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Sensors and Actuators B 104 (2005) 199–206
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Preparation and application on a kind of immobilization method of anti-diphtheria for potentiometric immunosensor modified colloidal Au and polyvinyl butyral as matrixes

Dianping Tang, Ruo Yuan*, Yaqin Chai, Linyan Zhang, Xia Zhong, Yan Liu, Jianyuan Dai

Chong Qing Key Laboratory of Analytical Chemistry, College of Chemistry and Chemical Engineering, Southwest China Normal University, Chongqing 400715, China

Received 25 March 2004; received in revised form 21 April 2004; accepted 26 April 2004
Available online 15 June 2004

Abstract

In this paper, two technologies named as the self-assembled technique and the opposite-charged adsorption are combined for a novel immobilization of diphtheria antibody (anti-Diph) molecules applied to an immunosensor for detecting diphtheria antigen (Diph). Anti-Diph was immobilized successfully on nanometer-sized Au colloid particles associated with polyvinyl butyral on a platinum electrode surface, and was characterized by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The immobilized anti-diphtheria was shown to keep its biological activity well and exhibited direct electrochemical behavior toward Diph. The factors influencing the performance of the resulting immunosensor were studied in detail. The resulting immunosensor exhibited fast potentiometric response (<3 min) and the linear range was from 24 to 1280 ng mL^{-1} with a detection limit of 7.8 ng mL^{-1}. Moreover, the studied immunosensor exhibited high sensitivity and long-term stability. The response mechanism of immuosensors was also preliminarily studied using AC impedance.

Keywords: Potentiometric immunosensor; Diphtheria; Platinum electrode; Colloidal Au; Polyvinyl butyral

1. Introduction

Immunosensors have attracted growing attention with expectation of obtaining quick and highly sensitive immunological response. In the last two decades, many reports have been published on the use of immunosensors for a wide range of applications in food industry, environmental monitoring, biotechnology, pharmaceutical chemistry and clinical diagnostics. There are numerous transduction techniques, such as quartz crystal microbalance [1], surface plasmon resonance [2], ellipsometry [3] and electrochemical techniques [4] etc. receiving much attention for direct monitoring of immune reaction at surface. Of these, optical detection methods are most developed in terms of commercial applications, but the electrochemical detection method using immunoreactions was not applied much by now. However, it appears very promising due to the relatively simple and inexpensive equipment required. So search for new immobilization method with improved potentiometric response characteristics is of considerable interest.

The immobilization of biomolecules or the design technology of sensing interface is one of the key factors for potentiometric immunosensors. To maximize the sensitivity of the assay and the quantity based on immobilization of electrode while maintaining a sufficient working signal, the bare platinum electrode was made cathodic prior to the anti-Diph-Au-PVB-modified electrode. Thus, it was held at a potential of −1.5 V in a 0.1 M NaOH stirred solution for 2 min followed by −0.5 V (versus SCE) for 45 s in the same medium in order to take on negative charge at the platinum surface, and then adopted the self-assembled technique on the platinum electrode based on immobilization of anti-Diph modified colloidal gold and polyvinyl butyral via a highly positively charged species of the anti-Diph and a negatively charged species of gold nanoparticles as a result of the adsorption of citrate in the fabrication process

* Corresponding author. Tel.: +86-23-68252277; fax: +86-23-68253195.
E-mail address: yuanruo@swnu.edu.cn (R. Yuan).

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doi:10.1016/j.snb.2004.04.116

in this paper. The self-assembled process of immunosensors could be validated by AC impedance measurement and cyclic voltammetry (CV) experiments. The immunosensor fabrication procedure was optimized with respect to the size of the gold nanoparticles and the assembling time. In addition, the factors influencing the performance of the resulting immunosensor have been studied in detail. It is hoped that the attractive properties of the anti-Diph-Au-PVB modified electrodes would find various practical applications.

2. Materials and methods

2.1. Reagent and materials

Anti-Diphtheria (anti-Diph) and Diphtheria antigen (Diph) (E.C. 1.1.3.4, 800 ng mL^{-1}) was purchased from Bioengineering Company (Chengdu, China), polyvinyl butyral (PVB) was bought from Shanghai Chemical Reagent Co. (China). Bovine serum albumin (BSA, 96–99%) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and solvents used were of analytical grade and were used as received. Double distilled water was used throughout this study. The standard Diphtheria stock solutions were prepared with phosphate buffer solution (PBS, pH 7.0) and stored at 4 °C. The anti-Diph was stored in the frozen state, and its standard solutions were prepared daily with PBS solution as in use.

The preparation of phosphate buffer solution of pH 7.0: NaCl 8.0 g, Na_2HPO_4 1.15 g, KH_2PO_4 0.2 g, KCl 0.2 g, were dissolved in 1000 mL double distilled water.

2.2. Apparatus

The AC impedance of the immunosensor membrane was measured with a Model IM6e (ZAHNER elektrick Co., Germany). Cyclic voltammetric measurements were carried out with a CHI 660A electrochemistry work station (Shanghai CH Instruments Co., China). A three-compartment electrochemical cell contained a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and anti-Diph-Au-PVB modified platinum electrode (Φ = 1 mm) as working electrode. The size of Au colloids was estimated from transmission electron microscopy (TEM) (H600, Hitachi Instrument Co., Japan). All potentiometric and pH measurements were made with a pH meter (MP 230, Mettler-Toledo Co., Switzerland) and a digital ion analyzer (Model PHS-3C, Dazhong Instruments, Shanghai, China).

2.3. Preparation of Au colloids

All glassware used in the following procedures was cleaned in a bath of $K_2Cr_2O_7$–H_2SO_4, rinsed thoroughly in double distilled water and dried in air. Gold colloids were prepared according to the literature [5]. Solution A: 1 ml of 1% $HAuCl_4$ solution was added to 99 ml water. Solution B: 4 ml of 1% Na_3-citrate solution. The two solutions were heated up to 60 °C, respectively. During mixing, solution B was added to solution A quickly. The mixture was heated for 35 min subsequently. The solution color was claret. The mean size of the prepared Au colloid was about 16 nm, which was estimated from transmission electron microscopy (see Fig. 1).

2.4. Preparation of the immunosensor

The platinum electrode (1 mm diameter) was used for electrochemical measurements. The platinum electrode was polished carefully with alumina slurries (1.0, 0.3, 0.05 μm), then rinsed thoroughly two times with water, boiled in nitric acid (1:1) for 10 min, ultrasonicated in acetone and washed in water two times, respectively, then dried in air before use. The clean platinum electrode was made cathodic prior to the anti-Diph-Au-PVB-modified electrode. Thus, it was held at a potential of −1.5 V in a 0.1 M NaOH stirred solution for 2 min followed by −0.5 V (versus SCE) for 45 s in the same medium. Then a sol–gel method was adopted to prepare the electrode. An appropriate amount (unless otherwise specified, 60 μL was used) of anti-Diph solution was mixed with 0.3 mL Au colloids into a beaker in ice water. Ten minutes later, 3 mL of polyvinyl butyral ethanol solution (w = 2%) was added to the beaker quickly. The mixture was stirred and disposed upon the surface of the platinum electrode. The platinum electrode was dipped into the solution to a depth of 10 mm for 10 min and then removed. After storing for about 24 h at 4 °C, the modified immunosensor was incubated in 0.25 wt.% BSA for 60 min at 37 °C in order to block out active positions. Then the immunosensor was formed and the

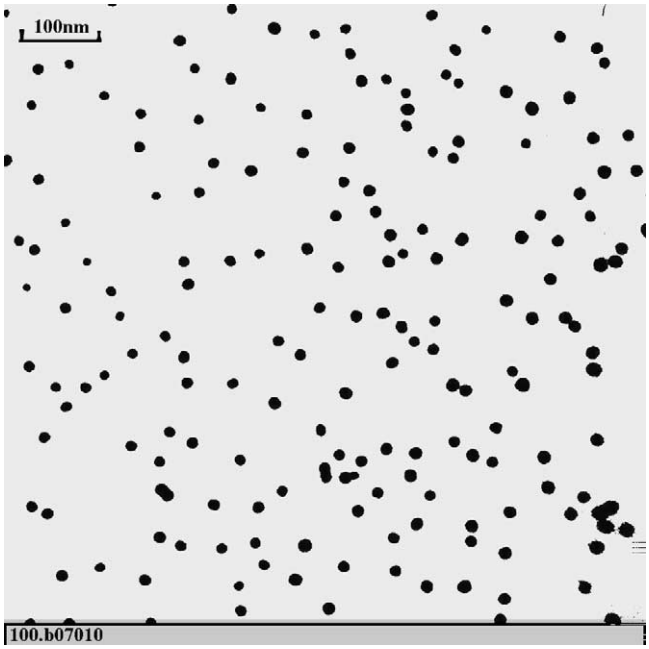

Fig. 1. The TEM images of Au colloid particles. The size of the Au colloid particles is from 8 to 20 nm.