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Electrochemical immunosensor based on gold nanoparticles deposited on a conductive polymer to determine estrone in water samples

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ABSTRACT

This paper describes the development of a simple and sensitive electrochemical immunosensor (EI) to quantify estrone (E) in water samples. This EI does not require the sample pre-treatment, to label neither the antigen nor the antibody, and its detection format is based on the fact that E is co-substrate of the horse radish peroxidase (HRP). Therefore, the EI was constructed by immobilization of the anti-E monoclonal antibody (mAbE) on a glassy carbon electrode (GCE) modified with gold nanoparticles (AuNPs) electro-synthesized on a 1-naphtylamine polymer (pNap) film. This format reduced significantly the time of EI preparation. Water samples were spiked with known E concentrations, and then incubated on mAbE-AuNPs-pNap-GCE disk electrode. The electrochemical response was proportional to the amount of pyrocatechol (H_2Q), another enzyme co-substrate, and inversely proportional to the amount of E presents in water samples. The immunosensor showed a linear range from 8×10^{-2} to 2×10^{4} pg mL^{-1}. The limit of detection (LOD) was 0.061 pg mL^{-1}. Recovery percentages obtained were very good, with values of 98.20, 105.50, and 100.85% for 50, 100 and 200 pg mL^{-1}, respectively. Tests were also conducted to evaluate the cross-reactive of E with other hormones of similar structure such as 17β-estradiol, progesterone and estriol. The EI showed a high selectivity to determine E in the presence of these hormones. Thus, this EI is an attractive tool to determine E in water samples.

1. Introduction

Both natural and synthetic estrogens are well known to be endocrine disrupting compounds (EDC), which constitute interfering compounds in the endogenous hormonal system of mammals, producing adverse effects.

Estrone (E) is a natural estrogenic hormone secreted by the ovary, and adipose tissue. It is the predominant hormone in post-menopausal women. In recent years, new biochemical functions have been found for E in different tissues, including the pituitary gland, breast, vascular and the colon [1].

The presence of hormones in the aquatic environment began to be important when a connection was established between a synthetic contraceptive drug and its impact on fish [2]. Steroid hormones can get into environment through discharge of wastewater, as they are excreted by humans and animals [3], and in different amounts [4], depending on gender, health status, age, diet or pregnancy [5].

Estrogens are released into the urine as a glucuronide conjugate complexes or as sulfates [6], which may be converted quickly to potent hormones by excision and/or during transport and treating wastewater [7].

In this sense, the first estrogenic contamination of aquatic environments was detected by the appearance of hermaphroditic fish in British rivers [8]. Therefore, exposure of aquatic life in polluted waters with EDC has important consequences [9].

Based on these results, there is a growing need for a continuous and fast monitoring of pollution levels. Therefore, different monitoring techniques have been developed; including capillary electrophoresis [10], receptor assays [11] and chromatographic techniques [12–18]. Chandra Bose et al. [19] have developed an assay for the simultaneous determination of dexamethasone, testosterone, and E using reverse phase HPLC chromatography. Recoveries were in the range from 98 to 102%. The disadvantages of chromatographic methods are the high cost of instrumentation and maintenance, high consumption of solvents and time, and the difficulty of conducting experiments outside the laboratory.

Pre-concentration methodologies such as the solid phase extraction (SPE) and the liquid-liquid extraction (LLE) may be used to identify and quantify hormones in water samples with limits of quantification (LOQ) between 0.02 and 1.02 ng L^{-1} [20]. Although the LOQ were good, SPE also involves a large consumption of organic solvents. In this sense, the solid phase micro extraction (SPME) has the advantage of

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consuming small amounts of solvents, but it also presents difficulties such as high cost, fragility, and a time useful limited of the fibers [21]. Regarding the extraction in liquid phase, the main weakness of the method is that it consumes a long period of time as well as large volumes of organic solvents. Thus, the liquid phase micro-extraction (LPME) appears as a good alternative to other methodologies [22]. The hollow fiber LPME (HF-LPME) was used in combination with GC–MS to detect steroid hormones in tap and sewage water samples, with limits of detection (LOD) from 1.6 to 10 ng L^{-1} [23].

In recent years it has become important the development of immunosensors to detect and quantify different analytes at very small concentrations. The importance of these devices is that they can be miniaturized and portable, making them a fast, sensitive and inexpensive technique.

In addition, the immunoassays based on the ELISA method have been developed to determine E in humans and animals fluids [24,25], and in environmental samples [26,27].

Li et al. [28] developed an ELISA method to analyze E in water samples. The LOD was 0.14 μg L^{-1}. However, the LOD was 1.25 ng L^{-1} when this methodology was combined with the SPE method.

On the other hand, the E electrochemical detection was reported by Brocenschi et al. [29]. The anodic oxidation of E was investigated at glassy carbon, nitrogen-incorporated tetrahedral amorphous carbon and boron-doped diamond electrodes.

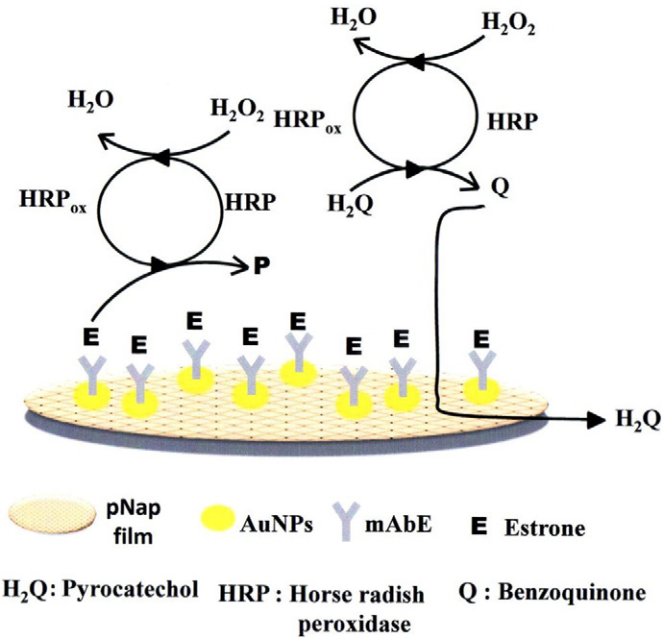
Yang et al. [30] detected E by linear sweep voltammetry (LSV) using surfactants and multiple-walled carbon nanotubes. The LOD was 1.35 μg L^{-1}, and the method was used to determine E in commercial pills.

Gao et al. [31] studied the interaction between E and the polyclonal antibody anti-E using an electrode modified with polypyrrole, doped with polyclonal antibody anti-E. The interaction between E and antibody-polypyrrole film was evaluated by voltammetric measurements based on the response of a probe redox couple (ferricyanide), being the response in current inversely proportional to the concentration of E.

Sun et al. [32] developed a method to detect E using an electrochemical detection platform based on bio-assembled nano-circuits covalently bound to the antibody anti E. The detection of E was performed using $[\mathbf{Ru}(\mathbf{NH_3})_6]^{+3/+2}$ as the probe redox couple to sense the antigen-antibody interactions. The LOD was 1.4 pg mL^{-1}.

We have recently developed an immunosensor to detect 17β-estradiol (17β-E) in bovine serum samples. The immunosensor showed a high analytical performance, and the LOD was 0.84 pg mL^{-1} [33].

In this work, we report a simple, and sensitive electrochemical immunosensor (EI) to quantify E in water samples. The EI does not require the sample pre-treatment, and to label neither the antigen nor the antibody. The EI was constructed by immobilization of the anti-E monoclonal antibody (mAbE) on a glassy carbon electrode (GCE) modified with AuNPs electro-synthesized on a 1-Naphtylamine (pNap) polymer film (AuNPs-pNap-GCE). These composite structures possess interesting properties arising from both the size effects of AuNPs as well as the exceptional properties of the polymer, which allows stacking of the particles and increase their stabilization [34–36]. Indeed, the (pNap) polymer can provide a large specific surface and a compact matrix for the incorporation of AuNPs, and leads to the improved stability of the resultant AuNP-pNap-GCE. Water samples were spiked with known concentrations of E and, then, incubated on mAbE-AuNPs-pNap-GCE. Then, the EI was transferred to an electrochemical cell containing pH 5.00 citrate buffer solutions (CBS), where given concentrations of HRP, pyrocatechol (H_2Q) and H_2O_2 were added. The E and H_2Q, both enzyme co-substrates, react with HRP. The HRP, in the presence of H_2O_2, catalyzes the oxidation of both the E to a given product and the H_2Q to benzoquinone (Q). The back electrochemical reduction of Q to H_2Q was performed on the modified electrode surface (mAbE-AuNPs-pNap-GCE) by square wave voltammetry (SWV) (Scheme 1). The electrochemical response is proportional to the amount of H_2Q that reacts with the enzyme, and inversely proportional to the amount of E in water samples. Therefore, the maximum electrochemical response is obtained in the absence of E at the electrode surface for a given H_2Q concentration. This EI showed a very high sensitivity to determine E at trace levels in water samples, compared to other conventional techniques.



Scheme 1. Schematic representation of the electrochemical immunosensor developed to determine estrone in water samples.

2. Materials and methods

2.1. Reagents and solutions

E, 17β-E, progesterone (P4), estriol (E1), anti estrone sheep monoclonal antibody (mAbE), HRP (E.C:1.11.1.7, H_2O_2-oxido-reductase), H_2Q, pNap and gold (III) chloride hydrate ($HAuCl_4$) were purchased from SIGMA, USA. AuNPs were synthesized using $HAuCl_4$. All reagents were used as received. The following buffer solutions were prepared from their salts (Merck, p.a.): 1×10^{-2} mol L^{-1} phosphate buffer solutions, 0.137 mol L^{-1} NaCl and 2.7×10^{-3} mol L^{-1} KCl (pH 7.00, PBS); 5×10^{-2} mol L^{-1} citrate, 5×10^{-2} mol L^{-1} phosphate buffer solution, (pH 5.00, CBS), and pH 7.00 PBS containing 0.05% Tween 20 (PBST). Ethanol, H_2O_2, and H_2SO_4 were Merck p.a. Toluene and water were Sintorgan, HPLC grade. Real samples (tap water) free of E were used without pre-treatment.

2.2. Instruments

Electrochemical measurements were performed in a Teflon microcell. The cell operates with a volume of 200 μL. The working electrode was a glassy carbon disk electrode (GCE) (BAS, 1.6 mm diameter). Previous to perform the experiments, the electrode was successively polished on BAS™ cloth with diamond paste of 15.3 and 1 μm and then, polished with wet alumina powder (0.3 and 0.05 μm, from Fischer), rinsed copiously with water and sonicated in a water bath during 2 min. The counter electrode (CE) was a platinum foil. A calomel saturated electrode (CSE) or a silver (Ag) wire were used as reference or pseudo-reference electrodes, respectively.

The measuring system for performing SWV and cyclic voltammetry (CV) was an Autolab PGSTAT 12 potentiostat, run with the GPES software, version 4.9 (Eco-Chemie, Utrecht, The Netherlands). All SWV