



Electrochemical detection *in vitro* and electron transfer mechanism of testosterone using a modified electrode with a cobalt oxide film



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ABSTRACT

The voltammetric oxidation of testosterone (4-androsten-17 β -ol-3-one) has been studied at an edge plane glassy carbon electrode modified with cobalt oxide. One sensitive and well-defined peak is obtained with a peak potential of 0.50 V vs. SCE attributed to the reduction reaction $\text{Co}^{\text{IV}}/\text{Co}^{\text{III}}$, which is responsible for oxidation of the steroid. A calibration curve was obtained for testosterone, with the measurements performed in triplicate, in the range from 0.33 to 2.00 μM , yielding a correlation coefficient >0.99 and detection limit of 0.16 μM , with relative standard deviations (R.S.D) from 1.33×10^{-5} to $4.00 \times 10^{-4}\%$ with 95% significance ($p < 0.05$). The modified electrode showed a stable and reproducible response toward the detection of this steroid. Thus, the results showed that the electrode modified with cobalt oxide acts as a promising alternative for simple, cheap and rapid analysis of testosterone.

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1. Introduction

Testosterone (4-androsten-17 β -ol-3-one) (Fig. 1) is a steroid hormone from the androgen group that plays important roles in male sexual differentiation, protein synthesis and human physical performance [1]. This compound and other anabolic androgenic steroids (AAS) have been used by athletes since the 1950s as doping substances to increase muscle mass. The World Anti-Doping Agency (WADA) prohibited their use to ensure fair play and protect athletes from possible adverse side effects [2]. Chromatographic methods including HPLC [3], GC–MS [4] and LC–MS [5] were developed for the detection and quantitation of testosterone in biological fluids. These analytical methods provide quantitative and confirmatory results with high sensitivity and selectivity, but require some expensive instrumentation and suffer from considerable time delays between sampling and obtaining the results. These

disadvantages limit their daily routine use in laboratory environments [6]. Electrochemical techniques using modified electrodes have proved to be a sensitive and versatile method for the determination of analytes undergoing biologically important reactions of oxidation and/or reduction, including isolated drugs and related molecules in pharmaceutical dosage forms. Due to their extreme simplicity, low cost, relatively short analysis time, high sensitivity and specificity compared to other techniques, electroanalytical techniques have arisen as a stand out in the field of steroid analysis [7–12]. However, some difficulties have arisen in modifying electrodes in order to analyze steroid hormones because they are small, rigid, hydrophobic molecules, and possess few functional groups for specific interaction with antibodies.

The electrochemical method presented in this work is a promising substitute for the frequently reported chromatographic methods due to its simplicity, rapidity, reliability and low cost of analysis. In recent years, the application of modified electrodes has been found to enhance the sensitivity of electrochemical determinations. In this work, a new electrochemical sensor for the detection of testosterone *in vitro* using a cobalt oxide-modified edge plane glassy carbon electrode is described. The preparation of a modified electrode combining the immobilization methods of cobalt

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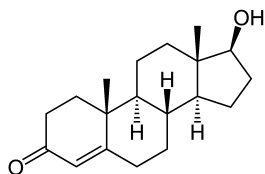


Fig. 1. Chemical structure of the testosterone molecule.

oxide on the glassy carbon electrode (GCE), works as a sensitive electrochemical transducer of testosterone that constitutes a challenge.

2. Experimental

2.1. Apparatus and electrodes

The electrochemical experiments were performed using an AUTOLAB PGSTAT302N electrochemical analyzer driven with NOVA 1.10 software. A glassy carbon electrode as a working electrode (BAS Model MF 2012; geometric area = 0.0774 cm²), Hg/Hg₂Cl₂/KCl_(sat.) (SCE) as reference electrode, a platinum plate as auxiliary electrode and a three-electrode standard cell with a capacity of 50 mL were used in the experiments.

2.2. Reagents and solutions

A solution of 20 mM Co(NO₃)₂·6H₂O (Sigma–Aldrich) and 0.10 M Na₂SO₄ (Sigma–Aldrich) was prepared with double distilled water. A solution of 0.10 M NaOH (Merck) was prepared and its pH was adjusted to 12.5 with the addition of several drops of 0.010 M HNO₃ (Merck). Testosterone (Fluka, >99%) was purchased from Biospecific. A stock solution of testosterone (1.0 mM) was prepared in methanol/water (1:1) (methanol from Merck, 99%). Specific amounts of the stock solution of testosterone were added into an electrochemical cell containing the support electrolyte (0.10 M NaOH).

A calibration curve based on the values of cathodic peak current from the reduction of Co^{IV}/Co^{III} species was obtained from the cyclic voltammograms. All measurements were performed in triplicate. Subsequently, the detection limits (LOD) were calculated at a significance level of 95% ($p < 0.05$). The LOD values corresponded to three times the standard deviation of the blank current signal, calculated from 21 measurements divided by the slope of the calibration curve. Blank signals were measured from the modified electrode with cobalt oxide in 0.10 M NaOH alone in the absence of testosterone.

2.3. Cobalt film preparation

Prior to modification, a glassy carbon electrode (GCE) was pretreated in two ways in order to obtain reproducible results: (1) mechanical polishing on a micro-cloth pad with a 0.05 μm α-alumina suspension, and (2) electrochemical treatment by applying a potential of 1.5 V for 4 s in the same solution in which the measurements were carried out. The electrode cleaning procedures were carried out for each experiment and this pretreatment required 5 min. All experiments were performed at room temperature.

Cobalt oxide films were deposited in a cyclical voltage process at 50 mV s⁻¹ between -1.0 and 1.0 V vs. SCE. The depositions were performed in an aerated environment of 20 mM Co(NO₃)₂·6H₂O containing 0.10 M Na₂SO₄ solution. The electrodeposited cobalt oxide films were removed from the glassy carbon electrode by polishing the surface with a 0.05 μm α-alumina suspension on a polishing micro-cloth and washing with double distilled water.

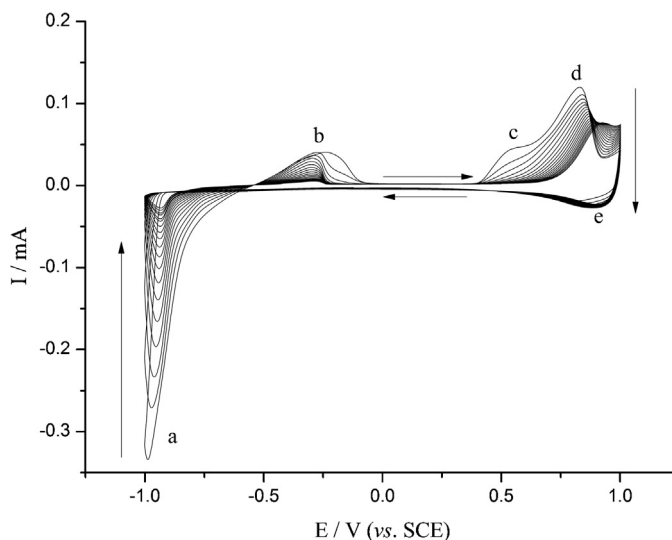


Fig. 2. Consecutive cyclic voltammograms of cobalt oxide film growth on a GCE in 20 mM Co(NO₃)₂·6H₂O + 0.10 M Na₂SO₄ electrolyte. Scan rate: 50 mV s⁻¹.

The nominal surface concentration of the cobalt oxide film electrodeposited on the glassy carbon surface was evaluated by cyclic voltammetry in aqueous 0.10 M NaOH and a 100 mV s⁻¹ scan rate between -1.0 and 0.5 V vs. SCE.

3. Results and discussion

3.1. Film deposition

Cobalt oxide films can be deposited electrochemically under anodic conditions onto a number of different conducting substrates such as glassy carbon, gold, platinum, transparent ITO electrodes, etc. Typical cyclic voltammograms of cobalt oxyhydroxide have growth at 50 mV s⁻¹ between -1.0 and +1.0 V vs. SCE in a 20 mM Co(NO₃)₂·6H₂O solution containing 0.10 M Na₂SO₄, examples of which are shown in Fig. 2. As can be seen, the bulk deposition of cobalt species increases at a constant rate. The maximum rate of cobalt deposition was observed only at high potentials (*i.e.* 0.9/1.1 V), where the formation of Co^{IV} species on the electrode surface is accompanied by simultaneous massive oxygen gas evolution. In fact, it has already been stated that oxygen evolution generally increases at significant rates only when potentials corresponding to higher oxidation states of the cobalt oxide are attained.

The variations in the curve profiles of the voltammograms shown in Fig. 2 during continuous cycling of the potential seem rather complex, indicating that the cobalt deposition process involves multiple simultaneous electrochemical processes on the electrode surface. The initial process involves the oxidation of Co^{II} with the formation of Co^{III} and Co^{IV} oxide species, which are insoluble at the experimental pH solution and remain deposited on the electrode surface. The appearance of a cathodic peak at -1.0 V (peak a) in the first scan is related to the reduction reaction of Co^{II} ions to metallic cobalt (Co⁰). With an increase in the number of cycles, there is a gradual decrease in this cathodic peak at -1.0 V, indicating that all the Co^{II} ions are being slowly reduced on the layer of deposited cobalt oxide on the GCE surface. Moreover, the appearance of a small peak in the range of -0.30 to -0.10 V (peak b) can be noted, which is related to the dissolution of the cobalt layer deposited on the electrode surface [13].

The formation of the first Co^{III} and Co^{IV} oxide species monolayers induces a noticeable increase in the anodic current related to

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