Imprinted Polymeric Film-Based Sensor for the Detection of Dopamine Using Cyclic Voltammetry

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The imprinted polymeric film was synthesized on the glass-carbon electrodes directly. The response to the template molecule-dopamine and other molecules with similar structure was measured by cyclic voltammetry. The response of dopamine on imprinted electrode was much higher than that of other molecules, because of the existing of micro-cavities in polymeric film fitting with the size and shape of dopamine in the imprinted polymer. Experimental results showed that dopamine can be enriched by the imprinted film, therefore increasing the sensitivity of the sensor. The imprinted film could also efface the interference of ascorbic acid, indicating that dopamine can be determined with a large excess of ascorbic acid.

Keywords dopamine, cyclic voltammetry, imprinted polymeric film

Introduction

In recent years bio-sensors have attracted considerable interest because of their potential to furnish a diverse range of new or improved measurement for biotechnology, food industry, medicine, and environmental monitoring. Bio-sensors generally rely on bio-molecules such as antibodies or enzymes as the specific recognition elements. Theoretically they have the potential to meet the analytical demands for almost any target analytes. However given the often-poor chemical and physical stability of biomolecules, artificial receptors have been gaining importance as a possible alternative to natural systems. Molecular imprinting increasingly become to be recognized as a versatile technique for the preparation of synthetic polymers containing tailor-made recognition sites. This is achieved by co-polymerized functional and cross-linking monomers in the presence of the analyse, which acts as a molecular template. After elution of the template, complementary binding sites are revealed allowing for specific rebinding of the analyse.

The technique has been recently reviewed, both generally[1-3] and with respect to analytical applications.[4-7] The recognition sites obtained in this manner can possess binding affinities approaching those demonstrated by antibody-antigen systems and have been dubbed antibody mimics.[8] These mimics display some clear advantage over real antibodies: they do not rely on animal experiments, and the resulting polymers are intrinsically stable and robust, facilitating their application in extreme environments, such as organic solvents, or at high temperature, which make them ideal recognition elements for sensors.

Another important aspect in the development of a sensor is the need for mass-produced and low-cost transducers.[9] For electrochemical sensors, glassy-carbon electrodes fulfill this need. Electrochemistry has been combined with pharmacological stimulation method to provide information about basic biology and transmitter diffusion on an on-line basis. However attempts to measure neutrotransmitters, particularly dopamine in the brain with voltammetric electrodes, require the use of several strategies to achieve the qualitative and quantitative aspects of these measurements. The main problem associated with measuring each of this mono-mine *in vivo* is the very low dopamine concentration and the large excesses of interfering anions such as ascorbic acid.[10] Unfortunately, at most solid electrodes, ascorbic acid oxidized at potentials similar to those of dopamine, resulting in an overlapped voltammetric response. The ability to measure dopamine in the presence of ascorbic acid has been a major target of electro-analytical research. Considerable efforts have been devoted towards improving the sensitivity and selectivity of dopamine against ascorbic acid. Thus polymer films, including Nafion,[11-13] stearic acid,[14,15] poly(estersulfonic acid),[16] poly(*N*-vinylpyrrolidone)[17] have been explored as electrode modifiers.

In the present paper, we aimed to combine the advantage of glassy-carbon electrodes with those of molecularly imprinted polymers for the development of an electrochemical dopamine sensor. A polymeric film molecularly imprinted with dopamine was employed as the recognition element. Also in this study, we developed imprinted membrane coated glass carbon electrode sensors for the determination in the presence of ascorbic acid. The method may

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Received April 30, 2003; revised August 7, 2003; accepted August 27, 2003.

Project supported by the National Natural Science Foundation of China (No. 20175009), the Doctoral Foundation of Education Ministry of China (No. 20020055002) and the Natural Science Foundation of Tianjin (No. 033603511).

have prospects for many analytical applications and significance in medicine and pharmacology.

Experimental

Reagents

Dopamine, adrenaline, ascorbic acid were from National Institute for the Control of Pharmaceutical and Biological Products, P. R. China. 2-(Trifluoromethyl)acrylic acid (TFMAA) was purchased from Aldrich Chem. Co., ethylene glycol dimethacrylate (EGDMA) was prepared from ethylene glycol and methylacrylic acid. 2,2′-Azobisisobutyronitrile (AIBN, Nankai University Special Reagent Factory) and others were of analytical grade. Working solutions of analytes were prepared daily with distilled, de-ionized water, which was previously deoxygenated with high-purity nitrogen. Phosphate buffer solution (0.01 mol/L, pH = 7.4) was used as the supporting electrolyte. The dissolved oxygen was removed by bubbling with high-purity nitrogen and a nitrogen atmosphere was maintained throughout.

Apparatus

Cyclic voltammetry was performed using an XJP-821 (B) polarograph (Jiangsu Electroanalytical Instrument Factory) and a 3036X-Y recorder was used to record the voltammograms. A conventional three-electrode system was used throughout. The working electrode was a polymeric film coated glassy-carbon electrode (Φ = 4 mm). The auxiliary electrode was a platinum wire and a saturated calomel electrode (SCE) was used as reference electrode.

Synthesis of the molecularly imprinted polymeric film on the surface of glassy-carbon electrode using a sandwich technique

Dopamine (template molecule, 0.0766 g, 0.5 mmol) and TFMAA (functional monomer, 0.2801 g, 2 mmol) were dissolved in methanol (porogen, 2.3 mL) for 2 h reactively. Then the mixture of EGDMA (cross-linking agent, 1.587 g, 10 mmol) and AIBN (initiator, 20 mg) was added. After nitrogen bubbled in the solution for 15 min, 1 μL of the reactive solution was dropped onto the surface of the glassy-carbon electrode using micro-pipette under the nitrogen atmosphere and covered with cover slip, and then polymerized 6 h under 365 nm UV irradiation at room temperature. After polymerization, the cover slip was separated and the imprinted polymeric film was left on the surface of glassy-carbon electrode. The electrode was soaked into the acetic acid methanol (1∶9, *V*/*V*) solvents to wash the template out and scan until stable baseline was obtained. The non-imprinted polymeric film was synthesized using the same method without the addition of the template.

Voltammetric determination of dopamine with polymeric film coated electrodes

The pre-concentration voltammetric determination-renewal scheme was used in all experiments. For the preconcentration step, the film-coated electrode was immersed into the solutions of different concentration of analytes in 0.01 mol/L phosphate buffer. After a given period of time, the electrode was taken out and rinsed briefly with de-ionized water to avoid carryover of the analytes. The electrodes were then transferred into the buffer solution for potential cycling between -1 and $+1$ V, and the first circuit of current was recorded. The cycling was allowed continuously until the background current was obtained again. The peak current was calculated by substracting the background current.

Results and discussion

Preparation of the polymeric films

To prepare dopamine-imprinted polymeric film, 2-(trifluoromethyl)acrylic acid (TFMAA) was chosen as the functional monomer. TFMAA was more acidic than methacrylic acid (MAA), a common but potentially functional monomer used in molecular imprinting technique, owing to the electron-withdrawing effect of the trifluoromethyl group. It can expected that TFMAA can interact with dopamine more strongly than MAA by ionic interaction between $-$COOH group of TFMAA and $-NH_2$ group of dopamine thus creating more higher affinity binding sites in the imprinted film, therefore the recognition in aqueous can thus be fulfilled.[18]

The polymeric film was formed between cover-slip and glassy carbon electrode through photo-initiated polymerization. After the separation of the cover-slip, the polymeric film remained on the surface of the electrode. As widely recognized, for effective performance, the imprinted polymer should be highly cross-linked thus enabling the selective cavities to retain their shape after removal of the template. However, a compromise should be found between the degree of cross-linking needed for polymer stability and a certain degree of polymer chain's flexibility which provides rapid equilibration with the template to be bound. Hence different amount of cross-linking agent was tried. The optimal amount of cross-linker (EGDMA) added was found to be 85%. The films obtained were transparent, uniform and smooth.

Cyclic voltammetry of dopamine, adrenaline and ascorbic acid at dopamine-imprinted film coated electrodes

The structures of the analytes are shown in Fig. 1. Cyclic voltammograms for the oxidation of dopamine, adrenaline and ascorbic acid in 0.01 mol/L phosphate

buffer (pH = 7.4) at bare glassy carbon electrode, dopamine-imprinted electrode and non-imprinted electrode were studied respectively. As illustrated in Figs. 2—5, the cyclic voltammogram of dopamine at dopamine-imprinted electrode exhibited the biggest peak current, it is much higher than that of dopamine at non-imprinted electrode and bare electrode, indicating that dopamine can be enriched by the imprinted film and thus increase the sensitivity of the sensor. The anodic peak is at 333 mV, the cathodic peak is at 27 mV, the peak difference is 306 mV. The peak difference indicates that dopamine shows quasi-reversible behavior on the imprinted film coated electrode.

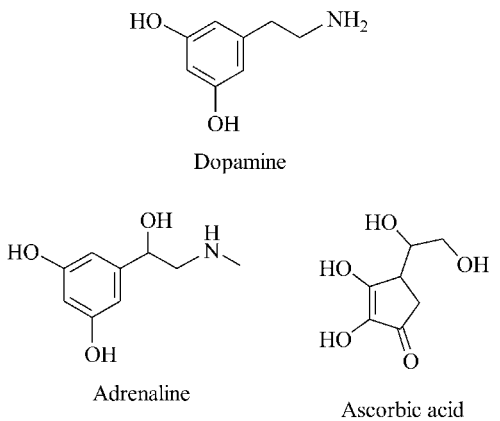

Fig. 1 Structures of analytes.

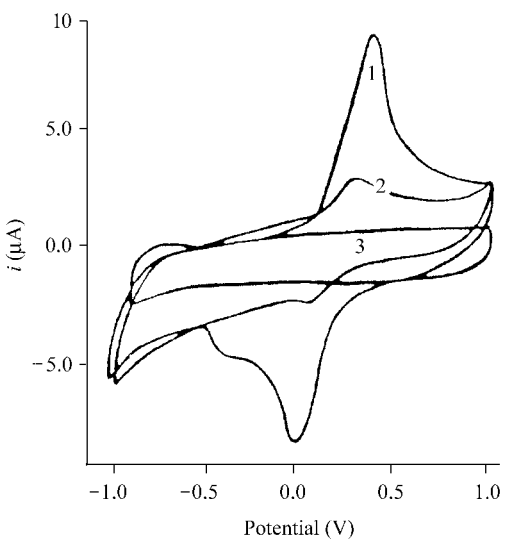

Fig. 2 Cyclic voltammogram of dopamine at imprinted-electrode and bare electrode in 0.01 mol/L phosphate buffer (pH = 7.4). The concentration of dopamine is 1×10^{-4} mol/L. (1) imprinted electrode; (2) bare electrode; (3) background of imprinted electrode.

The results inferred that the micro-environment of the imprinted and non-imprinted polymeric matrix must be different. During the preparation of imprinted polymeric film, the binding sites were formed around the dopamine by co-polymerizing functional and cross-linking monomers. When the template molecules are removed, a geometrically adapted polymer skeleton with fitting binding sites and dif-

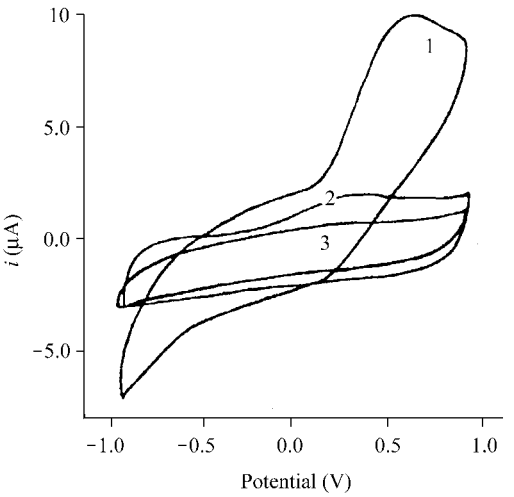

Fig. 3 Cyclic voltammogram of ascorbic acid at imprinted electrode and bare electrode in 0.01 mol/L phosphate buffer. Concentration of ascorbic acid is 1×10^{-3} mol/L. (1) Bare electrode; (2) imprinted electrode; (3) background of bare electrode.

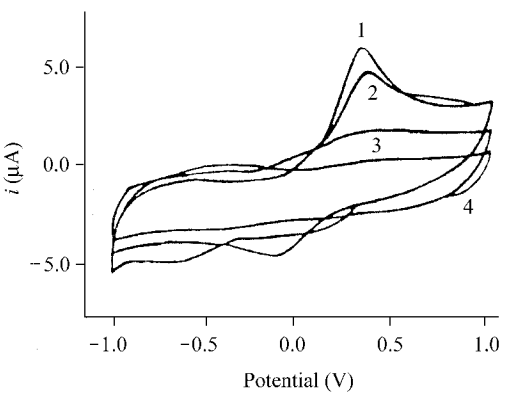

Fig. 4 Cyclic voltammogram of dopamine, adrenaline and ascorbic acid at non-imprinted electrode in 0.01 mol/L phosphate buffer. Concentration of dopamine and adrenaline is 1×10^{-4} mol/L, concentration of ascorbic acid is 1×10^{-3} mol/L. (1) Dopamine; (2) adrenaline; (3) ascorbic acid; (4) background of non-imprinted electrode.

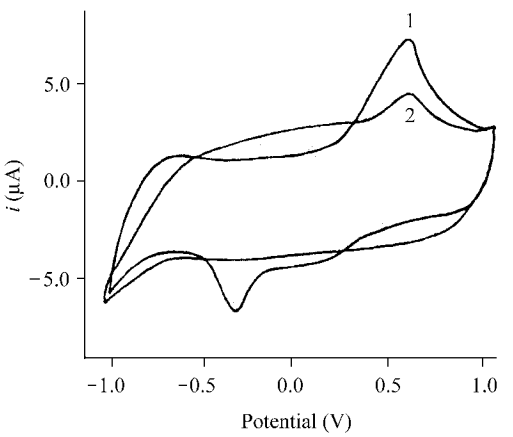

Fig. 5 Cyclic voltamogram of adrenaline at imprinted electrode and bare electrode in 0.01 mol/L phosphate buffer. Concentration of adrenaline is 1×10^{-4} mol/L. (1) imprinted electrode; (2) bare electrode.

fusion pathways for dopamine inclusion is left behind. The cavities can specifically rebind dopamine. Though the structure of adrenaline is similar with dopamine, its size is bigger than that of dopamine, adrenaline does not fit the micro-cavities complementarily with dopamine, that is, it is difficult for adrenaline to diffuse into the micro-cavities, and the peak currents of adrenaline are smaller at imprinted electrode. Ascorbic acid showed the biggest response at the bare electrode, whereas there were almost no responses at imprinted and non-imprinted film electrodes. In pH = 7.4 phosphate buffer, ascorbic acid is an anion. The carboxyl in the imprinted and non-imprinted film polymeric matrix is dissociated as $-COO^-$ in pH = 7.4 phosphate buffer, ascorbic acid is unable to diffuse into the polymeric matrix because of the charge repulsion between $-COO^-$ and ascorbic acid, thus there are no peak currents at the imprinted and non-imprinted electrodes. The experiment results indicate that the pre-concentration characteristics of the imprinted film make it suitable for voltammetric sensing. In particular, the enhanced selectivity obtained as a result of excluding anionic species could be valuable for *in vivo* electrochemistry.

Response time of the imprinted polymeric film

Up to now, the most severe problem for the use of molecular imprinting technique in chemical sensors is the long response time involved in the measurements, especially when the molecularly imprinted particles are used as sensitive materials. To overcome this problem, it is necessary to synthesize thinner polymeric film *in situ* on the surface of the transducers.

To investigate the response time of the dopamine-imprinted electrode, the peak current of 0.1 mmol/L dopamine in 0.01 mol/L phosphate buffer for different pre-concentration time was recorded using cyclic voltammograms. The peak current corresponding to different pre-concentration time is illustrated in Fig. 6.

Fig. 6 shows that the peak current reaches its equilibrium in about 20 min. The shorter response time of the imprinted film shows its potential for being used in practical measurement. The peak currents of dopamine at non-imprinted electrode were also determined. It can be seen from Fig. 6 that response of the imprinted sensors is much higher than that of non-imprinted sensors. The number of $-COOH$ in imprinted and non-imprinted polymers is the same, therefore, the higher response of imprinted electrode over the non-imprinted electrode should be attributed to the generation of substrate selective, high affinity binding sites in the polymer matrices during the template mediated cross-linking reaction.

The response of adrenaline at imprinted-sensor is smaller than that of dopamine, thus further verifying the existing of the micro-cavities matched the size and shape of dopamine in imprinted-film.

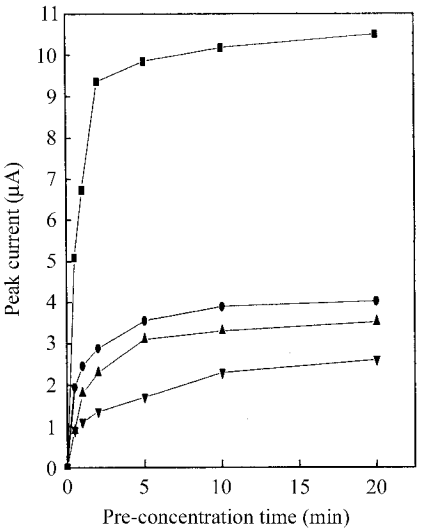

Fig. 6 Curve of response vs. pre-concentration time (background corrected, scan rate: 100 mV/s). Square: dopamine at imprinted electrode; Circle: adrenaline at imprinted electrode; Up-triangle: dopamine at non-imprinted electrode; Down-triangle: adrenaline at non-imprinted electrode.

Voltammetric determination of dopamine with a large excess of ascorbic acid

To determine the dopamine, it is necessary to establish a relationship between the concentration of dopamine and the peak current. Fig. 7 shows a plot of peak current of dopamine (background corrected) vs. concentration of dopamine in 0.01 mol/L phosphate buffer. It should be noted that there are two sections within the plot that can be regarded as straight line, and this is unusual compared with the other modified electrodes.

The results revealed that the binding sites in the imprinted membrane are heterogeneous. In the binding study of molecularly imprinted polymers, it has been found that different classes of binding sites often exist.[8,19,20] It was also found the existence of heterogeneous population of sites with various affinities for the imprinted molecule in the polymer film which was used as sensitive materials for thickness shear mode acousic sensor.[21]

All the dopamine molecules can bind into the higher affinity binding sites in the imprinted membrane at lower concentrations of dopamine, and the peak current increases quickly. When the concentration of dopamine increased, not only the higher affinity binding sites were fully occupied but also the lower affinity binding sites could be occupied. The peak current still increased, but the slope of the plot became smaller compared with the lower concentration.

The relationship between the response and the concentration of dopamine of non-imprinted membrane was also studied. As shown in Fig. 8, there is only one straight line in the plot, indicating that the microenvironment of the non-imprinted membrane is different with imprinted membrane.

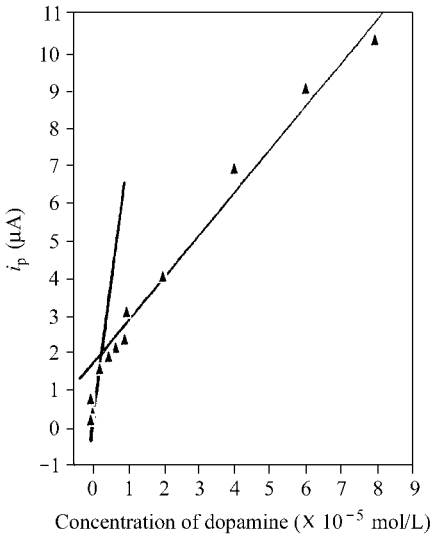

Fig. 7 Peak current for oxidation of dopamine (background corrected, scan rate = 100 mV/s) at imprinted-polymeric film modified electrode. Supporting electrolyte was 0.01 mol/L phosphate buffer (pH = 7.4) vs. concentration of dopamine (pre-concentration time 20 min for each measurement).

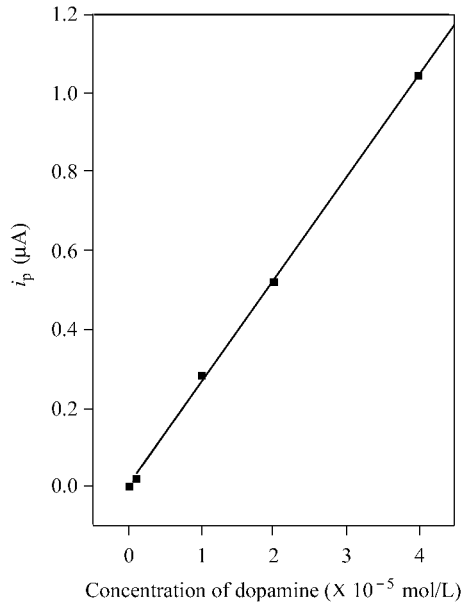

Fig. 8 Peak current for oxidation of dopamine (background corrected, scan rate = 100 mV/s) at a non-imprinted polymeric film modified electrode vs. concentration of dopamine. Supporting electrolyte was 0.01 mol/L phosphate buffer (pre-concentration time 20 min for each measurement).

The repeatability of the imprinted electrode was examined in six parallel determinations. For a 1×10^{-6} mol/L or 1×10^{-5} mol/L dopamine phosphate buffer solution, the relative standard deviation was 4.7% or 4.5%, respectively. The reproducibility of six imprinted electrodes synthesized was also investigated using the above concentration of dopamine. The relative standard deviation was 8.5% and 8.7%, respectively.

Voltammetric determination of dopamine with a large excess of ascorbic acid

Dopamine (1×10^{-6} mol/L) with 1×10^{-4} mol/L ascorbic acid in 0.01 mol/L phosphate buffer (pH = 7.4) was determined. The ratio of dopamine to ascorbic acid concentration is similar to that of biological system, *e.g.*, mammalian brain tissues. After immersion of the imprinted electrode in the solution for 20 min, the voltammogrames were obtained. The peak current is due to the oxidation of dopamine, and the peak potential of mixed solution is the same as dopamine, that is, the effect of oxidation of ascorbic acid can be eliminated by imprinted film. The peak currents were obtained by correcting the background current. The results of experiment are showed in Table 1.

Table 1 Determination of the mixed solution of dopamine (1×10^{-6} mol/L) and ascorbic acid (1×10^{-4} mol/L) in 0.01 mmol/L phosphate buffer (pH = 7.4)

Imprinted electrode	Peak current of mixed solution (μA)	Peak current of dopamine (μA)	Recovery (%)
1	0.429 ± 0.019	0.450 ± 0.017	95.3
2	0.393 ± 0.021	0.405 ± 0.19	97.0
3	0.589 ± 0.023	0.627 ± 0.026	94.0
4	0.319 ± 0.016	0.329 ± 0.016	96.8
5	0.508 ± 0.025	0.535 ± 0.021	95.0

± mean standard deviation ($n = 6$).

The results of Table 1 showed that the determination of dopamine in the presence of ascorbic acid is facilitated with imprinted polymer film coated electrodes, as ascorbic acid oxidation is almost entirely eliminated.

In summary, we investigated the pre-concentration characteristics of the dopamine imprinted polymer films as suitable sensors for the detection of dopamine in the presence of ascorbic acid. In particular, the enhanced selectivity obtained as a result of excluding anionic species could be valuable for *in vivo* electrochemistry. With its low cost and ease of preparation, the imprinted film appears to open up new opportunities for further sensor development.

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(E0304306 SONG, J. P.; FAN, Y. Y.)