–ED, the sensitivity for determination of these analytes was improved greatly at the MWNT-COOH CME compared to those at the GC electrode. Coupled with microdialysis sampling, the method appeared to be an appropriate analytical procedure for the routine determination of the monoamine neurotransmitters and their metabolites in real biological samples.

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