Polymeric bilayer modified microelectrodes for *in-vivo* determination of neurotransmitter dopamine

YANG, Li-Ju[a](杨丽菊) PENG, Tu-Zhi*,[a](彭图治) YANG, F. Catherine[b]

[a] *Department of Chemistry, Zhejiang University, Xixi Campus, Hangzhou, Zhejiang 310028, China*

[b] *Department of Chemistry, Rowan University, Glassboro, NJ 08028-1701, USA*

A composite polymer carbon fiber electrode modified with Nafion and cellulose acetate is described. The modified electrode discriminates against both anionic reactants and big molecular organic compounds. The bilayer configuration is prepared in two steps. First, the carbon fiber electrode is coated with Nafion, then followed by air evaporation of the solvent, the electrode is dipped in a cellulose acetate solution and hydrolyzed for a selected time. The permeability of the film is explored by use of rotating disk electrode measurements. Parameters affecting the film electrochemistry are investigated. The resulting electrodes show high selectivity and stability in body fluids. For *in-vivo* voltammetry, the composite polymer modified electrode has been used for detection of the oxidative current of neurotransmitter dopamine in rat brain, while it inhabits the oxidation of anionic neurotransmitter metabolites and some electroactive compounds.

Keywords Chemically modified electrode, neurotransmitters, *in-vivo* determination

Introduction

Carbon fiber is one of the mostly useful materials for preparing microelectrodes for *in-vivo* voltammetry.[1-3] In order to increase the sensitivity and avoid the interference of ascorbic acid (AA), different kinds of electrochemical procedures have been used to pretreat the electrodes, such as triangular potential,[4] cyclic voltammetry[5] and constant current.[6] For further improvement of the selectivity, the electrodes were chemically modified with different compounds or materials.[7,8] One of these materials is Nafion, which is a polycationic exchanger to be modified on the electrodes.[9,10] However, bilayer arrangement has not been so far reported previously. In this report, composite polymer carbon fiber electrodes modified with Nafion and cellulose acetate (CA) are described. The incorporation of ion exchanging polymer to the cellulose acetate domain allows the binding of counterionic reactants, while maintaining the size exclusion discriminative properties of cellulose acetate. The modified microelectrodes show high stability and inhabit the oxidation of some neurotransmitter metabolites and electroactive compounds, such as 3, 4-dihydroxyphenyl acetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), homovanillic acid (HVA), uric acid (UA) and AA. These composite polymer carbon fiber electrodes were used for *in-vivo* determination to detect the oxidation current of neurotransmitter dopamine (DA) in rat brain, when ischemia was induced to the brain by temporary occlusion of bilateral carotid arteries for 30 min.

Experimental

Reagents and materials

DA, 5-HT, DOPAC, 5-HIAA and HVA were purchased from Sigma and the concentration of stock solution was 1 mmol/L. CA (39.8% acetyl content) and Nafion (5%) were from Aldrich. The solution of gelatin (10^{-4} g/mL) was prepared daily. A phosphate buffered saline solution (pH 7.4) was used as supporting electrolyte. All solutions were prepared with redistilled wa-

* Received February 28, 2000; accepted April 24, 2000.

Project supported by the National Natural Science Foundation of China (No. 29975024), Zhejiang Province Natural Science Foundation and Instrumental Analysis Foundation.

ter.

Apparatus

A carbon fiber cylinder microelectrode (8 μm diameter, 1 mm long)[6] was used together with a Ag/AgCl reference electrode and a platinum auxiliary electrode (or stainless steel screw for *in-vivo* measurement). Semi-differential voltammograms were obtained with a BAS CV-37 voltammetric analyzer (scan rate, 100 mV/s) and a Houston Instrument *X-Y* recorder. In some experiments a 3 mm diameter glassy carbon disk electrode in conjunction with a BAS-100A electrochemical analyzer was employed.

Electrode modification

The bilayer film was constructed in two steps. First, the carbon fiber electrode was dip-coated 10 times in a 1% Nafion solution 5 s for each time, and allowed to remove the solvent for 2 min under a flow of hot air. Nafion was modified on the carbon fiber. Then, the Nafion coated electrode was dipped in a 5% CA solution (1 : 1 mixture of acetone and cyclohexanone) for 5 s. Following air evaporation of the solvent, the CA/Nafion electrode was hydrolysised in the KOH solution for the desired time.

In-vivo experiment

David Kopf stereotaxic instrument and Narishiqe micropusher were used for *in-vivo* determination. Anesthetized rats (with 100 mg choral hydrate) were prepared. The head of the rat was fixed in a David Kopf stereotaxic instrument and the exact position (AP 1.8, L 2.5, DV 5.1 mm) was located after removing the surface skin. A hole of 5 mm diameter was drilled carefully through the skull. The modified electrode was implanted through the dura to mater to the corpus striatum controlled by a micropusher. The reference microelectrode was placed in the burr hole with a little saline solution to get electrochemical contact. A stainless steel screw placed on the skull worked as a counter electrode. Voltammetric curves were recorded every 5 min until the stable base line was observed. BAS CV-37 analyzer was used for *in-vivo* experiments.

Results and discussion

Effect of hydrolysis time on stability and size discrimination

Many observations in different laboratories have shown that carbon fiber electrodes even coated with Nafion lost their sensitivity quickly with brain exposure.[1,9,11] This "contamination" of carbon fiber electrodes by brain tissue came from some forms of inclusion and adsorption of proteins and other big molecular species. In order to minimize the interference, Nafion modified electrodes were coated with CA film. After a certain period of hydrolysis in KOH solution, the polymeric chains of CA were broken down and numerous microholes were produced on the electrode surface.[12] The size of microholes depended on the hydrolysis time. The longer the hydrolysis time was, the bigger the size of microholes was.

The effect of hydrolysis time on the hole size and sensitivity was demonstrated on Table 1. If the hydrolysis time was short, the size of microholes was small, DA and other neurotransmitters were difficult to pass through CA film. If the time was too long, the hole size was large enough that some proteins and organic compounds could easily come in and foul the electrode. Table 2 showed the stability of Nafion coated electrodes and CA/Nafion electrodes with different hydrolysis time in gelatin solution. The response of Nafion electrodes for DA decreased rapidly while the CA/Nafion electrodes kept a stable one, indicating the protection effect of CA film on the bilayer modified electrodes. A balance between the sensitivity and stability was made. The optimum time of hydrolysis for CA film in KOH solution was 30 min. Table 2 showed that the protection effect of Nafion electrodes for the DA decreased rapidly while the response kept stable at CA/Nafion electrodes.

Table 1 Voltammetric responses of bare and CA/Nafion modified electrodes in 1×10^{-6} mol/L DA solution

Electrode	CA/Nafion modification					Bare
Hydrolysis time (min)	0	10	20	30	40	—
Peak current (nA)	0	1.8	4.2	10.4	10.6	11.6

Table 2 Sensitivity decay of Nafion and CA/Nafion modified electrodes in the solution containing 1×10^{-6} mol/L DA and 1×10^{-4} g/mL gelatin for different periods

Electrode	Sensitivity remaining (%)						
	0 min	10 min	20 min	30 min	40 min	50 min	60 min
Nafion	100	85	81	80	78	74	70
CA/Nafion[a]	100	98	97	95	94	94	94
CA/Nafion[b]	100	96	95	94	94	93	93
CA/Nafion[c]	100	88	82	80	78	74	72

[a] Hydrolysis time: 20 min; [b] Hydrolysis time: 30 min; [c] Hydrolysis time: 40 min.

Sensitivity and charge discrimination

In comparison with electrochemically modified electrodes, the sensitivities of CA/Nafion electrodes to neurocompounds changed obviously, while the peak potentials almost did not shift. The data in Table 3 showed these changes in determining some neurocompounds. The detection limits of endogenous neurotransmitters DA and 5-HT increased from 0.05 μmol/L to 0.1 μmol/L. However, the detection limits of their metabolites DOPAC, HVA, 5-HIAA and co-existed compounds AA and UA increased dramatically. It meant that CA/Nafion film inhibited the oxidation of these compounds while it kept fair sensitivity to DA.

Table 3 Detection limits of some compounds at bare and CA/Nafion modified electrodes

Electrode	Detection limits (10^{-6} mol/L)						
	DA	5-HT	AA	UA	DOPAC	5-HIAA	HVA
Bare	0.05	0.05	10	1	5	0.1	5
CA/Nafion	0.1	0.1	200	20	40	5	50

High concentration of AA (10^{-4} mol/L) and neurometabolite 5-HIAA in extracellular fluid of brain have been observed before.[6] The oxidation potential of AA was very close to that of DA and DOPAC, and the oxidation potential of 5-HIAA was almost the same as that of 5-HT. It was difficult to separate their oxidation currents in voltammetry. The peak potential of AA was moved negatively by the electrochemical pretreatment,[4,6] however, the method did not separate current peaks of DA, DOPAC and peaks of 5-HT, 5-HIAA. It was obvious that DOPAC and 5-HIAA interfered with the determination of DA and 5-HT seriously by using electrochemically treated electrodes for *in-vivo* voltammetry.

Fig. 1 showed semi-differential voltammograms of electrochemically treated electrodes and CA/Nafion modified electrodes in the solution containing AA, DA, 5-HT, DOPAC and 5-HIAA. For an electrochemically treated one, the peak oxidation currents of DA and DOPAC (p2), 5-HT and 5-HIAA (p3) were overloaded together and the width of peaks broadened. It was difficult to measure the concentration of the compounds quantitatively by these peaks. For a CA/Nafion electrode, the peaks of AA(p1), DOPAC and 5-HIAA were inhibited, only peaks of DA and 5-HT could be observed. This discrimination was dependent on the Nafion layer which is a polycationic exchanger. It screened out the ascorbate, DOPAC and 5-HIAA anions. So CA/Nafion electrodes obtained stability and charge discrimination due to these two layers. It demonstrated that CA/Nafion electrodes could be used to detect endogenous neurotransmitters DA and 5-HT directly.

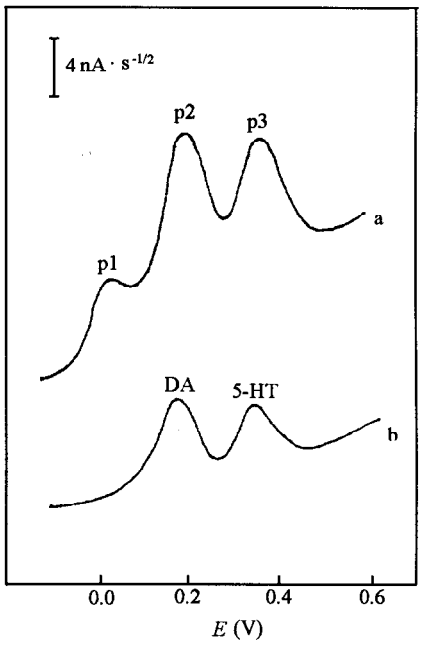

Fig. 1 Comparison of semi-differential voltammetric response for the electrochemically treated electrode (a) and CA/Nafion modified electrode (b) in the solution containing 1.0×10^{-4} mol/L AA, 2.0×10^{-5} mol/L DOPAC, 2.5×10^{-6} mol/L 5-HIAA, 5.0×10^{-7} mol/L DA and 5.0×10^{-7} mol/L 5-HT.

Transport characteristics

The diagnostic power of rotating disk electrode was used to evaluate the transport characteristics of the CA/Nafion film. A nonionic electroactive species, hydroquinone, was used as a neutral electrolyte to eliminate possible electrostatic effect. The limiting current with

different rotation speeds was recorded at glassy carbon electrodes coated with CA/Nafion film hydrolyzed (a) 0 min, (b) 20 min, (c) 30 min, and (d) 40 min, as well as (e) Nafion film and (f) bare electrode. Electrode rotation speeds were varied over 0—3600 rpm range. Koutecky-Levich plots were shown in Fig. 2. The current limited by the film permeability was the inverse of the intercept of these plots.[12] The magnitude of the intercept followed the expected order: 0 min hydrolysis CA/Nafion > 20 min hydrolysis CA/Nafion > 30 min hydrolysis CA/Nafion film > 40 min hydrolysis CA/Nafion film > Nafion film > bare. The relative magnitude of the limited current (intercept^{-1}), associated with different hydrolysis time, thus reflected changes in the structural characteristics of the films. According to the theory of active site rotating electrodes (equivalent to reactions through pores in a film),[13] the intercept was a function of ratio of pore diameter to spacing, when the thickness of diffusion layer in solution was larger than the diameter and spacing of active site. Obviously, the pore size of CA/Nafion films followed the order: 40 min hydrolysis > 30 min hydrolysis > 20 min hydrolysis > 0 min hydrolysis. The change in permeability might be attributed to the hydrolysis of KOH associated with different time to make different size of holes in CA film.

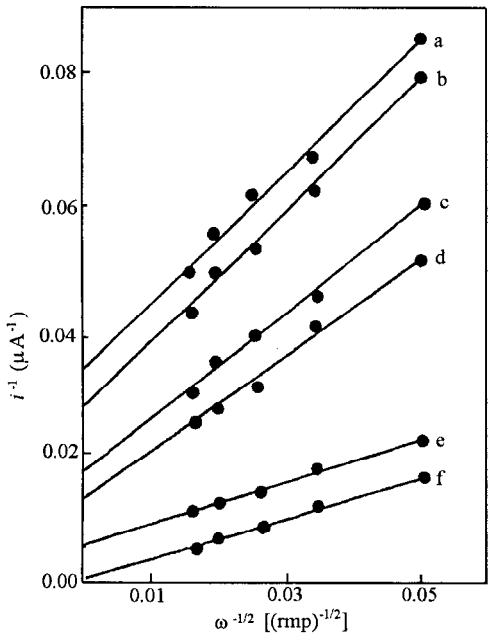

Fig. 2 Koutecky-Levich plots of the limiting current values for the oxidation of 1×10^{-3} mol/L hydroquione. CA/Nafion electrode hydrolyzed for 0 min (a), 20 min (b), 30 min (c), 40 min (d), as well as Nafion electrode (e) and bare electrode (f). Applied voltage: +0.9 V.

The experiments in Tables 1 and 2 demonstrated that the CA/Nafion modified electrodes hydrolyzed more than 30 min allowed DA molecules to pass through the film and rejected the big size molecules. The size exclusion of CA/Nafion film was very important for *in-vivo* determination to prevent electrodes from big biomolecules.

Reproducibility and calibration curves

At 1×10^{-6} mol/L DA concentration, the relative standard deviation of result was 1.5%, the average current was 10.6 nA, ranging from 10.4 to 10.9 nA for 7 successive determinations. Its electrochemical response to DA did not change obviously upon storage in a PBS solution for one week.

Calibration curves of electrochemically pretreated electrodes and chemically modified electrodes were measured in 10^{-7} mol/L DA and 5-HT solution. After modification of bilayer polymeric film to a carbon fiber, the electrode still kept good linear relationship between oxidative currents and concentrations. The slopes and correlation coefficients of two kinds of electrodes were shown in Table 4. It indicated that bilayer modified electrodes could be employed to measure neurotransmitters quantitatively.

Table 4 Slopes and corelation coefficients of calibration curves for DA and 5-HT

Compound	Slope (nA/10^{-7} mol/L)		Correlation coefficient	
	Bare	CA/Nafion	Bare	CA/Nafion
DA	1.16	1.05	0.999	0.998
5-HT	0.86	0.81	0.997	0.999

In-vivo determination

In *in-vivo* semi-differential voltammograms, there were four peaks found at 0.01, 0.18, 0.35, 0.54 V with an unmodified electrode, which represented the oxidation of AA, DOPAC, 80% 5-HIAA and 20% UA, HVA, respectively.[14] No peak was found in the semi-differential voltammogram with a CA/Nafion modified electrode (Fig. 3 curve a). The oxidative currents of anionic AA, UA and metabolites of neurotransmitters were inhabited by the modified film. In rat striatum, the concentrations of DA and 5-HT were lower than the de-

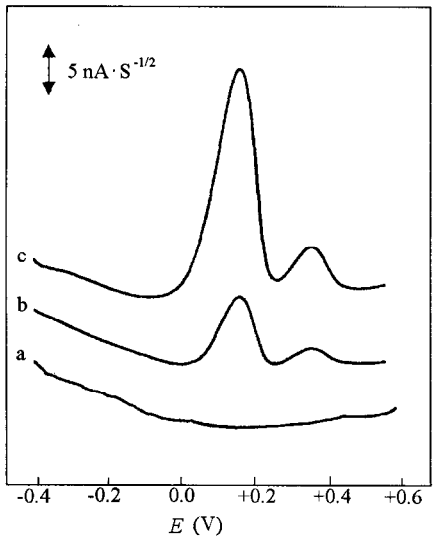

Fig. 3 Semi-differential voltammetric responses in *in-vivo* determination with a CA/Nafion carbon fiber electrode. a: a stable response after the electrode was inserted into the corpus striatum of rat brain. b: response after 6 min occlusion of bilateral carotid arteries. c: response after 18 min occlusion of bilateral carotid arteries.

tection limits of the electrode, and no current of DA or 5-HT was measured. Then bilateral carotid arteries of the rat were occlusioned for 30 min to make ischemia in rat brain, the change of neurotransmitters were observed by recording voltammetric responses every 3 min, which could reflect the physiological changes in the brain. After 6 min of occlusion of the arteries, two small peaks occurred (curve b), which were from the oxidation of DA (0.18 V) and 5-HT (0.35 V). The peak current of DA increased rapidly with occlusion time. After 18 min, the peak current of DA was nearly stable (curve c), and the rat gradually lost consciousness. Then, the rat was reperfused. And the concentration of DA returned to normal level gradually. The rat kept alive after the experiment. This phenomenon implied that when a rat lost its consciousness, the brain might be in a very active state. This is an important physiological change before death. CA/Nafion modified electrodes have shown good selectivity and reproducibility in *in-vivo* determination of neurotransmitters. It also demonstrated that electrochemical method may act as a useful technique in life science.

Acknowledgment

Analytical Center of Zhejiang University provided some instruments and useful results for our experiments.

References

1. Cahill, P.S.; Walker, Q.D.; Finnegan, J.M.; Mickelson, G.E.; Travis, E.R; Wightman, R.M., *Anal. Chem.*, **68**, 3180(1996).
2. O'Neill, R.D., *Analyst*, **119**, 767(1994).
3. Wightman, R.M.; May, L.J.; Michael, A.C., *Anal. Chem.*, **60**, 769A(1988).
4. Gonon, F.; Fombarlet, C.T.; Buda, M.T.; Pujoi, J.F., *Anal. Chem.*, **53**, 1386(1982).
5. Feng, J.; Brazell, M.; Renner, K.; Kasser, R.; Adams, R.N., *Anal. Chem.*, **59**, 1863(1987).
6. Peng, T.; Lü, H.; Liu, G.; Cao, Y., *Anal. Lett.*, **25**, 795(1992).
7. Broderick, P.A., *Electroanalysis*, **2**, 241(1990).
8. Downard, A.D.; Roddick, A.D.; Bond, A.M., *Anal. Chim. Acta*, **317**, 303(1995).
9. Capella, P.; Ghasemzadeh, B.; Mitichell, K.; Adams, R.N., *Electroanalysis*, **2**, 175(1990).
10. Brazell, M.; Kasser R.; Renner K.; Feng J.; Moghaddam, B.; Adams, R.N., *J. Neurosci. Meth.*, **22**, 167 (1987).
11. Stamford, J.A., *J. Neurosci. Meth.*, **17**, 1(1986).
12. Wang, J.; Peng, T., *J. Electrochem. Soc.*, **134**, 586 (1987).
13. Gueshi, T.; Tokuda, K.; Matsuda, H., *J. Electroanal. Chem.*, **89**, 247(1978).
14. Peng, T.; Wang, G.; Li, H.; Shen, B., *Anal. Lett.*, **28**, 395(1995).

(E200002039 JIANG, X.H.; LING, J.)