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Nanostructured electrode based on multi-wall carbon nanotubes/Pt microparticles nanocomposite for electrochemical determination of thiols in rat striatum by high performance liquid chromatography separation

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Abstract

In this paper, multi-wall carbon nanotubes (MWNTs)/Pt microparticles nanocomposite was prepared by electrodepositing Pt microparticles onto the MWNTs matrix. The surface of glassy carbon electrode was modified with this kind of nanocomposite for measurement of thiols, such as L-cysteine (L-Cys) and glutathione (GSH). Compared with the MWNTs or Pt microparticles modified electrode, the nanocomposite modified electrode exhibited high sensitivity and good stability for detection of thiols. According to the results of experiments, the peak currents of L-Cys and GSH are linear with their concentrations and the detection limits (S/N = 3) are 2.9×10^{-8} mol/L and 4.5×10^{-8} mol/L, respectively. Coupled with microdialysis, the method has been successfully applied to the determination of these two thiols in rat striatal microdialysates.

Keywords: Multi-wall carbon nanotubes; Pt microparticles; L-Cysteine; Glutathione

1. Introduction

L-Cysteine (L-Cys) plays an important role in the communication among immune system cells [1], and it is also critical to the metabolism of a number of essential biochemicals, such as coenzyme A, heparin, biotin, lipoid acid and glutathione. Glutathione (GSH) is body's key antioxidant and protectant to remove free radicals inside the body that will damage or destroy key cell components. Owing to the important physiological roles of Cys and GSH, it is necessary to develop sensitive and selective methods for measurement of these substances.

Various chemical and instrumental techniques for the determination of thiols have been reported, such as liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE) and enzyme-based assays. With LC system, there are numerous detection methods, such as fluorescence detection [2,3], UV detection [4], mass spectral detection [5,6], and electrochemical detection [7,8]. Each method has its advantages and limitations and may serve a particular need in analysis. For example, fluorescent detection with the need for derivatization may lack stability or the accuracy of measurement may be hampered by the presence of excess reagent and products from the process of derivatization; UV detection suffers from the difficulties with insufficient selectivity; mass spectral detection may be difficult to quantify all the thiols in a single chromatogram. In contrast, electrochemical detection (ECD) has attracted significant attention as an alternative method for the detection of electroactive species because of its inherent advantages of simplicity, ease of miniaturization, high sensitivity and relatively low cost.

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One initial challenge to the development of electrochemical detection for thiols was that direct oxidation of thiols at solid electrodes was often hampered by slow electron transfer and usually required a potential of at least +1.0 V to initiate [9,10]. This problem was overcome by taking advantage of indirect detections at mercury or gold–mercury amalgam electrode [11] to form stable mercury thiolate complexes. Moreover, much work has been done to develop chemically modified electrodes for measurement of thiols and eliminate the toxicity caused by mercury-based electrode. A series of modified electrodes base on inorganic compounds were designed to reduce the overpotential of thiols [12,13]. Organic compounds [14], enzyme [15] and metallo-phthalocyanines [16] were also used for the catalytic oxidation of thiols. Nevertheless, the poor long-term stability and sensitivity still circumscribe the application of these modified electrodes to biological analysis. Therefore, it is of considerable interest to develop novel electrochemical methodology for monitoring thiols in biological system.

Nanocomposites, where nanoscale inclusions are embedded within a matrix material, have attracted increasing research attention in recent years [17–23]. The discovery of nanotubes with unique mechanical, electrical, thermal properties has led to their uses in the development of the next generation of nanocomposite materials [24–29]. Carbon nanotubes had been used as modifier to fabricate modified electrode in HPLC–ECD system for the determination of some small molecules [30–32]. On the other hand, carbon nanotubes could act as support matrix to be filled with different materials, leading to 1D crystallization of salts with the confining nanotube walls [33,34]. In addition, metal microparticles have attracted extensive interest because of their unique electronic, optical, and catalytic properties [35–40]. It is known that metal microparticles dispersed on porous supports are the common form of solid catalyst, offering the advantages of high surface area per unit volume of the catalyst and a large fraction of the catalytically active material at surfaces, where it is accessible to reactants [41–44]. Owing to the particular characteristics of the metal microparticles and nanotubes, the novel nanocomposites may be developed by depositing the metal microparticles on nanotubes matrix. In this paper, we proposed to design nanocomposite by electrodepositing Pt microparticles on the surface of multi-wall carbon nanotubes (MWNTs), and then to develop a nanostructured electrochemical sensor for thiols by modifying the glassy carbon electrode with this nanocomposite. From the results of experiments, we find that the nanocomposite modified electrode shows some new characteristics: First, it exhibits lower overpotential and higher sensitivity towards thiols relative to MWNTs modified electrode [45], Pt microparticles modified electrode and some other existed modified electrode. Improved catalytic activity is observed when Pt microparticles are incorporated into MWNTs matrix. Second, the stability of this modified electrode is greater than Pt microparticles modified electrode. It is known that one of the limitations of EC detection is the insufficient stability of detector. The carbon nanotubes provide a large specific surface and compact matrix for Pt microparticles to incorporate, which leads to the improvement of the stability of Pt microparticles modified electrode. In brief, the combination of the highly efficient catalytic ability of Pt microparticles with the superior electrical conductivity and stability of MWNTs offers an attractive approach for sensitive and stable thiols detection.

2. Experimental

2.1. Chemicals and reagents

MWNTs with the diameter of 10–30 nm and the length of 1–10 μm were obtained from Sun Nanotech Co. Ltd. (China); K_2PtCl_6 was obtained from Shanghai Chemical Reagent Research Laboratory (China); GSH (reduced) and L-Cys were obtained from Sigma Co. Ltd. (USA); ascorbic acid (AA), uric acid (UA) were purchased from Shanghai Chemical Reagents (China). All chemicals were at least analytical-reagent grade and all solutions were prepared with double-distilled deionized water.

2.2. Apparatus

Electrochemical experiments were carried out on a CHI-830 Electrochemical station (CH Instruments, USA) with a three-electrode system, GC electrode (diameter, 2 mm; BAS Co., Japan) or GC/MWNTs/Pt microparticles modified electrode as the working electrode, a saturated calomel electrode (SCE) (Jiangsu Electroanalytical Instruments Factory, China) as the reference electrode and a platinum electrode as the auxiliary electrode. The experiments were carried out in 0.1 mol/L potassium phosphate buffer solution at pH 3.0. All the electrochemical experiments performed in this paper were carried out at the room temperature (25 °C).

Separations were performed on a liquid chromatography system that was equipped with a Waters 510 HPLC pump (Waters, USA) and a HP ODS Hypersil column (4.6 mm i.d. × 200 mm, 5 μm particle, HP, USA). The working electrode was the GC disk electrode (diameter, 5 mm), the MWNTs modified electrode, the Pt microparticles modified electrode or the MWNTs/Pt microparticles modified electrode. An Ag/AgCl (saturated KCl) electrode was used as reference electrode and the stainless steel as counter electrode. A 0.1 mol/L phosphate buffer solution (PBS) was used as the mobile phase and the separations were performed under the room temperature.

Microdialysis was accomplished using a CMA/101 microdialysis pump (CMA Microdialysis AB, Stockholm, Sweden) and a PES 12 microdialysis probe with a membrane diameter of 0.24 mm and a length of 4.0 mm (BAS Co.).

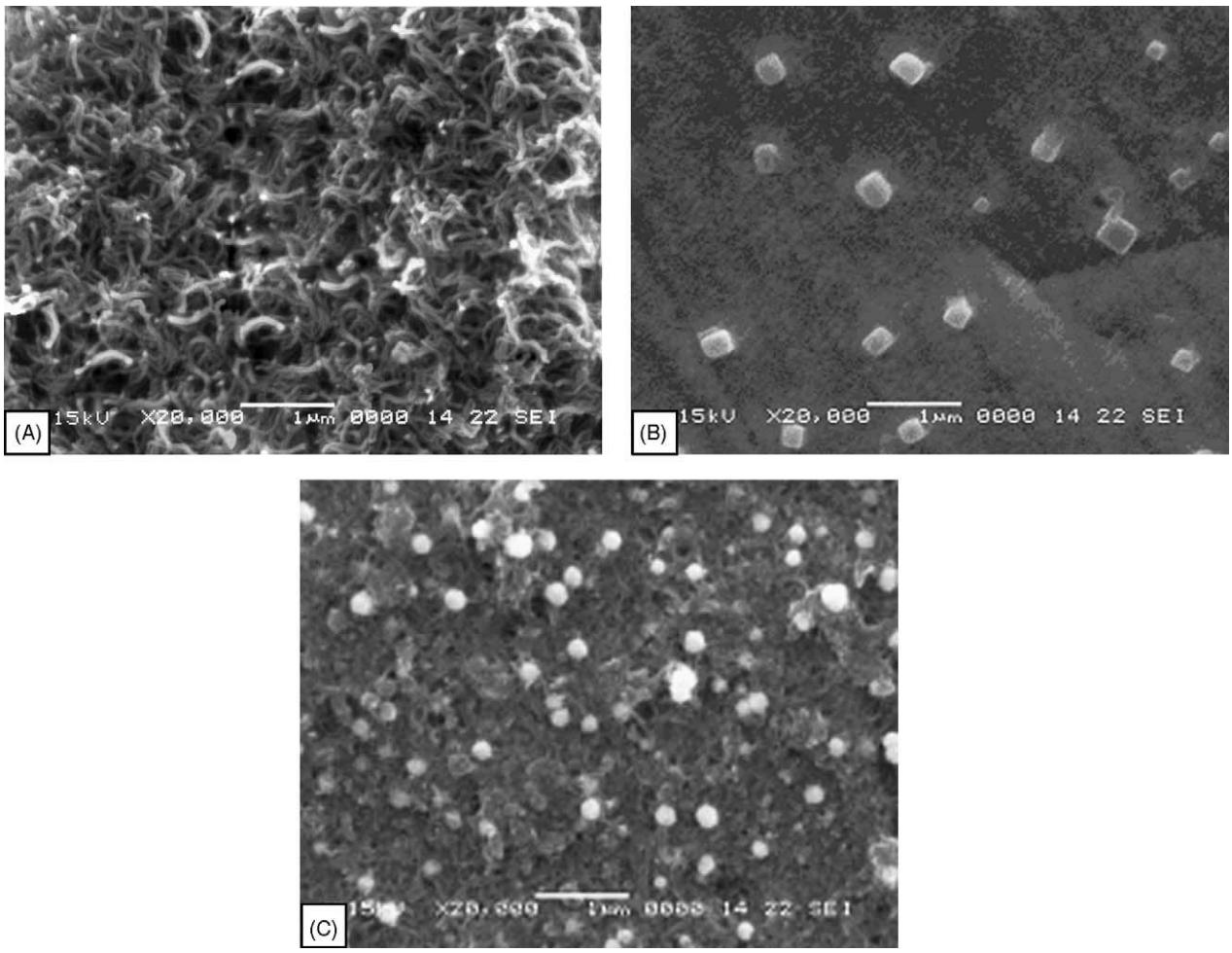

Fig. 1. SEM image of multi-walled carbon nanotubes on GC electrode (A), Pt microparticles on GC electrode (B), and Pt microparticles combined with MWNTs on GC electrode (C).

2.3. Preparation of GC/MWNTs/Pt microparticles modified electrode

The GC electrodes were first mechanically polished with alumina paste (0.5 μm) and rinsed with double-distilled deionized water, and then further cleaned ultrasonically with HNO_3($V_{H_2O} : V_{HNO_3} = 1 : 1$), 1 mol/L NaOH and double-distilled deionized water, respectively. MWNTs-DMF solution (4 μL, 0.1 mg/mL) was dropped on a cleanly polished GC electrode surface to form a homogeneous film. Fig. 1A is the SEM of MWNTs on the surface of GC electrode.

The Pt microparticles were prepared by electrodeposition. The MWNTs modified GC electrode was cycled in 0.5 mol/L sulfuric acid containing 2.0 mmol/L K_2PtCl_6 in a potential that ranged from −0.25 V to +0.4 V at a sweep rate of 100 mV/s for 15 cycles. Fig. 2 is the cyclic voltammogram for the deposition of Pt microparticles. As shown from the SEM in Fig. 1C, a high and homogeneous dispersion of spherical Pt metal particles was formed on MWNTs matrix. After modification, the electrode was rinsed with double-distilled water. According to Fig. 1, we could find that Pt microparticles deposited on GC electrode (Fig. 1B) were cubic with average diameter of about 250 nm; however, Pt microparticles deposited on GC/MWNTs modified electrode (Fig. 1C) were spherical with average diameter of about 100 nm.

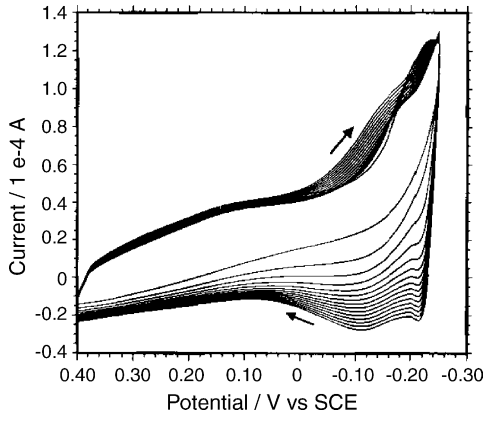

Fig. 2. Consecutive cyclic voltammograms of MWNTs modified electrode in 0.5 mol/L H_2SO_4 solution containing 2.0 mmol/L K_2PtCl_6 with a scan rate of 100 mV/s.

2.4. In vivo microdialysis

Before microdialysis experiment was carried out, in rat striatum, the relative recovery of the microdialysis probe was studied and determined. And then, five male Sprague–Dawley (S.D.) white rats weighing 270–290 g were anaesthetized with 25% urethane (1.5 g/kg) until the animal no longer exhibited limb reflex. After surgery, the rat was kept unconscious with subcutaneous administration of urethane as needed. Once the rat was fixed at a trestle, the microdialysis probe was implanted into the rat striatum (stereotaxic coordination: Bregma $x = +3.0$, $y = +0.6$, $z = -7.0$ mm) [46] and perfused with Ringer's solution (140.0 mmol/L NaCl, 2.4 mmol/L KCl, 1.0 mmol/L $MgCl_2$, 1.0 mmol/L $CaCl_2$, 5.0 mmol/L $NaHCO_3$) at a flow rate of 1.0 μL/min. Sampling started after equilibration with the perfusate for 90 min. All the operations were carried out at the condition of $37 \pm 1\,^{\circ}C$. Five samples with the volumes of 25 μL were collected and injected into the HPLC column successively.

3. Results and discussion

3.1. Electrochemical catalytic oxidation for L-Cys and GSH

The electrodeposition condition of Pt microparticles was studied by cycling the potential between −0.25 V and +0.4 V for 10 cycles, 15 cycles and 20 cycles, respectively. In our experiment, 15 cycles was chosen as the optimal number of deposited turns.

Fig. 3 shows the electrochemical behavior of L-Cys for different types of electrodes with cycle voltammetry. During scanning from −0.2 V to +1.0 V at a bare GC electrode, there was only a much smaller current response for 1×10^{-3} mol/L L-Cys (Fig. 3A). An increase of the current response was observed when a GC/MWNTs modified electrode (Fig. 3B) or a GC/Pt microparticles modified electrode (Fig. 3C) was used. A much larger increase could be observed on the GC/MWNTs/Pt microparticles modified

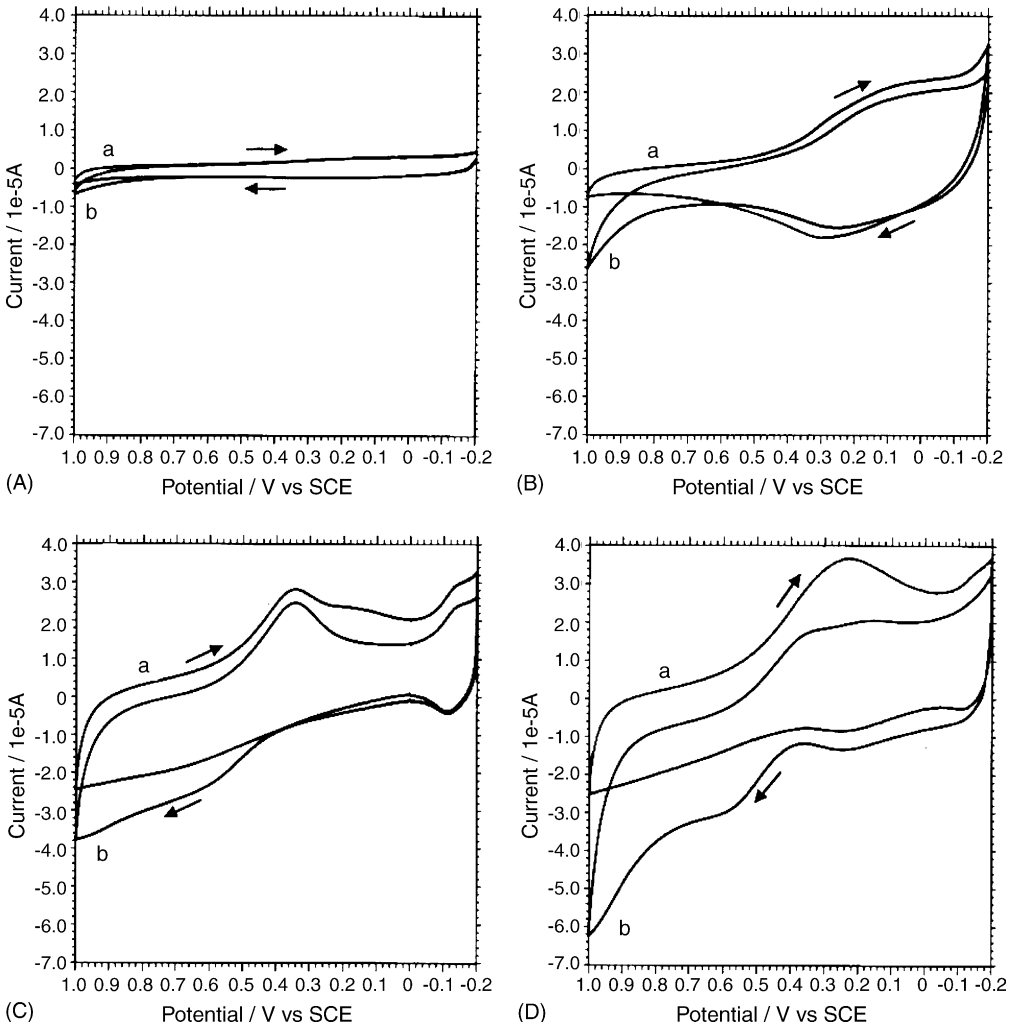

Fig. 3. Cyclic voltammograms of L-Cys on bare GC electrode (A), MWNTs modified electrode (B), Pt particles modified electrode (C), and MWNTs/Pt microparticles chemically modified electrode (D). Scan rate: 100 mV/s (a) in 0.1 mol/L PBS solution (pH 3.0); (a) in 0.1 mol/L PBS solution (pH 3.0); (b) in 0.1 mol/L PBS solution (pH 3.0) containing 1.0×10^{-3} mol/L L-Cys.

electrode (Fig. 3D). In addition, there is no oxidized current peak for L-Cys on the GC electrode, GC/MWNTs modified electrode and GC/Pt microparticles modified electrode over the potential range from −0.2 V to +1.0 V. However, an obvious oxidation peak with a potential at +0.57 V was observed on the GC/MWNTs/Pt microparticles modified electrode (Fig. 3D). Both the enhancement of current response and the decrease of oxidation potential were clear evidence of the improved catalytic effect of MWNTs/Pt microparticles nanocomposite towards L-Cys oxidization than that of MWNTs or Pt microparticles. The similar phenomenon could be observed for GSH. It was suggested that MWNTs/Pt microparticles accelerated the electron transfer during the process of electrochemical oxidation of L-Cys and GSH. The possible explain might be that the partially unsaturated surface d-orbitals of noble metal actorms could absorb and thereby stabilize free radical intermediate oxidation products. The catalytic mechanism was explained in detail in the papers of Johnson and coworkers [12,47], who developed the pulsed ameperometric detection to achieve much more reproducible and sensitive detection of the thiols [48–51].

On the other hand, with the large surface area and integrate surface structure, MWNTs provided a well-defined matrix to allow more densely distributed, more homogeneous and smaller Pt microparticles to deposit on. Therefore, the active surface area of the electrode was increased and the stability of Pt microparticles was improved. It is known that carbon nanotubes offer a good medium for electron transfer and Pt microparticles possess excellent catalysis ability. Hence, the best electrochemical responses of L-Cys and GSH were obtained after the GC electrode was modified with the nanocomposite of MWNTs and Pt microparticles.

3.2. Optimization of liquid chromatographic conditions for the determination of L-Cys and GSH

3.2.1. Optimization of mobile phase

3.2.1.1. Effect of organic modifier. In our experiments, a 0.1 mol/L phosphate buffer solution (PBS) was used as the mobile phase. The present of the additives, such as methanol or acetonitrile, led to the decrease of the retention time of L-Cys and AA. Therefore, these two compounds could not be completely separated. Taking into account the fact that the separation time was less than 12 min, we did not add any modifier to the mobile phase in order to obtain a good peak resolution.

3.2.1.2. Effect of pH value of mobile phase. A series of PBS with different pH values were tested. The appropriate pH range of ODS column was between pH 2.0 and pH 8.0. According to our experiments, when the pH value of the mobile phase ranged from 2.0 to 8.0, the variations of the peak currents were less than 8%. Moreover, with the increase of the pH value, the retention time of L-Cys and AA reduced, which resulted in the incomplete separation between L-Cys and AA.

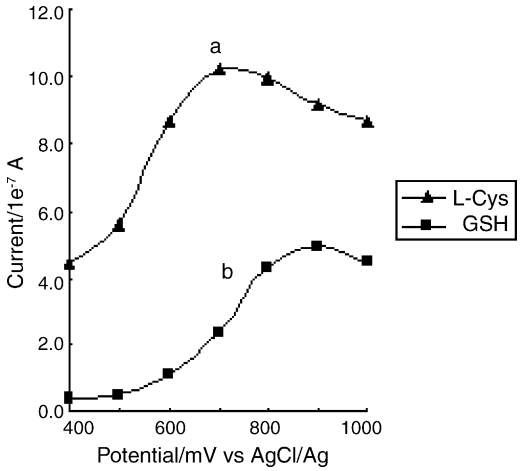

Fig. 4. Hydrodynamic voltammograms of 5.0×1.0^{-5} mol/L L-Cys (a) and GSH (b). (Working electrode: the GC/MWNTs/Pt microparticles modified electrode.)

In our experiment, the PBS with pH 3.0 was chosen as the best.

3.2.1.3. Effect of flow rate of mobile phase. In addition, the results of experiments showed that varying the flow rate of mobile phase from 0.5 mL/min to 1.0 mL/min had little influence on the separation of the analytes. To save the time of separation, the flow rate of 1.0 mL/min was used in our experiment.

In conclusion, 0.1 mol/L PBS (pH 3.0) with flow rate of 1.0 mL/min was used as mobile phase in order to realize the complete separation of analytes.

3.2.2. Optimization of working potential

The hydrodynamic voltammetry (HDV) of each compound was performed in order to select the optimized potential value for their detections. For this purpose, a potential scan in the range from +0.4 V to +1.0 V was applied to a solution containing 5.0×10^{-5} mol/L of each of the compounds. Fig. 4 is the HDV of L-Cys (a) and GSH (b). It shows that the peak current of L-Cys increases gradually when the potential ranges from +0.4 V to +0.7 V and then decreases slightly after +0.7 V. The peak current of GSH increases continuously when the potential ranges from +0.4 V to +1.0 V. In our experiments, +0.8 V was selected as the working potential for the optimal signal-to-noise ratio.

3.2.3. Optimization of working electrode

According to the static CV responses of L-Cys on different electrodes (Fig. 3), the GC/MWNTs/Pt microparticles nanocomposite modified electrode exhibited the highest activity toward catalytic oxidation for L-Cys and GSH among the four electrodes. However, we did not know which one was the best during the separation process. Therefore, a control experiment was conducted. In our experiment, a mixed standard solution of 5.0×10^{-5} mol/L L-Cys (a), GSH (c) and 1.0×10^{-5} mol/L AA (b), UA (d) was

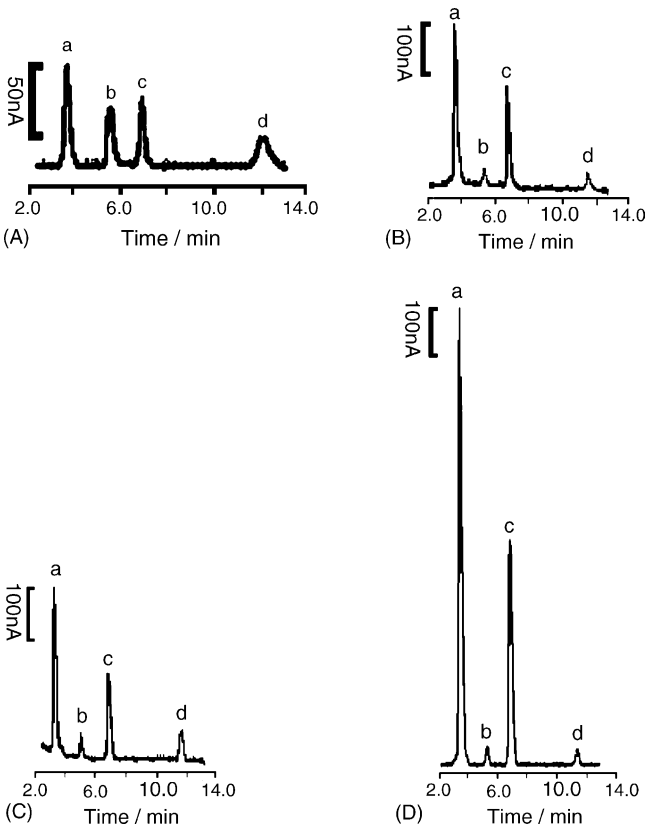

Fig. 5. Chromatograms of mixed standard solution of 5.0×10^{-5} mol/L L-Cys (a), GSH (c) and 1.0×10^{-5} mol/L AA (b), UA (d) on bare GC electrode (A), MWNTs modified GC electrode (B), Pt microparticles modified GC electrode (C), and MWNTs/Pt microparticles modified GC electrode (D). (Working potentials: (A) +1.2 V; (B) +1.0 V; (C) +0.9 V; (D) +0.8 V vs. AgCl/Ag; flow rate: 1.0 mL/min; flow phase: 0.1 mol/L PBS with pH 3.0; injection volume: 25 μL.)

separated and measured with bare GC electrode, MWNTs modified electrode, Pt microparticles modified electrode and MWNTs/Pt microparticles modified electrode, respectively. The retention time of the L-Cys, GSH, AA and UA was 3′30″, 5′23″, 6′46″, and 11′30″, respectively. We found that the bare GC electrode showed poor response to L-Cys and GSH (Fig. 5A), while there were obviously increased responses on MWNTs modified electrode (Fig. 5B) and Pt microparticles modified electrode (Fig. 5C), respectively. The highest peak currents of L-Cys and GSH in Fig. 5D indicated that the sensitivity was improved remarkably by nanocomposite. Except for the best electrochemical responses of L-Cys and GSH, the MWNTs/Pt microparticles nanocomposite modified GC electrode exhibited the best stability in HPLC–ECD system. In our experiments, when GC/Pt microparticles modified electrode served as working electrode in HPLC-EC system for 8 h, only 65% and 60% peak currents were kept for L-Cys and GSH, respectively. However, the peak currents were very stable when GC/MWNTs/Pt microparticles electrode was used in the same flowing system for continuous 24 h experiments. During the experimental process, the mixed standard solution of 5.0×10^{-5} mol/L L-Cys and GSH was injected every 30 min. About 4.2% and 5.3% peak currents for L-Cys and GSH were reduced, respectively. Due to the sensitivity and stability of the different electrodes, the GC/MWNTs/Pt microparticles modified electrode was selected as working electrode.

Moreover, the comparisons of response of L-Cys and GSH on the four different working electrodes in the HPLC–ECD were also made. The results were summarized in Table 1. Compared with the other working electrodes, the MWNTs/Pt microparticles modified electrode has the largest slope of the regression equation, which is related to the significantly increased sensitivity.

3.2.4. Linearity and detection limits, reproducibility

In HPLC–ECD system, a series of standard samples from 1.0×10^{-7} mol/L to 2.0×10^{-3} mol/L of the L-Cys and GSH were tested. The working electrode (MWNTs/Pt microparticles modified electrode) was maintained at +0.8 V against an

Table 1
Regression data of L-Cys and GSH on four different electrodes in HPLC–ECD[a] system

Electrodes	Regression equation ($Y = aX + b$[b])	Correlation coefficients (R)	Linear range (mol/L)	Detection limit[c] (mol/L)
(I) The data of L-Cys in HPLC–ECD system				
GC electrode	$Y = 0.0004X + 3 \times 10^{-10}$	0.9990	3.0×10^{-6}–2.0×10^{-3}	1.1×10^{-6}
MWNTs CME	$Y = 0.0044X + 5 \times 10^{-10}$	0.9987	3.0×10^{-7}–1.0×10^{-3}	1.2×10^{-7}
Pt microparticles ME	$Y = 0.0097X + 1 \times 10^{-10}$	0.9982	2.5×10^{-7}–1.0×10^{-4}	1.0×10^{-7}
MWNT/Pt microparticles ME	$Y = 0.0191X + 5 \times 10^{-10}$	0.9995	1.0×10^{-7}–1.0×10^{-4}	2.9×10^{-8}
(II) The data of GSH in HPLC–ECD system				
GC electrode	$Y = 0.0002X + 2 \times 10^{-10}$	0.9967	3.0×10^{-6}–2.0×10^{-3}	1.6×10^{-6}
MWNTs CME	$Y = 0.0022X + 3 \times 10^{-10}$	0.9990	3.0×10^{-7}–1.0×10^{-3}	2.2×10^{-7}
Pt microparticles ME	$Y = 0.0060X + 1 \times 10^{-10}$	0.9995	4.0×10^{-7}–1.0×10^{-4}	1.8×10^{-7}
MWNT/Pt microparticles ME	$Y = 0.0092X + 7 \times 10^{-10}$	0.9994	2.0×10^{-7}–1.0×10^{-4}	4.5×10^{-8}

[a] HPLC–ECD conditions: working electrode: bare GC electrode, +1.2 V vs. Ag/AgCl; or the GC/MWNTs modified electrode, +1.0 V vs. Ag/AgCl; or the GC/Pt microparticles modified electrode, +0.9 V vs. Ag/AgCl; or the GC/MWNTs/Pt microparticles modified electrode, +0.8 V vs. Ag/AgCl; mobile phase: 0.1 mol/L PBS with pH 3.0; injection volume: 25 μL; flow rate: 1.0 mL/min.

[b] Where Y and X represent the peak current (A) and the concentration of the analyte (mol/L), respectively.

[c] The detection limits of MWNTs/Pt microparticles modified electrode for L-Cys and GSH were calculated according to S/N = 3; the average of the noise values were determined from the blank injection ($n = 11$).

Ag/AgCl and the mobile phase was PBS with pH 3.0. The results showed that the peak currents of the analytes were all well linear with concentration. The linear ranges for the L-Cys and GSH were from 1.0×10^{-7} to 1.0×10^{-4} mol/L and from 2.0×10^{-7} to 1.0×10^{-4} mol/L, respectively. The linear ranges of the two analytes were found to be over three orders of magnitude and the correlation coefficients were larger than 0.999. Fig. 5D was the Chromatogram of mixed standard solution of 5.0×10^{-5} mol/L L-Cys (a), GSH (c) and 1.0×10^{-5} mol/L AA (b), UA (d). From Fig. 5D, a conclusion can be drawn that the two analytes and the two interferential compounds can be separated completely under these conditions. In other words, AA and UA did not interfere the determination of L-Cys and GSH.

The detection limit was calculated according to S/N = 3, and the average of the noise values was determined from the blank injection ($n = 11$). It was found that the detection limits of L-Cys and GSH on MWNTs/Pt microparticles modified electrode were about 2.9×10^{-8} mol/L and 4.5×10^{-8} mol/L, respectively. The detection limits were lower than that of other electrodes in HPLC–ECD system, such as Prussian blue modified screen-printed electrode [52], palladium field-decoupler modified electrode [53], and carbon disc electrode [54].

The repeatability of the MWNTs/Pt microparticles modified electrode was evaluated by repetitive injection ($n = 10$) of 5.0×10^{-5} mol/L L-Cys and GSH under the same conditions as in Fig. 5D. The relative standard deviation (R.S.D.) of the peak currents of L-Cys and GSH was found to be 1.8% and 2.0%, respectively. On the other hand, the same modified electrode was used as working electrode and the mixed standard solution of 5.0×10^{-5} mol/L L-Cys and GSH was injected into the same flowing system several times ($n = 10$) on the 1st day, 2nd day, 5th day, 10th day, 18th day, and 30th day. The results suggested that only $7.0 \pm 0.5\%$ decrease of the L-Cys signal and $8.0 \pm 0.5\%$ decrease of the GSH signal were observed after a month.

3.2.5. Relative recovery of microdialysis

The principle of microdialysis is based on passive diffusion into the probe due to a concentration gradient without alteration of the analyzed system. In the process of microdialysis, recovery is related to the quantities of sample that passed through the dialyzing membrane of microdialysis probe. The relative recovery of the microdialysis probe was defined as the ratio of the analyte's concentration in microdialysate (C_{out}) to its concentration in the medium surrounding around the probe (C_{in}), which can be expressed by a formula $R = C_{out}/C_{in}$. The relative recovery of microdialysis was mainly influenced by the perfusion rate. The lower the microdialysis rate was, the higher relative recovery was. However, lower microdialysis rate might lead to the longer collection time. In this paper, the relative recoveries of L-Cys and GSH at the rate of 5.0 μL/min, 4.0 μL/min, 3.0 μL/min, 2.0 μL/min, 1.0 μL/min were investigated. In order to detect the thiols rapidly and accurately, 1.0 μL/min was chosen as the optimum flow rate. During the experimental process, the microdialysis probe was implanted into the Ringer's solution containing 2.0×10^{-6} mol/L L-Cys and GSH (with the similar concentration to that of in rat striatum). The medium solution (the mixed solution of 2.0×10^{-6} mol/L L-Cys and GSH) and the collected microdialysates were injected into the HPLC–ECD system, respectively. The relative recovery of the probe was calculated by the formula according to the resulting chromatographic peaks. In this experiment, the relative recoveries of L-Cys and GSH were $34.0 \pm 0.6\%$ and $35.0 \pm 0.8\%$, respectively ($n = 5$).

3.2.6. Determination of L-Cys and GSH in rat striatum

Fig. 6 is the chromatogram of L-Cys and GSH in rat striatum microdialysates (a: L-Cys; b: AA; c: GSH). Basal level of L-Cys and GSH measured in this system were 2.5 ± 0.1 μmol/L and 2.7 ± 0.2 μmol/L ($n = 5$). These values and their precisions are in good agreement with previous estimates of 2.0–3.2 μmol/L L-Cys and 1.9–3.2 μmol/L GSH in the extracellular space of rat cortex [55,56]. The data were also in agreement with the results obtained in rat caudate nucleus using microdialysis on-line with capillary zone electrophoresis-laser induced fluorescence detection [57] (2.0 ± 0.1 μmol/L for L-Cys and 2.3 ± 0.2 μmol/L for GSH). Compared to capillary zone electrophoresis-laser induced fluorescence detection, the HPLC–ECD method was easy-to-operate without derivation and had less interference coming from the derivation reagent and derivation production. Meanwhile, this method gave quantitative results similar to those obtained previously using other modified electrodes in our groups (2.2–2.6 μmol/L for L-Cys and 2.0–2.8 μmol/L for GSH) [13,58].

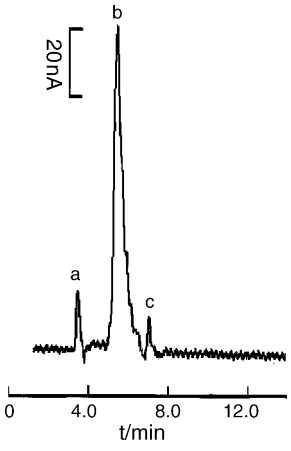

Fig. 6. Chromatograms of L-Cys and GSH in rat striatal microdialysates: (a) L-Cys; (b) AA; (c) GSH. (Working electrode: MWNTs/Pt microparticles modified GC electrode; working potential: +0.8 V vs. AgCl/Ag; flow rate: 1.0 mL/min; flow phase: 0.1 mol/L PBS with pH 3.0; injection volume: 25 μL.)

4. Conclusion

In this paper, the GC/MWNTs/Pt microparticles nanocomposite electrode was prepared and its catalytic oxidation for L-Cys and GSH was also studied. According to the experiment, the sensitivity and stability of the electrode were improved after the nanocomposite modified electrode was used. Coupled with microdialysis, the nanocomposite modified electrode was applied successfully to the HPLC–ECD system for the determination of L-Cys and GSH in rat striatum. Compared to many other modified electrodes, MWNTs/Pt microparticles modified electrode was more sensitive, stable and easier to prepare. The method has been proved to be simple, convenient, and sensitive. Therefore, it has the potential value in the fields of biology and medicine.

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