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Electrochemical detection of phenolic estrogenic compounds at carbon nanotube-modified electrodes

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Abstract

The use of a carbon nanotube-modified glassy carbon electrode (CNT-GCE) for the LC–EC detection of phenolic compounds with estrogenic activity is reported. Cyclic voltammograms for phenolic endocrine disruptors and estrogenic hormones showed, in general, an enhancement of their electrochemical oxidation responses at CNT-GCE attributable to the electrocatalytic effect caused by CNTs. Hydrodynamic voltammograms obtained under flow injection conditions lead to the selection of +700 mV as the potential value to be applied for the amperometric detection of the phenolic estrogenic compounds, this value being remarkably less positive than those reported in the literature using other electrode materials. Successive injections of these compounds demonstrated that no electrode surface fouling occurred. A mobile phase consisting of a 50:50 (v/v) acetonitrile:0.05 mol l^{-1} phosphate buffer of pH 7.0 was selected for the chromatographic separation of mixtures of these compounds, with detection limits ranging between 98 and 340 nmol l^{-1}. Good recoveries were obtained in the analysis of underground well water and tap water samples spiked with some phenolic estrogenic compounds at a 14 nmol l^{-1} concentration level.

Keywords: Carbon nanotube-modified electrodes; Phenolic estrogenic compounds; Amperometric detection; Liquid chromatography

1. Introduction

Nowadays, it is well known that some pesticides and industrial products called endocrine-disruptors chemicals (EDCs) exhibit hormones-like behaviour [1–3]. The effects of EDCs on human reproduction is probably due to their ability to mimic the function of natural estrogens as well as to disrupt the synthesis and metabolism of such hormones by binding the hormonal receptors. Some alkyl phenols produced in a high extent in many industrial applications have been identified as xenoestrogens. Examples are disinfectants such as hydroxybiphenyls, or bisphenols, i.e. BPA used for the fabrication of polycarbonate packages. Besides EDCs, natural or synthetic estrogens used for birth control that are excreted by women, such as 17α-estradiol (EE2) or β-estradiol (E2) are also matter of concern due to their environmental impact. Recently, the European Commission elaborated a list of prioritary endocrine disruptors, and the admissible concentration limits as diary doses were fixed, in a first approximation, in 15 mg/kg [4].

Gas chromatography is the commonly used technique for trace analysis of estrogenic phenols [5–9]. GC–MS provides useful analytical information for the identification and quantification of these compounds. However, the required sample treatment procedures, which involve pre-purification, fractionation, hydrolysis and derivatization, limit its application for complex real samples. Liquid chromatography coupled with mass spectrometry (LC–MS) has demonstrated to be a useful technique for the analysis of phenolic estrogenic compounds in environmental and biological samples [10–13]. Also, fluorescence [14] and UV [15] have been used as detection modes. HPLC with coulometric detection has been successfully applied for the determination of bisphenol A (BPA) in human serum [16] and urine [17]. This detection mode implied the use of multi-electrodes due to the good selectivity that can be achieved by profiting from the differences in the redox behaviour of phenol derivatives at the electrode array, each electrode being maintained at a different potential value. Amperometric detection has also been employed, and so the analysis of river waters containing a mixture of EDCs and estrogenic phenols was accomplished

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by using a glassy carbon electrode (GCE) at +1.0 V, after preconcentration by SPME [18].

Electrochemical studies of alkyl phenols with estrogenic properties have been recently reported. The voltammetric behaviour of xenoestrogens 4-nonylphenol (NP) and BPA at a platinum electrode has been compared with that of β-estradiol and other natural hormones [19]. Moreover, the electrooxidation of BPA was also studied at a glassy carbon electrode [20]. Carbon fiber electrodes were used to carry out the electrochemical removal of NP [21] and BPA [22]. Finally, an amperometric tyrosinase biosensor in which the enzyme was entrapped within a poly(thionine) film, has been used to detect phenolic endocrine disruptors [23].

As a consequence of the unique electronic and conducting properties of carbon nanotubes (CNTs), modified electrodes with CNTs have demonstrated to improve the electroanalytical performance of different species [24,25]. Concerning electrooxidation of phenols, it is well known that phenoxy radicals and non-conducting/passivating polymeric materials are produced causing an irreversible fouling at glassy carbon electrode surface [26]. However, using CNT-modified electrodes, an improvement in the stability of voltammetric and amperometric responses of such compounds [27], and an enhancement of the electron-transfer kinetics for catecholamines and NADH [28,29] has been observed.

In this article, we report on the development of a method for the determination of phenolic estrogens based on the amperometric monitoring of their oxidation responses at CNTs-modified glassy carbon electrodes. A high sensitivity, together with a good repeatability of the measurements and a good reproducibility of the responses obtained with different modified electrodes have been achieved. The method has been applied to the analysis of spiked water samples containing low concentration levels of these compounds previous separation by HPLC.

2. Experimental

2.1. Apparatus and electrodes

Voltammetric measurements were carried out with a BAS 100B potentiostat provided with a BAS C2 EG-1080 cell stand. A Metrohm 6.084.010 glassy carbon electrode (3 mm Ø) modified with carbon nanotubes or unmodified, was used as working electrode. Carbon paste (CPE) and carbon felt (CFE) electrodes prepared as reported previously [30,31], and a BAS MF 2013 Pt electrode (1.6 mm Ø), were also used for comparative studies. The reference electrode was a BAS MF 2063 Ag/AgCl 3 M electrode, and a BAS MW-1032 Pt wire was used as the auxiliary electrode. BAS VC-2 10 ml electrochemical cells were also used. Chromatographic and flow injection experiments were performed using a Knauer 1000 Smartline pump provided with a Knauer 5000 Smartline Manager stand, and a Knauer A 1357 injection valve provided with a 50 μl coil. A Luna C_{18} (150 mm × 4.6 mm i.d., 5 μm particle size) (Phenomenex) chromatographic column was used. The flow-cell was a Metrohm EA-1096 wall-jet cell provided with a Ag/AgCl reference electrode and a gold counter electrode. The potential values applied were controlled by means of a BAS Epsilon Multichannel detector, and the Chromgraph 1.0.01 software (Liquid Chromatography Control Software) from BAS was used to record data.

2.2. Reagents and solutions

Stock 5.0×10^{-2} mol l^{-1} solutions of bisphenol A (BPA), 2-hydroxybiphenyl (OPP), 4-nonylphenol (NP), 2,3-dimethylphenol (DMP), 17α-ethynylestradiol (EE2), β-estradiol (E2), estriol (E3), estrone (E1) and 4-terbutylphenol (TBP) (Sigma, 99%), were prepared in acetonitrile (SDS, HPLC reagent grade). More diluted solutions were prepared by suitable dilution with the supporting electrolyte solution. A 0.05 mol l^{-1} phosphate buffer solution of pH 7.0 or a 0.1 mol l^{-1} Britton–Robinson buffer solution were used as the supporting electrolytes. Other solvents and chemicals used were of analytical reagent grade and water was obtained from a Millipore Milli-Q purification system. Multi-wall carbon nanotubes (MWCNTs, 30 ± 15 nm Ø), with a 95% purity were obtained from NanoLab (Brighton, MA), and were used without any pretreatment.

2.3. Procedures

2.3.1. Preparation of the CNTs-modified GCE

Prior to the modification, the glassy carbon electrode surface was polished with 0.3 μm alumina slurries, rinsed thoroughly with double distilled water, sonicated 30 s into water and 30 s into acetone, and dried in air. One milligram of CNTs was dispersed with the aid of ultrasonic stirring for 45 min in an aqueous 0.1% Nafion solution. A 10 μl aliquot of this dispersion was dropped on the GC electrode surface and then the solvent was evaporated under an infrared heat lamp.

2.3.2. HPLC with amperometric detection at the CNT-GCE

Chromatographic separation of mixtures of estrogenic and xenoestrogenic compounds were performed using a 50:50 acetonitrile–0.05 mol l^{-1} phosphate buffer of pH 7.0 as the mobile phase, which was previously filtered through a 0.1 μm membrane. A flow rate of 0.8 ml min^{-1} and an injection volume of 50 μl were used. A detection potential of +0.7 V versus Ag/AgCl was applied.

2.3.3. Determination of estrogenic phenols in water samples

Water (500 ml) spiked with bisphenol A, 2,3-dimethylphenol, 17α-ethynylestradiol and 4-terbutylphenol, at a 14 nmol l^{-1} concentration level for each compound, was treated with 1% methanol and acidified to pH 6 with HCl. Then, the sample was passed through C_{18} and styrene divinyl benzene (SDB) disks which were previously conditioned with 10 ml acetonitrile, 10 ml methanol and 20 ml distilled water successively. The C_{18} disk was set on top of the SDB disk; the sample was percolated at a flow rate of 50 ml min^{-1}, and eluted with three portions of 3.0 ml acetonitrile. The SPE eluate was evaporated to dryness under nitrogen atmosphere and the dried residue was reconstituted in 500 μl of acetonitrile.

3. Results and discussion

3.1. Voltammetric behaviour of phenolic estrogenic compounds at CNT-GCE

Fig. 1 shows the comparison of cyclic voltammograms obtained at a CNT-GCE and at an unmodified GCE for 1.0×10^{-4} mol l^{-1} solutions of OPP (A), BPA (B) and NP (C) in 0.05 mol l^{-1} phosphate buffer of pH 7.0. As it can be seen, OPP and BPA exhibit well-defined oxidation peaks at +575 mV (OPP) and +590 mV (BPA) versus Ag/AgCl, at the CNT-GCE, whose peak currents are considerably larger than those obtained at the bare GCE. Moreover, a slight potential shift towards less positive potentials is also observed with the CNT-GCE (see Table 1). In addition, the cyclic voltammogram for OPP showed a small cathodic peak at +267 mV preceded by a shoulder at +350 mV. Background voltammograms (dotted line) showed a slightly higher capacitive current at the CNT-GCE, which becomes more evident at more positive potential values. However, at the potential values at which the oxidation peaks are developed, the difference in capacitive current between both electrodes is practically negligible. On the contrary to that observed for OPP and BPA, the oxidation peak obtained for NP at the CNT-GCE, with an E_p value of +606 mV, was poorly defined and the peak current was smaller than that at the bare GCE.

Table 1
Peak potential values (V) and current densities per concentration unit (μA cm^{-2} mol^{-1} l) (in brackets) for the voltammetric oxidation of some phenolic xenoestrogens at different electrode materials

Electrode	BPA	OPP	NP
CNT-GCE	+0.59[a] (1.0×10^6)	+0.58[a] (1.1×10^6)	+0.60[a] (2.1×10^5)
GCE	+0.62[a] (3.5×10^5)	+0.65[a] (3.5×10^5)	+0.60[a] (3.2×10^5)
PtE [19]	+0.90[b] (1.0×10^5)		+1.05[b] (1.7×10^5)
CPE	+0.60[a] (4.1×10^5)	+0.61[a] (6.1×10^5)	+0.59[a] (4.2×10^5)
CFE [31]	+0.54[a] (3.8×10^6)	+0.60[a] (2.2×10^6)	+0.51[a] (9.3×10^6)

Peak potentials obtained by CV vs. Ag/AgCl.
[a] 0.05 mol l^{-1} PBS pH 7.0.
[b] 0.05 mol l^{-1} TBAP/H_2O/acetonitrile (50:50).

Cyclic voltammograms recorded for other phenolic endocrine disruptors and estrogenic hormones: 2,3-dimethylphenol (DMP), 4-terbutylphenol (TBP), 17α-ethynylestradiol (EE2), β-estradiol (E2) and estriol (E3), at both CNT-GCE and unmodified GCE, are displayed in Fig. 2. As it can be seen,

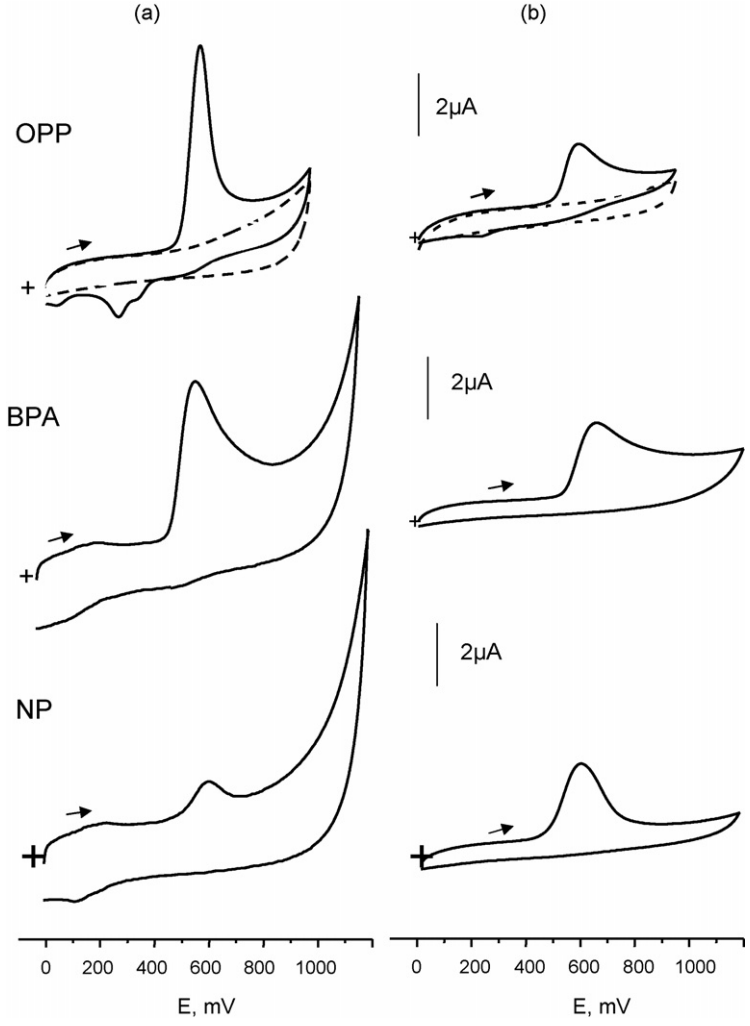

Fig. 1. Cyclic voltammograms at a CNT-GCE (a) and at a bare GCE (b) for 1.0×10^{-4} mol l^{-1} 4-hydroxybiphenyl (OPP), bisphenol A (BPA) and 4-nonylphenol (NP) in 0.05 mol l^{-1} phosphate buffer solution of pH 7.0. (- - -) Background voltammograms; $v = 50$ mV s^{-1}.

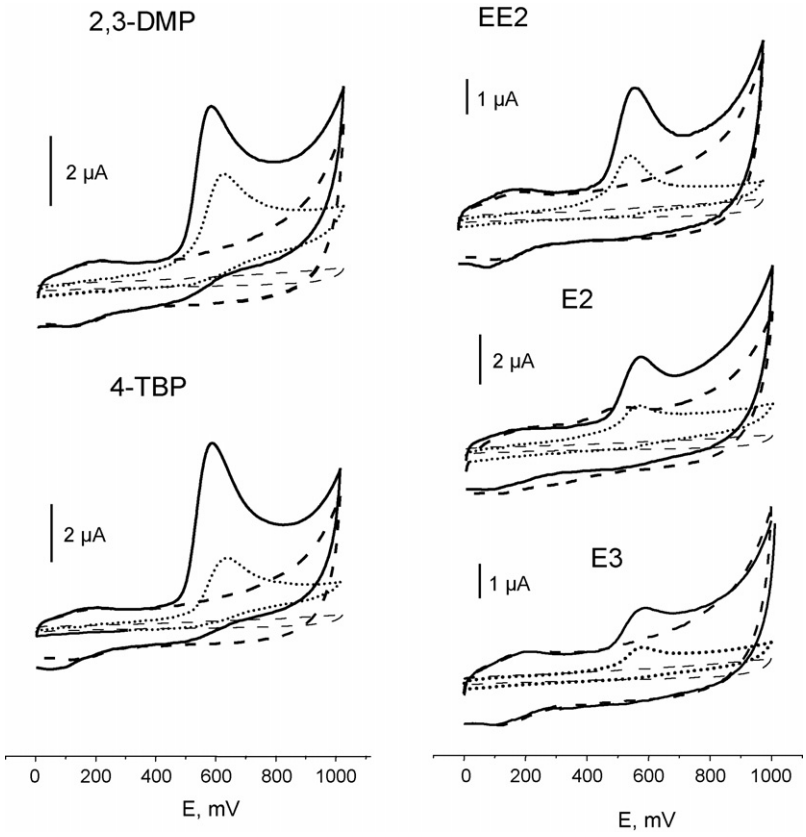

Fig. 2. Cyclic voltammograms at a CNT-GCE (—) and at a bare GCE (···) for 1.0×10^{-4} mol l^{-1} 2,3-dimethylphenol (2,3-DMP), 4-tertbutylphenol (4-TBP), 17α-ethynylestradiol (EE2), β-estradiol (E2) and estriol (E3) in 0.05 mol l^{-1} phosphate buffer solution of pH 7.0; (- - -) and (– – –) background voltammograms at CNT-GCE and GCE, respectively.

modification of the electrode surface with CNTs produced an enhancement of the electrochemical oxidation response for these compounds, particularly in the case of DMP and TBP.

Voltammetric parameters for OPP, BPA and NP at the CNT-GCE were also compared with those experimentally obtained or reported in the literature using other different electrode materials (Table 1). The structures of these phenols are presented in Fig. 3. BPA also showed an irreversible behaviour at Pt, carbon felt (CF) or carbon paste (CP) electrodes, the oxidation process involving one phenol moiety of the molecule with an overall transfer of two electrons and two protons followed by dimerization of the phenoxonium ion formed [19]. A similar irreversible behaviour is found for NP, which was also observed at CF [31], CP and Pt [32] electrodes. The more reversible electrochemical behaviour of OPP at the CNT-GCE was similar to that observed at other electrodes such as CF [31] or CP, and can be attributed to the oxidation of the phenolic compound to the corresponding quinone, which is subsequently reduced in the cathodic sweep. The oxidation peak potential values obtained by cyclic voltammetry, as well as the current density values per concentration unit ($I_p = i_p/AC$, where i_p is the oxidation peak current in μA, A the geometric electrode surface area in cm^2 and C is the concentration of the phenolic compound in mol l^{-1}) are summarized in Table 1. As it can be seen, the E_p values for each compound are relatively similar at the different electrode materials in phosphate buffer solution of pH 7.0. However, the current densities calculated at the CNT-GCE for BPA and OPP are approximately one order of magnitude higher than those obtained at bare GC, Pt or CP electrodes, and similar to those observed at carbon felt electrodes, which are quasi-three-dimensional electrodes with a high surface area at which practically quantitative electrolysis of the compounds is produced [31]. The enhanced current density obtained at the CNT-GCE is attributable to the electrocatalytic effect caused by CNTs towards the oxidation processes of the phenolic compounds, which is due to the structural characteristics and high conductivity of this material [26].

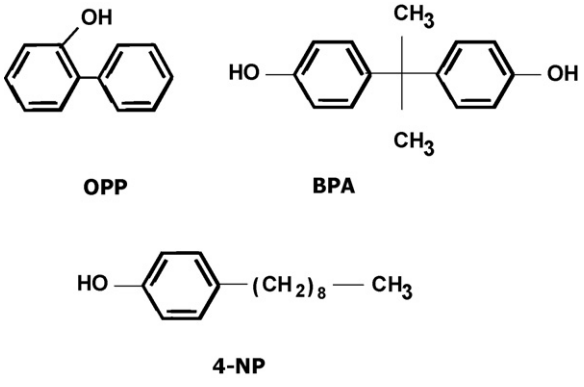

Fig. 3. Chemical structures of 2-hydroxybiphenyl (OPP), bisphenol A (BPA) and 4-nonylphenol (NP).

Regarding NP, the differences in its voltammetric behaviour with respect to BPA and OPP must be commented. As observed in Table 1, modification of the electrode surface with CNTs did not give rise to an enhancement in the current density when compared with other electrode materials, and, indeed this parameter was much lower that that measured at carbon felt electrodes. However, we have demonstrated recently [31] that the oxidation of NP at the CF electrode leads to a practically quantitative transformation of the compound to an NP oxidation product polymerized film which is accumulated on the electrode surface. The high tendency of NP to form this polymerized film can explain the inhibition of the electrocatalytic properties of the CNTs layer.

The effect of pH on oxidation peak current and peak potential values for OPP, BPA and NP at the CNT-GCE was checked over the pH range 2.0–12.0 using 0.1 mol l^{-1} Britton–Robinson buffer as supporting electrolyte. As expected, peak potential values shifted towards less positive values when the pH increased as it is usual for the oxidation of phenolic compounds. Fig. 4 shows the linear dependence ($r = 0.999$) found for OPP between pH 2.0 and 10.0, with a slope value of −61.7 mV. In the case of NP, this linear behaviour was observed between pH 2.0 and 7.0 ($r = 0.998$), with a slope value of −56.8 mV. These slopes values are consistent with the exchange of two electrons and two protons in the electrochemical oxidation of these compounds at the CNT-GCE. In the case of BPA, a linear E_p versus pH dependence was also observed over the 2.0–10.0 pH range ($r = 0.994$), but the slope value, −42.9 mV, moves away from the theoretical value, thus indicating a somewhat different oxidation mechanism as commented before. The intercept values of the two linear ranges obtained from the corresponding E_p versus pH plots (see Fig. 4 for OPP) should be related to the pK_a values of the phenolic compounds. So, the intercepts were 10.0, 9.9 and 7.0 for OPP, BPA and NP, respectively, which agree fairly well with literature data for their respective pK_a values (10.01, 9.8 and 7.2) [33,34]. On the other hand, peak current values also showed a decrease with increasing pH, similarly to that reported for phenolic compounds at different electrode materials. Therefore, in order to achieve a compromise between acceptably large peak currents and low detection potentials, a pH value of 7.0 was selected for the amperometric detection of these compounds.

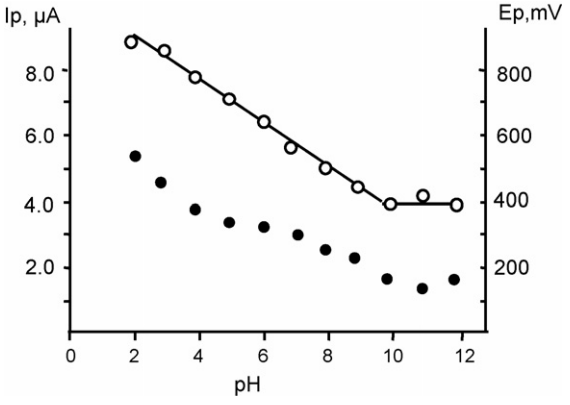

Fig. 4. Influence of pH on peak current (●) and peak potential (◯) values obtained by cyclic voltammetry at a CNT-GCE for 1.0×10^{-4} mol l^{-1} OPP.

The influence of the potential scan rate on the OPP, BPA and NP oxidation peaks was tested over the 2–5000 mV s^{-1} range, and for a phenolic estrogenic compound concentration of 5.0×10^{-5} mol l^{-1} in 0.05 mol l^{-1} phosphate buffer of pH 7.0. For BPA and NP, E_p values shifted to more positive values as the scan rate increased. However, OPP E_p values remained practically constant (with an absolute difference of 23 mV) between 2 and 100 mV s^{-1}, which is in good agreement with the more reversible electrochemical behaviour observed for this compound. Furthermore, a linear i_p versus $v^{1/2}$ plot was obtained for OPP in this scan rate range ($r = 0.997$), which is consistent with a diffusion control of the oxidation process. Similarly, a linear i_p versus $v^{1/2}$ plot was also obtained for NP ($r = 0.992$) in the 2–100 mV s^{-1} range. However, the use of faster scan rates for both compounds produced a deviation from this linearity, with higher i_p values with increasing v, which suggested the contribution of adsorption at these higher v values. In the case of BPA, a linear i_p versus v plot ($r = 0.997$) was obtained over the 2–2000 mV s^{-1}, with a slope value of the log i_p versus log v plot of 1.2. These results revealed that an adsorption process is involved in the electrochemical oxidation of BPA at CNT-GCEs.

In conclusion, it can be said that the oxidation mechanisms of OPP, BPA and NP at CNT-GCE show some differences attributables to their different structure. So, OPP exhibits a behaviour typical of the oxidation of phenolic compounds to the corresponding quinone, which is reduced in the reverse sweep. Furthermore, the oxidation current has demonstrated to be diffusion controlled. Concerning NP, its structure permits the formation of a quinoid derivative, but not the quinone because there is only an aromatic ring p-substituted. Such an oxidation process is also diffusion controlled. After this process, the quinoid derivative can suffer polymerization at the electrode surface. In the case of BPA, its structure does not allow the formation of a double bond to form the quinoid derivative. Therefore, the only possibility is polymerization after formation of the phenoxonium ion. This is supported by the adsorptive behaviour observed for this compound when the scan rate is varied.

3.2. Amperometric detection at the CNT-GCE

Flow injection with amperometric detection at the CNT-GCE, using OPP, BPA and NP as target compounds, was evaluated. Firstly, hydrodynamic voltammograms were constructed by measuring the corresponding peak current values at different applied potentials, after injection of 50-μl aliquots of 1.0×10^{-4} mol l^{-1} OPP, BPA or NP solutions into a carrier solution consisting of a 0.05 mol l^{-1} phosphate buffer of pH 7.0–acetonitrile (50:50) mixture (Fig. 5). This carrier solution was selected because it constituted an appropriate mobile phase for the chromatographic separation of estrogenic and xenoestrogenic compounds (see below). As it can be observed, well-defined sigmoidal shape plots were obtained for the three compounds, with peak current "plateaux" for potential values higher than +0.7 V. Moreover, very stable background currents were found over the whole potential range checked. According to these results, +700 mV was selected as the potential value to be applied for the amperometric detection of the phenolic

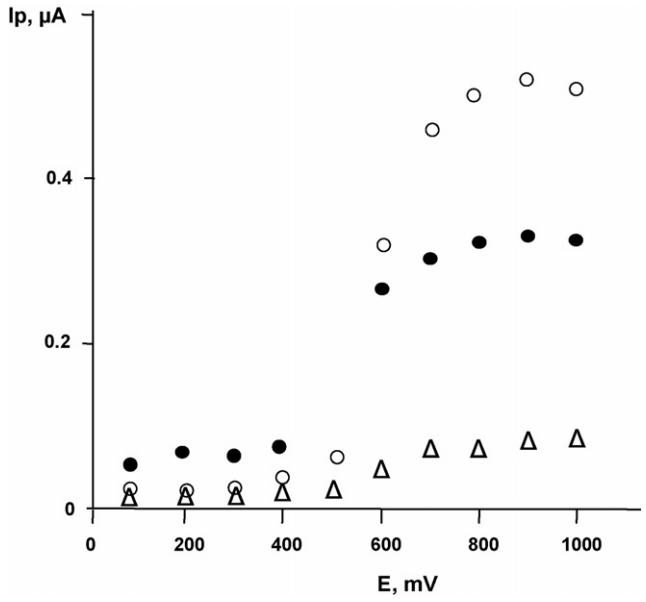

Fig. 5. Hydrodynamic voltammograms obtained by flow injection with amperometric detection at a CNT-GCE after injection of 50 μl aliquots of 1.0×10^{-4} mol l^{-1} OPP (◯), BPA (●) and NP (△) solutions into a carrier consisting of a 0.05 mol l^{-1} phosphate buffer of pH 7.0–acetonitrile (50:50) mixture.

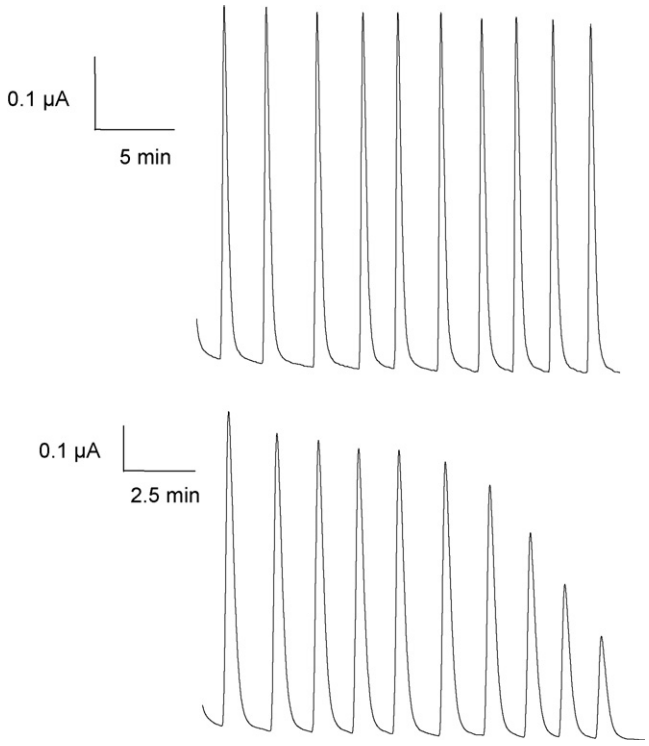

Fig. 6. Successive flow injection amperometric responses obtained at a CNT-GCE (a) and at a bare GCE (b) after injections of 50 μl of 1.0×10^{-4} mol l^{-1} OPP. Carrier solution as in Fig. 4; flow rate, 0.6 ml min^{-1}.

estrogenic compounds at CNT-GCE. It should be remarked that this value is considerably less positive than that reported in the literature for the amperometric detection of these phenolic compounds. For example, it is 300 mV less positive than that used for the determination of a mixture of estrogenic phenols in river waters at a conventional glassy carbon electrode, after a chromatographic separation [18].

The repeatability of the amperometric flow responses at this potential value was checked by successive injections of 50 μl of 1.0×10^{-4} mol l^{-1} OPP, BPA and NP and compared with that obtained at a bare GCE. As an example, fiagrams obtained for ten successive injections of OPP are displayed in Fig. 6. As it can be seen, reproducible amperometric signals were achieved using the CNT-GCE, with a R.S.D. value for i_p of 1.7% ($n = 10$). However, a continuous decrease of i_p is observed at the unmodified electrode, as a consequence of the surface fouling by the electrode reaction products under potentiostatic conditions. The anti-fouling properties of CNT-modified electrode surfaces are so corroborated. Similar results were obtained for BPA and NP, with R.S.D. values for i_p ($n = 10$) of 2.2 and 2.5%, respectively.

3.3. LC–EC detection at the CNT-GCE

HPLC with isocratic elution using a C_{18} reversed-phase column was employed to develop a method for the determination of several estrogenic compounds. The use of this stationary phase was recommended in the literature for the chromatographic separation of this type of compounds [16–18]. The mobile phase used to investigate the separation of mixtures of these compounds consisted of an acetonitrile:0.05 mol l^{-1} phosphate buffer solution of pH 7.0. Firstly, the influence of the acetonitrile percentage on the separation of BPA, DMP, EE2 and TBP, which were selected because of their differences in polarity, was evaluated. As expected, both the retention time and the peak current for these compounds decreased with the increase in the acetonitrile percentage over the whole range studied (10–60%, v/v). As a compromise between adequate retention times for the less polar compounds and a good sensitivity, when peak areas are measured, a mobile phase with a 50% (v/v) acetonitrile was selected.

The effect of the mobile phase flow rate was tested in the 0.2–1.0 ml min^{-1} range. As expected, both retention time and peak width decreased as the flow rate increased for all the compounds. However, a lower resolution was observed for the higher flow rates. Therefore, as a compromise, a flow rate of 0.6 ml min^{-1} was chosen for further work. Under these conditions, retention times of 9.5, 10.4, 12.5 and 16.7 min were obtained for BPA, DMP, EE2 and TBP, respectively. Fig. 7a shows a chromatogram obtained for a mixture of these compounds at a concentration of 1.0×10^{-5} mol l^{-1} each. Although there is not baseline resolution for BPA and DMP, their correct quantification is possible, as it will be demonstrated below. Fig. 7b shows the chromatogram for a mixture of six estrogens and xenoestrogens at the same concentration level. As it can be observed an adequate resolution, as well as retention times in a convenient range were achieved.

Calibration graphs constructed for BPA, DMP, EE2 and TBP using the peak areas as the analytical signals, exhibited the analytical characteristics summarized in Table 2. The detection limits were calculated according to the $3s_b/m$ criterion, where m is the slope of the calibration graph for each analyte and s_b was

Table 2
Analytical characteristics of the calibration graphs for different phenolic estrogens obtained by HPLC with amperometric detection at CNT-GCE

Species	Linear range (μM)	Slope (μC $M^{-1} \times 10^5$)	Intercept (μC)	r	R.S.D.[a] (%)	Detection limit[a] (μM)
BPA	0.3–100	1.53 ± 0.02	−0.09 ± 0.08	0.999	3.4	0.098
DMP	0.5–100	1.45 ± 0.03	0.1 ± 0.1	0.996	2.9	0.171
EE2	1.0–100	0.70 ± 0.01	0.10 ± 0.07	0.994	3.2	0.340
TBP	1.0–100	0.82 ± 0.02	0.07 ± 0.04	0.998	3.0	0.308

[a] For peak areas ($n = 5$) at a concentration of 1.0×10^{-5} mol l^{-1} each.

estimated as the standard deviation ($n = 10$) of the peak area values obtained for the lowest concentration of the corresponding range of linearity. Using this criterion, detection limits ranging between 98 nM (BPA) and 340 nM (EE2) were achieved. The detection limit obtained for BPA can be compared with those reported in the literature for this compound using fluorescence [14] and MS [11] detection. By applying the S/N = 3 criterion, the limits of detection reported were 1 and 0.1 ng ml^{-1}, respectively. Using this same criterion, the detection limit for BPA with amperometric detection at the CNT-GCE is 0.6 ng ml^{-1}, which is comparable to that obtained using the much more expensive MS detection mode, thus demonstrating the suitability of the electrochemical approach at the modified electrode for the determination of low concentrations of phenolic estrogens.

The usefulness of the chromatographic method with amperometric detection at the CNT-GCE for the determination of phenolic estrogens was evaluated by analysing different water samples spiked with these compounds. The sample treatment described in Section 2 is a modification of that proposed by Cargouët et al. for the treatment of surface water for the determination of estrogenic compounds by GC–MS [35]. Underground well water and tap water samples were adequately collected and stored at 4 °C, and extraction of phenols was accomplished in a maximum period of time of 48 h. These water samples were spiked with BPA, DMP, EE2 and TBP at a 14 nmol l^{-1} concentration level of each compound. Solid phase extraction using C_{18} and SDB disks was employed in order to extract non-polar and polar or moderately polar compounds, respectively. Moreover, a blank sample prepared with spiked Milli-Q water was also analyzed. As described in Section 2, a 500-ml sample volume was processed through the disks in all cases, and eluted with acetonitrile. Aliquots of 50 μl from the analytical solution in acetonitrile containing the phenolic estrogenic compounds were injected into the chromatographic system. The results obtained are summarized in Table 3. As it can be seen, recoveries were satisfactory, independently of the type of water analyzed, thus demonstrating the suitability of the method for the determination of phenolic estrogenic compounds in this type of samples.

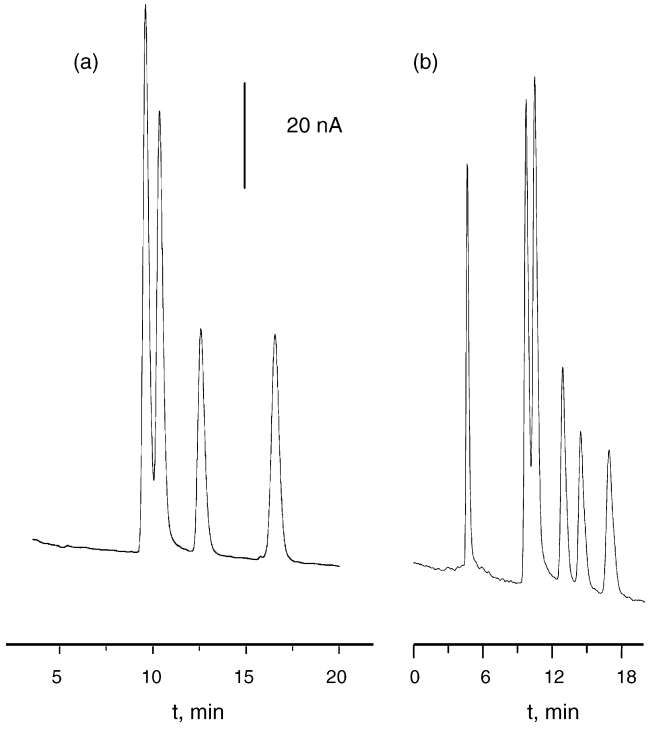

Fig. 7. Chromatograms obtained with amperometric detection at a CNT-GCE for a mixture of BPA, DMP, EE2 and TBP (a), and of E3, BPA, E2, EE2, E1 and TBP (b) at a concentration of 1.0×10^{-5} mol l^{-1} each. Mobile phase, 50:50 acetonitrile–0.05 mol l^{-1} phosphate buffer of pH 7.0; flow rate, 0.6 ml min^{-1}.

Table 3
Recovery studies of EDCs in spiked water samples using HPLC with amperometric detection at CNT-GCE

Compound	Well water	Tap water	Milli-Q water
BPA	97 ± 10	98 ± 2	102 ± 8
DMP	100 ± 6	93 ± 7	93 ± 7
EE2	88 ± 6	100 ± 6	100 ± 6
TBP	96 ± 1	98 ± 7	99 ± 3

Mean values from three determinations.

4. Conclusions

Modification of glassy carbon electrodes with carbon nanotubes produced an enhancement of the electrochemical oxidation responses obtained for phenolic estrogenic compounds. This allowed amperometric detection of these compounds at the CNT-GCE to be performed at an applied potential considerably lower than those previously reported using conventional electrode materials. Moreover, no significant fouling of the modified electrode surface was observed. This improved electroanalytical performance was profited to develop an HPLC–EC method with a high sensitivity for the determination of several estrogenic compounds in different water samples.

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