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Analytical methodology for the electro-catalytic determination of estradiol and progesterone based on graphene quantum dots and poly(sulfosalicylic acid) co-modified electrode

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ABSTRACT

The goal of this study was to develop an electroanalytical method for the simultaneous determination of steroid hormones for the first time. The key factor in the electrochemical methods is the choice of suitable electrode materials. For this purpose, graphene quantum dots (GQDs) doped poly(sulfosalicylic acid) (PSSA) was immobilized on a glassy carbon electrode (GCE). Apart from exhibition strong and stable electrocatalytic response towards estradiol (E2) and progesterone (P4), the proposed sensor was able to distinguish two hormone's oxidation peaks clearly. Under the optimal conditions, for selective determination of E2, good linear relationships were obtained in the range of 0.001–6.0 μmol L^{-1}, with detection limit of 0.23 nmol L^{-1}, and for P4 in the range of 0.001–6.0 μmol L^{-1}, with the detection limit of 0.31 nmol L^{-1}. The prepared sensor possessed accurate and rapid response toward E2 and P4 with an improved stability, selectivity and repeatability. More importantly, the facile and environment-friendly electrochemical construction strategy provided here, may be open a cost-effective way for setting up nanocomposites or nanohybrid-based sensing platform, which extend the application of electrochemical sensor for the green, facile and sensitive analysis of electroactive compounds in biological systems and pharmaceutical formulations.

1. Introduction

In the last years, there has been a worldwide interest in chemicals that might be disrupting the endocrine system of humans and wildlife. Herein, it is important to establish new techniques concerning the detection of biological compounds. In particular, the growing need for conventional detection tool with high selectivity and sensitivity is at the heart of modern technology in the field of sensors [1]. One of the primary substances that has high endocrine disruptor activity is estradiol (17 β-estradiol) [2]. Estradiol is the essential steroid hormone with estrogenic activity that is naturally present in mammalian [3]. It is an important bioactive substance, involved in reproduction in women, with relevant roles in many intracellular processes and takes part in women's fertility [4]. Herein, its concentration and changes are closely related to human's health status [5].

The quantification of estradiol levels in serum and urine is important in various clinical practices, in the prevention and treatment of related diseases that occur because of hormone dysfunction and breast cancer [6]. Furthermore, the determination of estradiol is also of interest in environmental monitoring because of its high endocrine disrupting potency and the extensive use of therapies with the synthetic hormone in humans and other animals [7]. Estradiol deficiency can cause diseases such as hyper-androgenism, cancer, heart disease, osteoporosis, and menopausal symptoms [8].

Another important bioactive substance, which belongs to a group of steroid hormones, is progesterone. Progesterone (pregn-4-ene-3,20-dione) is an unsaturated α, β ketone [9]. It plays a vital role in the stabilization and maintenance of pregnancy in mammals, acting in the synthetic route of various biologically active steroids [10,11] and participating in the regulation of the menstrual cycle [12]. Also in the pharmaceutical industry, P4 is the active principle of many drugs used in hormone replacement therapies for post-menopausal women [13,14].

Its imbalance in the human organism can cause malformation problems within the reproductive system [9,12]. P4 is secreted by the uterus to prepare it for pregnancy and maintain the pregnancy after conception secretes P4 [15]. Thus, it is very important to know P4 levels in different reproductive systems which is adequate to diagnose an early pregnancy. Moreover, monitoring of progesterone level in plasma or milk has a particular interest in application areas ranging

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from clinical fertility measurement to pregnancy diagnosis in veterinary and farming practices [16].

In the view of such an environmental and clinical importance of steroids, for the individual quantify of steroid hormones level, such as estradiol and progesterone in urine, serum and amniotic fluid several attempts have been made by HPLC using various detectors (including UV [17,18], fluorescence [19,20] and electrochemical (EC) [21]), GC–MS [22,23], micellar electrokinetic chromatography [24], enzyme-linked immunosorbent assay (ELISA) tests [25,26], and radioimmunoassay (RIA) [27,28] techniques so far.

Nevertheless, most of these methods, although highly accurate and possess high sensitivity [29], are time-consuming, require high-cost equipment, sometimes adversely affect the analytical results and often require several sample preparation steps which limited their applications. Moreover, these techniques usually generate waste-containing organic solvents, which makes the procedure more complicated and expensive [29,30]. In the case of immunoassays, for example, it is necessary to obtain a specific antibody for these steroids [31]. However, enzyme-based biosensors are hindered by the thermal and chemical instabilities of the enzyme [32].

From a contrasting point of view, electroanalytical techniques owing to fast response, low expense, operational simplicity, ability to allow the construction of simple and portable devices for fast screening purposes, in-field/on-site monitoring and high sensitivity, have been widely attracts great concern and a variety of modified electrodes have been reported for hormones determination [30].

Nowadays, the tendency in the field of electroanalytical devices, sensors and biosensors is substituting the organic redox mediators with different nanomaterials due to the growing miniaturization of microelectronic devices. Indeed, nanomaterials modified electrodes show effective catalysis for the reaction involving the analyte, fast mass transport and electron transfer kinetics, large active surface area and good control of electrode microenvironment [33].

In recent years, graphene-based nanomaterials such as graphene oxide (GO), graphene nanoribbons (GNRs) and graphene quantum dots (GQDs) have become a hotspot for various fields of research and shown a grand potential as enhanced materials to fabricate the electrochemical sensing interface, owing to the outstanding electronic, thermal and mechanical properties and good chemical stability [34,35]. In this work, an emerging class of the nano carbon family, ultrafine graphene quantum dots are proposed. GQDs are graphene fragments with lateral dimensions less than 100 nm [36–38] which clearly possess a graphene structure inside the dots. GQDs are superior to common carbon materials thanks to its low toxicity, easy preparation, high chemical stability, environmental friendliness, high solubility in many solvents and the possibility of functionalization at their edges [39]. It has shown great promising applications in the fields of sensors, bioanalysis, optical dyes and biological medicine [40]. Furthermore, GQDs expand the contact area with the analyte, which increases the electrochemical effective surface area to interact with some electroactive analytes. Since geometric surface area is a very important parameter in electrochemistry, modification of different substrates by GQDs can increase the rate of electrochemical reaction [41]. Therefore, attention has to be paid to a platform for the fabrication of electrochemical biosensors by using GQDs in order to obtain an efficient electron transport between the analyte and the surface of conventional electrodes.

Graphite oxide (GO), a two dimensional carbon material, has attracted considerable attention due to its importance in nano-science. GO is easily obtained by the harsh oxidative treatment of graphite powder. After oxidation process, a large number of oxygen-containing functional groups such as hydroxyl, carboxyl and epoxy groups are generated [42]. Moreover, the oxidation process often breaks the π-conjugation, and exfoliates or expands the graphene layers. Thus, the resulting GO product is water dispersible. Because of the functional groups present in GO, the sorption and intercalation of ions and molecules are possible. This feature, together with high specific surface area and easy dispersion, makes GO promising materials to construct novel electrochemical sensing film and to use widely as the supports [42,43].

Since the discovery of the conductance in conjugated polymers [44], these materials have come to the forefront of researches such as battery electrodes, corrosion protectives, and sensors [45] because of their potential application, good stability, good electrical conductivity, and easy preparation [44,46]. Moreover, the modification of electrodes with alternating deposition of functional compounds is a very simple way to experimentally produce complex layered structures with precise control of layer composition and thickness. It is established that thin layers of conducting polymers (CPs) on the surface of substrate electrodes show an improvement of response for the determination of various important biological and clinical species and be able to enhance the kinetics of various electrode processes [47]. CPs have therefore been considered to be useful matrices for the immobilization of dispersed nanomaterials. Thus porous structure of conducting polymer nanocomposite was achieved, which may provide sufficient active sites for the adsorption of target analyte and enhanced charge transport properties compared to the conventionally synthesized CPs [48].

Among the different CPs, poly(sulfosalicylic acid) known as one of the most stable and successful commercially available CPs today. PSSA is ideal for improving the chemo/biosensors materials' sensitivity because of its easy preparation, good environmental and thermal stability, low cost of starting or raw materials, operation at room temperature and friendly biocompatibility [47]. Moreover, PSSA provides better electrical conductivity [44] and more active sites than the modified electrodes fabricated through covalent bonding or adsorption [49]. Recent developments of facile as well as effective method of synthesis of PSSA nanocomposite with high aspect ratio and increased surface area have generated tremendous interest as sensors. Superior properties of GQDs and the advantages of PSSA have received more attention for preparation of high performance CP/QDs hybrid materials. When GQDs fill the polymer matrix results in conductive polymer nanocomposite. Moreover, the incorporation of GQDs with the sufficient oxygen based functionalities led to significant morphological changes in PSSA layer that improves the carrier conductance [50]. In this context, the synergistic contribution from PSSA and GQDs makes the new material, a capable candidate to prepare of high performance modified electrode materials for the determination of trace levels of biological compounds.

As compared to other methods, electrochemical deposition is a versatile technique for producing surface coatings, owing to its precise controllability, room temperature operation, rapid deposition rate, strong adherence to electrode surface, no need of vacuum, no wastage of chemicals and relatively low cost [51,52]. As far as we know, there have been very few reports about PSSA film-modified glassy carbon electrode.

Hitherto, simultaneous determination of estradiol and progesterone was performed by HPLC [53], SPE/LC-(ESI) MS-MS [54] and LC-MS/MS [55] methods. To the best of our knowledge, no electroanalytical technique was found in the literatures for simultaneous quantitative determination of these hormones.

This paper reports on the novel strategy based on the proposed sensor for the simultaneous electrochemical determination of E2 and P4. Because of the mentioned characteristics of GQDs and CPs, we considered GQDs doped PSSA that would possess large electroactive surface area, better conductivity and more active sites for electrochemical sensing. There are three main objectives in the following sections. The first was to investigate the performance of GQDs-PSSA/GO modified GC electrodes for the electrochemical oxidation of E2 and P4; the second was optimizing the experimental parameters to improve the method efficiency during the experiment; and the third and last aim was to demonstrate the practical application of the present modified electrode by simultaneously determining of E2 and P4 content in clinical and combined pharmaceutical formulations.