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A novel electrochemical sensor based on electropolymerized molecularly imprinted polymer and gold nanomaterials amplification for estradiol detection

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ABSTRACT

A novel molecularly imprinted electrochemical sensor for the rapid detection of estradiol (E_2) was reported in this paper. Glassy carbon electrode (GCE) was modified by gold nanoparticles (AuNPs) and molecular imprinted polymer (MIP). AuNPs were electrodeposited on the surface of GCE and were used to increase the electrode surface area and to amplify the sensor signal. *p*-Aminothiophenol (ATP) was then combined with AuNPs through Au–S bonds. Moreover, the E_2 template was further assembled onto the ATP monolayer by the formation of hydrogen bonds with the amino group in ATP. The modified electrode was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The parameters of the detection process were also optimized. The electrochemical sensor exhibits a linear detection range from 1.0×10^{-7} to 1.0×10^{-12} mg/mL, and a detection limit of 1.28×10^{-12} mg/mL under the optimal conditions. The sensor had good reproducibility, stability and selectivity for E_2 detection. The recovery rate of E_2 in the untreated milk samples was 84.7–102.9%, which had no obvious difference with that of the samples extracted with ethyl acetate (92.7–104.8%). The sensor can be potentially utilized for the detection of other deleterious chemicals.

1. Introduction

Estradiol is a natural estrogen and is a typical environmental endocrine disruptor (EED). It can be obtained from numerous sources with a wide distribution. It can promote animal growth, improve lean meat proportion of livestock, and increase milk yield by improving the milk secretion capability of animals. As such, it is widely used in the livestock and poultry industry [1,2]. However, estradiol residues in animal food can cause premature puberty in children and increase the risk of ovarian and breast cancer in women.

Currently, a large number of highly sensitive estradiol detection techniques, such as GC, HPLC, GC-MS, and LC-MS [3–6], have been extensively developed. However, the rapid detection of estradiol in food is difficult because of the complex sample pre-treatment process, high cost, and technical constraints. The electrochemical method has received extensive attention from researchers because it is rapid and sensitive. The enzyme immunoassay and electrochemical methods based on enzyme and antigen antibody reactions have the advantages of ease of operation [7,8] and high specificity, but the whole reaction process still has low controllability and high demands on the detection environment [9–12] because of the poor stability of the enzyme and the antibody.

Thus, choosing synthetic counterparts to replace the biological receptor as a recognition element in biological and chemical sensors has gradually becoming the focus of many studies. Sensors fabricated via the molecular imprinting technique (MIT) [13–18]

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have attracted extensive attention because this method can efficiently synthesize specific binding substances and the polymer material is very stable.

MIT [19–25] is a method used in functional polymer research, and it is a well-established technique for producing specific and selective absorbents [26]. The imprinting process involves the copolymerization of functional monomer with a cross-linker agent in the presence of a template molecule in order to design 3D polymeric matrices with specific interaction ability [27]. The template molecule was introduced into the polymers through the polymerization process. The functional groups are then "frozen" in the polymer bulk. After removal of the template molecule, the nanocavities which are complementary to the template in shape and functionality are formed. These imprinted nanocavities allow for the selective recognition of the template molecules and their analogs. MIP with high selectivity and affinity has been used as a chemical/biological sensing element of the sensor and is widely used in estrogen detection because it is chemically and mechanically stable, low cost, and easy to prepare [28,29]. Recently, some electrochemical sensors based on MIPs were fabricated for the determination of estradiol and also obtained good experiment results [30,31]. However, the electrons transfer rate and the diffusion of the analyte to the recognition site are both obstructed in a particular degree because of the highly cross-linked characteristics of the imprinted polymer. As such, the detection efficiency, sensitivity, and accuracy all decrease [32]. Therefore, in the present study, GCE was modified with AuNPs to increase its surface area [33–36], and the molecularly imprinted membrane was assembled onto the AuNP layer to construct MIP-based electrochemical sensors via in situ polymerization [37].

This study analyzed the function of a molecularly imprinted electrochemical sensor in the rapid and sensitive detection of E_2. Self-assembly of functional monomers resulted in the connection of MIP to the surface of the AuNP-modified GCE. The detection results indicated that the AuNP layer could amplify the signals of the electrochemical sensor, thereby extending the measurement range. This sensor presented good stability and selectivity in real sample measurements.

2. Material and methods

2.1. Reagents and materials

p-Aminothiophenol (ATP) and chloroauric acid were purchased from Sinopharm Chemical Regent Co., Ltd. (Shanghai, China). The EEDs used in this study, which include E_2, estriol (E_3), diethylstilbestrol (DES), bisphenol A (BPA), and bisphenol acid (DPA), were all purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Potassium hexacyanoferrate ($K_3[Fe(CN)_6]$), potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$), KCl, $Na_2HPO_4\cdot 12H_2O$, $NaH_2PO_4\cdot 2H_2O$, H_2SO_4, HCl, hydrogen peroxide (30% V/V aqueous solution), ethanol, acetonitrile, acetone, dimethyl sulfoxide (DMSO), and ethyl acetate were of analytical purity. Ultrapure water was obtained from a Millipore-Milli-Q system (Millipore Corporation, MA, USA).

The buffers or solutions used in this study were as follows: phosphate buffer solutions (PBS) (0.01 mol/L, pH 6.97), elution buffer (90% acetonitrile aqueous solution containing 0.5 mol/L HCl), regeneration buffer (90% DMSO aqueous solution containing 0.5 mol/L HCl), and detection solution (2.5 mmol/L $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]\cdot 3H_2O$ in 0.01 mol/L PBS containing 0.1 mol/L KCl).

2.2. Apparatus

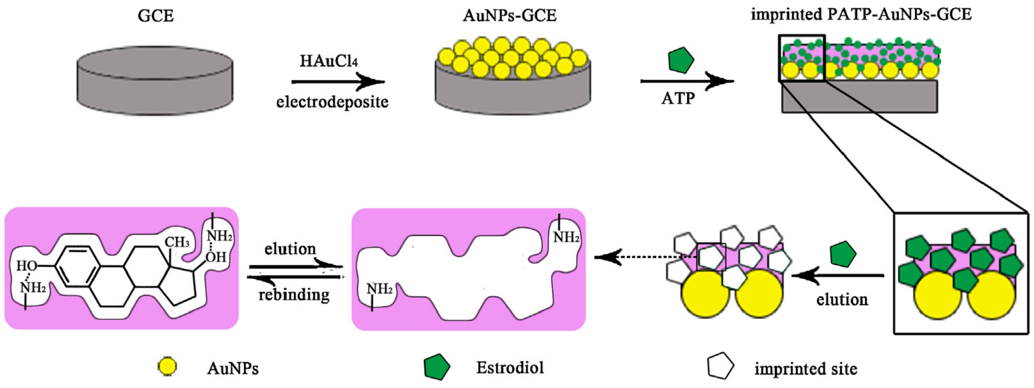
All electrochemical experiments were performed using an IviumStat Instrument (Ivium Technologies BV, The Netherlands). A three-electrode system composed of a platinum foil as the auxiliary electrode, Ag/AgCl saturated KCl electrode as the reference electrode, and a modified glassy carbon electrode (GCE) (3 mm in diameter) as the working electrode were employed for the electrochemical measurements. All experiments were carried out at room temperature.

2.3. Preparation of AuNP-modified electrode

Prior to electrode modification, GCE was carefully polished to achieve a mirror-like surface by applying 50 nm alumina aqueous slurry on the chamois and by washing the surface with water and then with ethanol. The bare electrode was characterized via cyclic voltammetry (CV) at a scan rate of 100 mV/s and at a potential range from −0.3 to 0.8 V in 10.0 mL of 0.01 mol/L PBS solution containing 2.5 mmol/L $Fe(CN_6)^{3-/4-}$ (1:1) and 0.1 mol/L KCl. CV scanning was terminated when a pair of oxidation reduction peaks with clear boundaries was obtained. The GCE was immersed in 0.5 g/L $HAuCl_4$ solution (Milli-Q ultrapure water as a solvent) and treated at a constant potential of −0.2 V for 200 s to electrodeposit the AuNPs on the surface of the GCE. The AuNP-modified electrode was submerged in fresh piranha solution (H_2SO_4/H_2O_2, 3:1, v/v) for 5 min after washing with ethanol and double distilled water. The electrode was carefully washed again with double distilled water then dried with nitrogen.

2.4. Preparation MIP and non-MIP (NIP) modified sensor

Scheme 1 gives the stepwise preparation procedures of the MIP modified sensor. The MIP modified sensor was prepared according to a reported method developed by Xie et al. [37] with minor modifications. The pretreated AuNP-GCE was transferred into a 10 mmol/L ATP ethanol solution and left in the dark overnight at



Scheme 1. Schematic illustrations of the fabrication procedure for the MIPs-AuNPs-GCE.