



Short communication

Electrochemical sensor for the detection of estradiol based on electropolymerized molecularly imprinted polythioaniline film with signal amplification using gold nanoparticles

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ABSTRACT

A sensitive electrochemical molecularly imprinted sensor was developed for the detection of estradiol, by electropolymerization of *p*-aminothiophenol functionalized gold nanoparticles in the presence of estradiol as template molecule. The extraction of the template leads to the formation of cavities that are able to recognize and bind estradiol with high affinity. The performance of the developed sensor for the detection of estradiol was investigated by linear sweep voltammetry using a hexacyanoferrate/hexacyanoferrite solution as redox probe. The molecularly imprinted sensor exhibits a broad linear range, between 3.6 fM and 3.6 nM and a limit of quantification of 1.09 fM. Compared to the non-imprinted sensor, the imprinted sensor exhibits high affinity for the binding of estradiol. Moreover, selectivity studies, performed towards binding of testosterone, a hormone with similar chemical structure, proved high sensor selectivity. Furthermore, the molecularly imprinted sensor was applied for the analysis of spiked river samples with good recoveries.

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1. Introduction

Estrogens, including 17 β -estradiol (17 β -estradiol), play critical roles in mammalian female development, fertility, and behavior, being involved in the growth and maturation of the female reproductive tract, regulation of menstrual cycle and ovule implantation [1]. The presence of 17 β -estradiol in the environment has raised serious concerns on human health; 17 β -estradiol is an endocrine disruptor chemical (EDC), which interferes with the normal endocrine function, having side effects on growth, metabolism and reproduction of the organisms, and increasing the incidence of cancer and tumors [2]. It can be present in wastewaters, due to its industrial and agricultural applications, and in animal food products, being used to fatten the animals due to its anabolic effect [3]. Thus there is an urge for the development of sensitive and accurate techniques for the detection of 17 β -estradiol. The commonly used techniques, such as HPLC [4] or GC/MS [5], are time-consuming and expensive. Electrochemical sensors gained interest lately due to their inherent advantages such as low cost, fast response, ease of use and real-time analysis.

Molecularly imprinted polymers (MIPs) have been widely applied in recent years as biomimetic receptors in the development of sensors [6–8]. The molecularly imprinting technique involves a polymerization

process of one or more functional and crosslinking monomers in the presence of the analyte of interest, which serves as the template, in a proper solvent. The further removal of the template leads to the formation of selective recognition cavities towards the analyte. The selectivity of the polymer depends on various factors like the size and shape of the cavity and binding interactions [9]. MIPs have several advantages, over biological molecules, such as simple synthesis, low cost, high selectivity, mechanical strength and resistance at various temperatures and pH [10]. Zhang et al. reported on an electrochemical sensor based on electropolymerized MIP with electrodeposited AuNP amplification for the detection of 17 β -estradiol in milk samples that exhibited a detection limit of 1.28×10^{-12} mg/mL [11]. In another approach Yuan's group fabricated an MIP sensor for 17 β -estradiol based on the electropolymerization of 6-mercaptopuric acid on Pt nanoparticles-modified electrode. The sensor was able to detect concentrations of 17 β -estradiol as low as 1.6×10^{-8} M [12].

The work herein presents a biomimetic sensor based on MIP for the detection of 17 β -estradiol. The potentiodynamic electropolymerization was then carried out using solution containing *p*-aminothiophenol (PATP) in the presence of 17 β -estradiol as template molecule. PATP was first self-assembled at the surface of gold electrodes to assure a close contact with the transducer. The electropolymerization was then carried out by cyclic voltammetry in a solution containing PATP functionalized gold nanoparticles and estradiol. The removal of estradiol from the resulted polymer matrix leads to selective recognition cavities that are

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able to bind the analyte from sample solutions. The developed sensor was also tested on spiked river water samples with high recoveries.

2. Materials and methods

2.1. Chemicals and instrumentation

17 β -estradiol (17 β -estradiol), testosterone, potassium hexacyanoferrate, potassium hexacyanoferrite, *p*-aminothiophenol (PATP), tetrachloroauric acid trihydrate, sodium boron hydride, methanol and phosphate buffered saline (PBS) tablets, were purchased from Sigma-Aldrich. All solutions were prepared using Milli-Q water with a resistivity of 18.2 M Ω cm at 25 °C.

Electrochemical data was obtained with a VOLTALAB-80 PGZ/402 potentiostat-galvanostat (Radiometer Analytical, France), in a three-electrode electrochemical cell, with a gold working electrode (0.19 cm²), a platinum plate (0.54 cm²) as auxiliary electrode and a saturated calomel electrode (SCE) as reference.

2.2. Preparation of molecularly imprinted polymer sensors

In order to obtain a polymer matrix with selective recognition cavities for 17 β -estradiol, AuNPs were firstly synthesized according to the procedure described in the literature [13]. For a typical preparation of gold nanoparticles, 31.6 mg (8×10^{-5} M) of tetrachloroauric(III) acid trihydrate was dissolved in 30 mL of methanol in a round flask of 0.1 L equipped with a condenser. A solution of *p*-aminothiophenol (1.6×10^{-4} mol) in 12 mL of a mixture methanol/water (v/v) was added dropwise to the gold salt solution under stirring, which changes its color from yellow to dark brown. After 10 min, 30.4 mg of NaBH₄ (8×10^{-4} mol) dissolved in 2.2 mL of water was added dropwise to the mixture under vigorous stirring. After 10 min, stirring was stopped and the solution was kept in darkness for 1 h. The suspension was then filtered on a polymer membrane and washed successively with water and ether, the resulting black powder being then dried.

Gold working electrodes were cleaned by sonication in acetone for 5 min, followed by exposure to UV-Vis for 20 min and piranha solution for 5 min. The electrodes were then immersed in 50 mM PATP ethanolic solution for 12 h at 4 °C. After the self-assembly of PATP, electropolymerization of MIP was performed in a mixing solution containing 0.1 mg/mL PATP-functionalized AuNPs prepared as previously described and 36 mM 17 β -estradiol in the supporting electrolyte of 10 mM [Fe(CN)₆]^{-3/-4} in PBS containing acetone (4:1 v/v), by cycling the potential between -350 mV and 600 mV, for 10 cycles, with a scan rate of 100 mV/s. To confirm the imprinting process, a control experiment, non-imprinted polymer modified electrode (NIP), was prepared under the same experimental conditions but without the addition of 17 β -estradiol. The modified electrodes were then washed with a solution of acetone:PBS pH = 7.2 (1:1) for 30 min to remove the template molecules embedded in the film and to form the selective recognition cavities.

2.3. Electrochemical measurements for the recognition and binding of 17 β -estradiol by MIP

In order to assess the rebinding of 17 β -estradiol in the selective cavities, MIP films were immersed in solutions of various concentrations of 17 β -estradiol for 20 min and linear sweep voltammetry (LSV) was carried out in 10 mM [Fe(CN)₆]^{-3/-4} in PBS pH = 7.2 in the range of potential between 800 mV and 350 mV vs SCE, at a scan rate of 50 mV/s. Based on the height of the obtained peak, a calibration curve was constructed upon the analysis of various concentrations of 17 β -estradiol ranging from 3.6 fM to 3.6 nM.

2.4. Detection of 17 β -estradiol in river water samples

The prepared MIP sensors were applied for the detection of 17 β -estradiol in river water samples collected from Rhone River in Lyon area. Normally the analyzed water samples did not contain 17 β -estradiol, therefore they were spiked with different concentrations of 17 β -estradiol and the resulted samples were left in contact with the MIP sensor for 20 min. The experiments were performed as previously described in Section 2.3 and the recovery rate was calculated.

3. Results and discussions

3.1. Preparation of molecularly imprinted polymers by electropolymerization

The 17 β -estradiol imprinted polymer was obtained by electrodeposition on gold electrodes using CV for 10 cycles in a solution containing PATP-functionalized AuNPs and 17 β -estradiol in the supporting electrolyte of 10 mM [Fe(CN)₆]^{-3/-4} in PBS pH = 7.2 containing acetone. Acetone, a miscible organic solvent, was used to ensure the solubility of 17 β -estradiol and to decrease the surface tension and minimize hydrophobic interactions, since MIPs are usually hydrophobic [13]. During electropolymerization the molecules of 17 β -estradiol are trapped in the polymer matrix through non-covalent interactions between -NH₂ groups of the polymer and O atoms in the structure of 17 β -estradiol. The formation of the polymeric film is investigated through its permeability, using [Fe(CN)₆]^{-3/-4} redox probe. The peak current for the oxidation/reduction of the redox probe decreases with each cycle, due to the continuous formation of a polymer film that hinders the electron transfer to the surface of the electrode. The presence of AuNPs in the polymeric film enhances the number of accessible complementary cavities, the catalytic activity of the surface and the fast equilibration with the analyte [14].

3.2. Detection of estradiol by the molecularly imprinted polymer sensor and selectivity studies

The imprinted polymer film was washed for 30 min with a solution of PBS:acetone 1:1 in order to remove the template molecules and obtain selective recognition cavities. The solvent acetone:PBS was used for washing, in order to obtain the same swelling effect as in the electropolymerization step. In order to check if the resulted cavities are able to recognize and bind 17 β -estradiol, LSV measurements have been performed, sweeping the potential between 800 mV and -350 mV with a scan rate of 50 mV/s. [Fe(CN)₆]^{-3/-4} was used as mediator between imprinted films and substrate solutions. As shown in

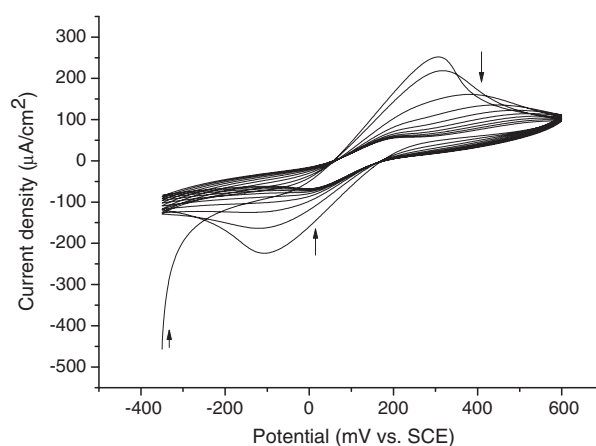


Fig. 1. Cyclic voltammogram for the electropolymerization of MIP in a solution of 10 mM [Fe(CN)₆]^{-3/-4} in PBS:acetone 4:1 (v/v), 36 mM 17 β -estradiol and 0.1 mg/mL PATP-AuNPs.

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