Preparation and Electrochemical Behavior of L-Glutamate Electrochemical Biosensor

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Using the nano-porous pseudo carbon paste electrode (Nano-PPCPE) as the working electrode, mixing L-glutamate oxidase (L-GLOD), catalase (Cat) and bovine serum albumin (BSA) with phosphate buffer (PB, pH = 7.4), followed by cross-linking with glutaraldehyde, a novel L-glutamate electrochemical biosensor was successfully formed. It was demonstrated that the modified nano-PPCPE exhibits a high selectivity and sensitivity in comparison with the modified CPE. The L-glutamate biosensor showed a linear range from 5 × 10⁻⁷ M to 1 × 10⁻⁵ M with the detection limit of 2.5 × 10⁻⁷ M.

Keywords: L-Glutamate, Biosensor, Electrochemical, Nano-Porous Pseudo Carbon Paste Electrode.

Electrochemical biosensors are a kind of sensors for detecting electrical signals in bioassay,¹–⁴ which are based on potential, current or capacitance for feature detection signal of the biosensors. Neurotransmitters biosensors as a special form of electrochemical biosensors for neural biology at the molecular level of brain research provide a convenient approach and reliable data.⁵–⁸ The L-glutamate is the major excitatory neurotransmitter in the central nervous system (CNS),⁹–¹⁰ and it plays an important role in maintaining the normal brain function. If the glutamate is dysfunctional,¹¹–¹² it would lead to a series diseases, such as drug addiction, epilepsy, schizophrenia, etc. So, the real-time online detection of the glutamate has a great significance in clinical studies. At present, there are many methods about the detection of the glutamate, such as high performance liquid chromatography, capillary electrophoresis, spectrophotometry, polarimetry, and acid-base titration, etc. But these methods are difficult to achieve detecting in vivo, while the glutamate oxidase modified electrodes are easy to implement for real-time on-line testing purposes in the body.¹³–¹⁵

Glutamate oxidase (GLOD, 5.0 units/mg), bovine serum albumin (BSA) and catalase (Cat, 2000~5000 units/mg) were purchased from Sigma. 25% glutaraldehyde (biochemical reagent) was purchased from Sinopharm Group Chemical Reagents Co., Ltd. (Shanghai, China). Glutamate was purchased from Sunshine Biotechnology (Nanjing) Co., Ltd. (Jiangsu, China). 5% Nafion-CH₃OH solution was purchased from Shanghai Hesen Electric Co., Ltd. (Shanghai, China). A 0.1 M phosphate buffer at pH = 7.4 was prepared from Na₂HPO₄·2H₂O and NaH₂PO₄·12H₂O in an appropriate proportion. All other reagents were of analytical grade.

Cyclic voltammetry and linear sweep voltammetry measurements were carried out on an electrochemical workstation PGSTAT302N (Metrohm) in 0.1 M phosphate buffer at pH = 7.4. The measurements were carried out in a three-electrode cell consisting of a homemade nano-PPCPE or CPE electrode as the working electrode in reference to our previous reports,¹⁶–¹⁸ a saturated calomel electrode as the reference electrode, and a Pt electrode as the counter electrode. All electroanalytical measurements were made at room temperature.

The carbon paste electrode (CPE) was prepared by mixing 1 g of graphite powder and 0.3 mL of mineral oil into a uniform paste, followed by filling the paste into the hollow glass tube (3 mm inner diameter) and inserting it with a copper wire. The CPE electrode was polished with weighing paper prior to use.

The nano-porous pseudo carbon paste electrode (Nano-PPCPE) was prepared by mixed 1 g of graphite powder and 2 g of poly styrene (PS) microspheres into a 10 mL centrifugal tube which was then oscillated in order to get

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Preparation and Electrochemical Behavior of L-Glutamate Electrochemical Biosensor

Deng et al.

A uniform mixture. Then 1.5 mL of pyrrole was added to grind the mixture into a paste, the paste was then packed into a hollow glass tube (3 mm inner diameter) with a copper wire inserted, which formed as an electrode. And then the electrode was immersed in FeCl₃ solution for several hours until the pyrrole was fully polymerization. Finally, the as-prepared electrode was immersed in the toluene solution for several hours in order to dissolve PS microspheres to successfully obtain a nano-PPCPE. The Nano-PPCPE electrode was polished with weighing paper prior to use.

100 μL of PB (0.1 M, pH = 7.4) containing 10 mg of BSA, 2 mg of L-GLOD and 2 mg of Cat were mixed with 20 μL of 5% glutaraldehyde solution (mixed 25% glutaraldehyde solution with PB at 1:4, and formed 5% glutaraldehyde solution). 3 μL of the resulting enzyme solution was coated onto the nano-PPCPE or CPE surface and air-dried at room temperature. Finally, 3 μL of 5% Nafion-methanol solution was dripped onto those electrodes, allowing the solvent to air dry to form the L-glutamate electrochemical biosensor.

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) response of glutamate at the two electrodes were checked. Shown in Figure 1 are the CV curves of glutamate solution (1 × 10⁻⁵ M) at Nano-PPCPE (the black line) and CPE (the red line) in 0.1 M PB at pH = 7.4, and we could see that the electrochemical response and current intensity of the glutamate at the Nano-PPCPE is stronger than that at CPE, indicating that the nano-PPCPE has greater electrochemical activity because of its larger surface area and porous structure which can allow more glutamate molecules adsorbed.

Linear range and detection limit of nano-PPCPE were tested by LSV. Shown in Figure 2, the LSV results showed that the oxidation peak currents of glutamate at nano-PPCPE are linear with the concentration of glutamate ranging from 5 × 10⁻⁷ M to 1 × 10⁻⁵ M. The regression equation is $y = 2.09336x - 1.55232$, and the regression coefficient ($R$) of the linear curve is 0.9945 (Fig. 3), with the detection limit as low as 2.5 × 10⁻⁷ M ($S/N = 3$).

In 0.1 M PB solution containing 1 × 10⁻⁵ M glutamate, adding large concentrations of some interfering materials such as glucose, urea, ascorbic acid, glycine and lactic acid does not interfere with the determination of glutamate. The result shows that these substances almost have no effect on glutamate determination. So, the response of glutamate at nano-PPCPE has good selectivity. This is because the glutamate oxidase catalysis is specifically catalytic, and the Nafion membrane has significant inhibition effect on glutamate.

![Fig. 1. The CV curves of glutamate (1 × 10⁻⁷ M) in 0.1 M PB (pH = 7.4) at nano-PPCPE (the black line) and CPE (the red line), scan rate: 0.12 v/s.](image1)

![Fig. 2. The LSV curves of different concentrations of glutamate solution (5 × 10⁻⁷ M–1 × 10⁻⁵ M) in 0.1 M PB (pH = 7.4) at nano-PPCPE, scan rate: 0.1 v/s.](image2)

![Fig. 3. The relationship between different concentrations of glutamate at nano-PPCPE in 0.1 M PB (pH = 7.4) and the current response, scan rate: 0.1 v/s.](image3)
ascorbic acid. It implies that the glutamate electrochemical biosensor has good selectivity, and has great significance in practical applications.

In summary, we have developed a novel, simple, and applicable biosensor for neurotransmitter assay and it has been shown that the electrochemical behavior of nanomaterials is much better than that of the CPE, and this kind of electrode has larger surface, higher sensitivity, and better selectivity, etc. With the application of nanotechnology in biosensing and biomedical area, we believe this kind of approach will find promising application.

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References and Notes

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