Graphene Supported Platinum Nanoparticles Modified Electrode and Its Enzymatic Biosensing for Lactic Acid

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Lactic acid is a metabolic product of human physiological activity. It is of great importance in the diagnosis and treatment of diseases and scientific sports management. It is necessary to build a lactate biosensor that can realize rapid and accurate detection of lactic acid. Graphene supported platinum nanoparticles (GPNs) were prepared by the redox reaction between graphene oxide, chloroplatinic acid and ethylene glycol. Pt nanoparticles were highly dispersed on the graphene, and the surface platinum content of the prepared GPNs was as high as 28%. The GPNs showed excellent electrochemical performance compared to graphene modified electrodes or platinum modified electrodes. An enzymatic lactic acid biosensor (ELB) was prepared by immobilizing lactate oxidase (LOX) on the GPNs modified glassy carbon electrode. The response current of the sensor at +0.4 V in the presence of lactic acid showed a wide linear relationship in the range of 0–1.0 mM, and the limit of detection (LOD) was calculated to be 8.0 μM according to S/N = 3. The ELB was free from common interfering species, and exhibited excellent stability. From the 1st day to the 15th day, the response signal lost less than 2%.

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Experimental

Chemicals and apparatus.—Lactate oxidase (LOx) and Nafion (5 wt%) were purchased from J&K Scientific company. The activity of LOx was 37 units mg−1. The LOx solution was prepared using water and 7% of bovine serum albumin (BSA). Nafion was diluted to 0.5% with anhydrous ethanol. Ethanol was offered by Zhenglong biochemical products laboratory in Sichuan. Glutaraldehyde (50 wt%) was diluted to 0.1 wt% before use. Chitosan was purchased from Sigma-Aldrich and was dissolved in 2% acetic acid to prepare 1% chitosan. The pH value of the phosphate buffer solution (PBS) was 7.0. All other chemicals were of analytical reagent grade, and the water used in this work was ultrapure water (18.2 MΩ).

The scanning electron microscopy (SEM) photographs were obtained using a Hitachi SU-70 electron microscope, the accelerating voltage of the electron beam was 5 kV. Mapping analysis was performed by energy dispersive spectrometer (EDS) with 15 kV accelerating voltage. All electrochemical measurements were performed using an electrochemical workstation from Shanghai Chenhua Instrument Company (CHI 660E). A three-electrode system was used with a modified glassy carbon electrode (GCE) as the working electrode, saturated calomel electrode (SCE) as the reference electrode, and platinum electrode as the auxiliary electrode.

Preparation of GPNs.—Firstly, graphite oxide was prepared from flake graphite according to the method reported by Hummers.30 Then, 50 mg of graphite oxide powder were dispersed in 50 mL water and stirred for 15 min to ensure dispersing sufficiently. Next, 2 mL of H2PtCl6 solution (0.01 M) were added 100 mL of ethylene glycol solution and mixed evenly by stirring for 30 min. Then, all the solutions were heated with an oil bath at 100°C for 6 h under magnetic stirring. The resulting precipitate was GPNs. GPNs were cleaned 5 times with water using the centrifugal method, and then were cleaned one more time with anhydrous ethanol. The washed GPNs were dried in a vacuum oven at 60°C for 12 h.

Preparation of modified electrodes.—The bare GCE was placed vertically and 5 μL of Nafion solution with GPNs was added on the surface of the GCE. After it was naturally dried, the GPNs modified GCE (GPNs/GCE) was obtained. Similarly, Pt and graphene were separately dispersed in 0.5% Nafion. The Pt modified electrode (Pt/GCE) and graphene modified electrode (Gr/GCE) were prepared by dropping 5 μL of the corresponding solutions on the surfaces of the GCEs. Then, the modified electrodes were used after drying naturally.

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Preparation of ELB.—The enzymatic lactic acid biosensor (ELB) was obtained by adding LOx, chitosan, and glutaraldehyde to GPNs/GCE (LOx/GPNs/GCE). LOx was dissolved in water at a concentration of 1 unit μL⁻¹. A pipette was used to take out 5 μL of LOx that was poured on the surface of the GPNs/GCE. After the electrode had dried naturally, 5 μL of chitosan solution was poured dropwise on the surface of the electrode. The mass percentage of chitosan was 1%, dissolved in 2% acetic acid. Finally, 5 μL of glutaraldehyde were added and the mass percentage of glutaraldehyde was 0.1%. The prepared ELB was placed in PBS (pH 7.0) at 4°C.

Characterization of GPNs.—The SEM photographs were taken using a Hitachi SU-70 electron microscope. The accelerating voltage used was 5 kV. The SEM photograph of graphite flakes was magnified 10000× (Figure 1A) and GPNs was magnified 20000× (Figure 1B). From the photographs, it was apparent that the flake graphite had good lamellar structure, while the surface morphology of the GPNs changed significantly compared to that of the flake graphite. In order to better characterize the dispersion of Pt in the material, part of the GPNs was selected (Figure 1C) to conduct mapping analysis by EDS (Figure 1D). The Pt was evenly distributed in GPNs (Figures 1E and 1F). The element content analysis result showed that the mass fraction of Pt is as high as 28%.

Electrochemical behavior of modified electrodes.—Cyclic voltammetry (CVs) of ferricyanide system is a valuable and convenient tool to monitor the characteristic of the surface of modified electrode. Figure 2 shows the CVs of GCE, Pt/GCE, Gr/GCE, and GPNs/GCE, which were recorded in 5 mM of Fe(CN)₆⁴⁻ in PBS with 0.1 M KCl. The CV of GPNs/GCE reveals the redox behavior of Fe(CN)₆⁴⁻ with a peak separation of 0.10 V at a scan rate of 100 mV/s. The CV of GPNs/GCE shows a smaller peak separation and a higher peak current compared to the CVs of other modified electrodes. GPNs/GCE has the highest peak current and is twice than that of Gr/GCE. It is evident that the CV of GPNs/GCE has the best performance, which explains the fact that GPNs have better electrochemical conductivity compared to single graphene or Pt.

Detection of lactic acid by ELB.—The pKa of lactic acid is 3.86. So in neutral solution or in weak acid solution (pH > 4), the lactic acid exists as lactate. In this work, lithium lactate is used as the substrate of ELB. The CV curves of the ELB (LOx/GPNs/GCE) in 0, 0.1 and 1 mM lithium lactate were measured and were presented in Figure 3. It can be observed that the oxidation current of the sensor in 0.1 mM, especially in 1 mM lactate increased more rapidly than that without lactate when the applied potential is over 0.3 V. This indicates that the biosensor could response to lactate when the applied potential is over 0.3 V.

The amperometric i-t method was used to detect the ELB’s response current in the presence of lactic acid in PBS at +0.4 V. During testing, certain amount of lithium lactate solution were added into 10 mL PBS solution every 100 s. The ELB was measured on the 1st, 7th, and 15th day, respectively (Figure 4). The step currents shown in Figure 4 for the concentration of lactic acid has a linear range from 0 to 1.0 mM. The corresponding regression equation of the linear plot is: i/μA = 0.18 + 3.52 c/mM, R = 0.996. The sensitivity is thus estimated as 3.52 μA/mM. The detection limit is estimated to be 8.0 μM (S/N = 3) according to the calibration curve. Table I shows the linear range, limit of detection, and applied potential (V vs. SCE) in some typical amperometric lactate acid biosensors. The above parameters in this work are comparable with the aforementioned lactate acid biosensors.

Stability and selectivity of ELB.—The amperometric i-t curves of ELB at 0.3, 0.5, and 0.7 mM of lactic acid were detected, respectively. At each concentration, the i-t curve was detected five times. The average relative standard deviation (RSD) of the corresponding current of ELB. The CV curves of the ELB (LOx/GPNs/GCE sensor could response to lactate when the applied potential is over 0.3 V. This indicates that the biosensor could response to lactate when the applied potential is over 0.3 V.

![Figure 1. The SEM image of flake graphite (A); GPNs (B); partial magnification of GPNs (C); the elements mapping of GPNs (D), carbon mapping (E), platinum mapping (F).](image1)

![Figure 2. Cyclic voltammograms (scan rate: 100 mV/s) of GCE, Pt/GCE, Gr/GCE, and GPNs/GCE in 5 mmol L⁻¹ Fe(CN)₆⁴⁻ solution, respectively.](image2)

![Figure 3. Cyclic voltammograms of the LOx/GPNs/GCE sensor in the absence and presence of lactate.](image3)
The i-t measurement was conducted in diluted serum sample at a constant potential of 0.4 V vs. SCE. During the measurement, 0.3, 0.6 and 0.9 mM of lactic acid were added into the solution, respectively. The measurement was repeated five times. The calculated recovery and the relative standard deviation (RSD) were shown in Table II. The recoveries of the samples ranges from 98% to 105%, indicating the validation of the developed lactic acid biosensor. In order to further measure the absolute content of lactate in the purchased serum sample by using the as-prepared ELB sensor, the amperometric i-t method was applied to detect the response current of serum in PBS (2 mL of serum was added into 8 mL of PBS). The average response current of five times measurements is 1.56 μA. According to the regression equation of the sensor, the content of lactate in PBS is 0.39 mM. Thus the measured content of lactate in serum is 1.95 mM, which is consistent with the nominal value of 1.88 mM. These data show that the ELB sensor could be used to detect lactate in real samples.

### Conclusions

In this work, graphene supported platinum nanoparticles (GPNs) were successfully synthesized. The mass percentage of Pt in the GPNs was as high as 28%. Based on the results obtained by the CV method, it can be concluded that the GPNs showed the best electrochemical performance among graphene, Pt, and GPNs. An enzymatic lactic acid biosensor (ELB) was fabricated based on GPNs, and it showed excellent performance. The corresponding i-c curve of the ELB in lactic acid solution showed a wide linear relationship from 0 to 1.0 mM. It also showed very high stability, the respond signal lost less than 2% in 15 days. This biosensor was not susceptible to common interfering species. In one word, the ELB prepared can be applied to the detection of lactic acid with excellent detectability, high stability, and good anti-interference performance.

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