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The analysis of estrogenic compounds by flow injection analysis with amperometric detection using a boron-doped diamond electrode

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ABSTRACT

We report on the use of flow injection analysis with amperometric detection (FIA-EC) to evaluate the potential of using diamond electrodes for the analysis of three estrogenic compounds: estrone, 17-β-estradiol, and estriol. Amperometric detection was performed using a cathodically pretreated boron-doped diamond electrode that offered low background current, relatively low limits of detection, and good response reproducibility and stability. For all three compounds, response linearity was observed over the concentration range tested, 0.10 to 3.0 μmol L^{-1}, the sensitivity was *ca.* 10 mA L mol^{-1}, and the minimum concentration detection ($S/N \geq 3$) was 0.10 μmol L^{-1} (~27 μg L^{-1}). The response variability with multiple injections was *ca.* 10% (RSD) over 20 injections. For estrone, the oxidation reaction on diamond does not proceed through an adsorbed state like it does on glassy carbon. After an initial current attenuation, the diamond electrode exhibited a stable response (oxidation current) for 3 days of continuous use, indicative of minimal surface contamination or fouling by reaction intermediates and products. The method for estrone was assessed using spiked city tap and local river water. Estrone recoveries in spiked city and river water samples presented standard deviations of less than 10%. In summary, the FIA-EC method with a diamond electrode enables sensitive, reproducible, stable, quick, and inexpensive determination of estrogenic compounds in water samples.

1. Introduction

Declining water quality has become an issue of global concern as human populations grow, industrial and agricultural activities expand, and climate change threatens to cause major alterations to the hydrological cycle. As a consequence, water quality is one of the most important topics in environmental chemistry. Considerable attention has been focused in recent years on one class of organic pollutants, the so-called endocrine disrupting compounds (EDCs). These comprise a wide group of environmental pollutants that are able to mimic or antagonize the effects of endogenous hormones [1]. In humans and animals, endocrine disruption is related to interferences in the functioning of their endocrine and homeostatic systems. Animal and human exposure to environmental EDCs can have consequences, such as decreased sperm counts in human males, sexual disruption of fish populations, and eggshell thinning in bird populations [1,2].

Estrogen and estrogen-like compounds represent a class of EDCs that enter reservoirs, ground water, rivers and streams, and remain there even after passage through water treatment plants [3]. These pollutants are introduced into the environment through human and animal excretion. The three main natural estrogens (sexual hormones) are estrogen, estradiol, and estriol, and all are found in the environment. The use of oral contraceptives for birth control, which contain estrogen, is a significant source of this pollutant [2]. Other synthetic estrogens, such as 17α-ethinylestradiol and diethylstilbestrol, can also be found in the environment. The environmental levels are linked to population growth and intensive farming [4]. Sexual hormone molecules have at the base a steroidal structure [3]. They also have a phenolic moiety that is crucial for high affinity binding to estrogen receptors. The long-term effects of exogenous estrogenic compounds on human health are not fully understood. As mentioned above, they may affect male fertility by interfering with sperm production. Links have also been suggested between estrogenic compounds and some types of cancer. Trends are emerging for increased endocrine-related diseases and disorders among children. A growing body of evidence suggests that exposure to EDCs through consumer goods, personal care products, and

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drinking water may adversely affect child development through altered endocrine function [5].

The analytical challenges and recent advances in the determination of estrogenic compounds in water supplies have recently been reviewed [3]. Several methods have been used for the separation and detection of estrogenic compounds. For example, mass spectrometry [6–13] and UV–vis absorption [14–18], both coupled with either gas or liquid chromatography, have been successfully employed to measure these pollutants in aqueous media. State-of-the-art analysis involves the application of solid phase extraction before final determination by LC-MS [3]. Limits of detection with this method are generally in the low ng L^{-1} range [3]. In general, the determination of natural and synthetic estrogenic compounds in water supplies is a challenging and time-consuming analytical task because of the low detection limits required and the complexity of matrices [3]. Developing inexpensive, portable, and easy-to-use assays for these and other pollutants would be of great value.

Electrochemical detection can be incorporated into portable and easy-to-use assays. The key with this method of detection is having an electrode that exhibits excellent response sensitivity, reproducibility, and stability over time, even in complex matrices. Electrochemical detection coupled with liquid chromatography has been employed for the analysis of estrogenic compounds in different sample types [19]. Carbon nanotube-modified glassy carbon [7,20,21] and nano-Al_2O_3 dispersed onto the surface of glassy carbon (GC) [22] are two examples of modified carbon electrodes that have been used.

Boron-doped diamond (BDD) is another type of carbon electrode that could prove useful in electrochemical assays for estrogenic compounds, since this material generally offers significant improvements in linear dynamic range, limit of detection, response reproducibility, and response stability, as compared to conventional sp^2 carbon electrodes, like GC. BDD possesses among its principal characteristics high stability and hardness, chemical inertness, low background current, and a wide potential window ($\sim$3.2 V) in aqueous solutions, which allows for the detection of electroactive species without the interference of water decomposition [23]. Another beneficial property is its resistance to molecular adsorption and electrode fouling, making it useful for measurements in complex environments. The principal reason for this is the fact that the diamond surface is relatively nonpolar when hydrogen terminated and contains no extended π-electron system [24]. In prior work, we demonstrated using cyclic voltammetry that estrogenic compounds could be electro-oxidized at diamond electrodes [25]. We expand herein on prior work and demonstrate that flow injection analysis with electrochemical detection (FIA-EC) using diamond is a viable method for the analysis of three estrogenic compounds.

FIA-EC has been coupled with diamond electrodes for the detection of pharmaceuticals and other pollutants. For example, a multicommutation stopped-flow system was successfully developed and applied for the simultaneous determination of sulfamethoxazole and trimethoprim in pharmaceutical formulations by differential pulse voltammetry, using a cathodically-pretreated boron-doped diamond electrode [26]. A method for the simultaneous determination of two phenolic antioxidants (butylated hydroxyanisole and butylated hydroxytoluene) in food was also developed using FIA with pulse amperometry along with a cathodically pretreated boron-doped diamond electrode [27]. More recently, a similar analytical method was developed for the simultaneous determination of two pairs of food colorants, tartrazine and sunset yellow (SY), and brilliant blue and SY [28].

Here we report on the use of FIA-EC to measure three estrogenic compounds: estrone, 17-β-estradiol, and estriol. Amperometric detection was performed using a cathodically pretreated BDD electrode. The electrode performance for real sample analysis was assessed using (i) city tap and (ii) local river water spiked with estrone.

2. Methods and materials

2.1. Reagents

All solutions were prepared using ultrapure water (>17 MΩ cm) from a Barnstead E-pure system. The electrochemical measurements were performed using aqueous 0.5 mol L^{-1} H_2SO_4 (95%–98%, EMD Chemicals) as the supporting electrolyte, to which appropriate volumes of methanolic estrone (E1) stock solution (200 μmol L^{-1}) ($\geq$99%, Sigma Aldrich) were added. This supporting electrolyte was chosen after comparison with other possible ones (data not shown): 0.1 mol L^{-1} Britton–Robinson buffer (pH 2), 0.1 mol L^{-1} phosphate buffer (pH 2–10), 0.1 mol L^{-1} sodium sulfate, and H_2SO_4 (0.01, 0.1, and 0.25 mol L^{-1}). The dependence of the E1 oxidation peak potential on the pH was previously reported for the pH range of 2 to 12 with a 0.1 mol L^{-1} phosphate buffer [25].

2.2. Equipment, electrode and apparatus

A BDD thin film (8000 ppm doping level, 1.0–1.5 μm thickness, lot no. WD1390-4) was used as a working electrode (1.2 cm × 1.2 cm). It was prepared on a silicon wafer by Adamant Technologies SA (Switzerland). The GC electrode (GC-20 Tokai Ltd.) was pretreated by polishing for 20 min on separate felt pads with slurries of successively smaller grades (1.0, 0.3, and 0.05 μm) of alumina powder in ultrapure water. After each polish, the electrode was thoroughly rinsed with and ultrasonically cleaned in ultrapure water for 10 min. Cyclic voltammetric and chronocoulometric measurements were performed with a CHI 650B potentiostat/galvanostat (CH Instruments, Austin, TX). For these measurements, a three-electrode, single-compartment glass cell (V=50 mL) was used. The working electrode had an exposed geometric area of 0.32 cm^2, the counter electrode was a Pt foil (1.0 cm × 0.5 cm), and a home-made reference electrode Ag/AgCl (3 mol L^{-1} KCl) was employed. The amperometric detection and quantification of the estrogenic compounds were carried out using a homemade, flow-through electrochemical detection cell ($\sim$100 μL), constructed out of two acrylic blocks. The design is described in detail elsewhere [26,29]. In this detector, a miniaturized Ag/AgCl "no leak" (3 mol L^{-1} KCl, 66-EE009 – Cypress Systems a Division of ESA Biosciences, Inc.) commercial electrode was used as the reference.

Prior to use, the BDD electrode was submitted to a two-step electrochemical pretreatment that first involved cleaning the electrode using an anodic polarization (0.5 A cm^{-2} for 60 s), followed by a cathodic polarization (-0.5 A cm^{-2} for 360 s), both in a 0.5 mol L^{-1} H_2SO_4 solution. The anodic pretreatment increases the surface's oxygen content while the cathodic pretreatment reduces it, presumably through the formation of hydrogen surface termination. This supposition is based on previously reported results including XPS analysis [23,30,31]. We performed no surface analysis on the pretreated diamond electrode used in this work.

The FIA system consisted of an Alltech HPLC pump, Model 301 (Grace, Deerfield, USA), a Rheodyne injection valve, and a flow-through (cross-flow) electrochemical cell, which are described in detail elsewhere [26,29]. A pulse dampener (Model LP-21, Grace) was used in series to reduce the pump noise. The carrier solution for the estrogenic compound analysis was 0.5 mol L^{-1} H_2SO_4.

2.3. Standard solutions

For the generation of a calibration curve, a methanolic stock solution (200 μmol L^{-1} estrogenic compound) was first prepared. From that, standard methanolic solutions were prepared: from 0.10 to 300 μmol L^{-1} of the estrogenic compound in 0.5 mol L^{-1} H_2SO_4. Three standard estrone solutions (100, 1000, and 1700 nmol L^{-1})

were prepared for the recovery studies from spiked city tap and river water (collected at the Red Cedar River on campus, location: +42° 42′ 38.70″, −84° 28′ 39.09″, Michigan State University–East Lansing, USA). The general water chemistry of the Red Cedar River has been reported elsewhere [32]. Prior to the measurements, the river water was filtered once using 1 μm pore filter paper, while the tap water was used without any filtration.

3. Results and discussion

Fig. 1 shows voltammograms obtained for the pretreated BDD electrode in 0.25 mol L^{-1} H_2SO_4 in the absence and presence of 110 μmol L^{-1} estrone. For the supporting electrolyte (solid line), the voltammogram presents a capacitive current in the potential range 0.6–1.1 V, with a faradaic current at more positive potentials due to oxygen evolution reaction. In the presence of estrone (dashed line), a well-defined irreversible oxidation current peak appears at *ca.* 0.95 V. As previously described by Ngundi et al. [33] and more recently by Brocenschi et al. [25], an equal number of electrons and protons are transferred during the estrone oxidation reaction. Usually, the oxidation of undissociated phenolic molecules involves the transfer of two electrons, whereas that of dissociated molecules (phenolates) involves only one electron. Hence, under the conditions in which the voltammogram was obtained, two electrons and two protons are presumed to be transferred per molecule. Clearly, the pretreated diamond electrode is responsive for estrone, providing a well-defined oxidation peak.

Next, hydrodynamic curves (current and charge) were generated with the cathodically-pretreated BDD electrode using 250 μL injections of 4.0 μmol L^{-1} estrone in 0.5 mol L^{-1} H_2SO_4. The carrier solution flow rate was 1.0 mL min^{-1}. The applied potential was increased in 50 mV steps and each signal response was recorded at the end of a 25–30 min period. Fig. 2A shows the hydrodynamic curves for both current and charge as a function of the applied potential. Each point corresponds to the average response for 3 injections. Standard deviations are shown and are generally within the size of the marker. The estrone curve (Fig. 2A) was obtained after subtracting the background current signal (Fig. 2B). For estrone, a well-defined, sigmoidally shaped curve is seen with a mass transport limited current response at *ca.* 1.1 V and a half-wave potential of 0.98 V. This sigmoidal curve is similar to the response observed for other new diamond electrodes (data not shown). It should be noted that, positive of 1.1 V, the current increases due to the contribution of oxygen evolution. Taking these data into account, an electrode potential of 1.15 V (with a current response of about 225 nA for 4.0 μmol L^{-1} estrone) would be useful for detection.

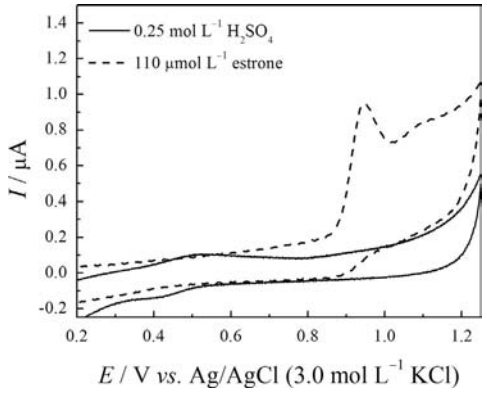

Fig. 1. Cyclic voltammograms (50 mV s^{-1}) in a 0.25 mol L^{-1} H_2SO_4 solution obtained with a cathodically pretreated BDD electrode in the absence (solid line) or presence (dashed line) of 110 μmol L^{-1} estrone.

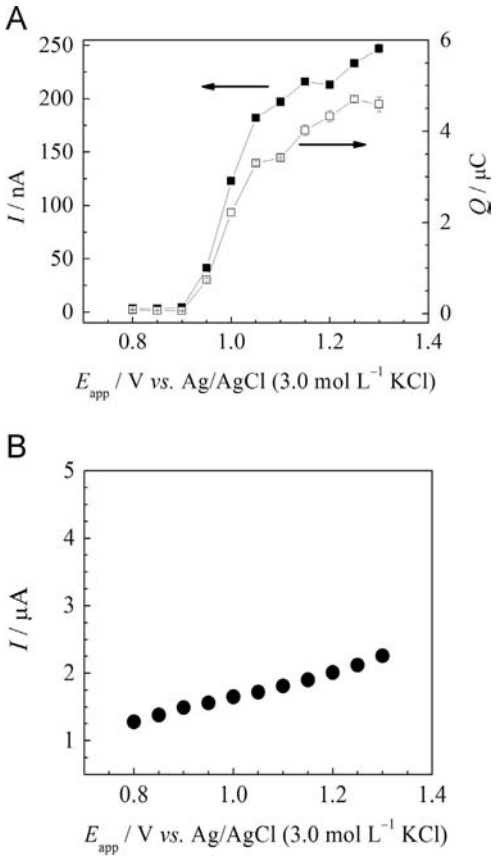

Fig. 2. (A) Hydrodynamic voltammograms for 250 μL injections of 4.0 μmol L^{-1} estrone in 0.5 mol L^{-1} H_2SO_4 using a cathodically pretreated BDD electrode. (B) Background currents at different potentials for a 0.5 mol L^{-1} H_2SO_4 solution. Each datum corresponds to the average of three injections. The carrier solution was 0.5 mol L^{-1} H_2SO_4, with a flow rate of 1 mL min^{-1}.

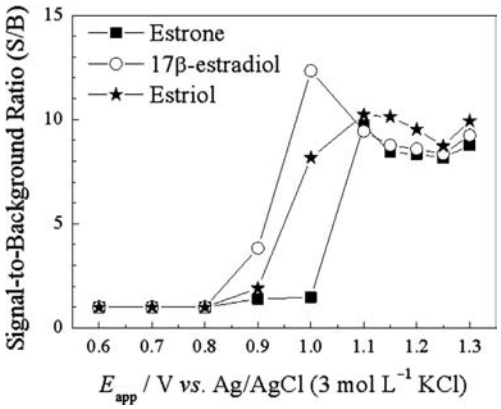

Fig. 3. Hydrodynamic voltammograms (background current corrected—I_{bkg}) obtained with a cathodically pretreated BDD electrode, for 250 μL injections of 4.0 μmol L^{-1} estrone (E1), 17-β-estradiol (E2) or estriol (E3) in 0.5 mol L^{-1} H_2SO_4 (also used as carrier solution; flow rate of 1 mL min^{-1}).

Fig. 3 shows the hydrodynamic responses for all three estrogenic compounds plotted as the signal-to-background ratio (S/B, $(I_p - I_{bkg})/I_{bkg}$) as a function of the applied potential. Each point corresponds to the average response for 3 injections. Standard deviations are not shown because they are within the size of the marker. The analysis was performed using 250 μL injections of a 4.0 μmol L^{-1} estrone (E1), 17-β-estradiol (E2) or estriol (E3)

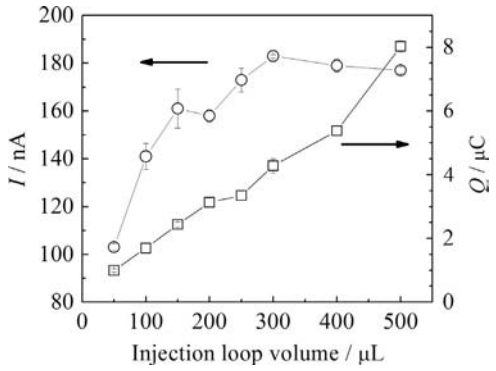

Fig. 4. Current and charge responses for estrone oxidation obtained with a cathodically pretreated BDD electrode as a function of the injection loop volume. The estrone concentration injected was 4.0 μmol L^{-1} in 0.5 mol L^{-1} H_2SO_4. Each datum is the average of three injections. The carrier solution was 0.5 mol L^{-1} H_2SO_4 at a flow rate of 1 mL min^{-1}.

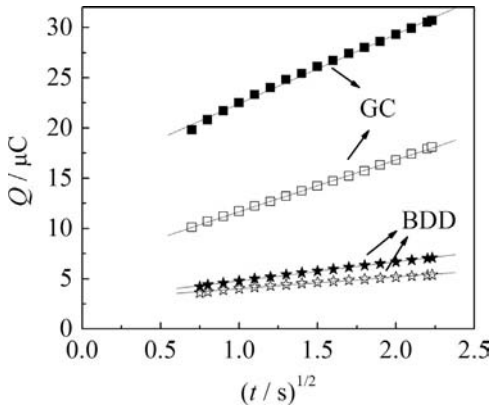

Fig. 5. Chronocoulometric plots of Q_{total} *versus* $t^{1/2}$ for the oxidation of 4.0 μmol L^{-1} estrone (filled circles) in 0.5 mol L^{-1} H_2SO_4 obtained with GC and BDD electrodes. The oxidation charge was recorded during a 5 s potential step from 0.6 to 1.2 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The responses for both electrodes in just the supporting electrolyte are shown with the open symbols.

solution in 0.5 mol L^{-1} H_2SO_4 (also used as carrier solution; flow rate of 1 mL min^{-1}). Consistent with the data shown in Fig. 2A, these results indicate that a detection potential of 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl) would be appropriate for all three compounds.

The effect of the injected volume was investigated to determine how the oxidation current (after stabilization) depends on the amount of analyte passing through the electrochemical cell. Detection was made at 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). Fig. 4 shows the corresponding current and charge responses when different volumes of 4.0 μmol L^{-1} estrone solution in 0.5 mol L^{-1} H_2SO_4 were injected. Two different responses were observed. The current response for estrone oxidation tends to be sigmoidal, whereas that for the charge tends to be linear. It is known that $Q=I\times t$, such that, when the injected volume of the estrone solution increases, the number of molecules passing through the detector with time proportionally increases, thereby having a direct influence on the charge value. The current response actually increases until the injection volume approaches 250 μL, above which a limiting response is seen. Hence, this volume provides the optimum response for estrone oxidation in terms of current magnitude.

Electrodes used in electrochemical detectors, in general, should exhibit good response sensitivity, reproducibility, and stability. One factor that will affect these detection figures of merit is site-blocking adsorption of reaction intermediates and products that cause current attenuation. In this regard, prior work revealed that GC electrodes exhibit some undesirable properties related to signal instability, higher background current, and surface adsorption when used for the detection [25]. In the first example of BDD outperforming GC, Xu et al. [34] compared two types of BDD electrodes with GC for the amperometric detection of the azide anion in FIA. Like the estrogenic compounds, detection of azide occurs at positive potentials, ~1.2 V. GC exhibited a larger and less stable background current at the detection potential than BDD did. This led to higher S/B ratios, higher limits of detection, greater response variability, and inferior response stability for the former, as compared to BDD. Hormone molecules, as previously noted, have a phenolic ring in their structure. Consequently, their electro-oxidative detection is often plagued by strong, irreversible adsorption of reactants, intermediates and/or products of the anodic reaction [24]. BDD electrodes, generally speaking, are relatively immune to electrode fouling. This is because these electrodes typically contain low levels of non-diamond carbon impurity, have no extended π-electron system and have a relatively non-polar surface when hydrogen is terminated [34]. We believe these characteristics contribute to the weak adsorption of hormones on the diamond surface, at least at a level that does not produce a totally attenuated current response [25]. The adsorption of estrone on GC and BDD was quantified using chronocoulometry. In this measurement, the total electrolysis charge is given by the following equations [35,36]:

$$Q_{total} = Q_{faradaic} + Q_{dl} + Q_{ads} \quad (1)$$

$$Q_{total} = \frac{2nFAD^{1/2}Ct^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma \quad (2)$$

where Γ is the adsorbate surface coverage (mol cm^{-2}) and the other terms have their usual meaning (*e.g.*, C=conc., mol/cm^3, A=area, cm^2, D=diffusion coefficient, cm^2/s). The first term in the expression is the faradaic charge associated with the semi-infinite linear diffusion-controlled current, the second term is the double-layer charge, and the third term the charge associated with any adsorbed analyte [35–37]. Fig. 5 shows the corresponding chronocoulometric Q *vs.* $t^{1/2}$ curves for the oxidation of estrone on the GC and BDD electrodes, obtained using A=0.32 cm^2 and c=4.0 μmol L^{-1}. The difference between the intercepts of plots (Q_{total} (filled markers) and Q_{dl} (open markers)) in the presence and absence of estrone is equal to Q_{ads}, *i.e.* the charge required to instantaneously oxidize all adsorbed estrone molecules, from which the value of Γ can be calculated.

The data reveal that there is significantly greater adsorption of estrone on GC than on BDD. We did not measure the other two estrogenic compounds but presume that both would exhibit adsorption on GC. An estrone surface coverage (*i.e.*, electroactive surface coverage) of 167 pmol cm^{-2} was found for GC, which is three orders of magnitude higher than the apparent coverage found for BDD, 0.65 pmol cm^{-2}. The GC surface consists of a significant fraction of exposed edge plane where polar carbon–oxygen functionalities exist and where the density of electronic states is high [38]. Both contribute, as well as the extended π-electron system, to the strong adsorption of estrone [25]. In related work, McDermott and McCreery [38] proposed that different quinone molecule adsorption on GC depends on electronic effects such as electrostatic attraction with partially charged carbon atoms near a surface defect. In contrast, Xu et al. [37] have shown that adsorption of one of these same anthraquinone molecules (2,6-anthraquinonedisulfonate) is virtually non-existent on hydrogen-terminated BDD. The strong estrone adsorption on GC is likely due, in part, to the presence of polar carbon–oxygen functional groups terminating edge plane and defect sites [37,38]. These functionalities promote strong dipole–dipole and ion–dipole

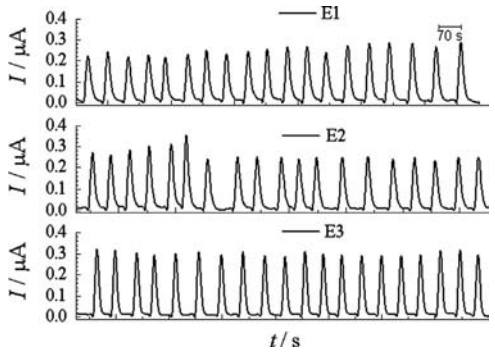

Fig. 6. FIA-EC responses obtained with a cathodically pretreated BDD electrode for 20 repetitive 250 μL injections of 10 μmol L^{-1} estrone (E1), 17-β-estradiol (E2), or estriol (E3) in 0.5 mol L^{-1} H_2SO_4 (also used as carrier solution; flow rate of 1 mL min^{-1}). Detection was made at E_{app} = 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl).

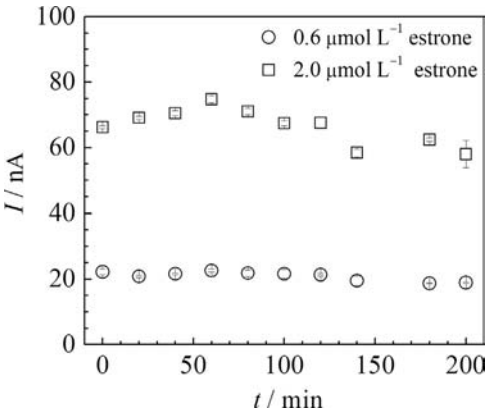

Fig. 7. FIA-EC results (n = 3) showing the short-term response stability for multiple injections of two different concentrations of estrone (indicated in the figure) in 0.5 mol L^{-1} H_2SO_4. Detection was made using a cathodically pretreated BDD electrode at E_{app} = 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The injection volume of the estrone solution was 250 μL, the carrier solution was 0.5 mol L^{-1} H_2SO_4, and the flow rate was 1 mL min^{-1}.

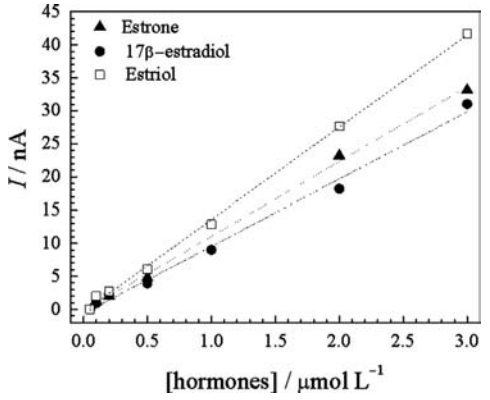

Fig. 8. Analytical response curves (n = 3) for estrone (E1), 17-β-estradiol (E2), and estriol (E3) obtained using a cathodically pretreated BDD electrode. Detection was made at E_{app} = 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The solutions of the estrogens (0.1, 0.20, 0.5, 1.0, 2.0 and 3.0 μmol L^{-1}) were prepared in 0.5 mol L^{-1} H_2SO_4 (also used as carrier solution; flow rate of 1 mL min^{-1}). The injection volume was 250 μL.

interactions with estrone. Very recently, using similar experimental conditions, we reported low estrone adsorption values (Γ = 12 pmol cm^{-2}) for a BDD electrode with a boron-doped level of *ca.* 2000 ppm [25]. For that electrode, we did not use any hydrogen plasma treatment of the surface prior to use, only electrode cleaning by immersion in ultraclean isopropanol for 20 min. According to Salazar-Banda et al. [23], a hydrogen surface termination on cathodically pretreated BDD electrodes is produced and resistant to air oxidation when the boron-doping level is high. In our present work, the boron-doping level of the cathodically pretreated diamond electrode was high (*ca.* 8000 ppm), consequently a stable hydrogen surface termination is expected. The low adsorption on diamond is presumably due to (i) the hydrogen-terminated surface, produced by the cathodic pretreatment (which gives the surface a nonpolar character), and (ii) the absence of an extended π-electron system.

Fig. 6 shows the responses for repetitive 250 μL injections of 10 μmol L^{-1} estrone (E1), 17-β-estradiol (E2), or estriol (E3) in 0.5 mol L^{-1} H_2SO_4 (also used as carrier solution; flow rate of 1 mL min^{-1}) obtained using a cathodically pretreated BDD electrode. Detection was made at E_{app} = 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The response reproducibility (RSD) over 20 injections was 9.6% for estrone, 11.4% for 17-β-estradiol and 10.8% for estriol. These variabilities are higher than those desired and are generally higher than the typical values for diamond of < 5% [34]. Actually, these data are the worst-case scenario for the electrode as we have other data for estrone showing a 2.3% response variability over 10 injections. We believe that these response variabilities can be consistently lowered with a change in the flow cell design and use of high-precision analyte injection. At this point it should be mentioned that there is generally some response attenuation (30%–40%) for these estrogenic compounds when using a freshly pretreated BDD electrode for the first time. The response, however, stabilizes after few injections. These reproducibility data were obtained after the initial response attenuation.

The longer-term stability of the BDD electrode response was evaluated by making repeated injections of estrone over ~3 h period. Measurements were made using two different estrone concentrations (0.60 and 2.0 μmol L^{-1}, both in 0.5 mol L^{-1} H_2SO_4). Detection was made at 1.15 V. The results are shown in Fig. 7. Each marker represents the average response for three injections using a 2 min interval between each. It can be seen that the BDD electrode exhibits excellent response stability over this period, with maximum RSD values of 7.5 and 2.0% for 0.60 and 2.0 μmol L^{-1} concentrations, respectively.

Analytical response curves (n = 3) for estrone (E1), 17-β-estradiol (E2) and estriol (E3), obtained using the cathodically pretreated BDD electrode, are shown in Fig. 8. Detection was made at E_{app} = 1.15 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The estrogen solutions (0.1, 0.20, 0.5, 1.0, 2.0, and 3.0 μmol L^{-1}) were prepared in 0.5 mol L^{-1} H_2SO_4 (also used as the carrier solution; flow rate of 1 mL min^{-1}). The injection volume was 250 μL. Table 1 shows some of the detection figures of merit. The sensitivities range from 9 to 14 mA L mol^{-1}, with the most sensitive response seen for estriol. Good linearity was observed for all the compounds over the whole concentration range probed ($R^2 \geq 0.990$). The lowest concentration measured ($S/N \geq 3$) for all three analytes was 0.10 μmol L^{-1} (~27 μg L^{-1}), which was close to the limit of detection.

To evaluate the intra- and inter-day repeatability of estrone determination using the cathodically pretreated BDD electrode, five successive injections in the FIA system of 0.10, 1.0, and 1.7 μmol L^{-1} estrone solutions were carried out for over three days. From the obtained results (see Table 2), we can conclude that the new method here reported for estrone detection using FIA in the amperometric mode with a cathodically pretreated BDD electrode shows excellent accuracy (recovery) and precision (standard deviation), with values in the range 94%–110% and 0.8%–7.1%, respectively.

Table 1
Summary of the analytical detection figures of merit for the FIA-EC determination of estrogenic compounds using a cathodically pretreated BDD electrode.

Compounds	Linear range/(μmol L^{-1})	Sensitivity/(mA L mol^{-1})	Lowest conc. detected (μmol L^{-1})	R^2
Estrone (E1)	0.10–3.0	11	0.10 ($S/N \geq 3$)	0.998
17-β-estradiol (E2)	0.10–3.0	9	0.10 ($S/N \geq 3$)	0.990
Estriol (E3)	0.10–3.0	14	0.10 ($S/N \geq 3$)	0.998

One measurement of each sample set was used to generate these data.

Table 2
FIA-EC results for accuracy and day-to-day reproducibility for estrone (E1) detection using a cathodically pretreated boron-doped diamond electrode.

	1st day		**2nd day**		**3rd day**	
$[E1]_{prepared}$/μmol L^{-1}	$[E1]_{found}$/μmol L^{-1}	SD/%	$[E1]_{found}$/μmol L^{-1}	SD/%	$[E1]_{found}$/μmol L^{-1}	SD/%
0.10	0.11	4.8	0.11	5.2	0.11	7.1
1.0	1.1	1.8	1.0	4.8	1.0	5.9
1.7	1.7	0.8	1.7	2.4	1.6	2.2

SD = standard deviation ($n = 5$)

Table 3
Recovery studies of estrone (E1) in spiked city tap and river water samples using FIA-EC detection with a cathodically pretreated BDD electrode.

	Tap water			**River water**		
$[E1]_{added}$/μmol L^{-1}	$[E1]_{found}$/μmol L^{-1}	SD/%	Recovery/%	$[E1]_{found}$/μmol L^{-1}	SD/%	Recovery/%
0.10	0.11	3.0	110	0.098	9.0	98
1.0	0.94	2.4	94	0.97	6.9	97
1.7	1.6	1.5	94	1.5	4.0	88

SD = standard deviation ($n = 5$)

The BDD electrode response was further evaluated in FIA-EC using two real samples ($n = 5$) to carry out recovery studies using city tap and river water spiked with estrone. As can be seen in Table 3, recovery values for estrone in two types of water matrices ranged from 94 to 110% and 88 to 98%, respectively. These results indicate that the diamond electrode provides an accurate response for estrone in these two contrasting real matrices, and that the complexities of the river water do not adversely affect the electrode response.

While improvement is possible, the current detection figures of merit for BDD compared well with other electrochemical detection methods reported in the literature, especially when considering most of the other reported methods made use of some form of sample pre-concentration prior to analysis. Our minimum concentration detected for the three estrogenic compounds, 0.10 μmol L^{-1} (~27 μg L^{-1}), is comparable to limits of detection reported in the literature for other estrogenic compounds [19,20,39]. Liu et al. [20] and Vega et al. [39] compared the electrochemical detection of phenolic estrogens at Ni (II)tetrakis(4-sulfonatophenyl) porphyrin (NITPPS)/carbon nanotube composite and carbon nanotube-modified GC (CTN-GCE) electrodes, respectively, using HPLC-EC. Their reported limits of detection for ethinylestradiol were higher (0.120 and 0.340 μmol L^{-1}, respectively) than our value. Yamada et al. [19] used a multi-electrode electrochemical detector to determine estradiol using HPLC-EC and obtained limits of detection of 8 ng mL^{-1} (0.03 μmol L^{-1}) for a standard solution and 24 ng mL^{-1} (0.089 μmol L^{-1}) for a rat plasma sample. Solid phase microextraction has been used with HPLC-EC for the determination of estrogenic compounds [40]. The method enabled limits of detection of 0.06–0.08 μg L^{-1} with 17-β-estradiol being detected in wastewater at 1.9–2.2 μg L^{-1}. Gan et al. [41] reported on a voltammetric study of different estrogenic compounds (including E1, E2, and E3) at an oxidized BDD electrode. They also reported on an electroanalytical study of estradiol by square-wave voltammetry using this electrode. Limits of detection were reported in the 1–100 μmol L^{-1} range (~0.27–27 mg L^{-1} for estrone). The limits of detection were lowered by three orders of magnitude, 5–100 nmol L^{-1} (~1.4–27 μg L^{-1} for estrone) by modifying the diamond electrode with nanoscopic carbon powder, which served to pre-concentrate the analytes for detection. Kanso et al. [42] reported on the use of electrochemical immunosensors for 17-β-estradiol and ethinylestradiol in spiked and natural water samples. The sensing platform used pre-concentration with modified magnetic beads on a screen-printed electrode. Limits of detection were in the 1–10 ng L^{-1} range. Finally, Martinez et al. [21] reported on the detection of ethinylestradiol (EE2) at a multi-walled carbon nanotube-modified GC electrode. Pre-concentration prior to detection was achieved using modified magnetic nanoparticles. They reported that the method can be used to detect the analyte in the 0.035–70 ng L^{-1} range with a limit of detection of 0.01 ng L^{-1} and a response variability of 4.2%.

Finally, while FIA-EC is an excellent method for assessing the BDD electrode response for these estrogenic compounds individually, it would be of limited use in the analysis of real samples that contain multiple analytes. HPLC-EC would be needed for such an analysis. To this end, we present preliminary HPLC-EC measurements of five estrogenic compounds including E1, E2, and E3. The measurements were made using a cathodically pretreated BDD electrode different from the one used in the FIA-EC studies but similar in terms of electrical conductivity. Fig. 9A shows a typical reversed-phase chromatogram for the compounds and Fig. 9B shows the hydrodynamic voltammograms obtained by HPLC-EC, which were used to determine the detection potential of $E_{app} = 1.35$ V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl) used for detection in Fig. 9A. The BDD electrode was cathodically pretreated prior to injection of 250 μL of the analytes solution. The analytes were dissolved in a phosphate buffer (pH 3.0)/acetonitrile (55:45 v/v) mixture, the same solution that was used as the mobile phase. The analyte solution consisted of 4.0 μmol L^{-1} E1, E2, E3, 17α-ethinylestradiol (EE2) and diethylstilbestrol (DES). The flow rate was 1 ml min^{-1}. Separation of all five analytes with baseline resolution was achieved isocratically in about 16 min. The

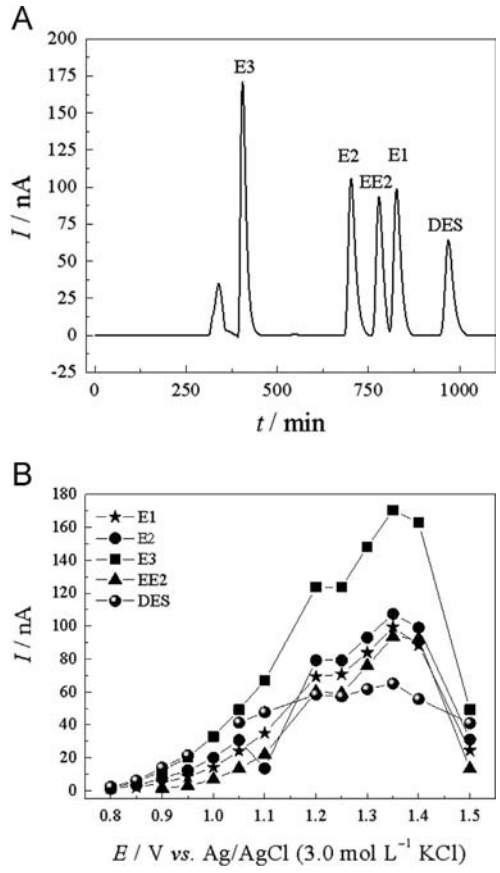

Fig. 9. (A) Reversed-phase HPLC chromatogram for 4.0 μmol L^{-1} estrone (E1), 17-β-estradiol (E2), estriol (E3), 17-α-ethinylestradiol (EE2), and diethylstilbestrol (DES) in a phosphate buffer (pH 3.0)/acetonitrile (55:45 v/v) solution. This solution also served as the mobile phase. The flow rate was 1 mL min^{-1}, the injection volume was 250 μL and the detection was made at E_{app} = 1.35 V *vs.* Ag/AgCl (3.0 mol L^{-1} KCl). The diamond electrode was cathodically pretreated prior to use. (B) The corresponding hydrodynamic voltammograms for these estrogenic compounds obtained by HPLC using the same conditions as indicated above.

hydrodynamic voltammograms reveal that the optimum detection potential for all five analytes is 1.35 V. This potential in the mixed organic/aqueous electrolyte mobile phase is a little more positive than that seen for the FIA measurements in 0.5 mol L^{-1} H_2SO_4. These preliminary results suggest that BDD electrodes can provide a useful analytical response for these estrogenic compounds when coupled with HPLC. Future work will focus on determining the detection figures of merit in the HPLC-EC mode and applying the method to the analysis of endogenous levels of estrogenic compounds in water samples and biological fluids.

4. Conclusions

The initial testing and application of an FIA-EC method for the determination of estrogenic compounds revealed that cathodically-pretreated boron-doped diamond could provide good detection figures of merit. Importantly, the response attenuation (*i.e.*, fouling) seen for a commonly used carbon electrode, like glassy carbon, is not seen for diamond. The pretreated diamond electrode provided low and stable background current, a relatively low minimum detectable concentration without any analyte preconcentration, and good response stability. Unlike for glassy carbon, the estrone oxidation reaction on the pretreated diamond does not involve significant adsorption. After an initial response attenuation, the diamond electrode exhibited short-term response reproducibility for estrone as low as 2.3% RSD and good longer-term response stability (3 h) with response changes of 7.5% and 2.0% RSD, respectively, for injections of 0.6 and 2.0 μmol L^{-1}. For estrone, 17-β-estradiol, and estriol, response linearity was observed over the concentration range tested, 0.10–3.0 μmol L^{-1}, the sensitivity was *ca.* 10 mA L mol^{-1}, and the minimum concentration detection ($S/N \geq 3$) was 0.10 μmol L^{-1} (~27 μg L^{-1}). Estrone recoveries in spiked river and city tap water samples presented less than 10% RSD at different concentrations, indicating that the diamond electrode provides an accurate response for estrone in these two contrasting real matrices, and that the complexities of the river water do not adversely affect the electrode response. Finally, preliminary results indicate that diamond electrodes can provide a useful analytical response for these estrogenic compounds when coupled with HPLC.

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References

[1] E. Diamanti-Kandarakis, J.-P. Bourguignon, L.C. Giudice, R. Hauser, G.S. Prins, A.M. Soto, R.T. Zoeller, A.C. Gore, Endocr. Rev. 30 (2009) 293–342.
[2] P.K. Jjemba, Pharma-ecology: The occurrence and fate of pharmaceuticals and personal care products in the environmentWiley, Hoboken, 2008.
[3] R. Dominica Briciu, A. Kot-Wasik, J. Namiesnik, J. Chromatogr. Sci. 47 (2009) 127–139.
[4] N.A. Martínez, R.J. Schneider, G.A. Messina, J. Raba, Biosens. Bioelectron. 25 (2010) 1376–1381.
[5] J.D. Meeker, Arch. Pediatr. Adolesc. Med. 166 (2012) 952–958.
[6] J.Q. Jiang, Q. Yin, J.L. Zhou, P. Pearce, Chemosphere 61 (2005) 544–550.
[7] Y. Zhou, J. Zha, Y. Xu, B. Lei, Z. Wang, Environ. Monit. Assess. 184 (2011) 1719–1729.
[8] Z. Jin, S. Daiya, H.I. Kenttämaa, Int. J. Mass Spectrom. 301 (2011) 234–239.
[9] N. Migowska, M. Caban, P. Stepnowski, J. Kumirska, Sci. Total Environ. 441 (2012) 77–88.
[10] O.T. Komesli, S. Bakirdere, C. Bayören, C.F. Gökçay, Environ. Monit. Assess. 184 (2012) 5215–5224.
[11] S. Liu, G.-G. Ying, J.-L. Zhao, F. Chen, B. Yang, L.-J. Zhou, H.J. Lai, J. Chromatogr. A 1218 (2011) 1367–1378.
[12] D. Matějíček, J. Chromatogr. A 1231 (2012) 52–58.
[13] M. Muz, M.S. Sonmez, O.T. Komesli, S. Bakirdere, C.F. Gokcay, Analyst 137 (2012) 884–889.
[14] M.J.L. de Alda, D. Barceló, J. Chromatogr. A 911 (2001) 203–210.
[15] R.T. Verbinnen, G.S. Nunes, E.M. Vieira, Quim. Nova 33 (2010) 1837–1842.
[16] C. Ribeiro, M.E. Tiritan, E. Rocha, M.J. Rocha, Arch. Environ. Contam. Toxicol. 56 (2009) 1–11.
[17] C. Almeida, J.M.F. Nogueira, J. Pharm. Biomed. Anal. 41 (2006) 1303–1311.
[18] A.P. Fonseca, D.L.D. Lima, V.I. Esteves, Water, Air, Soil Pollut. 215 (2011) 441–447.
[19] H. Yamada, K. Yoshizawa, T. Hayase, J. Chromatogr. B 775 (2002) 209–213.
[20] X. Liu, H. Feng, X. Liu, D.K.Y. Wong, Anal. Chim. Acta 689 (2011) 212–218.
[21] N.A. Martínez, S.V. Pereira, F.A. Bertolino, R.J. Schneider, G.A. Messina, J. Raba, Anal. Chim. Acta 723 (2012) 27–32.
[22] Q. He, S. Yuan, C. Chen, S. Hu, Mater. Sci. Eng. C 23 (2003) 621–625.
[23] G.R. Salazar-Banda, L.S. Andrade, P.A.P. Nascente, P.S. Pizani, R.C. Rocha-Filho, L.A. Avaca, Electrochim. Acta 51 (2006) 4612–4619.
[24] G.W. Muna, N. Tasheva, G.M. Swain, Environ. Sci. Technol. 38 (2004) 3674–3682.
[25] R.F. Brocenschi, R.C. Rocha-Filho, L. Li, G.M. Swain, J. Electroanal. Chem. 712 (2014) 207–214.
[26] L.S. Andrade, R.C. Rocha-Filho, Q.B. Cass, O. Fatibello-Filho, Anal. Methods 2 (2010) 402–407.
[27] R.A. Medeiros, B.C. Lourencao, R.C. Rocha-Filho, O. Fatibello-Filho, Anal. Chem. 82 (2010) 8658–8663.

[28] R.A. Medeiros, B.C. Lourencao, R.C. Rocha-Filho, O. Fatibello-Filho, Talanta 99 (2012) 883–889.
[29] L.S. Andrade, M.C. de Moraes, R.C. Rocha-Filho, O. Fatibello-Filho, Q.B. Cass, Anal. Chim. Acta 654 (2009) 127–132.
[30] H.B. Suffredini, V.A. Pedrosa, L. Codognoto, S.A.S. Machado, R.C. Rocha-Filho, L.A. Avaca, Electrochim. Acta 49 (2004) 4021–4026.
[31] G.R. Salazar-Banda, A.E. de Carvalho, L.S. Andrade, R.C. Rocha-Filho, L.A. Avaca, J. Appl. Electrochem. 40 (2010) 1817–1827.
[32] S.P. McElmurry, D.T. Long, T.C. Voice, Appl. Geochem. 25 (2010) 650–660.
[33] M.M. Ngundi, O.A. Sadik, T. Yamaguchi, S. Suye, Electrochem. Commun. 5 (2003) 61–67.
[34] J. Xu, G.M. Swain, Anal. Chem. 70 (1998) 1502–1510.
[35] F.C. Anson, Anal. Chem. 38 (1966) 54–57.
[36] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, Wiley, New York, 2000.
[37] J. Xu, Q. Chen, G.M. Swain, Anal. Chem. 70 (1998) 3146–3154.
[38] M.T. McDermott, R.L. McCreery, Langmuir 10 (1994) 4307–4314.
[39] D. Vega, L. Agüí, A. González-Cortés, P. Yáñez-Sedeño, J.M. Pingarrón, Talanta 71 (2007) 1031–1038.
[40] A. Peñalver, E. Pocurull, F. Borrull, R.C. Marcé, J. Chromatogr. A 964 (2002) 153–160.
[41] P. Gan, R.G. Compton, J.S. Foord, Electroanal 25 (2013) 2423–2434.
[42] H. Kanso, L. Barthelmelo, N. Inguimbert, T. Noguer, Anal. Chem. 85 (2013) 2397–2404.