Journal of Electroanalytical Chemistry 756 (2015) 153–160

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jeac

Development of a glucose biosensor based on electrodeposited gold nanoparticles–polyvinylpyrrolidone–polyaniline nanocomposites

Zhuling Miao [a], Peiyu Wang [a], AiMing Zhong [b], Minfeng Yang [a], Qin Xu [a,*], Shirong Hao [a], Xiaoya Hu [a,*]

[a] College of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, China
[b] Yangzhou Polytechnic institute, Yangzhou, 225127, China

ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 18 August 2015
Accepted 19 August 2015
Available online 22 August 2015

Keywords:
Polyaniline
Gold nanoparticles
PVP
Electrodeposition
Glucose
Sensor

ABSTRACT

In this work, the fabrication and performances of a glucose biosensor based on the electrodeposited gold nanoparticle–polyvinylpyrrolidone–polyaniline (AuNP–PVP–PANI) nanocomposites have been demonstrated. The nanocomposites were electrodeposited on a glassy carbon electrode (GCE) in a homogeneous three-component solution consisting of aniline, PVP and AuNPs. Glucose oxidase (GOx) was subsequently immobilized on the as-prepared AuNP–PVP–PANI nanocomposites using Nafion as the preventing layer. The electrochemical and electrocatalytic properties of the Nafion/GOx/AuNP–PVP–PANI modified GCE were researched. It exhibited good electrocatalytic performances with a large linear range from 0.05 mM to 2.25 mM, a low detection limit of 1.0×10^{-5} M (S/N = 3), and a high sensitivity of 9.62 μA mM^{-1} cm^{-2}. This sensor with excellent stability and reproducibility was also allowed for the detection of glucose in human serum samples with satisfactory results.

1. Introduction

Composites of conducting polymers (CPs) and metal nanoparticles have received much attention in the last decades because of their various applications such as catalysts, biosensors and capacitors [1,2]. Incorporation of metal nanoparticles would preserve the properties of low dimensional conductors, enhance the conductivity of polymers, and obtain high surface areas. Among the well-known CPs, polyaniline (PANI) and noble nanoparticle composites are especially of great research interest because of their straightforward polymerization process, relatively high conductivity, good electrochemical stability, and interesting electrochemical properties [3]. Furthermore, PANI is compatible with most enzymes and provides a suitable matrix for enzyme immobilization [4,5].

The incorporation of gold nanoparticles (AuNPs) into PANI matrix has been extensively studied because AuNP–PANI composite reveals characteristics through the combination of both components including good biocompatibility, large surface areas, good electrochemical stability and enhanced electrocatalytic activity [6–10]. Electrochemical or chemical methods are always used to incorporate AuNPs into the PANI matrix [11,12]. Yang et al. [13] have prepared AuNP@PANI nanocomposites by the polymerization of aniline using AuNPs as the seeds and $HAuCl_4$ as the oxidant. This nanocomposite exhibited excellent electrochemical catalytic activities towards dopamine and ascorbic acid. Compared with the chemical preparation method, the electrochemical polymerization process permits the synthesis without any oxidizing agent, and the resulting thin polymeric film can be directly modified at the electrode surface by a one-step process [14]. The other advantage of electropolymerization is that the organic and inorganic ions can be doped simultaneously into the film. Moreover, the thickness of the film and localization of biologically active molecules in the film can be controlled [15]. Wang et al. [16] have decorated AuNPs on electropolymerized PANI film via the direct electroreduction of the adsorbed $AuCl_4^-$ ions on a glassy carbon electrode. However, little work reported the direct incorporation of AuNPs into PANI matrix by electrochemical methods. During the electrochemical doping process, the remarkable stability of nanoparticles in the deposition medium is important. This issue can be addressed effectively by appropriate nanoparticle surface modification.

Polyvinylpyrrolidone (PVP) is a water-soluble homopolymer with a long and soft polyvinyl backbone and an amide group in each monomer. It has been widely used as a steric stabilizer or capping agent with a major role to protect the metal colloid against agglomeration in many solvents and wide pH ranges [17–19]. In addition to the role as protecting agent, PVP molecule was also found to increase the conductivity of polyaniline [20]. In this research, the AuNP–PVP–PANI nanocomposites were prepared through a simple one-step electrochemical polymerization process using aniline as reactant and PVP-stabilized AuNPs as doping agent. This work also reports the further application of the AuNP–PVP–PANI composites for enzyme immobilization and biosensor construction. Glucose oxidase (GOx) as a model protein was immobilized on the AuNP–PVP–

---

* Corresponding authors.
E-mail addresses: xuqin@yzu.edu.cn (Q. Xu), xyhu@yzu.edu.cn (X. Hu).

http://dx.doi.org/10.1016/j.jelechem.2015.08.025
1572-6657/

PANI modified electrode with Nafion to ensure its adhesion to the electrode. Nafion is a sulfonated tetrafluoroethylene copolymer that has been widely used as a proton conductor for proton exchange membrane, in fuel cells, and for biosensor applications [21,22]. The main advantages of Nafion in biosensor applications are its biocompatibility, excellent thermal and mechanical stability, and film formation ability. Most work has reported the application of Nafion as the protecting layer to maintain the stability of biosensors [23]. Direct electron transfer characteristics and the electrocatalytic behavior of GOx immobilized on AuNP–PVP–PANI composites were also investigated in detail.

2. Experimental

2.1. Reagents and apparatus

Trisodium citrate, poly(N-vinyl-2-pyrrolidone) (PVP, MW = 40,000), aniline, concentrated sulphuric acid (H_2SO_4), and β-D-(+)-glucose were all purchased from Shanghai Chemical Reagent Company. Aniline was distilled under reduced pressure and stored in the dark prior to use. Other reagents were used without further purifications. Nafion (5 wt.% in ethanol and water) and hydrogen tetrachloroaurate ($HAuCl_4 \cdot 4H_2O$) were purchased from Sigma-Aldrich. Glucose oxidase (GOx, EC 1.1.3.4, type II from *Aspergillus*, activity ≈ 100 U/mg) was purchased from Amresco. Phosphate buffer solution (PBS, 0.1 M) was prepared by mixing the stock solutions of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4, and its pH was adjusted either with 0.1 M H_3PO_4 or 0.1 M NaOH. All the chemicals were of analytical grade, and all solutions were prepared with deionized water.

Morphological and element analytical data were obtained using a Hitachi S-4800 scanning electron microscope (Japan). The transmission electron microscopy (TEM) images were obtained by a Philips Tecnai-12 microscope (Netherlands). Zeta potentials were determined using Zetasizer Nano-ZS (Malvern Instruments Ltd., UK). Each data has an average of at least 15 measurements. An inductively coupled plasma optical emission spectroscopy (ICP-OES, Optima 7300 DV, PerkinElmer, USA) was used to detect the content of Au in the nanocomposite. The Fourier transform infrared (FT-IR) spectra were recorded on a FT-IR spectrophotometer (IFS66/S, Bruker, USA). The UV–vis spectra were recorded using a Cary 5000 UV–vis-NIR spectrophotometer (Varian). Electrochemical experiments were performed with a CHI840B electrochemical workstation (CHI, Shanghai Chenhua, China). All experiments were carried out with a three-electrode system using a glassy carbon electrode (GCE, d = 3 mm) as the working electrode, a platinum wire as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference electrode. All experiments were carried out at room temperature (~25 °C).

2.2. Preparation of AuNPs

Gold nanoparticles (AuNPs) were prepared by the traditional citrate reduction method [24]. Typically, 1 mL of 1% $HAuCl_4$ aqueous solution was added into a 250 mL round-bottom flask followed by the addition of 100 mL deionized water. Then, the obtained solution was heated to boiling under constant stirring. Subsequently, 2.5 mL of 1% trisodium citrate solution was added rapidly. While the solution was boiled for another 10 min, its color changed from pale yellow to deep red. After stirring for another 15 min at room temperature, a transparent red colloidal solution of AuNPs was obtained. It was stored in a brown glass bottle at 4 °C for further use.

2.3. Electrodeposition of AuNP–PVP–PANI nanocomposite and its application for glucose sensor construction

Prior to electrodeposition, the GCE was carefully polished to a mirror-like surface with 0.3 μm and 0.5 μm alumina powders, followed by sonication in 1:1 nitric acid–water (v/v), ethanol and water for 5 min, respectively. Then the prepared electrode was dried using ultrapure nitrogen and used for modification. 0.04 g PVP was dispersed in 5 mL of the as-prepared gold solution containing 10 μL distilled aniline under ultrasonication, and the pH was adjusted to 2.0 using 0.5 M H_2SO_4 solution. The pre-treated GCE was then immersed into the mixed solution and the electropolymerization process was carried out by applying twenty potential cycles between −0.2 and +0.75 V vs. SCE at 100 mV/s. After the electropolymerization, the obtained AuNP–PVP–PANI electrode was rinsed with water and dried in air. Afterwards, 5 μL of 8 mg/mL GOx solution was deposited on the AuNP–PVP–PANI modified electrode and dried at 4 °C overnight. Finally, 5 μL of 0.5% Nation was dropped on the whole surface to improve operational and storage stability of the biosensor.

3. Results and discussion

3.1. Characterizations of AuNP–PVP–PANI nanocomposite modified electrode

In this work, AuNPs were tried to be incorporated into PANI film by a simple electrodeposition method. Its well-dispersion and stability in solution are of great importance. TEM was utilized to check the dispersion and stability of AuNPs in solution. Fig. 1 displays the TEM images of the initial citrate stabilized AuNPs (A), AuNPs dispersed in aniline solution in the presence (B) and absence of PVP (C) at pH 2.0. Compared with the initial citrate stabilized AuNPs (Fig. 1A), good dispersion of AuNPs was found in the presence of PVP and aniline (Fig. 1B). However, the aggregation of the citrate stabilized AuNPs was observed in the presence of aniline but the absence of PVP (Fig. 1C). This suggested that PVP could increase the aqueous-stability of AuNPs. The good dispersion of AuNPs in aniline solution in the presence of PVP can also be checked by the naked eyes. Inset of Fig. 1B shows the photograph of AuNPs in the presence of aniline and PVP. No color change and aggregation of AuNPs were observed, indicating that PVP served as an effective stabilizing agent for AuNPs. In contrast, AuNPs dispersed in the solution without PVP formed aggregates. The color of the AuNP solution turned into purple. Black precipitations were found on the bottom of the cell (inset of Fig. 1C). Zeta potentials were measured to confirm the successful coating of AuNPs with PVP. The zeta potential of the initial citrate stabilized AuNPs was −37.8 mV because the nanoparticles were coated by negative citrate ions. When pH of AuNPs was adjusted to pH 2.0, the zeta potential of AuNPs was increased to −13.3 mV because of the protonation of some charged citrate ions. The hydrogen bonds between the protonated citrate ions caused the aggregation of AuNPs [25,26]. To prevent the aggregation of AuNPs, it is necessary to modify the surface of AuNPs. The zeta potential of PVP and aniline at pH 2.0 was 0.162 mV and 6 mV, respectively. Rapid adsorption of large numbers of positively charged PVP or aniline would take place onto the surface of the negatively charged AuNPs, causing charge neutralization. When AuNPs were modified with aniline or PVP, the values of zeta potential became 0.11 mV and −0.65 mV, which clearly confirmed the successful coating of AuNPs by aniline or PVP. Though aniline monomer could adsorb on citrate-modified AuNPs, the aggregation of AuNPs based on the dipole–dipole interaction and hydrogen bonds at pH 2.0 could not be prevented owing to the short chain length of aniline monomers [27]. PVP is a hydrophilic polymer with a long and soft polyvinyl backbone and its individual monomer contains an amide group. It can act as a capping agent for citrate modified AuNPs through the electrostatic interaction, intermolecular hydrogen bonding between —OH groups in AuNPs and C—N groups in PVP [28,29]. The long polyvinyl backbone groups of PVP adsorbed on AuNPs hinder the nanoparticles from close contact and enhance their stability via steric stabilization mechanisms [29].

SEM images were used to study the morphologies of PANI and AuNP–PVP–PANI composites deposited onto the surface of the glassy carbon electrode (Fig. 2A–D). Fig. 2A–B shows the deposited PANI film on GCE in the absence of PVP and gold nanoparticles, which are