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Preparation of Ag–Au nanoparticle and its application to glucose biosensor

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Abstract

In this paper, Ag–Au nanoparticles are produced in sodium-bis(2-ethylhexyl)-sulfosuccinate (AOT)–cyclohexane reverse micelle system. The properties of the obtained nanoparticles are characterized with transmission electron microscope (TEM) and UV–vis absorption spectrophotometer. Glucose biosensors have been formed with glucose oxidase (GOx) immobilized in Ag–Au sol. GOx are simply mixed with Ag–Au nanoparticles and crosslinked with a polyvinyl butyral (PVB) medium by glutaraldehyde. Then a platinum electrode is coated with the mixture. The effects of the various molar ratios of Ag–Au particles with respect to the current response and the stability of the GOx electrodes are studied. The experimental results indicate the current response of the enzyme electrode containing Ag–Au sol increase from 0.32 to 19 $\mu A\,cm^{-2}$ in the solution of 10 mM β-D-glucose. In our study, the stability of enzyme electrodes is also enhanced.

Keywords: Ag–Au nanoparticles; Immobilization enzyme; Glucose oxidase; Biosensor

1. Introduction

Glucose biosensor is one of the most important biosensors. The ability to obtain rapid, accurate and precise glucose measurement is essential to the appropriate administration of insulin therapy [1]. Glucose biosensors would offer the possibility of carrying out analytical and diagnostic procedures simply and rapidly [2]. They allow greater ease of use with opportunities for self-monitoring by user groups such as diabetics. With improved glucose control, it is possible that some of the long-term diabetic syndromes, such as retinal and kidney damage, would be avoided.

In biosensor the biological substances are used as recognition elements, which can convert the analytic substances concentration with the measurable electrical signals. The immobilization of the enzymes is one of the crucial factors in biosensor preparation. Many methods have been used to immobilize enzymes and to improve the enzymatic activity [3–9]. Miniaturization is one of the important developments in biosensor technology [10]. Miniaturization, however, may result in low current. To overcome this problem, nanoparticles have been introduced to the immobilization of the enzymes.

Metal nanoparticles have many unique properties: large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency, and strong adsorption ability. They have possible applications in many areas such as nonlinear optical switching [11], immunoassay labeling [12], and Raman spectroscopy enhancement [13]. The immobilization of proteins in nanoparticles sol has been reported in Refs. [14–17]. Recently, Xiao et al. [18] report the reconstitution of an apo-flavoenzyme, apo-glucose oxidase, on a 1.4 nm gold nanocrystal functionalized with the cofactor flavin adenine dinucleotide and integrated into a conductive film that yields a bioelectrocatalytic system with exceptional electrical contact with the electrode support. Their work shows that electron transfer through the Au nanoparticles is much faster than electron transfer to O_2.

In this paper, Ag–Au nanoparticle is introduced to the research of the glucose electrode. We use Ag–Au nanoparticle because silver is the best conductor among metals and gold has good biocompatibility. Different from other papers,

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we use the Ag–Au nanoparticle prepared in reverse micelle. Numerous articles have given the proof that enzymes have higher activity in reverse micelles than in aqueous systems [19–21]. Our experiments show that these Ag–Au particles can significantly enhance the current sensitivity of GOx enzyme electrodes.

2. Experimental

2.1. Chemicals and reagents

Glucose oxidase (GOx) was extracted from *Aspergillus niger* (100 U mg^{-1}; Toyobo Co. Ltd., Japan). Sodium-bis(2-ethylhexyl)-sulfosuccinate (AOT) was obtained from Nacalai Tesque (Kyoto Inc., Japan). β-D-Glucose was from Sigma. Silver nitrate ($AgNO_3$), chlorauric acid ($HAuCl_4$) and sodium citrate were obtained from Beijing ShiJi Company.

All the chemicals were used without further purification. All solutions were prepared with redistilled water.

2.2. Preparation of Ag–Au sol

The preparation of the Ag–Au nanoparticles was achieved by mixing two sets of reverse micelle solutions (0.2 mol/kg AOT/cyclohexane), with $AgNO_3$ and $HAuCl_4$ ($[AgNO_3] + [HAuCl_4] = 0.8$ mM) solubilized in one set and sodium citrate ($[Na_3C_6H_5O_7] = 2$ mM) as the reduction agent in the other set. The micelles in cyclohexane were stabilized by the surfactant. The concentration of the AOT and the micellar size were the same in both sets. These reversed micelles were then stirred thoroughly at room temperature until the solution turned to red. Varying the molar ratios of $HAuCl_4$ and $AgNO_3$ aqueous solutions, we could achieve different nanoparticles.

2.3. Characterization of the metal colloids

The samples for transmission electron microscopy (TEM) were prepared by putting one drop of the Ag–Au colloid on a formvar-coated copper grid followed by drying in a desiccator. Electron micrographs were taken with a NEC JEM-100CX electron microscope, operating at 100 kV. Absorption spectra were recorded using a Hitachi U-2001 diode array spectrophotometer at 1 cm path length.

2.4. Preparation of enzyme electrode

In order to simplify production process of the enzyme electrode, the self-assembly method was used to prepare GOx electrode. Platinum wire with a diameter of 1 mm was polished and boiled in nitric acid for 5 min, washed in redistilled water and boiled in redistilled water. When it became cool, platinum wires was washed in acetone and redistilled water by ultrasound.

The aqueous solution of six units GOx was added to a 10 ml beaker. An amount of 200 μl Ag–Au nanoparticle sol was added to the GOx solution. Several minutes later, 2 ml PVB solution (2%) in anhydrous alcohol and 100 μl of glutaraldehyde solution (1%) were added to the beaker. After the mixer was uniform, the platinum wire was dipped into the solution to a depth of 1 cm for several minutes and dried in air. A thin membrane was formed on the electrode. The electrode was stored in a refrigerator at 277 K.

The enzyme electrode without Ag–Au nanoparticle, which was used as compare, was prepared by dipping platinum wire into the mixture containing GOx, PVB and glutaraldehyde. This method and amount were as same as described above.

2.5. Cyclic voltammetry

Cyclic voltammetry studies used a PARC EG&G Model 283 potentiostat and three electrode cells equipped with a Pt-wire as counter electrode and a Ag/AgCl electrode as reference electrode. The applied scan rate was 50 mV/s. The cyclic voltammograms was taken at 33 mmol/L glucose concentrations.

2.6. Amperometric measurement

The sensitivity of the glucose biosensor was tested by measuring the current response. The experiment was carried out using a two-electrode cell consisting of an enzyme working electrode and a reference electrode of Ag/AgCl [22]. Measurements were conducted in a 5 ml phosphate buffer ($KHPO_4-NaOH$, pH 6.8) cell at 308 K. A fixed potential of 0.4 V was applied to this electronic cell. Firstly, working electrode and reference electrode were put into a phosphate solution at 308 K. When background current reached a constant value, different concentrations (from 2.7 to 33 mM) of β-D-glucose solution were added. Then response current was noted down, and background current was deducted, and the correlation between response currents and different concentrations of glucose solution was obtained.

3. Results and discussion

3.1. Preparation of Ag–Au particles

The Ag–Au particles were prepared with the various mixtures of $HAuCl_4$ and $AgNO_3$ aqueous solutions. Except the pure silver, the color of the other solutions turned red at last. Transmission electron micrographs of the Ag–Au nanoparticles are in Fig. 1. Fig. 2 shows the UV–vis absorption spectra of Ag–Au composite particles. A remarkable shift in the absorption band at 530 nm was observed for silver (75)–gold (25); the number in metal colloids indicate molar percent of the metal salt incorporated. But with increasing molar ratio of $HAuCl_4$, the absorption peak