Electrochemical Biosensor Based on Nano TiO2 Loaded with Highly Dispersed Photoreduced Nano Platinum

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Precious metal nanomaterials have been widely used in electrochemical sensors. Further improving the dispersion of nanomaterials is beneficial to improving sensor performance and reducing the usage of noble metals. In this work, platinum nanoparticles (NPs) were loaded onto titanium dioxide nanoparticles (TiO2) by the photoreduction method. The morphology, content, and distribution of NPs were determined by high resolution transmission electron microscopy (HRTEM) and energy dispersive spectroscopy (EDS). The method has simple steps and a short synthesis time (14 min). NPs with an average particle size of about 5 nm were uniformly dispersed on the surface of TiO2 nanoparticles. The Pt loaded TiO2 (Pt/TiO2) nanocomposites were modified and used as a carrier of lactate oxidase to prepare an amperometric lactate biosensor. The enzyme-free H2O2 sensor has a linear detection range of 0.003–0.7 mM and a detection limit of 3 μM toward lactate.

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Hydrogen peroxide (H2O2) has important applications in many fields such as the light industry, electronic technology, health care, and environmental engineering. It is the intermediate product of many oxidase catalysis process. The accurate and rapid detection methods of H2O2 have a huge significance in scientific practice. Although many methods have been applied to the quantitative detection of H2O2, such as spectrophotometry, titration analysis, and fluorescence analysis, electrochemical sensors have attracted widespread attention due to their rapid detection, cost-effectiveness, accuracy, and reliability for H2O2 quantitative analysis. On the other hand, lactic acid (LA) is an intermediate product of the body tissues’ anaerobic metabolism, and is of great significance in the diagnosis and treatment of diseases and scientific sports management. Therefore, it is important to construct a disposable LA sensor that has a rapid detection rate and a low cost. Nanoparticles have unique chemical and physical properties and have been widely used to construct high performance biosensors.

The precious metal nanoparticles (NPs) have large surface areas for enhanced biorecognition and receptor immobilization, good reaction catalysis, rapid electron transfer capability, and good biocompatibility. These capabilities not only improve the sensitivity of the sensor, but they can further expand the types of test substances and the miniaturization of sensors. Precious metal platinum (Pt) has stable chemical properties as well as excellent catalytic activity, and has an indisputable position in energy, materials, and chemical industries.

Titanium dioxide (TiO2) was discovered as the first generation of photocatalytic materials and has led to a broad interest in semiconductor-based photocatalysis. Although there are many crystal forms of titanium dioxide, there are only four crystal structures in the natural world: anatase, rutile, brookite, and TiO2 (B). It is well known that the P25, a commercial nano TiO2, is composed of anatase and rutile crystallites with a typically reported ratio of 70:30 or 80:20. Nanosized TiO2 exhibits higher reactivity and chemical stability under ultraviolet (UV) light [wavelength (λ) < 387 nm].

In this study, TiO2 (P25) was used as a photoreductant to reduce PtCl6− to fine nano platinum particles under the irradiation of ultraviolet (365 nm). The average particles size of the base TiO2 (P25) particles are about 25 nm. Analysis by HRTEM showed that the reduced nano platinum was uniformly dispersed on the TiO2 nanoparticles, and the particle size of the reduced nano platinum was only 5 nm. The atom ration of Pt on Pt/TiO2 is only 1.49%.

The highly dispersed photo reduced nano platinum shows very high electrochemical activity, and the electrochemical sensors using the as-prepared Pt/TiO2 nanocomposites have the characteristics of large linear range and lower limit of detection.

Materials and Methods

Reagents and instruments.—Nafion (5 wt%) and lactate oxidase (LOx) were purchased from J&K Scientific Ltd. The activity of LOx was 37 units/mg. The LOx solution was prepared using water and 7% of bovine serum albumin (BSA). N-methyl-2-one Pyrolidone was purchased from Xilong Chemical Co., Ltd. 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 deionized water.

The SEM photographs were obtained using the Hitachi SU-70. The morphologies and microstructures of the samples were obtained using the HRTEM (JEM-2100). All electrochemical measurements were performed using an electrochemical workstation from Shanghai Chenhua Instrument Company (CHI 660E). A bare electrode or modified electrode was used as the working electrode, a platinum electrode was used as an auxiliary electrode, and a saturated calomel electrode was used as a reference electrode to form a three-electrode system. Cyclic voltammetry (CV) and current time (I-T) curves were measured and recorded.

Preparation of Pt/TiO2 nanocomposite.—17.4 mg of TiO2 were dispersed in 10 mL water and stirred constantly. 25 mg of glucose were dissolved in the above suspension. 3 mL of H2PtCl6, 6H2O solution (0.01 g/mL) were added into the suspension dropwise and magnetically stirred at room temperature for 30 min. After adding 145 μL of N-methyl-2-pyrrolidone as a polymerization inhibitor, the suspension was magnetically stirred at room temperature for 30 min. The suspension was placed in a quartz glass bottle, and this bottle was placed in an ultraviolet synthesizer for 14 min under ultraviolet light (365 nm). After several centrifugation cleanings, the resulting product was vacuum dried for use.

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Preparation of modified electrodes and lactic acid biosensor.—The bare glassy carbon electrode (GCE) (Ø 3 mm) was polished to a mirror surface with 0.3 μM and 0.05 μM Al2O3 powder, and the GCE was washed with deionized water and anhydrous ethanol for 5 minutes. 10 mg of Pt/TiO2 nanocomposite were dissolved in 1 mL mixture solution (750 μL of deionized water, 210 μL of ethanol, and 40 μL of 5% Nafion solution) and sonicated for 60 min. 5 μL of this turbid solution were taken and placed on the treated GCE surface to make Pt/TiO2 electrode (Pt/TiO2/GCE) for the detection of H2O2. For comparison, the TiO2/GCE also was made using pure TiO2 nanopowder according to the same procedure.

A LA biosensor was prepared by dropping 5 μL of 1 U/μL lactic acid oxidase onto a Pt/TiO2/GCE followed by crosslinking with 5 μL of 0.1% GA (LOx/Pt/TiO2/GCE). This process is shown in Scheme 1.

Results and Discussion

Characterization of the TiO2 and Pt/TiO2.—Titanium dioxide could produce electrons and holes with the excitation of ultraviolet light. With the excitation of ultraviolet light, electrons and holes appears on the surface of TiO2. Glucose was added to catch these holes,26 and PtCl62− was reduced by electrons to form nano Pt particles and loaded onto TiO2. The wavelength of the ultraviolet lamp used in this work was 365 nm. Fig. 1 shows the UV-visible absorption spectrum of TiO2. It can be seen that the TiO2 used in this work can absorb ultraviolet light with a wide wavelength, including the wavelength $\lambda = 365$ nm.

In order to investigate the morphology and the distribution of nano Pt on TiO2, Pt/TiO2 nanomaterials were analyzed by HRTEM and EDS. Fig. 2a is the TEM image of Pt/TiO2. It can be clearly seen that the TiO2 used in this work can absorb ultraviolet light with a wide wavelength, including the wavelength $\lambda = 365$ nm. Figure 1. UV absorption spectrum of TiO2 (P25).
Figure 2. TEM images of Pt/TiO$_2$ (A); HRTEM images and electron diffraction patterns of TiO$_2$ (B) and Pt (C); The EDS analysis (D) and elements mapping of Pt/TiO$_2$ (E and F).

is the HRTEM image and electron diffraction pattern of Pt that was loaded onto the TiO$_2$. It can be seen from the HRTEM images that the particle size of Pt is about 5 nm, and the lattice spacing of the nano Pt is 0.2 nm. The content of Pt in the sample and the mapping analysis were analyzed by EDS (Figs. 2d–2f). It was found that the Pt was evenly distributed on TiO$_2$. The atom ratio of Pt is only 1.49%, showing very high dispersity.

Electrochemical properties of the modified electrode (Pt/TiO$_2$/GCE).—In order to investigate the prospective applications of Pt/TiO$_2$ nanomaterials in electrochemical detection, an enzyme-free H$_2$O$_2$ electrochemical sensor and a lactic acid biosensor based on the photoreduced Pt/TiO$_2$ nanomaterials were constructed, respectively. Fig. 3a shows the CV of TiO$_2$ modified glassy carbon electrode (TiO$_2$/GCE), Pt/TiO$_2$ modified glassy carbon electrode (Pt/TiO$_2$/GCE) and LOx/Pt/TiO$_2$ modified glassy carbon electrode (LOx/Pt/TiO$_2$/GCE) between the potential range from −0.2 to 0.6 V in 5 mM potassium ferricyanide solution with a sweep rate of 50 mV/s. The CV of the bare GCE is also shown in Fig. 3a as a comparison. The CV of the Pt/TiO$_2$/GCE shows a smaller peak separation and a higher peak current compared to the CVs of TiO$_2$/GCE and bare GCE. The closer oxidation-reduction peak and the higher oxidation-reduction peak current indicate that this sensor has excellent electrochemical performance. Since the two insulating films of LOx and GA hinder the transfer of electrons, the oxidation peak current and the reduction peak current of the LOx/Pt/TiO$_2$/GCE was reduced greatly. Fig. 3b shows the CVs of Pt/TiO$_2$/GCE at various scan rates. It can be seen that both anodic peak potential (Epa) and cathodic peak potential (Epc) remained almost unchanged with the increase of potential scan rate, indicating the good electrochemical reaction ability and fast electron transfer kinetics of the electrode. The inset of Fig. 3b shows the relationship between the scan rate and the anode or cathode peak current. The anodic and cathodic peak current increase linearly with the square root of scan rate in the range from 0.01 to 0.1 V/s, implying the dominance of a diffusion controlled process. In order to explore the electrocatalytic activity of Pt/TiO$_2$/GCE for H$_2$O$_2$, the CVs of bare GCE, TiO$_2$/GCE, and Pt/TiO$_2$/GCE (Fig. 3c) were carried out with 10 mM H$_2$O$_2$. The bare GCE and TiO$_2$/GCE have lower electrocatalytic activity toward H$_2$O$_2$ compared to the Pt/TiO$_2$/GCE. The highly dispersed nano Pt on TiO$_2$ significantly lowers the overpotential for the oxidation of H$_2$O$_2$, improves the oxidation current and shows an obvious oxidation peak. In addition, as can be seen from Fig. 3d, the anode peak current increases as the H$_2$O$_2$ concentration increases from 1 mM to 9 mM. These results strongly suggest that Pt/TiO$_2$ nanocomposites exhibit excellent electrocatalytic activity for the oxidation of H$_2$O$_2$. In order to achieve quantitative detection of H$_2$O$_2$ conveniently, an amperometric method was used to test the Pt/TiO$_2$/GCE. Fig. 3e shows the current-time response curve of H$_2$O$_2$ on the Pt/TiO$_2$/GCE and the TiO$_2$/GCE in 0.1 M PBS under a potential of 0.5 V. With
Figure 3. (a) CVs of bare GCE, TiO2/GCE, Pt/TiO2/GCE and LOx/Pt/TiO2/GCE in 5 mM potassium ferricyanide solution at a scan rate of 50 mV/s. (b) CVs of the Pt/TiO2/GCE in 5 mM potassium ferricyanide solution at different scanning rates from 0.01 to 0.1 V/s. Inset shows the linear relationship between the square root of scan rate and the anode and the cathode peak current. (c) CVs of blank GCE, TiO2/GCE, and Pt/TiO2/GCE in 0.1 M PBS (pH = 7.0) with the absence and presence of 10 mM H2O2 at a scan rate of 50 mV/s. (d) CVs of the Pt/TiO2/GCE in 0.1 M PBS (pH = 7.0) in different concentrations of H2O2 (1 to 9 mM) at a scan rate of 50 mV/s. (e) i-t curves of Pt/TiO2/GCE and TiO2/GCE toward H2O2 at 0.5 V potential. (f) Amperometric responses of Pt/TiO2/GCE to the successive addition of low concentration of H2O2. The upper inset is the enlarged part of the amperometric response curve. The lower inset shows the corresponding calibration plot for the Pt/TiO2/GCE.

the increase of the concentration of H2O2, both of the response currents increase continuously. However, the response current of the Pt/TiO2/GCE is obviously higher than that of TiO2/GCE, showing that the photoreduced nano Pt has excellent catalytic ability. In addition, the response signal of Pt/TiO2/GCE changes rapidly toward the change of H2O2. It can reach a steady state current within 2 s with the addition of H2O2. Fig. 3f shows the response current curve with the successive addition of different concentrations of H2O2 in 0.1 M PBS (pH = 7.0). The Pt/TiO2/GCE has a lower detection limit (0.92 μM) and a wider linear range (0.002–15 mM) toward H2O2 and could be used as a highly performance enzyme-free sensor. These results are comparable or superior to the recent reported results, as shown in Table I.

Good stability is one of the indispensable factors of excellent sensor. The enzyme-free H2O2 sensor (Pt/TiO2/GCE) was tested 5 times in 0 mM, 10 mM, and 20 mM of H2O2 solution, respectively. The relative average deviation (RAD), standard deviation (S), and relative standard deviation (RSD) were used to estimate the stability of the sensor. The results are shown in Table II. The enzyme-free H2O2 sensors have lower RAD and RSD values (less than 3%), showing good stability.

Electrochemical properties of the lactic acid biosensor.—A lactic acid biosensor was obtained by immobilizing LOx on Pt/TiO2/GCE. Fig. 4 shows the i-t response curve of LA in a 0.1 M PBS at a voltage of 0.5 V. The sensor has a significant current response toward LA with a linear range from 0.003 to 0.7 mM. The inset in Fig. 4 is the corresponding current-concentration curve. The corresponding regression equation of the linear plot is: i/μA = 0.007 + 0.136 c/mM, n = 8, R2 = 0.996. The sensitivity is 0.136 μA/mM. The detection
photoreduction method. Though the amount of loaded platinum is very low (1.49%), it has very high electrochemical activities because of the large surface area of the highly dispersed nano Pt. The as-prepared enzyme-free H$_2$O$_2$ sensor has good linear response, low detection limit (0.92 μM), wide detection range (0.002–15 mM), and excellent repeatability. A lactic acid biosensor was prepared by immobilizing LOx on Pt/TiO$_2$/GCE. It could detect lactic acid in serum samples with a linear range from 0.003 to 0.7 mM and a detection limit of 3.0 μM. The results showed that the enzyme-free sensor and the biosensor based on photoreduced Pt/TiO$_2$ nanomaterials have great potential application in the detection of H$_2$O$_2$ and LA in biological samples.

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