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# Simultaneous determination of the monoamine neurotransmitters and glucose in rat brain by microdialysis sampling coupled with liquid chromatography-dual electrochemical detector

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**Abstract**

A new type of chemically modified electrode based ring–disk electrode as the dual electrochemical detector (DECD) for high-performance liquid chromatography (HPLC) to simultaneously determine the monoamine neurotransmitters and glucose is described. The ring electrode was modified with an ion-exchange polymer-overoxidized polypyrrole (OPPy) and the disk electrode was modified with nano Au colloid and glucose oxidase (GOD). The electrochemical behaviors of dopamine (DA) and ascorbic acid (AA) at the OPPy chemically modified electrode (CME) were investigated by differential pulse voltammetry (DPV). It was found that the CME could permeate dopamine cations and repelled the ascorbate anions, which could be used to determine the monoamine neurotransmitters and avoid the interference of AA. The electrochemical behavior of glucose at the Nafion/GOD–Au colloid/GC CME was investigated by amperometry and flow injection analysis (FIA). It was found that the sensitivity of the CME increased apparently in determination of glucose. In order to obtain better separation and current responses of the analytes in HPLC-DECD, several operational parameters have been investigated. Under the optimum conditions, the method showed good stability and reproducibility. The application of this method coupled with microdialysis sampling for in vivo simultaneous determination of monoamine neurotransmitters and glucose in rat brain was satisfactory.
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*Keywords:* Microdialysis sampling; Monoamine neurotransmitters; Glucose

## 1. Introduction

The monoamine neurotransmitters and glucose are important components in the brain and play critical roles in neuronal metabolism. Monoamine neurotransmitters, such as dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT) are a kind of neurotransmitter, which are released to extracellular fluids from the cerebral neurons and have important biological, pharmacological and physiological functions [1]. For example, an abnormal loss of DA rich neurons of the substantia nigra may lead to Parkinsons disease [2,3]. As for glucose, it is a major fuel for body energy metabolism and an essential metabolic fuel for brain. Glucose deficit (glucoprivation)

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elicits a variety of physiological and behavioral responses crucial for survival [4]. Previous study has shown that glucose can regulate dopamine release from substantia nigra neurons [5]. Recently, a new therapy for Parkinsons disease was based on the use of glucose as a transportable agent to deliver dopamine into the central nervous system [6]. Therefore, in order to realize their mutual physiological and pharmacological roles, it is important to develop a method that can separate and determine the monoamine neurotransmitters and glucose simultaneously.

There are many approaches to determine the monoamine neurotransmitters and glucose respectively. The methods for determination of the neurotransmitters include spectrophotometer [7], fluorescence [8], chemical luminescence [9], polarography [10], voltammetry [11] and capillary electrophoresis [12]. However, because the monoamine neurotransmitters are absence of spectrum groups, the applications of fluorometric or other photometric methods for their determination were limited. In recent years, high-performance liquid chromatography (HPLC) with electrochemical detector (ED) has been used widely for the analysis of biological samples [13–17]. As an important aspect of HPLC-ED, chemically modified electrodes (CMEs) have received rather extensive interest since they can be designed to make great improvements in sensitivity and selectivity while remaining versatile enough to detect some monoamine analytes [18–20]. Hsueh and Brajter [21] and Pihel et al. [22] reported overoxidized polypyrrole (OPPy) film as an electrode modifier for application in chemical sensors or biosensors. Previous investigations showed that OPPy film could be made quite permeable and had ionic conductivity. During overoxidation of polypyrrole (Ppy), carbonyl groups are introduced into the polymer backbones. The high electron density of the carbonyl group acts as a barrier to hinder the diffusion of anions in the film and has excellent cation permselectivity. Since the OPPy repels anions with the neutral carbonyl groups instead of negative charge sites, electrostatic binding does not occur, the OPPy has fast response time and fewer memory effects and is free from the problem of saturation of the binding sites [23]. Therefore, when modified by OPPy film, the CME can be used as the HPLC detector to determine the cations and repel the anions of biological samples, in which the neurotransmitters interested are present in very low concentrations, while the ascorbic acid is present at higher concentrations that can bring interference to the signal of interest.

Several methods have also been developed to determine the glucose, such as chemical luminescence [24], UV–visible spectrophotometry [25] and electrochemistry [26,27]. Among these methods, the amperometric biosensors for hydrogen peroxide based on electron transfer between an electrode and immobilized enzyme have attracted more attention for the fabrication of selective and sensitive glucose sensors [28,29]. A very important factor in enzyme-based electrode development is the immobilization of enzyme. Recently, the adsorption of enzyme with gold colloid as a biosensor has been reported [30,31]. Because the gold colloid has good biocompatibility and provides a similar native environment and amplifies the surface coverage of the functional layer [32], the electrode modified by the enzyme and gold colloid can be a promising biosensor in glucose determination.

In this paper, a new type of carbon film based ring–disk electrode was used as the dual electrochemical detector (DECD) for LC to determine the monoamine neurotransmitters and glucose simultaneously. The ring electrode was modified with overoxidized polypyrrole (OPPy) film. Differential pulse voltammetry (DPV) and LC also showed that the OPPy CME could effectively permeate DA cations and repel the ascorbic acid anion. NE, E, DA, DOPAC and 5-HT had good and stable current responses at the OPPy CME. The disk electrode was modified by immobilization of glucose oxidase in the Au colloid matrix. To immobilize the enzyme firmly, glutaraldehyde was employed as a conventional cross-linking agent. On the disk electrode surface modified by the Au colloid–GOD, Nafion film was used to prevent interference from compounds such as ascorbate and urate. The CME electrode showed excellent stability, sensitivity, selectivity and long-life time towards determination of the glucose. The application of the LC-DECD coupled with microdialysis sampling for in vivo simultaneous determination of the monoamine neurotransmitters and glucose in rat brain was satisfactory.

## 2. Experimental

### 2.1. Reagents

Dopamine (DA), norepinephrine (NE), 5-hydroxytryptamine (5-HT), epinephrine (E), 3,4-dihydroxyhenylacetic acid (DOPAC), ascorbic acid (AA), glucose oxidase (GOD) (EC 1.1.3.4, type VII-S from *Aspergillus niger*, 229.2 U/mg), glucose, 1% Nafion (methanol solution) and bovine serum album (BSA) were analytical grade (Sigma Company, St. Louis, MO, USA). Pyrrole was analytical grade (Fluka Chemie AG, Switzerland) and purified by distillation, stored in a refrigerator and protected from light. $HAuCl_4$, sodium citrate (Shanghai Chemical Reagents Institute, Shanghai, China). All buffer components were analytical reagents or better quality. Double-distilled deionized water was used for all solutions. Prior to use, all solutions were deaerated with prepurified nitrogen for 15 min.

### 2.2. Apparatus

Electrochemical measurements were performed on a CHI 832 Electrochemical System (CHI, USA). All experiments were employed by a three-electrode cell with an Ag/AgCl as the reference electrode, a gold wire electrode as counter electrode and a glassy carbon (GC) electrode as working electrode.

Liquid chromatographic experiments were conducted on a model 510 pump and a U6K injector (Waters Assoc., USA). The injection volume was 20 μl. The column was Zorbox C8 (4.6 mm×25 cm) (Du Pont, USA). A homemade thin-layer flow cell (see Fig. 1) was used as the detector with two CH-I potentiostats (Jiangsu Electroanalytical Instruments Limited Company, Jiangsu, PR China). The working electrode was a glassy carbon film based ring–disk electrode, which came from BAS Co. (Japan). The mobile phase was 0.20 mol/l phosphate buffer solution (PBS)(pH 5.0), which was delivered at a constant flow-rate of 1.0 ml/min. All the experiments were performed at room temperature and the pH value was accommodated with a B-212 pH meter (Horiba Co., Japan).

Microdialysis was accomplished by using a CMA 101 microdialysis pump (CMA Microdialysis AB., Stockholm, Sweden) and a CMA 12 microdialysis probe (dialysis length, 3 mm; diameter, 0.24 mm) (BAS Co., Japan). The probe was perfused with the Ringer solution (147 m*M* $Na^+$, 4.0 m*M* $K^+$ and 2.2 m*M* $Ca^{2+}$) [33] at a flow-rate of 1.0 μl/min.

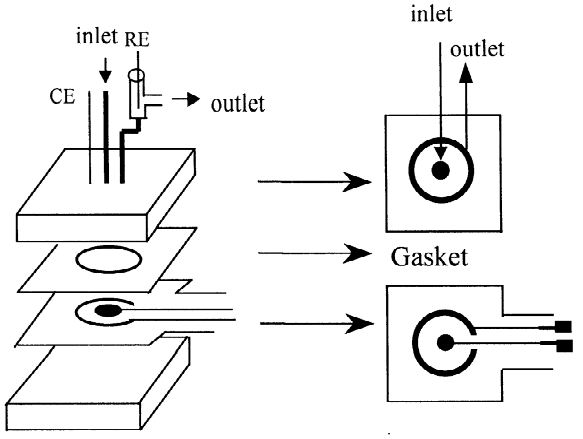


Fig. 1. Schematic diagram of a thin-layer flow cell with the carbon film based ring–disk electrode.

### 2.3. Preparation of Au colloid

Glassware used in the following procedures was cleaned in a bath of freshly prepared $HNO_3$: HCl (3:1), rinsed thoroughly in double distilled water and dried in air. $HAuCl_4$ and sodium citrate solutions were filtered through a 22 μm microporous membrane filter prior to use. The preparation of Au colloid was according to the literature [34]. Atomic force microscopy showed that the size of Au colloid particles was from 7 to 14 nm.

### 2.4. Modification of electrode

#### 2.4.1. The modification of ring electrode with OPPy

Electropolymerization of pyrrole was carried out in 0.2 mol/l KCl solution (pH 7.4) containing 0.1 mol/l pyrrole by cycling the potential from 0.0 to 1.0 V at 0.1 V/s for eight times. Then it was washed with distilled water and air-dried, and the PPy film CME was obtained. The PPy CME was overoxidized in stirred 0.1 mol/l NaOH solution by holding their

potential at +1.0 V for 4 min. Then the stable OPPy CME was obtained and stored in the buffer solution.

*2.4.2. The modification of disk electrode with Au colloid and GOD*

An enzyme solution was prepared by dissolving 2 mg of GOD and 10 mg BSA in 0.20 ml Au colloid solution. An aliquot of 50 μl of the enzyme solution and 10 μl of 5% glutaraldehyde solution were then mixed thoroughly and 3 μl of the mixture solution was dropped on the disc electrode. After drying in air, it was cross-linked at room temperature for 2 h. Then 5 μl 1% Nafion was dropped on the surface of the GOD electrode. Allowed to air-dry, the electrode was kept in phosphate buffer solution at 4 °C.

*2.5. In vivo microdialysis experiment*

Animal care was in accordance with the guide for the Care and Use of Laboratory Animals (NIH Publication No. 86-23, 1985, Beheads, MD, USA). The experiment was performed on Sprague–Dawley male rats weighing 200–250 g. The animals were anesthetized by 20% urethane (1.25 g/kg), and placed in a stereotaxic frame. The hole was drilled in the skull and then the microdialysis probe was implanted in the striatum. The microdialysis probe was stereotaxically implanted into the striatum at coordinates 0.2 mm posterior to the bregma, 4.0 mm lateral from midline and the middle of the probe 4.5 mm below the dura [35]. Microdialysis samples were collected at the microdialysis rate of 1.0 μl/min for 20 min and analyzed at the ring–disk dual electrode of the LC-DECD. The dialysate samples collected during the first 60 min were discarded to allow recovery from the acute effects of the surgical procedure.

## 3. Results and discussion

*3.1. DPV of DA and AA at GC electrode and OPPy CME*

Fig. 2 shows the differential pulse voltammograms of DA and AA at the bare GC electrode and the OPPy CME respectively. At the bare GC electrode, AA and DA showed oxidative peaks at 0.2874 V and 0.2445 V respectively. At the OPPy CME, there was no obvious oxidative peak for AA. However, DA showed an oxidative peak at 0.1123 V. Compared with the data at the bare GC electrode, the peak current of DA increased and its overpotential decreased at the CME, while the current response of AA decreased apparently at the OPPy CME. Because the p$K_a$ of ascorbic acid is 4.2 and the p$K_a$ of

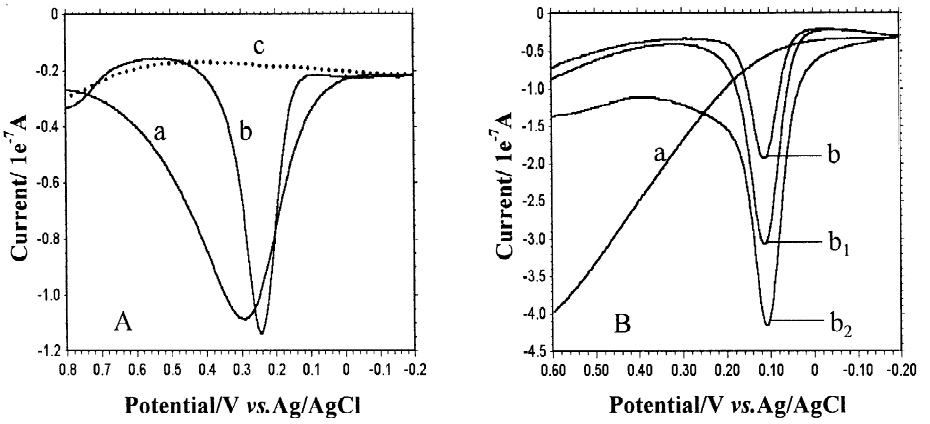


Fig. 2. Differential pulse voltammogram of DA and AA at the bare GC(A) electrode and the OPPy CME (B): (a) containing $1.0\times10^{-6}$ mol/l AA; (b) containing $1.0\times10^{-6}$ mol/l DA, ($b_1$) containing $2.0\times10^{-6}$ mol/l DA, ($b_2$) containing $3.0\times10^{-6}$ mol/l DA; (c) the blank solution: electrolyte: 0.20 mol/l phosphate solution (pH 5.0).

dopamine is 8.92, ascorbic acid is anion and dopamine is cation in phosphate buffer solution (pH 5.0). The results showed that the overoxidized PPy film could effectively exclude the negative ascorbate ions from the electrode but increase the current response of positive dopamine species.

During the overoxidation of PPy film in 0.1 mol/l NaOH, nucleophilic attack of the radical cationic pyrrole units in the polymer by hydroxide ions took place, resulting in loss of the conjugated structure and electrical conduction. Then the polymer was converted from an electronic/ionic conductor into a non-electronic but purely ionic conductor. Meanwhile, the doping ions of the polymer were also expelled from the polymer film, forming a porous structure on the film. Electroactive species and electrolyte still diffused through the porous film to the underlying electrode. The hydroxyl and carbonyl groups present in the overoxidized pyrrole units were confirmed by Fourier transform IR spectra [36]. Therefore the overoxidized PPy could be regarded as a negatively charged polymer film. The negative charges in the polymer effectively repelled negative anions from the film and attracted the positive cations to the film giving an electrostatic effect, which could be seen in the much lower current of ascorbic acid and much larger current of dopamine at the OPPy CME. Therefore the OPPy CME was very robust and could be used to determine the neurotransmitters and selectively retard the response of ascorbic acid, which is the main interference in in vivo determination.

### *3.2. Relationship between the OPPy film thickness and the current responses of DA and AA*

Fig. 3 shows the thickness of the OPPy film has an important effect on the permselective transport for the dopamine cations and against the ascorbic acid anions. When the scan cycles of electropolymerization were few, the film was also thin, then the sensitivity of OPPy CME for dopamine was higher than that of the bare GC electrode. Meanwhile the selectivity of the OPPy CME for dopamine with respect to ascorbic acid was higher than that of the bare GC electrode. With the increase of the scan cycles of electropolymerization, the film thickness

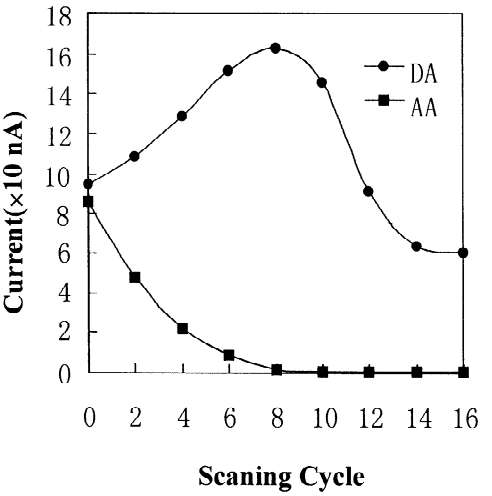


Fig. 3. The relationship between the scan times of electropolymerization and the current responses of $1.0\times10^{-6}$ mol/l DA and AA at the OPPy CME in the pH 5.0 phosphate buffer solution.

increased too. When the scan cycles were over eight, a substantial decrease in sensitivity of the OPPy CME for dopamine could be observed. In order to get better selectivity and sensitivity, the scan cycles of electropolymerization PPy film were selected as eight.

### *3.3. Hydrodynamic voltammetry (HDV) of the OPPy CME*

Fig. 4 is the hydrodynamic voltammograms of the mixed sample containing $1.0\times10^{-4}$ mol/l NE, E, DA, DOAPC and 5-HT at the OPPy CME. When the applied potential was larger than +0.20 V, the current responses of NE, E, DA, DOPAC increased apparently. But the current response of 5-HT was still very low. When the potential increased continuously, the current response of 5-HT increased quickly and reached a high value at +0.50 V. When the potential increased larger than +0.60 V, the baseline current became high and the other substances maybe responded on the electrode. In order to obtain the best selectivity and signal/noise ratio, +0.50 V was chosen as the optimum detection potential.

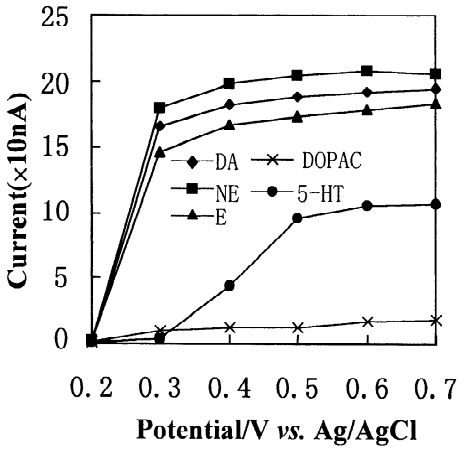


Fig. 4. Hydrodynamic voltammogram of a mixture of $1.0\times10^{-6}$ mol/l NE, E, DA, DOPAC and 5-HT at the OPPy CME in LC-DECD. Column, Zorbax C8 (4.0 mm×25 cm); injection volume: 20 μl; mobile phase: 0.2 mol/l phosphate solution; pH 5.0; flow-rate: 1.0 ml/min.

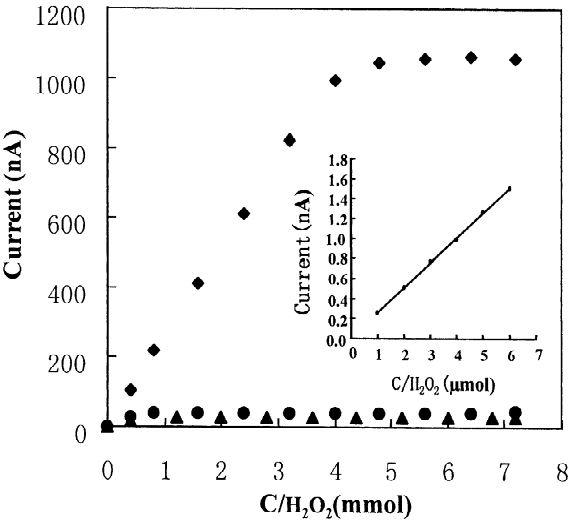


Fig. 5. Amperometric responses of Nafion/GOD–Au colloid/GC (♦), Nafion/GOD/GC (▲) and Nafion/Au colloid/GC (●) CME for successive additions of $H_2O_2$ in the pH 5.0 phosphate buffer solution.

### *3.4. Amperometric responses of Nafion/GOD–Au colloid/GC, Nafion/GOD/GC and Nafion/Au colloid/GC CME upon successive additions of $H_2O_2$*

The measurement of glucose has been intensively studied and is usually based on the following reaction:

$$\text{Glucose} + 1/2O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

The produced $H_2O_2$ can be directly measured via electrochemical determination. Therefore, the approach for hydrogen peroxide determination is investigated regarding its suitability to the measurement of glucose.

The glassy carbon electrodes were modified by Nafion/GOD–Au colloid, Nafion/GOD, Nafion/Au colloid respectively, and the amperometric responses of these electrodes to $H_2O_2$ are shown in Fig. 5. From the figure, it was found that the Nafion/GOD–Au colloid/GC CME had excellent responses to $H_2O_2$ concentrations, whereas the two glassy carbon electrodes modified with Nafion/GOD or Nafion/Au colloid had no catalytic current in the determination of $H_2O_2$. The inset in Fig. 5 showed the linear range of the calibration curve.

The GOD–Au colloid modified electrode has much more activity owing to the nano Au particles. Gold colloid particles have a very high surface to volume ratio. Uncontaminated gold particle surfaces have a high surface energy and so are very active. The interaction with enzyme molecules can be very strong. The small size of the colloidal particles gives the enzyme molecule more freedom in orientation and so increases the possibility that the functional group is closer to the metal particle surface. This makes the distance for electron transfer between the enzyme and the metal particles shorter and the electron transfer occurs directly between a redox enzyme and an electrode surface. With the GOD–Au colloid deposited onto an electrode surface, the gold colloid particles with GOD can function as electron-conducting pathways between the enzyme and the electrode surface and therefore facilitate the electron transfer process.

### *3.5. Hydrodynamic voltammetry of the glucose at the Nafion/GOD–Au colloid/GC CME*

Fig. 6 is the hydrodynamic voltammogram of glucose at the Nafion/GOD–Au colloid/GC CME. With the change of the applied potential from 0.0 V

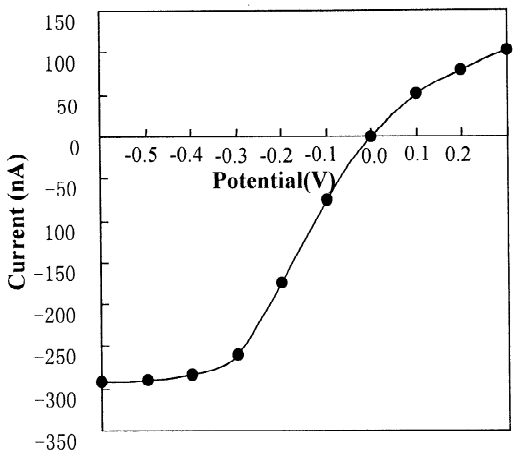


Fig. 6. Hydrodynamic voltammogram of $1.0\times10^{-3}$ mol/l glucose at the Nafion/GOD–Au colloid/GC CME in LC-ED. Column, Zorbax $C_8$ (4.0 mm×25 cm); injection volume: 20 μl; mobile phase: 0.2 mol/l phosphate solution; pH 5.0; flow-rate: 1.0 ml/min.

to −0.5 V, the current response of the glucose enhanced apparently, but if the potential was too negative, the baseline of the CME increased. Furthermore, other substances could be responded at the CME. As seen in Fig. 6, the optimum potential at the Nafion/GOD–Au colloid/GC was selected as −0.3 V (vs. Ag/AgCl).

### *3.6. Flow injection analysis of glucose at the Nafion/GOD–Au colloid/GC CME as HPLC detector*

Fig. 7 shows the flow injection analysis of glucose on the Nafion/GOD–Au colloid/GC CME as the amperometric detector of LC. From the chromatogram, it was found that glucose had a large amperometric response at the Nafion/GOD–Au colloid/GC, and the amperometric response of glucose on the Nafion/GOD–Au colloid/GC CME had a good linear correlation with glucose concentrations.

### *3.7. Effect of mobile phase pH*

In order to obtain the optimum amperometric responses of the neurotransmitters and the glucose, it is important to examine the effect of pH of the mobile phase. Fig. 8 shows the pH effect of the

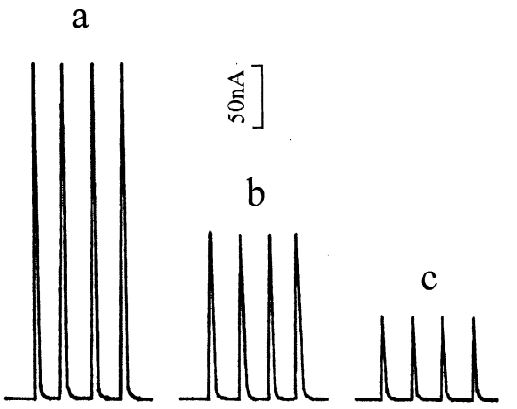


Fig. 7. Flow injection analysis of glucose on the Nafion/GOD–Au colloid/GC CME as the amperometric detector, (a) 1 m*M*, (b) 0.5 m*M*, (c) 0.25 m*M*. Applied potential −0.3 V vs. Ag/AgCl; other experimental conditions as in Fig. 6.

mobile phase on the amperometric responses of the neurotransmitters and glucose at the ring–disc electrode. In consideration that all the neurotransmitters and glucose would have good amperometric responses simultaneously, the mobile phase pH value was selected as 5.0.

### *3.8. Linearity detection limits and reproducibility*

To determine the linearity for the neurotransmitters and glucose at the ring–disc modified electrode

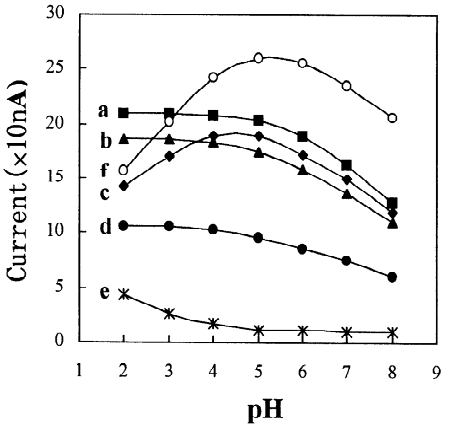


Fig. 8. The pH effect of the mobile phase on the amperometric responses of $1\times10^{-6}$ mol/l (a) NE, (b) E, (c) DA, (d) DOPAC, (e) 5-HT and (f) $1\times10^{-3}$ mol/l glucose at the ring–disc electrode. Other experimental conditions as in Fig. 4.

in LC-DECD, a set of standard solutions containing DA, E, NE, DOPAC, 5-HT ranging from $1.0\times10^{-9}$ to $3.0\times10^{-4}$ mol/l and glucose ranging from $1.0\times10^{-6}$ to $4.0\times10^{-3}$ mol/l were tested. The linear ranges were found to be over three orders of magnitude and the correlation coefficients were larger than 0.995. The analytical data for the six analytes were summarized in Table 1.

The reproducibility was estimated by eight times repetitive injection of 5.0 μmol/l mixture of the six analytes under the same conditions every 30 min. The relative standard deviations of the peak currents were found to be 1.0% for DA, 1.7% for NE, 1.5% for E, 2.2% for DOPAC, 1.6% for 5-HT and 2.6% for glucose.

In addition, the long-term stability of the biosensor stored at 4 °C was examined by checking its relative activity periodically. No apparent change in the response to glucose was observed and more than 95% activity of the biosensor was still retained over a 1 month period.

### *3.9. Different liquid chromatograms of monoamine neurotransmitters at the ring GC electrode and at the modified ring GC electrode*

Fig. 9 shows the liquid chromatograms of AA, NE, E, DA, DOPAC and 5-HT at the bare ring GC electrode and at the OPPy CME. From the chromatograms, the neurotransmitters of NE, E, DA, DOPAC and 5-HT had good responses at the bare GC electrode and at the OPPy CME. But the chromatographic peak of NE was interfered with by the peak of AA at the bare GC electrode. However, at the OPPy CME, the response of AA was eliminated and did not interfere with the determination of NE in LC. Moreover, the current responses of DA, NE, E and 5-HT at the OPPy CME were much larger than those at the bare GC. It could be ascribed to the permselectivity of the OPPy CME increasing the current responses of cations, such as DA, NE, E, 5-HT, and reducing the current response of AA anions at the OPPy CME. So the OPPy CME showed good selectivity and sensitivity for the determination of most neurotransmitters. However, the current response of DOPAC at the OPPy CME was little lower than that at the bare GC electrode. This is because DOPAC is also an anion in pH 5.0 buffer solution and the negatively charged OPPy CME suppressed the response of DOPAC on the CME. But because the DOPAC has a higher concentration than DA, NE, E and 5-HT in rat brain, and perhaps the hydrophilic interaction between DOPAC and the OPPy CME was

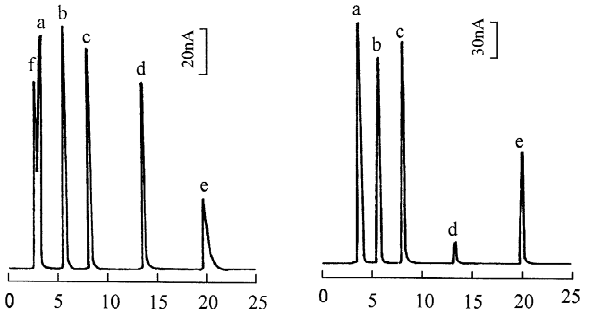


Fig. 9. Chromatograms of $1.0\times10^{-6}$ mol/l: (a) NE; (b) E; (c) DA; (d) DOPAC; (e) 5-HT; (f) AA; at (A) the bare GC electrode and (B) the OPPy CME. Applied potential: +0.50 V; other conditions are as in Fig. 4.

Table 1
Analytical data of the six analytes at the LC-DECD[a]

| Analytes | Regression equation[b] | Correlation coefficient ($R^2$) | Range (mol/l) | RSD(%) | Detection limit (mol/l)[c] |
|---|---|---|---|---|---|
| NE | $Y=204.6X-0.0124$ | 0.999 | $1.0\times10^{-9}$–$2.0\times10^{-5}$ | 1.7 | $0.5\times10^{-9}$ |
| E | $Y=174.1X-0.0169$ | 0.998 | $1.0\times10^{-9}$–$2.0\times10^{-5}$ | 1.5 | $0.5\times10^{-9}$ |
| DA | $Y=188.9X-0.0006$ | 0.999 | $5.0\times10^{-9}$–$1.0\times10^{-5}$ | 1.0 | $2.5\times10^{-9}$ |
| DOPAC | $Y=20.05X-0.0184$ | 0.997 | $1.0\times10^{-7}$–$3.0\times10^{-4}$ | 2.2 | $0.5\times10^{-7}$ |
| 5-HT | $Y=96.3X-0.0005$ | 0.998 | $2.0\times10^{-9}$–$2.0\times10^{-5}$ | 1.6 | $1.0\times10^{-9}$ |
| Glucose | $Y=0.2619X-0.0005$ | 0.998 | $1.0\times10^{-6}$–$4.0\times10^{-3}$ | 2.6 | $0.5\times10^{-6}$ |

[a] LC-DECD conditions as in Fig. 9.
[b] Where $Y$ and $X$ represent the peak current (nA) and the concentration of the analytes (μmol), respectively.
[c] The detection limits of the analytes were investigated using a signal-to-noise ratio of 3 ($S/N=3$).

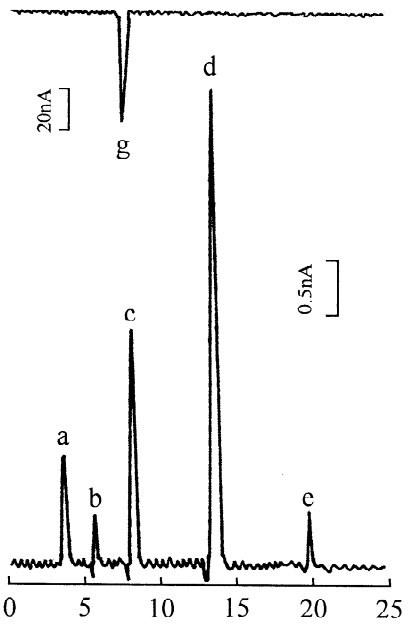


Fig. 10. Chromatogram of (a) NE; (b) E; (c) DA; (d) DOPAC; (e) 5-HT; (g) glucose, at the striatum of the rat brain obtained by LC-DECD coupled with microdialysis sampling. Other conditions as in Fig. 9.

favorable for the permeation of DOPAC [37], the OPPy CME was suitable for the determination of the monoamine neurotransmitters and their metabolites in real samples and could effectively remove the interference of AA.

### *3.10. In vivo experiment*

Fig. 10 shows the chromatograms of monoamine neurotransmitters, their metabolites and the glucose at the striatum of rat brain using the microdialysis sampling. From the chromatogram, NE, E, DA, DOPAC, 5-HT and glucose showed clear peak current responses, but AA did not show an obvious peak current response. Therefore, although AA had a much higher concentration than DA in rat brain, it did not interfere with the determination of monoamine neurotransmitters and their metabolites. The microdialysis relative recovery was found to be 30.8% for DA, 47.6% for NE, 48.2% for E, 37.8% for 5-HT, 34.5% for DOPAC and 42.9% for glucose under the microdialysis rate of 1.0 μl/min. The concentrations of analytes in brain microdialysate were calculated according to the literature [38]. The related analytical results were given in Table 2. The concentrations of DA, NE, E, 5-HT, DOPAC and glucose detected were within the normal range [39–43]. All these results suggested that the chemical modified dual electrode was very reliable and sensitive to determine neurotransmitters and glucose simultaneously in rat brain.

## 4. Conclusion

In this paper, the chemical modified carbon film based ring–disc electrode was prepared and used as the amperometric detector for liquid chromatography to determine the monoamine neurotransmitters and glucose simultaneously. All the differential pulse voltammetry and liquid chromatographic experiments showed that the ring electrode modified with OPPy effectively responded to the monoamine cations and repelled the ascorbic acid anions, which could be used in the determination of neurotransmitters and removed the interference of AA. The amperometry and the flow injection analysis showed that the disc electrode modified with Au colloid and GOD increased the sensitivity and the bioactivity when detecting glucose. In LC-DECD, the ring–disc dual modified electrode showed good and stable current responses to NE, E, DA, DOPAC, 5-HT and glucose. The application of this method coupled with microdialysis sampling for the in vivo simultaneous

Table 2
Content of monoamine neurotransmitters and their metabolites and glucose at the striatum of the rat brain[a]

| NE ($10^{-8}$ mol/l) | E ($10^{-8}$ mol/l) | DA ($10^{-8}$ mol/l) | DOPAC ($10^{-8}$ mol/l) | 5-HT ($10^{-8}$ mol/l) | Glucose (m*M*) |
|---|---|---|---|---|---|
| 1.02±0.02 | 0.56±0.01 | 3.68±0.04 | 106.00±2.30 | 1.32±0.02 | 0.46±0.03 |

[a] The values shown are calculated from the calibration curves and are the mean of $n=3$ in each case. LC-DECD as in Fig. 9.

determination of the monoamine neurotransmitters and glucose in rat brain was satisfactory.

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## References

[1] B.H.C. Westerink, W. Timmerman, Anal. Chim. Acta 379 (1999) 263.
[2] M.C. Kurth, C.H. Adler, Neurology 50 (5 suppl5) (1998) S3.
[3] T. Hashitani, K. Mizukawa, M. Kumazaki, H. Nishino, Neurosci. Res. 30 (1998) 43.
[4] S. Ritter, I. Llewellyn-Smith, T.T. Dinh, Brain Res. 805 (1998) 41.
[5] B.E. Levin, Brain Res. 874 (2000) 158.
[6] C. Fernandez, O. Nieto, E. Rivas, G. Montenegro, A. Jose, F.M. Alfonso, Carbohydrate Res. 37 (2000) 353.
[7] P.B. Issopouloas, S.E. Salta, Farmaco 56 (1996) 673.
[8] H. Nohta, T. Yukizawa, Y. Ohkura, M. Yoshimura, J. Ishida, M. Yamaguguchi, Anal. Chim. Acta 344 (1997) 233.
[9] O. Nozaki, T. Iwaeda, Y. Kato, J. Biolumin. Chemilumin. 11 (1996) 309.
[10] Y. Wu, R. Fan, J. Di, Chinese J. Anal. Chem. 24 (1996) 873.
[11] A.J. Downard, A.D. Roddick, A.M. Bond, Anal. Chim. Acta 317 (1995) 303, TrAC Trends in Analytical Chemistry.
[12] K. Thorre, M. Parvda, S. Sarre, G. Ebinger, Y. Michotte, J. Chromatogr. B: Biomed. Appl. 694 (1997) 297.
[13] O. Niwa, T. Horiuchi, M. Morita, T. Huang, P.T. Kissinger, Biosensors and Bioelectronics 11 (1996) ix.
[14] R.J. Slingerland, A.B.P. Van Kuilenburg, J.M. Bodlaender, H. Overmars, P.A. Voute, A.H. Van Gennip, J. Chromatogr. B Biomed. Sci. Appl. 716 (1998) 65.
[15] J. Auger, R. Boulay, B. Jaillais, S. Delion-Vancassel, J. Chromatogr. A 870 (2000) 395.
[16] Y.L. Liu, A.T.A. Cheng, H.R. Chen, Y.P.P. Hsu, Biomed. Chromatogr. 14 (2000) 544.
[17] E.C.Y. Chan, P.Y. Wee, P.C. Ho, J. Pharm. Biomed. Anal. 23 (2000) 239.
[18] F. Xu, M.N. Gao, L. Wang, L.T. Jin, Anal. Biochem. 307 (2002) 33.
[19] P.V.A. Pamidi, C. Parrado, S.A. Kane, J. Wang, M.R. Smyth, J. Pingarron, Talanta 44 (1997) 1929.
[20] M. Pravda, C. Petit, Y. Michotte, J.M. Kauffmann, K. Vytras, J. Chromatogr. A 727 (1996) 47.
[21] A. Witkowski, A. Brajter-Toth, Anal. Chem. 64 (1992) 635.
[22] K. Pihel, Q.D. Walker, R.M. Wightman, Anal. Chem. 68 (1996) 2084.
[23] C.C. Hsuek, A. Brajter-Toth, Anal. Chem. 66 (1994) 2458.
[24] B.X. Li, Z.J. Zhang, Y. Jin, Anal. Chim. Acta 432 (2001) 95.
[25] C. Matsubara, N. Kawamoto, K. Takamura, Analyst 117 (1992) 1781.
[26] J. Li, L.S. Chia, N.K. Goh, S.N. Tan, J. Electroanal. Chem. 460 (1999) 234.
[27] P.G. Osborne, O. Niva, T. Kato, K. Yamamoto, J. Neurosci. Methods 77 (1997) 143.
[28] P.G. Osborne, K. Yamamoto, J. Chromatogr. B 707 (1998) 3.
[29] J. Li, L.S. Chia, N.K. Goh, S.N. Tan, J. Electroanal. Chem. 460 (1999) 234.
[30] Y. Xiao, H.X. Ju, H.Y. Chen, Anal. Chim. Acta 391 (1999) 73.
[31] J.G. Zhao, R.W. Henkens, J. Stonehuerner, J.P. O'Daly, A.L. Crumbliss, J. Electroanal. Chem. 327 (1992) 109.
[32] L.A. Lyon, M.D. Musick, P.C. Smith, B.D. Reiss, D.J. Pena, M.J. Natan, Sensors and Actuators 54 (1999) 118.
[33] T.H. Tsai, C.F. Chen, J. Chromatogr. A 762 (1997) 269.
[34] A. Doron, E. Katz, I. Willner, Langmuir 11 (1995) 1313.
[35] X.M. Bao, Q.Y. Su, The Stereotaxic Atlas of the Rat Brain, Renmin Weisheng Publisher, China, 1991.
[36] F. Beck, P. Braun, M. Oberst, B. Bunsenges, Phys. Chem. 91 (1987) 967.
[37] F. Palmisano, C. Malitesta, D. centonze, P.G. Zambonin, Anal. Chem. 67 (1995) 2207.
[38] C.T. Huang, K.C. Chen, C.F. Chen, T.H. Tsai, J. Chromatogr. B 716 (1998) 251.
[39] T.H. Tsai, C.F. Chen, Neruosci. Lett. 166 (1994) 175.
[40] T. Hashitani, K. Mizukawa, M. Kumazaki, H. Nishino, Neurosci. Res. 30 (1998) 43.
[41] J.A.M. Mckenzie, C.J. Watson, R.D. Rostand, I. German, S.R. Witowski, R.T. Kennedy, J. Chromatogr. A 962 (2002) 105.
[42] R. Ramakrishnan, A. Namasivayam, Neruosci. Lett. 186 (1995) 200.
[43] L.K. Fellow, M.G. Boutelle, M. Fillenz, J. Neurochem. 59 (1992) 2141.