Sensitive determination of 17β-estradiol in river water using a graphene based electrochemical sensor

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HIGHLIGHTS

- A novel material for the electrochemical sensing was developed.
- Sensor based on metallic porphyrin supported on reduced graphene oxide was applied.
- The proposed sensor was suitable and sensitive for the determination of 17β-estradiol in river water.

GRAPHICAL ABSTRACT
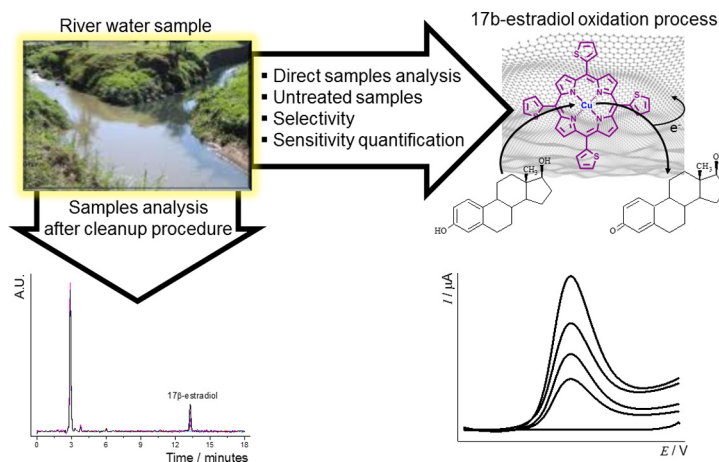
ABSTRACT

In this study, a novel material for the electrochemical determination of 17β-estradiol using an electrode based on reduced graphene oxide and a metal complex porphyrin has been applied to environmental monitoring. The electrochemical profile of the proposed electrode was analyzed by differential pulse voltammetry, which showed a shift of the oxidation peak potential of 17β-estradiol to 150 mV in a less positive direction compared to the bare reduced graphene oxide electrode. DPV experiments were performed in PBS at pH 7.0 to determine 17β-estradiol without any previous step of extraction, cleanup, or derivatization, in the range of $0.1–1.0\,\mu mol\,L^{-1}$ with a detection limit archived at $5.3\,nmol\,L^{-1}$ ($1.4\,\mu g\,L^{-1}$). The proposed sensor was successfully applied in the determination of 17β-estradiol in a river water sample without any purification step and was successfully analyzed under the standard addition method. All the obtained results were in agreement with those from the HPLC procedure.

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1. Introduction

The incidence of severe environmental contamination is due to the increasing number of artificial or natural compounds that are discarded indiscriminately into air, soil and water. In many cases, the environmental damage is highly relevant and can be

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irreversible. Certain of these contaminants are highly damaging to human health, and their use is controlled by government regulatory agencies. One class of substances that has been indiscriminately disposed of in water springs is endocrine disruptors (EDs). These contaminants are chemicals that may interfere with the natural functioning of the endocrine system of animal species, including humans. EDs can also be defined based on their effects in the organism; even at nanomolar levels, they are capable of changing the functioning of the endocrine system, causing different types of cancer and damage to the reproductive systems of humans [1,2].

One of the primary substances that has high endocrine disruptor activity is 17β-estradiol [3]. This hormone is a steroid that interferes with animals by causing abnormal development of thyroid function in birds and fish. The compound can also reduce fertility and lead to sexual disorders and immunological damage in crustaceans, fish, birds and reptiles. In humans, there is evidence that the development of certain diseases, such as breast and prostate cancer, has been caused by intense exposure to EDs. In addition, 17β-estradiol can reduce male fertility and can generate severe congenital malformations in children [4]. The route of contamination of 17β-estradiol in the aquatic environment is through the excretions of animals and humans and the subsequent transport of the chemical by sewage effluent. Thus, the bioaccumulation of 17β-estradiol in the environment is a problem that must be solved. Many regions, such as Brazil, the European Union, Japan and the United States, are concerned with limiting actions in the short, medium and long terms to identify the primary sources of contamination. In particular, the U. S. Environmental Protection Agency (EPA) has proposed requirements to monitor the ED classes of contaminants in wastewater samples [5]. In this context, it is a challenge to research groups to determine the mechanisms of action of these substances on the endocrine system of animals and humans and primarily develop analytical methodologies to identify and quantify these substances in the environment [6,7].

The major methodologies employed in 17β-estradiol determination in environmental samples are based on chromatographic methods, such as the HPLC [8], LC [9], LC/MS [10], and ELISA tests [11]. Chromatographic methods, although highly accurate, do not easily allow continuous *in situ* analysis and often require several previous sample preparation steps, including extraction and cleanup procedures, to obtain a final extract fully compatible with chromatographic determination. These techniques usually generate waste-containing organic solvents, which makes the procedure more complicated and expensive. From a contrasting point of view, electrochemical methods have a number of advantages, such as low cost, high sensitivity, easy operation, potential for miniaturisation and automation, ability to allow the construction of simple portable devices for fast screening purposes, and in-field/on-site monitoring.

The electrochemical determination of 17β-estradiol is based on the irreversible oxidation of the phenol group present in the molecule. However, this electrochemical process has been reported to present a low current response over some surfaces such as platinum and glassy carbon [12]. In this sense, several methods have been reported for the use of nanostructured materials as surface modifiers to increase the 17β-estradiol electrochemical response. For example, such applications as an aptamer optical biosensor based on 6-(O-carboxymethyl)oxime-BSA [6], molecularly imprinted membranes on platinum nanoparticle-modified electrodes [13], CdSe quantum dots based on indirect competitive immunoassay [14], and nanoporous polymeric film bearing the poly{1-butyl-3-[3-(N-pyrrole)propyl]imidazole dodecyl sulfonate} moiety have been reported [15].

A recently reported nanostructured material that has also attracted considerable attention in the development of

electrochemical sensors is graphene nanosheets. In particular, reduced graphene oxide (RGO) is a single layer of hybridised carbon atoms sp^2 that has attracted attention due to its two-dimensional structure of carbon atoms with a π-conjugated structure with excellent electron mobility ($2.0 \times 10^5 \, cm^2 \, V^{-1} \, s^{-1}$). These characteristics allow intrinsic electronic properties, such as fast charge transfer [16]. In this sense, the RGO can act as a support for immobilisation of several species, allowing it to demonstrate its applicability to different electrochemical sensors applied in environmental analysis, such as the determination of 17β-estradiol using quantum dot modified graphene [17], quantification of isoproturon and carbendazim pesticides [18], and determination of tetrabromobisphenol A [19].

One special substance used as an RGO modifier is metal complex porphyrins (MPs). MPs have been used extensively in the preparation of electrochemical sensors due to their catalytic activity for a wide range of redox processes [20] and their well-defined redox activity in sensing applications [21]. The electrocatalytic activity attributed to MPs is highly dependent on the central metal atom. The change in geometry of the complex during the redox process is one of the most important factors to consider [22]. Porphyrins can act as biomimetic molecules [23] because they can act as the active centre of an enzyme with the same efficiency and selectivity, particularly for phenolic compounds [24].

MPs can be adsorbed over the RGO surface *via* π-stacking interactions [25]. However, the MP/RGO composite has been observed to present the excellent catalytic properties of MPs with no loss of any of the electronic properties of RGO. Thus, this work outlines the development and application of an electrochemical sensor based on the Cu(II)-*meso*-tetra(thien-2-yl)porphyrin supported over a reduced graphene oxide surface to be applied to the identification of 17β-estradiol in river water. This novel material yielded excellent sensitivity for 17β-estradiol electro-oxidation, as well as a high electrocatalytic activity and a low detection limit. The proposed electrochemical device was successfully applied to the determination of 17β-estradiol in river water, and the results were compared with those of the official USEPA method [26], demonstrating the accuracy and efficiency of the proposed sensor.

2. Experimental

2.1. Chemicals and solutions

All chemicals were of analytical grade and were used without further purification. Analytical-grade 17β-estradiol was obtained from Sigma–Aldrich (Germany). Phosphate buffer solution (PBS) was used as a supporting electrolyte with ionic strength at 0.1 mol L^{-1}. For the graphene oxide synthesis, we used graphite powder (<20 μm), sodium nitrate, sulfuric acid, nitric acid, and potassium permanganate purchased from Sigma–Aldrich (Germany). For the synthesis of the Cu(II)-*meso*-tetra(thien-2-yl) porphyrin (CuTthP), we used the following reagents purchased from Sigma–Aldrich (Germany): dichloromethane, 2-thiophencarboxaldehyde, pyrrole, methanol, ethyl acetate, boron trifluoride diethyl etherate, *p*-chloranil and copper(II) acetate monohydrate. All organic solvents used on the HPCL experiments and graphene suspension such as methanol and ethanol of analytical grade were obtained from Mallinckrodt (Xalostoc, Edomex, Mexico). All solutions were prepared with water purified in a Millipore Milli-Q system (resistivity >18.2 MΩ cm).

2.2. Apparatus and procedures

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) experiments were performed using a model PGSTAT 302

Autolab electrochemical system (Eco Chemie, the Netherlands) monitored with NOVA software. The electrochemical cell was assembled with a conventional three-electrode system: a glassy carbon electrode modified with a composite (RGO/CuTthP), an Ag/AgCl electrode in KCl ($3.0 \, mol \, L^{-1}$) as a reference electrode, and Pt wire as an auxiliary electrode. All electrochemical measurements were performed in $0.1 \, mol \, L^{-1}$ PBS (pH 7.0) at a controlled temperature (25 °C). CV measurements were carried out in a potential range from 0.0 to +0.9 V with a scan rate of $100 \, mV \, s^{-1}$. DPV experiments were performed using an amplitude of 60 mV and a step potential of 2 mV. The potential was varied from +0.2 to +0.9 V for 17β-estradiol analytical responses. The 17β-estradiol was determined quantitatively by HPLC using a Shimadzu (Kyoto, Japan) Prominence LC-20AT modular system comprising two CBM-20A pumps, a CTO-10AS oven, a SIL-20A auto sampler, a SPD-20A variable wavelength detector and an LC-10 Workstation Class data processor. Separations were carried out on a Phenomenex Luna C-18 column (250 × 4.6 mm i.d.; 5 μm) protected by a Supelcosil C-18 guard column (4.0 × 3.0 mm i.d.; 5.0 μm), eluted with mixtures of 65% methanol and 35% water (acidified with formic acid). The chromatographic conditions were oven temperature 35 °C, flow rate $1.0 \, mL \, min^{-1}$, injection volume 20 μL (Rheodyne loop), and UV detection at 280 nm.

2.3. Synthesis of graphene oxide

The graphene oxide (GO) was prepared from graphite through a modified Hummers method [27]. A mixture of graphite powder (10.0 g) and sodium nitrate (10.0 g) was oxidized by using 400.0 mL of 1:3 volume concentrated solution of HNO_3/H_2SO_4 maintained in an ice bath under magnetic stirring. Next, 50.0 g of $KMnO_4$ was slowly added under vigorous stirring for 2 h. Then, the solution was stirred at 60 °C for 30 min, and 75 mL of H_2O_2 (30% v/v) was slowly added under magnetic stirring. Next, 100.0 mL of HCl solution (10% v/v) and 900.0 mL of cooled purified water were added, and the mixture was kept in a refrigerator at 4 °C for 24 h. The light brown supernatant was collected, and the graphene oxide was separated by centrifugation at 10,000 rpm. Thus, the graphene oxide was dried under lyophilisation for 24 h. The reduced graphene oxide (RGO) was prepared using sodium borohydrate. For this preparation, 100.0 mg of GO was mixture with 10.0 mg of sodium citrate, 40.0 mg of sodium borohydrate, and 10.0 mL of ethanol. The mixture was sonicated for 1 h. Then, the supernatant was discarded and the black solid was washed 5 times with water (using centrifugation). Finally, the RGO was washed with ethanol. The RGO was dried at 60 °C overnight.

2.4. Synthesis of metal porphyrin

The synthesis of the CuTthP was carried out following the literature described by Rochford et al. [28]. Dry CH_2Cl_2 (80 mL), pyrrole (2.0 mmol) and 2-thiophenecarboxaldehyde (2.0 mmol), were added to a 100 mL round-bottom flask equipped with adequate constant magnetic stirring and then purged with argon for 10 min. Then, the reaction temperature was adjusted to 0 °C and boron trifluoride diethyl etherate (0.2 mmol) was added. The resulting mixture was kept under magnetic stirring for 24 h. Next, the oxidant *para*-chloranil (1.0 mmol) was added, and the resulting mixture was kept under constant stirring for 12 h at room temperature. The product that was obtained was recrystallized from ethyl acetate three times and then washed in a 3:1 MeOH: CH_2Cl_2 mixture. The obtained free metal *meso*-tetra(thien-2-yl) porphyrin was metallated with Cu(II) using the following procedure: Under magnetic stirring, we added the *meso*-tetra (thien-2-yl)porphyrin (0.080 mmol), 33 mL of CH_2Cl_2, and a solution of copper(II) acetate monohydrate (0.29 mmol) in 4 mL

of methanol. The resulting reaction mixture was kept at constant temperature. The workup was performed by the addition of distilled water (50 mL), and the CuTthP was extracted with CH_2Cl_2. The organic layer was washed with distilled water and dried over anhydrous sodium sulfate. Next, the solvent was distilled under reduced pressure, and the crude product was purified by recrystallization from a mixture of MeOH:CH_2Cl_2 (3:1).

2.5. Electrode preparation

Prior to modification, the glassy carbon (GC) electrode surface was polished with 0.5 μm alumina slurry, rinsed thoroughly with ultrapure water, sonicated 3 min in acetone and 3 min in water, and dried in air. For graphene oxide (GO) and reduced graphene oxide (RGO), a mass of 2.0 mg of GO or RGO was dispersed in 1.0 mL of ethanol using ultrasonic stirring for 20 min. For RGO/CuTthP, 2.0 mg of RGO plus 1.0 mg of CuTthP were dispersed by ultrasonic stirring for 20 min in 1.0 mL of ethanol. A 15 μL aliquot of one dispersion or the other was placed as a droplet on the GC electrode surface, and the solvent was then left to evaporate at room temperature.

2.6. River water sample preparation

A sample of natural water from a river located in São Carlos (São Carlos/SP–Brazil) was collected in February 2014 at 22°01′13″S, 47°54′48″W. The environmental sample was previously filtered and used directly (without any other treatment) in the preparation of the $0.1 \, mol \, L^{-1}$ of PBS (pH 7.0), and the sample was later spiked with 15 μg L^{-1} of 17β-estradiol.

3. Results and discussion

3.1. Study of the electro-oxidation of 17β-estradiol

The electrochemical behaviour of 17β-estradiol at the GC/RGO/CuTthP electrode was carried out in $0.1 \, mol \, L^{-1}$ PBS (pH 7.0) containing 25.0 μmol L^{-1} of 17β-estradiol by cyclic voltammetry experiments using several scan rates in a range varying from 10 to $100 \, mV \, s^{-1}$, and the results obtained are presented in Fig. 1. Using a scan rate of the $50 \, mV \, s^{-1}$ (open circle), a single oxidation process can be observed for 17β-estradiol at +0.54 V over the RGO/CuTthP surface. This oxidation is attributed to irreversible oxidation of the hydroxyl group present in the aromatic ring of the 17β-estradiol molecule to give the corresponding ketone derivative [30], as
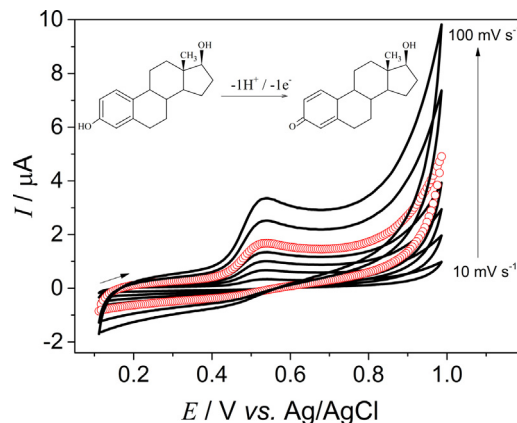

Fig. 1. Cyclic voltammograms for GC/RGO/CuTthP electrode in $0.1 \, mol \, L^{-1}$ of PBS (pH 7.0), containing 25.0 μmol L^{-1} of 17β-estradiol at the following scan rates: 10, 20, 30, 40, 50 (open circles), 75, and $100 \, mV \, s^{-1}$. Inset: proposed electrochemical mechanism for 17β-estradiol oxidation.

shown in the proposed electrochemical mechanism inset on Fig. 1. This irreversible behavior is attributed to an electron transfer control for the 17β-estradiol reaction on the electrode surface. This fact was in agreement with the linear dependence between the I_{pa} and the scan rate, suggesting a surface-controlled process on the modified electrode surface. The linear equation was I_{pa} (μA) = 0.242 (μA) + 3.32 (mV s^{-1}) (scan rate) (mV s^{-1}) (R^2 = 0.9995). This result also suggested that 17β-estradiol presents a good affinity to the RGO/CuTthP structure.

The oxidation of 17β-estradiol at the electrode surface was successfully carried out with 0.1 mol L^{-1} of PBS (pH 7.0) containing 25.0 μmol L^{-1} of 17β-estradiol by DPV experiments with a pulse amplitude of 100 mV, a step potential of 2 mV and a scan rate of 10 mV s^{-1}. The results obtained from the investigation of the three electrodes are presented in Fig. 2. In the potential range studied, there is only one oxidation process. On the GC/GO electrode, 17β-estradiol oxidation occurred at +0.75 V, which is 0.15 V less positive than on a GC/RGO electrode, demonstrating the electro-catalytic effect of the reduction process of the graphene. Moreover, the oxidation on this catalytic surface showed a factor increase of 1.7 in the peak current compared with the electrode fabricated with only graphene oxide. On the GC/RGO/CuTthP electrode, the 17β-estradiol oxidation potential decreased to +0.54 V, i.e., by a further 0.05 V with respect to the GC/RGO electrode. The extra factor increase of 2.2 in peak current can be associated with more efficient adsorption due to the porphyrin presence on the surface. This behaviour is similar to the behaviour described by Moraes et al. [21], in which the oxygenated groups contained in the target molecule adsorbed preferentially on the metal centre of the porphyrin ring, then allowing an increase of the current peak. Additionally, the geometry of the complex molecule adsorbs over the RGO surface *via* π-stacking interactions [25]. Then, the copper complex first acts as an anchor site for the 17β-estradiol adsorption and then, the CuTthP mediates the charge transfer to the RGO surface, leading to a synergistic effect which improves the sensitivity of the method.

To confirm the mechanism of 17β-estradiol oxidation at the GC/RGO/CuTthP electrode, as displayed in Fig. 1 inset, the dependence on the electrochemical oxidation of the hormone on pH was studied by DPV experiments at the pH range from 4.0 to 9.0 in PBS containing 25.0 μmol L^{-1} of 17β-estradiol. The results are shown in Fig. 3, which is a plot of the DPV peak current (I_{pa}) and peak potential (E_{pa}) as a function of pH. The variation of E_{pa} with pH can provide valuable information on the 17β-estradiol oxidation process. Reducing the hydrogen ionic concentration of the electrolyte causes a shift in the peak potential toward more negative values, as illustrated in Fig. 3. The slope of the E_{pa} vs. pH

linear relationship was found to be 57.5 mV per pH unit. Therefore, use of the Nernst equation proposes an electrochemical process involving the same number of protons and electrons during the electro-oxidation of 17β-estradiol. This result is in agreement with the work developed by Cincotto et al. [17] and Ngundi et al. [29], which explains that the electro-oxidation of this hormone is accomplished by the transfer of equal numbers of electrons and protons. The plot of I_{pa} vs. pH in Fig. 3 shows that the peak current has a maximum value at pH 7.0 and decreases for both higher and lower pH values. Based on this information, pH 7.0 was selected for subsequent experiments using the GC/RGO/CuTthP electrode.

3.2. Optimization studies of 17β-estradiol on the GC/RGO/CuTthP electrode

To maximize the DPV analytical signal, the effects of the experimental parameters (pre-treatment cleaning potential and time, pulse amplitude, and potential step) were studied at the GC/RGO/CuTthP electrode using 0.1 mol L^{-1} PBS (pH 7.0) containing 25.0 μmol L^{-1} of 17β-estradiol. As discussed, the electrochemical oxidation of 17β-estradiol is accompanied by adsorption of the endocrine disruptor or its oxidation products. Thus, there is a fouling of the electrode surface and a consequent decrease of the analytical signal. To overcome this drawback, the influence of applied potential pre-treatment after each scan was investigated. The following pre-treatment potential values were investigated: +0.2, 0.0, −0.2, −0.4, −0.6, −0.8, and −1.0 V. Complete recovery of the original response was obtained at −0.8 V. At more positive cleaning potentials, the peak current obtained during the following experiment, I_{pa}, began to decrease, reaching only 20% at +0.2 V pre-treatment cleaning potential. Thus, −0.8 V was chosen as the pre-treatment cleaning potential for further studies. The influence of the pre-treatment time was also evaluated, varying from 10 to 60 s, and no difference was observed. Hence, 10 s was chosen as the time of the pre-treatment for cleaning the electrode surface.

The influence of differential pulse voltammetry parameters was investigated. The effect of potential step increments was studied in the range of 1–10 mV. For potential steps greater than 2 mV, the deformation of the voltammetric profile was observed, and the peak current decreased in height. By fixing the potential step increment at 2 mV, the amplitude was varied in the range of 10–100 mV. The I_{pa} increased as the amplitude increased in this studied range. No deformation of the peak or any significant increase in peak width was observed, even for amplitudes greater than 50 mV. Hence, 100 mV was chosen as the DPV amplitude.
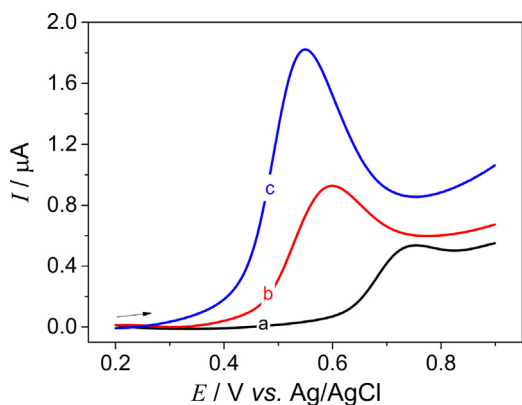

Fig. 2. Differential pulse voltammograms 0.1 mol L^{-1} of PBS (pH 7.0), containing 25.0 μmol L^{-1} of 17β-estradiol for the electrodes: (a) bare GC, (b) GC/RGO and (c) GC/RGO/CuTthP.
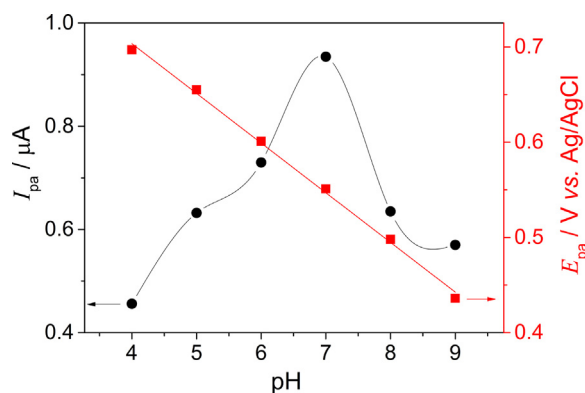

Fig. 3. Effect of pH on the peak potential (square) and peak current (circle) for 17β-estradiol oxidation on the GC/RGO/CuTthP electrode using PBS electrolyte of ionic strength at 0.1 mol L^{-1} containing 25.0 μmol L^{-1} of 17β-estradiol.

3.3. Analytical characteristics

Using DPV experiments with the optimised parameters described earlier, the proposed GC/RGO/CuTthP electrode was applied to investigate the electrochemical response as a function of the 17β-estradiol concentration. All measurements were made in triplicate, and the results are indicated as mean values. The analytical response shown in Fig. 4 has a linear response in the range from 0.01 to 1.00 μmol L^{-1}, in accordance with the following equation:

$$I_{pa}(\mu A) = 0.39(\mu A) + 0.51\,(\mu A\,\mu mol\,L^{-1})\,[17\beta - estradiol]\,(\mu mol\,L^{-1})$$

With a correlation coefficient of 0.996 (n = 12). The limit of detection (LOD) obtained was 5.4 nmol L^{-1} (1.9 μg L^{-1}), determined using a 3σ/slope ratio, where σ is the standard deviation of the mean value for 10 voltammograms of the blank.

The reproducibility of the GC/RGO/CuTthP electrode was measured from seven experiments (in different days), each of which consisted of five sequential DPV voltammograms. In each weekday, a set of five sequential voltammograms were performed. During a complete week (seven sequential days), the reproducibility tests were carried out. Prior to each experiment, the electrode surfaces were rinsed thoroughly with double-distilled water. Thus, the DPV voltammograms were performed in 0.1 mol L^{-1} PBS at pH 7.0 containing 25.0 μmol L^{-1} 17β-estradiol. The relative standard deviation (RSD) was calculated as 2.8%. In addition, intra-assay precision tests were performed from 10 DPV voltammograms of that same solution. The RSD was found to be 2.4%.

3.4. Selective determination of 17β-estradiol in the presence of interferents

The ability to determine 17β-estradiol in the presence of other endocrine disruptors was investigated. The pesticides carbendazim and carbaryl are the most widely used pesticides due to their high insecticidal activity [30]. These pesticides have been used extensively in sugarcane and orange crops. In addition, both pesticides are reported to be environmental contaminants and could promote high endocrine interference [31]. In this sense, the significance of the interference of these species in the electro-oxidation process of the 17β-estradiol on the GC/RGO/CuTthP electrode was evaluated. The experiments were carried out using DPV with optimised parameters in solutions with 0.1 mol L^{-1} of PBS (pH 7.0) containing concentrations of carbendazim and
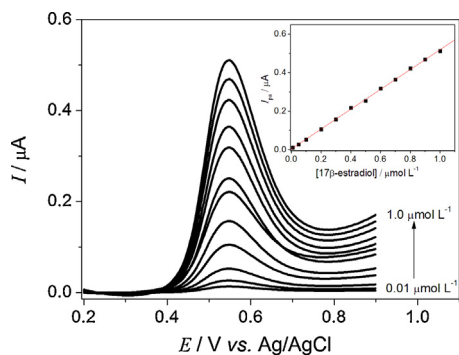
carbaryl fixed at 25.0 μmol L^{-1}, with sequential additions of 12.5, 25.0, and 50.0 μmol L^{-1} of 17β-estradiol. In all DPV experiments (figure in Supplementary information), no overlapping process of the 17β-estradiol oxidation peaks and the oxidation peaks of the interfering substances studied was observed. In addition, the large separation of the peak potentials enabled the selective and simultaneous determination of carbendazim, carbaryl, and 17β-estradiol in the mixture. The oxidation peak potentials of all the mixture components were well-resolved at the GC/RGO/CuTthP electrode with peak potentials at +0.54 for 17β-estradiol, +0.93 for carbendazim and +1.1 V for carbaryl. In the concentration range studied for the interferents, the decrease or increase in the 17β-estradiol peak height was observed to be negligible. In addition, the interfering substances did not shift the 17β-estradiol oxidation peak, indicating that the analytical signal does not suffer interference from the tested interferents in the sample.

To demonstrate the enhanced properties of the proposed sensor, the observed detection limit was compared with the detection limits reported in the literature. Several papers in the literature describe the electrochemical determination of 17β-estradiol. Those methods have used several strategies for hormone determination such as direct analysis based on the 17β-estradiol oxidation process or indirect methods based on immunosensors using bioconjugation. Comparing the detection limit of the electrodes presented in this work (5.4 nmol L^{-1} (1.9 μg L^{-1})), this work shows a lower detection limit than the detection limits published in papers using a multi-wall carbon nanotube-nafion modified glassy carbon electrode [32] (10.0 nmol L^{-1}, i.e., 2.72 μg L^{-1}). The use of a boron-doped diamond electrode in flow injection analysis [33] achieved an even higher detection limit: 100 nmol L^{-1} (27.3 μg L^{-1}). In these comparisons, the use of an electrode modifier is an advantage of the method proposed in this study.

From a different point of view, it is important also to compare our data with the data published using other types of modified electrodes. An electrochemical sensor based on molecular imprinted membranes at platinum nanoparticles [34] exhibited a detection limit of 16.0 nmol L^{-1} (4.5 μg L^{-1}). Another example in the literature describing a sensor based on reduced graphene oxide and dihexadecyl phosphate [35] achieved a detection limit of 77.0 nmol L^{-1} (20.9 μg L^{-1}). Boron-doped diamond modified with carbon black [36] exhibited a lower detection limit compared with the sensors proposed here (2.2 nmol L^{-1} or 0.60 μg L^{-1}). In all of these cases, the system described in this study, the GC/RGO/CuTthP electrode, yielded detection limits comparable with those described in the literature. However, in this work, the electrode has been tested in environmental samples with high reproducibility, stability, and precise results.

The indirect methods based on bioconjugated immunosensors could reach a wider linear range and lower detection limit compared with the GC/RGO/CuTthP electrode. The immunosensor based on graphene–polyaniline composites and carboxylated graphene oxide [37] reached a detection limit of 73.0 pmol L^{-1} (0.02 μg L^{-1}). A immunosensor using magnetic beads (MBs) as the solid support and screen-printed electrodes [38] achieved a detection limit even lower at 3.6 pmol L^{-1} (10 ng L^{-1}). However, the GC/RGO/CuTthP electrode presents a high sensitivity, good reproducibility, simple instrumentation, ease of preparation, and low timing data acquisition, which are also important advantages in the development of new sensors. In those papers described above [37,38], no descriptions were presented concerning the lifetime and robustness of the electrodes. Considering also that the electrode preparation in those papers [37,38] is time-consuming, this is also another advantage of the sensor described here once the preparation of an electrode can be executed in only 10 min. Immediately after the preparation, the electrode is ready for use. In

Fig. 4. DPV voltammograms for GC/RGO/CuTthP electrode, with the optimized parameters. The 17β-estradiol concentrations in μmol L^{-1} are: 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 10.0. Inset: linear dependence of the peak current with 17β-estradiol concentration.

addition, the electrodes prepared here were used for at least 60 determinations with no changes in their behavior. Finally, the electrodes were in storage for 30 days without loss of their electrochemical response capabilities.

3.5. Analysis of 17β-estradiol in river water

The sensor that we developed was used for the quantification of 17β-estradiol in river water. The 17β-estradiol determinations were performed in triplicate, without any treatment procedure, using the standard addition method. The analyses were using DPV voltammetry with optimised parameters, and the results are presented in Fig. 5A. The GC/RGO/CuTthP electrode showed recovery of 17β-estradiol from water samples between 98.7 and 101.2% when 30, 45, and 60 μg L^{-1} of 17β-estradiol standard were added and were extrapolated to the resulting straight line. The analysis of 17β-estradiol in water was also performed by HPLC. In all chromatograms in Fig. 5B, the solvent peak was observed at 2.8 min. Two small peaks were also detected at 3.8 and 5.9 min that could be species content in the water matrix. Regarding the presence of 17β-estradiol in the water sample, no significant signals were observed in the time range studied, indicating that the water was not polluted with the hormone. To evaluate the ability of the proposed sensor to detect the 17β-estradiol, the river water was spiked with 15.0 μg L^{-1} of 17β-estradiol. The analysis of the sample solution spiked with 17β-estradiol revealed a hormone peak recorded at 13.3 min. The standard additions of 30, 45, and

Table 1
Determination of 17β-estradiol in river water.

	GC/RGO/CuTthP		HPLC	
	Detected (μg L^{-1})	Recovery (%)	Detected (μg L^{-1})	E_r[a] (%)
Sample 1	14.5 ± 0.012	96.6	15.0 ± 0.003	−3.44
Sample 2	14.7 ± 0.015	98.0	15.1 ± 0.008	−2.72
Sample 3	14.9 ± 0.009	99.3	15.0 ± 0.003	−0.67

[a] E_r = detected proposed GC/RGO/CuTthP electrode *vs.* detected HPLC.

60 μg L^{-1} showed recoveries between 96.6 and 99.3%. The results obtained for the samples using the proposed method were compared with the results for the same samples using HPLC, and these results are presented in Table 1. According to Student's *t*-test, there was no significant difference between the HPLC and electrochemical methods at a 95% confidence level, indicating that the proposed sensor can be used for the determination of endocrine disruptors in water samples.

Several chromatographic techniques have been used to detect traces of estradiol in environmental samples. In many cases, those methods allowed detection limit levels at few ng L^{-1} [39–41] level. However, in all reported methods, it was necessary to execute many previous steps, such as pre-concentration, second-order calibration based on multivariate curves, ultrasonic extraction, use of different and large amount of solvents, cleanup procedures, which are time- and reagent-expensive, to obtain a sample that can be analysed. In contrast, the electroanalytical method can be used directly in the sample without any pre-procedural steps. Therefore, considering the ease of electrode preparation, price and stability are important advantages that make the electrode prepared in this paper a promising sensor for various applications.

4. Conclusions

An alternative electrochemical method has been proposed that enables the monitoring of 17β-estradiol concentrations in river water samples and does not require the separation, cleanup or derivatisation steps that are necessary for several other analytical methodologies. The well-known electrocatalytic properties of reduced graphene oxide associated with the excellent capability of CuTthP to mediate the decrease of 17β-estradiol oxidation potential resulted in a notably sensitive and selective sensor for the detection of this endocrine disruptor. This method provided values for LOD even lower than the values required to detect the hormone in natural water. Finally, the application of the analytical methodology to environmental samples did not exhibit any interference of the water matrix. The GC/RGO/CuTthP electrode was applied successfully to the determination of 17β-estradiol in river water samples, and the results obtained by the proposed methodology were similar to the results obtained using the HPLC procedure, implying that it is possible to develop an electrochemical sensor that would work *in situ* and in real time to monitor environmental contaminants.

Supplementary information

DPV experiments for GC/RGO/CuTthP electrode electrodes in solution with 0.1 mol L^{-1} of PBS (pH 7.0) containing concentrations of carbendazim and carbaryl fixed at 25.0 μmol L^{-1}, and sequential additions of 17β-estradiol standards in the following concentrations: 12.5 μmol L^{-1}, 25.0 μmol L^{-1} and 50.0 μmol L^{-1}.
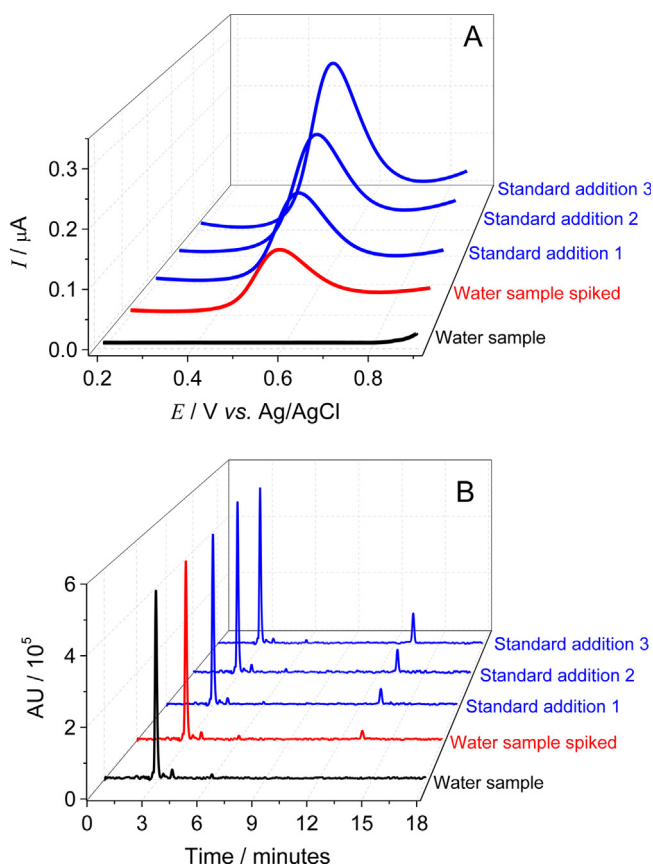
Acknowledgements

Fig. 5. (A) DPV voltammograms for river water samples: (black line) pure sample, (red line) sample spiked with 15.0 μg L^{-1} of 17β-estradiol and (blue line) sequential standard additions of 17β-estradiol in the following concentrations: 30.0, 45.0 and 60.0 μg L^{-1}. (B) Chromatograms in the same conditions described. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aca.2015.04.043.

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