Electrochemical determination of hormones using sensor based on graphene nanosheets

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Endocrine disruptors has become the subject of growing concern in the environment. Although the hypothesis that environmental chemicals may exhibit endocrine disrupting effects is not new, the issue has been a growing level of concern due to reports of increased incidences of endocrine related disease in humans, including declining male fertility, and more significantly, to adverse physiological effects observed in aquatic wildlife. 17β-estradiol is the most potent steroidal estrogen. It is produced endogenously by all mammalian species, but in high level by female species. Recently there have been many studies suggesting that estrogens are being released to aqueous environments such as sewage and river water. However, estradiols in environmental sources are present at very low concentrations, and are difficult to detect. Various technologies such as GC-MS, HPLC-MS, immunoassay and electroanalysis have been used to determine the hormone.

Graphene-based materials, for their superb properties, attracted more and more attentions due to their excellent properties and potential applications in the areas of catalysis, biosensors, spectroscopy, and energy storage. Graphene nano-sheets (GNs), is a single layer of sp2-hybridized carbon atoms arranged in a two-dimensional hexagonal lattice, is a promising material for use in nanoelectronics, sensors and optoelectronics. Graphite oxide was prepared from natural graphite powder by the modified Hummers method [1]. The graphene oxide (GO) was prepared by dispersing 0.5 mg/mL of graphite oxide in DMF, followed by ultrasonic treatment (12 h) to achieve full exfoliation from graphite oxide to graphene oxide. After stabilization for 48 h, the upper supernatant was separated carefully from the bottom sediment to obtain the GO solution. Furthermore, the GO solution was subjected to a high-speed centrifugation at 3500 rpm to remove some GO pieces.

Preparation of the modified electrode, GO dispersion was dropped onto the surface of glassy carbon; 3 drops of 5 μL, with a drying time of 2 hours between each drop. The modified electrode was dried at room temperature for 12 h. All electrochemical measurements was using a model PGSTAT 30 Autolab electrochemical system (Eco Chemie, Utrecht, Netherlands) equipped with the GPES software (Eco Chemie, Utrecht, Netherlands). The electrochemical cell was assembled using a conventional electrochemical cell with three electrodes (a platinum auxiliary electrode, Ag/AgCl as reference and glass carbon modified electrode as working electrode).

The DPV experiments were performed in the potential range of 0.2 to 0.8 V (vs. Ag/AgCl) in 0.1 mol L^{-1} PBS pH 7.0. The Graphene/GC electrode exhibited a well-defined oxidation peak at a potential value of +436 mV. This peak is attributed to an irreversible oxidation of the hydroxyl group present in the aromatic ring of the 17-β-estradiol to form phenoxy radical [2]. In Fig. 1 it was observed that the Graphene/GC surface caused a shift of 104 mV in the oxidation potential of 17β-estradiol to a less positive value when compared showed to the GC

electrode (+0.619 V). Additionally, the oxidation on the catalytic surface showed an increase in peak current compared to the electrode prepared with GC electrode unmodified.
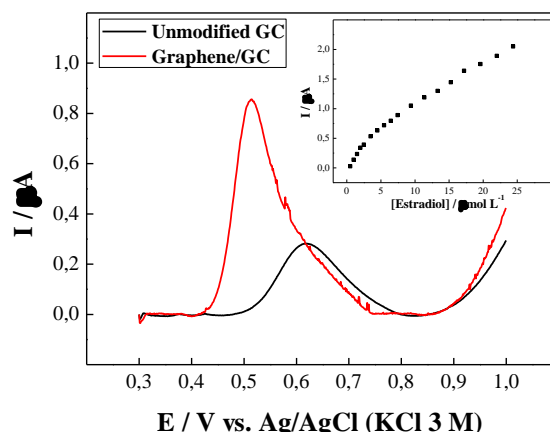

Fig. 1 – DPV of the (black line) unmodified GC electrode and (red line) Graphene/GC electrode in 0.1 mol L^{-1} of PBS containing 2.5 x 10^{-5} mol L^{-1}. Inset: linear dependence of the peak current with 17-β-Estradiol concentration.

The influence of DPV voltammety parameters was investigated First, the amplitude varied over the range from 10 to 100 mV, fixing the step potential at 1 mV. It was not observed any significant increase in peak width, including amplitudes greater than 50 mV. As the peak current was proportional to the increase in amplitude, the value of 50 mV was chosen as the optimized DPV amplitude. The follow step was, fixing amplitude in 50 mV, study the effect of potential step increment whthin the range 1 to 10 mV. For potential steps greater than 2 mV, deformation of the voltammetric profiles was observed and the current peak decreased in height. Hence a 1 mV potential step increment was chosen.

The dependence on the electrochemical oxidation of E_P on pH was studied at pH range from 3.0 to 8.0 in PBS containing 2.5 x 10^{-5} mol L^{-1} 17β-Estradiol. Reducing the hydrogenionic concentration of the electrolyte causes a shift in the peak potential towards more negative values. Therefore the pH 8.0 was selected for the subsequent experiments using the Graphene/GC electrode.

Based on the optimized conditions, i.e. a pulse amplitude of 50 mV and step potential of 1 mV, DPV was used to investigate the electrochemical response as a function of the 17β-Estradiol concentration. The inset in Fig. 1 presents the DPV signals recorded for the Graphene/GC electrode. The analytical signals exhibited a linearity range from 0.25 to 7.44 μmol L^{-1} (R^2 = 0.9954) and the limits of detection (LODs) values were determined using 3σ/slope ratio (where σ is the standard deviation of the mean current for 10 DPV voltammograms of the blanck, and the current values were collected at the 17β-Estradiol oxidation peak potential). The calculations were performed according to the recommendations of IUPAC [3] and by Miller and Miller method [4]. The LODs values obtained were 8.3 x 10^{-7} and 0.38 x 10^{-8} mol L^{-1} for recommendations of Miller and Miller method and IUPAC, respectively.

[1] J. of the Am. Chem. Soc., 80 (1958), 1339-1339.
[2] Sensors and Actuators B 177 (2013) 14–18.
[3] Analyst, 112 (1987), 199–204.
[4] Anal. Methods, 4, (2012), 2348-2354.