



Graphene oxide for rapid determination of testosterone in the presence of cetyltrimethylammonium bromide in urine and blood plasma of athletes



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ABSTRACT

Electro-reduction behavior of testosterone at reduced graphene oxide/glassy carbon electrode (rGO/GCE) was studied. Cationic surfactant cetyltrimethylammonium bromide (CTAB) enhanced the reduction peak of testosterone. In borate buffer (pH 5.4) CTAB–testosterone showed a reduction peak at -1.1 V (versus, Ag/AgCl). The increment of peak current obtained by deducting the reduction peak current of the CTAB–testosterone was rectilinear with testosterone concentration in the range of 2.0 to 210.0 nM, with a detection limit of 0.1 nM. The sensor was used for quantification of testosterone in biological fluids and drug.

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1. Introduction

Anabolic androgenic steroids (AAS) are a group of chemical compounds that mimic the activity of endogenous testosterone. In addition to their use in medicine for the past 50 years a wide range of AAS used by athletes, athletics, hoping to improve stability and performance. The use of AAS was prohibited in sport since the mid-70s [1]. Analysis of AAS in biological fluids due to the metabolism in the body and low concentrations in the urine is not an easy task. In the recent decades, gas chromatography coupled to a mass spectrometer (GC/MS) was the base diagnostic test for AAS analysis. Although optimized to allow relatively large-scale screening, more efficient instrumental techniques such as high-resolution mass spectrometry (HRMS), tandem mass spectrometry (MS/MS) and liquid chromatography/mass spectrometry (LC/MS) are used for analysis of AAS [2,3]. In addition to instrumental techniques, sample preparation techniques and data processing methods may be useful to increase the sensitivity of analytical methods. In any case, the method must be carefully selected to reduce the associated skills and prevent additional costs. In the recent years, progress in the field of electrochemical methods due to sensitivity, low cost and relatively short time analysis, in comparison with

other techniques was increased. Electrochemical methods may be used for highly sensitive and selective detection of drugs [4–6]. At this time, limited reports are available on the voltammetry behavior of testosterone.

The electrochemical adsorptive stripping voltammetry behavior of testosterone hormone family at a static mercury drop electrode (SMDE) is reported by Wang et al. [7]. It is shown that the SMDE method achieves a detection limit of 0.16 nmol L⁻¹ for 15 min of preconcentration. Hu et al. [8] by using adsorptive stripping voltammetry (AdSV) for the reduction of testosterone at hanging mercury drop electrode (HMDE) obtained an assay with the detection limit as 5 nmol L⁻¹. Square wave adsorptive stripping voltammetry (SWAdSV) by using lead film electrode in medium of acetate buffer is used by Tyszcuk which achieved a detection limit of 9 nmol L⁻¹ [9]. However, due to their toxicity and environmental pollution issues, mercury electrode is replaced with more safety and environment-friendly materials such as carbon. Today, electrodes with the carbon matrix such as fullerene, multi walled and single walled carbon nanotubes, graphite and graphene are designed to voltammetry assay. Single walled carbon nanotubes in the mode of square wave achieved the detection limit of 2.8 mM for determination of testosterone [10]. Recently, surfactant modified glassy carbon electrode in the Britton–Robinson buffer applied for determination of testosterone [11]. In this rod, Levent et al. [12] fabricated bismuth film electrode on the surface of a glassy carbon electrode achieving 0.3 nM as detection limit.

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Graphene with novel properties such as high electrical conductivity as well as exceptional mechanical and thermal properties have received considerable attention for potential applications in nanocomposites [13], photonic devices and photoluminescence [14], chemical sensors [15] and energy storage [16,17]. This nanosheet due to its high specific surface area, superior chemical and thermal stability has great preconcentration efficiency to conventional materials. Specially, strong π - π stacking interaction between the graphene and biological/organic materials made graphene as a superior adsorbent.

Electrocatalytic ability and antifouling properties of graphene sheets on the electrochemical devices have been studied carefully. It is interesting that electrodes modified with graphene material show excellent electrocatalytic activity toward molecules/biomolecules [18–21]. Because of these unique structural and electrical properties of graphene, we used reduced-graphene oxide (rGO) modified glassy carbon electrode as simple and rapid electrode for voltammetry of testosterone without any preconcentration or/and separation steps.

2. Experimental

2.1. Apparatus

All electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, Netherland). GPES software was used to control the experimental conditions. A conventional three-electrode system and a personal computer for data storage were used. All potentials are referred to silver/silver chloride/potassium chloride electrode. All experiments were performed at room temperature. pH measurements were made with a Metrohm pH meter model 780 (Switzerland).

2.2. Materials

Testosterone is obtained from ORGANON Company in Iran and used without further purification. Stock standard solution of testosterone was prepared by diluting appropriate amount of testosterone in water/ethanol (1:1 v:v). Natural graphite powder (particle diameter $\leq 40 \mu\text{m}$) was purchased from Sigma Aldrich. CTAB was purchased from Merck (Germany). Borate buffer (pH 5.4) was prepared. De-ionized water was used throughout.

2.3. Synthesis of graphene oxide

Natural graphite powder (particle diameter $\leq 40 \mu\text{m}$, Sigma Aldrich) was utilized to synthesize graphite oxide suspension through an improved Hummers' method [22,23]. In a typical method, 0.5 g graphite powder was mixed by 20 mL H_2SO_4 at 80 °C for 5 h. After that, 0.5 g NaNO_3 was added and the mixture was stirred in an ice bath for 10 min. Then, 3.0 g KMnO_4 was slowly added and the mixture was stirred for 2 h. After stirring, the mixture was warmed up to room temperature, while stirred continuously in a water bath at 35 °C for 1 h. The obtained suspension was diluted by 40 mL deionized (DI) water. During dilution, temperature of the suspension was controlled <60 °C. Then, 3 mL H_2O_2 (30%), already diluted by 100 mL DI water, was added into the suspension to reduce the residual permanganate to soluble manganese ions and cease the gas production of the suspension. The residual acids and salts of the suspension were removed by filtering through an anodic membrane filter (47 mm in diameter, 0.2- μm pore size, Whatman). The filtered graphite oxide material was dispersed in DI water to achieve an aqueous suspension with yellow-brownish color. The aqueous suspension was centrifuged (by an Eppendorf 5702 centrifuge with a rotor radius of 10 cm) at 2000 rpm for 15 min and 8000 rpm for 20 min to remove any unexfoliated graphitic materials and small particles, respectively. In the final step, GO suspension was obtained through sonication of the centrifuged graphite oxide suspension at frequency of 40 kHz and power of 100 W for 30 min.

For reduction of the GO sheets in the prepared aqueous suspension, at first, pH of 100 mL of the GO suspension with concentration of 0.5 mg/mL was adjusted ~ 9.0 using a diluted ammonia solution. Then, 100 μL of hydrazine solution (35%) was added to the suspension while stirring. After that, the suspension was refluxed at 90 °C for 3 h in an oil bath. Finally, the prepared rGO suspension was centrifuged at 8000 rpm for 5 min, filtered (to remove the residual ammonia and hydrazine), and resuspended in DI water for the next usages.

2.4. Material characterization

Surface topography and height profile of the graphene sheets were examined by atomic force microscopy (AFM, Digital Instruments NanoScope V) in tapping mode. The AFM samples were prepared by drop-casting a diluted suspension (~ 0.01 mg/mL) onto a cleaned Si(100) substrate. X-ray photoelectron spectroscopy (XPS) was used to investigate the chemical states of GO and rGO sheets. The data were acquired through a hemispherical analyzer equipped by an Al K α x-ray source ($h\nu = 1486.6$ eV) operating at a vacuum better than 10^{-7} Pa. For more analyses, the XPS peaks were deconvoluted by using Gaussian components after a Shirley background subtraction. Also, quantitative elemental compositions were obtained using peak area ratio of the XPS core levels and the sensitivity factor of each element in XPS. Raman spectroscopy was performed at room temperature using a HR-800 Jobin–Yvon system supplied by a 532 nm Nd–YAG excitation source. The samples used in the XPS and Raman spectroscopy were prepared through casting conc. graphene suspension onto the Si substrate and removing the solvent at 200 °C in air for 30 min.

2.5. Electrode preparation

Briefly, glassy carbon electrode was polished with polishing cloth. In order to remove adsorbed particles on the glassy carbon surface, the electrode was sonicated in water bath for several minutes. Reduced Graphene oxide (rGO) solution (0.5 mg/mL in ethanol) was cast on the surface of glassy carbon electrode. Solvent evaporation on the electrode surface was made with heating for 4 min at 45 °C. Finally, the prepared electrode was rinsed with deionizer water.

To immobilize the rGO on the electrode surface, the potential cycling (25 cycles) at a scan rate of 120 mV s^{-1} in a PBS solution of pH 7.0 was made. Potential cycling step, activates the aromaticity or functional groups such as π bond and carbonyl, alcohol and acid functional groups between rGO and glassy carbon. SEM image of the prepared electrode is shown in Fig. 1. The effective surface area of the electrode measured using the Brunauer–Emmett–Teller (BET) method was 451 m^2/g .

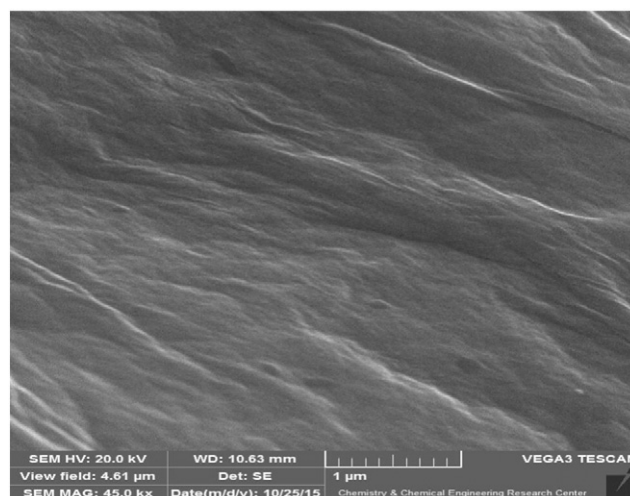


Fig. 1. SEM image of rGO/GCE.

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