

**Electroanalytical thread-device for estriol
determination using screen-printed carbon
electrodes modified with carbon nanotubes**

Abstract

Microflow systems are powerful analytical tools that explore similar principles of typical flow injection analysis driven to in a microfluidic device. Generally, microfluidic devices can promote a low consumption of reagents and samples, high speed of analysis and possibility of portability. Several advances have been reached applying a simple and low cost device based on cotton thread as microfluidic channel where the transportation of solutions is based on capillary force helped by gravity. In the present work, we have demonstrated the versatility of thread-based electroanalytical devices (μ TED) constructed using a cotton thread as the solution channel and screen-printed electrodes (SPE) surface modified with carbon nanotubes (CNT) as electrochemical detectors for the amperometric determination of estriol hormone in pharmaceutical samples. The parameters involved in the amperometric detection and microflow system were studied and optimized, using the best experimental conditions (flow rate of $0.50 \mu\text{L s}^{-1}$, 10 mm of analytical path, $2.0 \mu\text{L}$ of volume of injection and potential of detection of 0.75 V) a linear response was observed for concentration range (LDR) of 1.0 to $1000 \mu\text{mol L}^{-1}$ with limits of detection (LOD) and quantification (LOQ) of $0.53 \mu\text{mol L}^{-1}$ and $1.77 \mu\text{mol L}^{-1}$, respectively, and frequency of injection of 32 per hour. The proposed methodology was applied for determination of estriol in commercial samples and results were compared with those provided by spectrophotometric method (official methodology). The obtained results are in agreement at a 95% of confidence level.

The microfluidic systems have experienced explosive growth since its development in the 1970s and 1980s, also they are known as micro total analysis system (μ TAS). These devices have been fabricated using materials such as silicon/glass, elastomers, hydrogels, paper and other materials [1] ; [2]. The μ TAS have been used for various applications in chemical, biological, and medical research areas, showing a great potential due to its characteristics such as miniaturization, portability, low manufacturing costs, low consumption of samples and reagents, detections with high resolution and sensitivity and short times for analysis [2]; [3]; [4] ; [5].

The use of paper-based analytical devices (μ PAD) has been reported by several authors due to its versatility, abundant availability and disposability [5] ; [6]. Nonetheless, there are few limitations related to the use of μ PAD such as low efficiency of sample delivery, low mechanical strength of the wet paper, construction of hydrophobic barriers and, consequently, an extra fabrication process to define channels, and the leakage of sample with low surface tensions from channels [7]; [8] ; [9]. An alternative material is the cotton thread, its use result in a fabrication of the thread-based analytical devices (μ TAD). Recently, Agustini et al. [8] reported the construction of a low cost microfluidic thread-based electroanalytical device (μ TED) where the microfluidic channels were built with cotton threads for simultaneous amperometric determination of acetaminophen and diclofenac. The advantages of the use of this material over existing alternatives (e.g. paper) such as: (1) no need to define hydrophobic and hydrophilic regions; (2) the sample routes can be replaced easily; (3) greater tensile strength and flexibility; (4) no need of precise control of sample volumes introduced into the device; (5) different fibre materials and combinations can be chosen to make thread into wearable materials, etc [8] ; [9].

Among the several techniques used for the detection system, the electrochemical techniques are widely used for the fabrication of microfluidic devices, mainly due to features that include remarkable sensitivity (comparable to the fluorescence sensitivity), low cost, low-power requirements, direct electronic interface, use of simpler and smaller detectors and high compatibility with micromachining and microfabrication technologies. The miniaturization is possible since the electrochemical detection is based on the redox reaction occurring at the electrode surface and not on an optical path length, resulting in a miniaturized system without loss in sensitivity [10]; [11] ; [12].

Several kinds of disposable electrochemical sensors have been developed due economic and practical feasibility. Among this class of sensors, screen-printed electrodes (SPEs) have offered high-volume production of extremely inexpensive, and yet highly reproducible and reliable single-use sensors. The SPEs can be constructed by printing various types of inks on different substrates, such as ceramic, plastic or circuit boards. The composition of the several inks used for the printing process determines the selectivity and sensitivity required for the analytical procedure [13] ; [14]. In order to improve the analytical characteristics/performance, modifications over SPE surfaces with a variety of nanomaterials such as carbonaceous materials (carbon nanotubes (CNTs), graphene (GR), carbon black (CB), etc) have been exploited and showed successful results [15]; [16] ; [17]. Due to its features that include large surface area, unique structure, and remarkable mechanical and electrical properties, the CNTs have been widely applied in electroanalytical chemistry [18] and recently it have been used to improve the analytical performance of microfluidic systems [19]; [20] ; [21].

Therefore, we present in this work for the first time a thread-based electroanalytical device (μ TED) constructed using a cotton thread as the solution channel and a SPE modified with CNT as electrochemical detector for the amperometric determination of estriol hormone in pharmaceutical samples. Estriol can be found in pregnant mammals, once is one of the most abundant estrogens, also oral estriol tablets have been used for the treatment of postmenopausal women [22] ; [23]. The continuous ingestion of estriol is associated with an increased risk of endometrial cancer and endometrial hyperplasia when administered orally in doses from 1 to 2 mg per day [24]. Furthermore, this hormone is found as water pollutant and it is classified as an endocrine disruptor, which may cause adverse effects by interfering with the function of hormones in the human body and on aquatic organisms [25]; [26] ; [27]. In this context, the determination of estriol in human and environmental samples has been performed using several methods including chromatographic techniques [28] ; [29], immunoassays [30]; [31] ; [32], electrophoresis [33] ; [34] and electroanalytical methods [35]; [36] ; [37]. Some characteristics of electroanalytical methods including its operational simplicity, low cost, fast response, high sensitivity, potentiality for miniaturization and automation, have raised the interest in the applicability of the electroanalytical methods for estriol determination.

Experimental section

All chemicals used were of analytical grade and used without previous purification. Sodium acetate and acetic acid were purchased from Vetec (Duque de Caxias, RJ, Brazil) and were used to prepare the acetate-buffered solution. The acetate buffered solutions were prepared in ethanol: water (7:3 v:v) in concentration of 0.5 mol L⁻¹. Hormone standard solutions were prepared in ethanol: water (1:1 v:v) using estriol purchased from Sigma-Aldrich (St. Louis, Missouri, EUA). All solutions were prepared with deionized water (specific resistivity >18 MΩ cm) obtained with a Millipore Direct-Q3 water purification system (Bedford, MA, USA) and ethanol purchased from Neon (São Paulo, SP, Brazil).

The multiwall carbon nanotubes (CNT) samples (purity of 95%, length 1.5 μm, diameter 10 nm) were purchased from DropSens and the acid treatment was realized with sulfuric acid and nitric acid, purchased from Neon and Proquimios (Rio de Janeiro, RJ, Brazil), respectively. The isopropanol used for preparation of CNT dispersion was purchased from Neon.

The materials used to manufacture the μTED were purchased in a local market (Curitiba, PR, Brazil). These materials include double sided tape, hydrophilic cotton gauze, glass plates and polymeric reservoirs. The cotton threads were obtained from the hydrophilic cotton gauze.

The SPEs utilized consist of a graphite working electrode, a graphite counter electrode and an Ag/AgCl reference electrode. Throughout this work, only the graphite working electrode surface was utilized and external reference and counter electrodes were employed for consistency, vide supra. The SPEs, which have a 3 mm diameter working electrode, were fabricated in-house with appropriate stencil designs using a microDEK 1760RS screen-printing machine (DEK, Weymouth, UK). For the case of each fabricated electrode, first a graphite ink formulation (Product Code: C2000802P2; Gwent Electronic Materials Ltd, UK), which is utilized for the efficient connection of all three electrodes and as the electrode material for both the working and counter electrodes, was screen-printed onto a polyester (Autostat, 250 μm thickness) flexible film. After curing the screen-printed graphite layer in a fan oven at 60 °C for 30 min, a Ag/AgCl reference electrode was included by screen-printing Ag/AgCl paste (Product Code: C2040308D2; Gwent Electronic Materials Ltd, UK) onto the polyester substrates, which was subsequently cured once more in a fan oven at 60 °C for 30 min. Finally, a dielectric paste (Product Code: D2070423D5; Gwent Electronic Materials Ltd, UK) was screen-printed onto the polyester substrate to cover the connections and define the active electrode areas, including that of the working electrode. After curing at 60 °C for 30 min, the SPEs were ready to be used. The SPEs have been

electrochemically characterized previously and exhibit a heterogeneous electron transfer rate constant, k_{eff} , of ca. $1.1 \times 10^{-3} \text{ cm s}^{-1}$ using 1 mmol L^{-1} hexaammineruthenium (III) chloride and 0.1 mol L^{-1} KCl.

The acid functionalization of the CNTs consisted in an oxidation treatment by refluxing the CNTs in a mixture of 3.0 mol L^{-1} $\text{HNO}_3/\text{H}_2\text{SO}_4$ (1:1 v/v) by 6 h under magnetic stirring. After this period, the mixture was cooled to room temperature and the functionalized materials were filtered by vacuum filtration and rinsed until pH 7.0. They were dried in an oven at 50° C for 12 h and a CNT powder was obtained. For the SPE modification, a CNT dispersion was obtained using 0.5 mg of the dried CNT powder in 1.0 mL of a mixture of isopropanol and a solution of Nafion[®] (0.01% m/m). Then, SPE working electrode surface was modified with an aliquot of $4.0 \mu\text{L}$ of the functionalized CNT dispersion by drop-casting method.

The proposed device was developed by simple assembly of components and can be constructed by non-professional people. Glass plates (25 mm of wideness, 6.5 mm of thickness and 90, 60 and 30 mm of length) were used as the substrate for assembly of the μTED . The scheme presented in [Fig. 1](#) shows the device structure. The device construction steps consists in the (1) fixation of inlet and outlet reservoirs (both with a capacity of $\sim 4 \text{ mL}$) near the edges of the glass plates; (2) placement of two pieces of double sided scotch tape near the inlet and outlet reservoirs; (3) accommodation of the SPE modified with functionalized CNT on the double-sided tape next to the outlet reservoir; (4) fixation of arrangement of microchannels which are formed hydrophilic threads (9 parallel threads without twisting) throughout the device, from the inlet reservoir to the outlet reservoir; (5) placement of two pieces of double sided tape on the ends of the hydrophilic gauze. Detection zone was covered by pieces of cotton thread in order to maintain all electrodes immersed in solution during amperometric measurements.

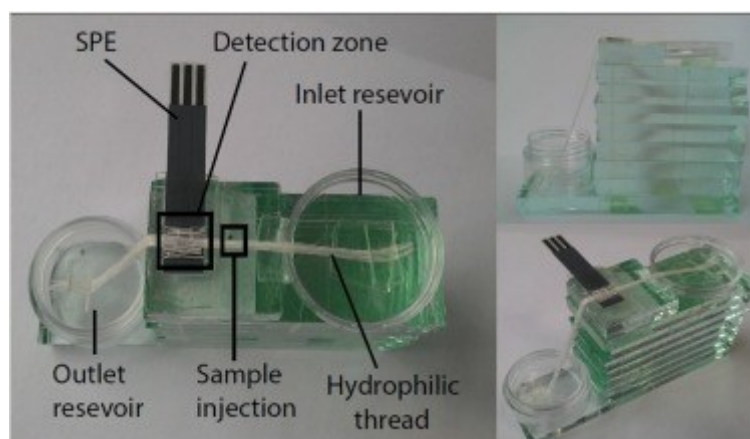


Fig. 1. Illustration of the μ TED after the assembly process.

The electrochemical measurements were performed at 20 °C and using a potentiostat/galvanostat μ Autolab type III (Metrohm Autolab, Utrecht, Netherlands), controlled by Nova 1.1 software. The voltammetric behaviour of estriol was studied through cyclic voltammetry, using a conventional three-cell electrode. For this step, the working electrode was a glassy carbon electrode (GCE), the reference electrode an Ag/AgCl/KCl (3.0 mol L⁻¹) and the counter electrode a platinum electrode. For the amperometric detection, the SPE was used combined with the μ TED. The design of the screen-printed carbon electrode (SPCE) used in all electrochemical experiments is based on plastic base, the working and the auxiliary electrodes are made of carbon conducting ink and the reference electrode was prepared with silver ink. The SPCE was connected to the potentiostat using a cable and used without any pretreatment. Acetate buffer solutions were added to the inlet reservoir and the analyte solution was injected (aliquot of 2.0 μ L) using a micropipette directly onto the microfluidic channel (threads) at a distance of 10 mm from the detection cell.

The spectrophotometric measurements were performed at a Femto Spectrophotometer (São Paulo, SP, Brazil) with a quartz cuvette and controlled by the FemtoScan Software. Commercial formulation Ovestrion[®] was analyzed as specified in European Pharmacopeia [38]. A mass of 25.0 mg of the estriol commercial sample were weighted and grinded with pistil in a mortar. The sample powder was dissolved in ethanol and diluted to 50.0 mL with the same solvent. Then, 10.0 mL of this solution were diluted to 50.0 mL with the same solvent and the resulting solution was filtrated using a paper filter. The measurements were carried out at 281 nm.

Results and discussion

For the voltammetric measurements, a GCE was used as the working electrode and the influence of the surface modification with the functionalized CNT can be illustrated in Fig. 2. In the voltammogram Fig. 2 – Curve (a) can be seen a typical cyclic voltammogram of a solution of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of estriol in acetate buffer solution (pH 5.0) on a GCE without modification using Ag/AgCl/KCl (3.0 mol L^{-1}) as the reference electrode. Cyclic voltammogram show that the electrochemical oxidation of estriol is characterized by an anodic peak at 0.74 V, which can be attributed to phenolic groups [22]. Another aspect that can be observed is the absence of a reduction peak in reverse scan, which suggests that the overall oxidation reaction is an irreversible process under the conditions of these experiments. The irreversible oxidation of the estriol at higher potentials leads to the formation of strongly bound carbon species at the electrode surface. Besides that, the oxidation of phenolic groups result in the generation of phenoxy radicals which are highly reactive and can cause a polymerization reaction, leading to the formation of a polymer film, resulting in a strongly adherent and non-electroactive deposit [39]; [40]; [41].

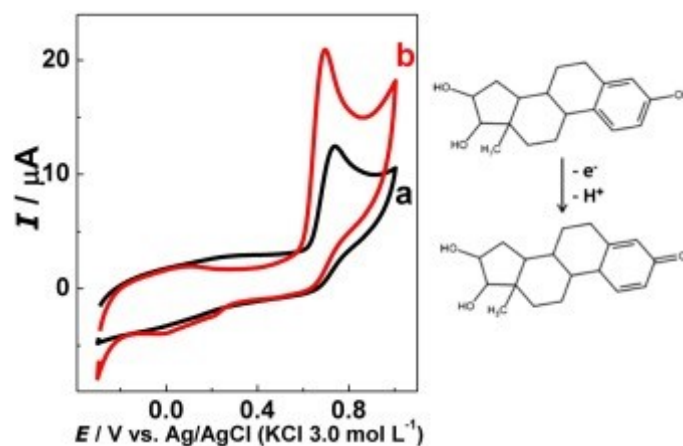


Fig. 2. Cyclic voltammograms using a GCE unmodified (a) and a GCE/CNT (b) as the working electrode. The voltammograms were carried out in presence of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of estriol in acetate buffer solution (pH 5.0). Scan rate: 50 mV s^{-1} . Possible electrochemical reaction of estriol.

Using a modified GCE with CNT treated an electrocatalytic effect on the oxidation of estriol is observed (Fig. 2 – Curve (b)). The oxidation process takes place at a less positive potential value (+0.69 V) and the peak current is two times higher than on the bare electrode. The peak current increase can be related to the presence of functionalized CNT, owing to its characteristics such as higher superficial

area, mainly due to nanometric structures and increased exposure of step edge sites (mainly responsible for the electronic transfer). Furthermore, the CNTs have major affinity to aromatic hydrocarbons and can promote an effective hormone adsorption at the electrode surface, resulting in higher values of peak current [18] ; [26]. Therefore, there is a great potentiality of using CNTs as electrode surface modifiers, aiming improved analytical performances.

The adherence of the film was also studied through consecutive cyclic voltammetry (10 cycles). A decrease in peak current values was observed for both electrodes used, suggesting that there is a fouling tendency probably due to the formation of a polymer film resulted from the oxidation of phenolic groups present in estriol structure [26]. Although the fouling tendency was observed for both electrodes, anodic current stabilization can be observed at higher peak current values for the GCE/CNT which suggests the presence of CNT on electrode surface minimizes the surface passivation. The minimization of such fouling due to the presence of CNT is reported in literature [43]; [44]; [45] ; [46].

The influence of the potential scan rate on the estriol oxidation was studied over the range 5.0–100 mV s⁻¹ and a peak potential shift to more positive values (with the scan rate increase) was observed in agreement with an irreversible electrochemical behavior [22]. Oxidation peak current increased linearly with square root of scan rate indicating that the initial oxidation process of the phenolic groups occurs through diffusion of the electroactive species from solution to the electrode surface. [26].

In order to optimize the carrying solution, which was used in the μ TED system, the influence of pH on the anodic peak potential and peak currents was studied over the range 2.0–8.0. Potential of oxidation peak shifted to less positive potentials with the increase of pH, which is a typical behaviour for the oxidation of phenolic estrogenic compounds as reported in literature [22]; [42] ; [43]. The linear equation that relates oxidation peak and pH was $E/mV = 944.8 - 52.87 \text{ pH}$ ($R^2 = 0.998$) and the slope approximately of 59 mV per pH indicates the transference of an equal number of protons and electrons during estriol oxidation reaction. Furthermore, higher value for anodic peak current was found in pH between 2.0 and 3.0, for pH values above 3.0 a decrease of peak current was observed. In order to obtain most sensitive results, the pH value 3.0 was selected for further amperometric measurements.

Aiming for the application of the SPCE as amperometric detector in the μ TED system, the electrochemical behaviour of estriol at modified electrode was carried out in a three-cell conventional system. The voltammetric behaviour observed using SPCE unmodified and modified with CNT was very similar to those obtained using glassy carbon electrodes, which confirms the analytical potentiality of SPCE/CNT for the estriol determination. SEM representative images of unmodified and modified SPCE (Fig. 3A and B) revealed a significant morphological difference between the surfaces due to presence of carbon nanotubes. TEM analysis (Fig. 3C) shows the CNTs with diameter medium of 10.2 nm which are presenting straightness of sidewalls and arranged as bundle. Raman spectrum (Fig. 3D) showed a typical response of CNT where bands relate to graphitic material were found at $\sim 1350\text{ cm}^{-1}$ (D band), which is due to defects present on carbon structure, at $\sim 1580\text{ cm}^{-1}$ (G-band) attributed to Csingle bondC symmetric stretching in graphene-like structure and at $\sim 2700\text{ cm}^{-1}$ (D' band) which is induced by structural disorder and defects.

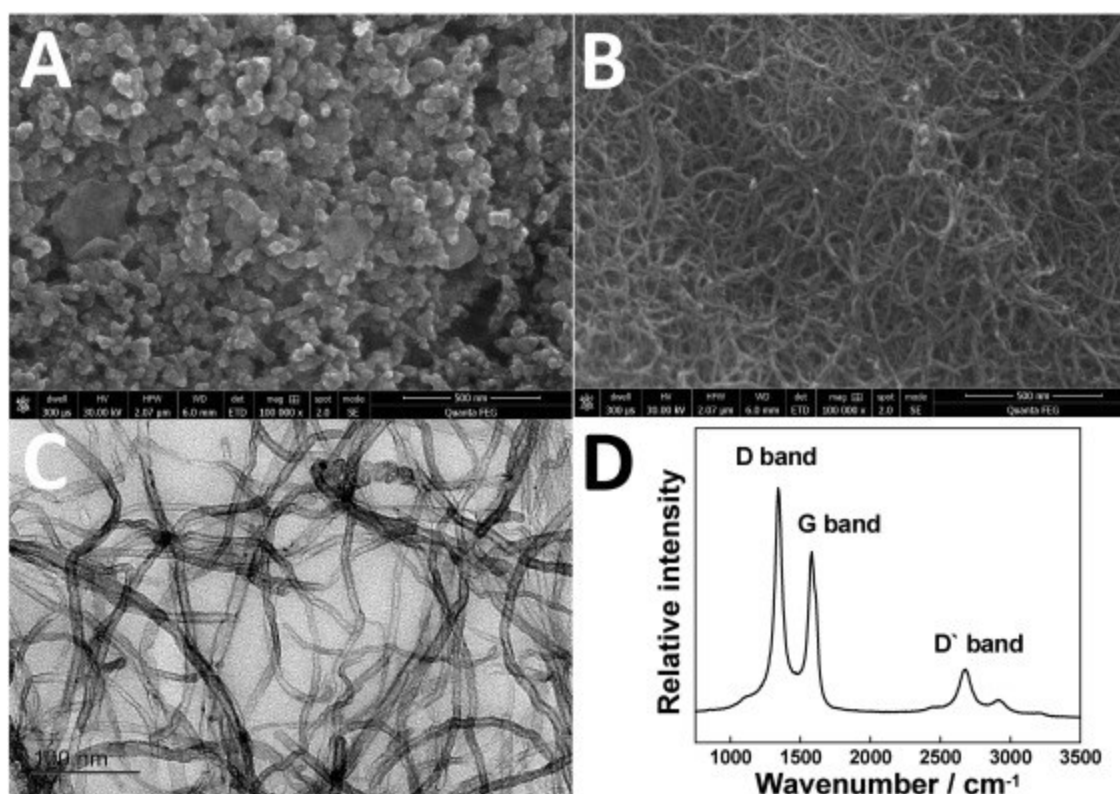


Fig. 3. SEM images of SPCE unmodified (A) and modified with CNT (B). TEM images (C) and Raman spectrum (D) of CNT sample used for electrode modification.

Several parameters relate to μ TED system were used based on previous works [8]. The amperometric response was strongly affected by microchannel, detection zone, device dimensions and sample injection. Thus, some experimental conditions such as flow rate of $0.50 \mu\text{L s}^{-1}$, nine cotton threads arranged in parallel for microchannels preparation, height difference of 52 mm between reservoirs of inlet/outlet and $2.0 \mu\text{L}$ of volume of injection were selected with the aim of obtaining a stable and constant flow rate of the solution and an adequate signal-noise ratio for determination of estriol.

The SPCE/CNT was combined with the μ TED as presented in Fig. 1 and amperometric technique was carried out with the mixture of acetate buffer solution and ethanol as the carrier solution. The influence of applied potential was investigated for values varying between 0.60 V to 0.90 V. The values of peak current for each applied potential were plotted and the results are presented in Fig. 4A. There was an increase in the peak current until the potential of 0.75 V which is in agreement with voltammetric behaviour observed for estriol. Using higher potentials, a significant variation in the noise of baseline was observed which was adverse for current sampling. This effect could be related to gas formation that occurs at electrode surface promoted by high potential values. Based on best relation signal/noise a potential of 0.75 V was chosen for detection step. The repeatability of the μ TED was evaluated with nine successive injections of $2.0 \mu\text{L}$ of estriol $1.0 \times 10^{-4} \text{ mol L}^{-1}$ aliquots (Fig. 4B). The standard deviation for the peak currents were 7.7%, which was considered a satisfactory repeatability confirming that under flow conditions there is no blocking of electrode surface.

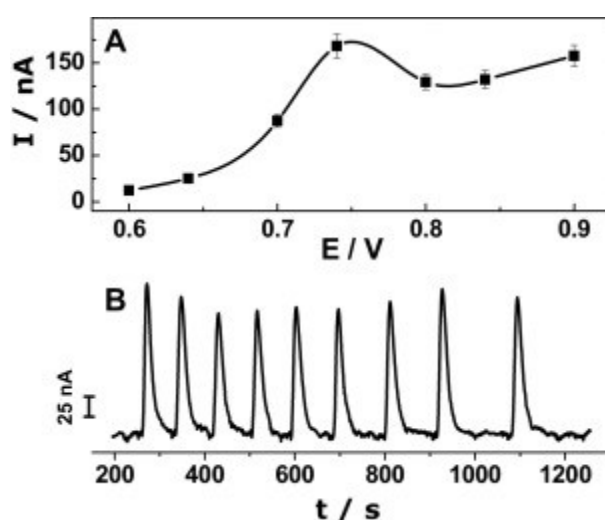


Fig. 4. (A) Variation of peak currents obtained from amperograms generated in μ TED under applied potential varied over the range of 0.60–0.90 V. Each point represents the average

response for 10 successive injections. (B) Amperometric responses generated in μ TED obtained for injections of 2.0 μ L of estriol 1.0×10^{-4} mol L⁻¹ aliquots, with a flow rate of 0.50 μ L s⁻¹. Applied potential: 0.75 V.

Typical amperometric response obtained for estriol standard solutions injections using proposed device is presented in Fig. 5. The estriol concentration varied from 1.0 to 1000 μ mol L⁻¹ and three consecutive injections were realized for each concentration evaluated. Aiming to verify the fouling tendency of the μ TED, the estriol concentration was varied from the minimal to the maximal concentration value and in sequence to the minimal concentration value again. Besides the increase of peak current with the estriol concentration, as the peak current for each concentration remained similar even after the injection of high estriol concentrations, no fouling tendency was observed for this device. This fouling tendency was not observed probably to the use of continuous support electrolyte flow over the electrode surface, which results in the cleaning of the surface after each injection and low concentrations of estriol used in μ TED system. In addition, chemical pretreatment of CNT under acid conditions promotes the oxidation of materials yielding hydrophilic groups (mainly hydroxyl and carboxyl) at CNT surface which can to minimize the surface passivation. The analytical response curve obtained is presented in Fig. 5 (inset). The μ TED showed considerable sensitivity (1.13 nA L μ mol⁻¹) with a satisfactory linear correlation ($R^2 = 0.992$). A linear dynamic range (LDR) from 1.0–1000 μ mol L⁻¹ estriol was observed. Limit of determination (LOD, three times the SD of base line/slope) was estimated as 0.53 μ mol L⁻¹ and of quantification (LOQ, ten times the SD of base line/slope) [8] was calculated as 1.77 μ mol L⁻¹. Frequency rate of 32 injections per hour was obtained due by efficient transport of the analyte to detector and for short time to cleaning the sensor under optimized features of μ TED. These results confirm that the SPCE combined with μ TED system described in this work has an excellent analytical performance for estriol determination. In order to compare the figures of merit of proposed sensor Table 1 summarizes the analytical characteristics of several electroanalytical methodologies employed for determination of estriol.

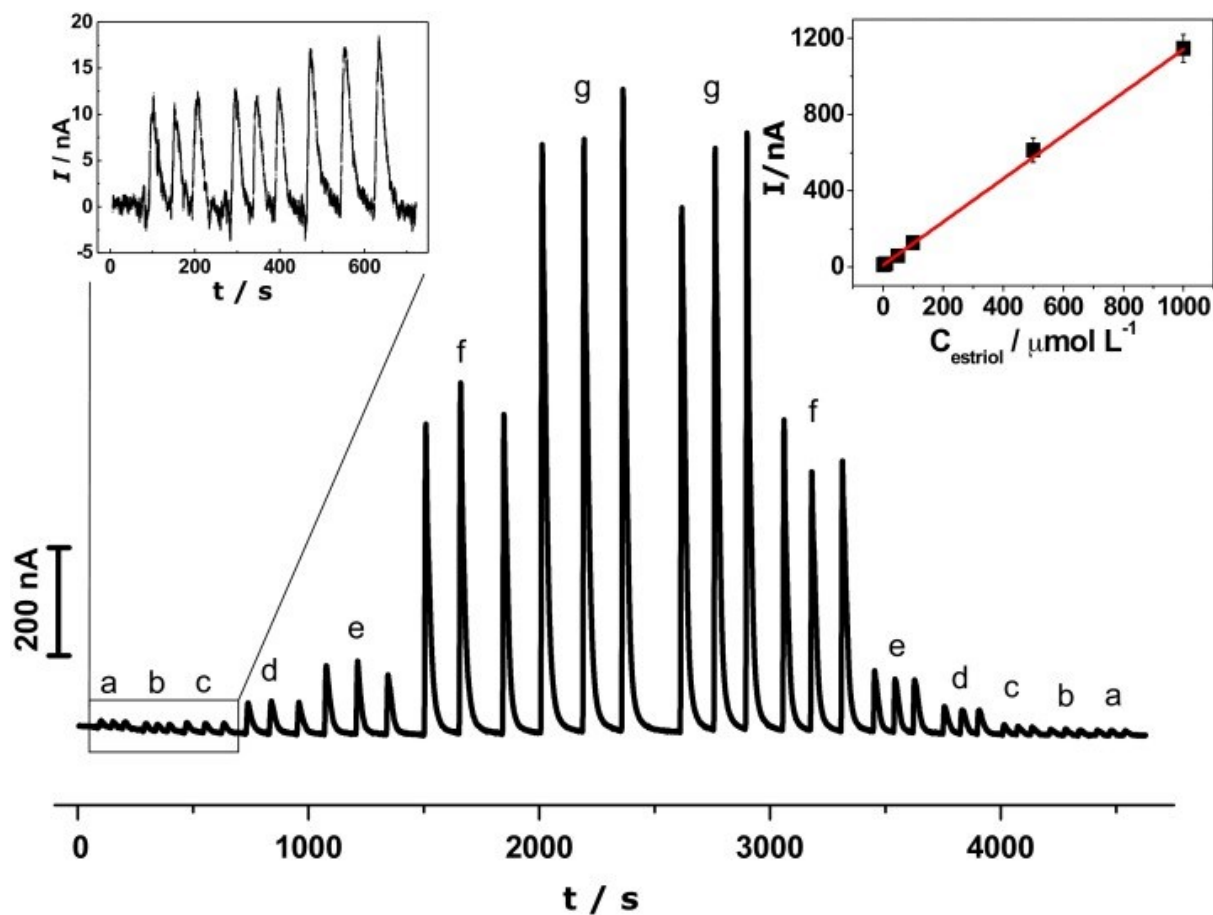


Fig. 5. Successive amperometric response in μ TED obtained for injections of 2.0 μ L of estriol standard solutions aliquots, varying over a range (a) 1.0; (b) 5.0; (c) 10.0; (d) 50.0; (e) 100.0; (f) 500.0 and (g) 1000.0 μ mol L⁻¹, with a flow rate of 0.50 μ L s⁻¹. Applied potential: 0.75 V. Analytical curve for the amperometric responses in μ TED (in detail). Each point is the average value of the 6 injections for each concentration.

Table 1.

General characteristics of the electrochemical methods used for estriol determination in several samples.

Electrode	Technique	S (nA L μ mol ⁻¹)	LDR (mol L ⁻¹)	LOD (mol L ⁻¹)	Ref.
GCE/Pt/MWCNTs	SWV	790	1.0×10^{-6} – 7.5×10^{-5}	6.2×10^{-7}	[35]
GCE/rGO–SbNPs	DPV	2.13	2.0×10^{-7} – 1.4×10^{-6}	5.0×10^{-10}	[25]
BDDES	SWV	2.35×10^{-3}	2.0×10^{-7} – 2.0×10^{-5}	1.7×10^{-7}	[22]
GCE/Lac/rGO/Sb ₂ O ₅	Chronoamperometry	275	2.5×10^{-8} – 1.03×10^{-6}	1.1×10^{-8}	[46]
SPCE/MWCNT	Amperometry	1.13	1.0×10^{-6} – 1.0×10^{-3}	5.3×10^{-7}	This work

S: Sensitivity; GCE/Pt/MWCNTs: Pt nanoclusters and multi-wall carbon nanotubes composite glassy carbon electrode; SWV: square-wave voltammetry; rGO – SbNPs: reduced graphene oxide and antimony nanoparticles composite; DPV: differential pulse voltammetry; BDDE: boron doped diamond electrode; Lac/rGO/Sb₂O₅: reduced graphene oxide doped with Sb₂O₅ film and with immobilized laccase enzyme.

The electroanalytical methodology proposed was applied for estriol determination in commercial pharmaceutical sample. Amperometric responses for the estriol standard solutions and the commercial samples are presented in Fig. 6. In order to verify the possible interference of excipients present in commercial sample, such as amido, lactose, magnesium stearate and amilopectine [22], two different pharmaceutical preparations were used for this study. One pharmaceutical preparation consisted in a mixture of commercial sample and estriol standard solution (S2) and the other contained exclusively the commercial sample (S1). Both pharmaceutical preparations contained an equal quantity of commercial estriol sample and the peak current for both samples were used for recovery calculations, which resulted in estriol recovery ranging from 92.9 to 104.7%. The results obtained suggest that the estriol can be successfully determined in pharmaceutical formulations using μ TED system without any significant matrix interference effects. Even though a high potential was applied during the amperometric measurements, good recovery values were found which indicate an adequate selectivity for estriol determination for pharmaceutical sample analysis.

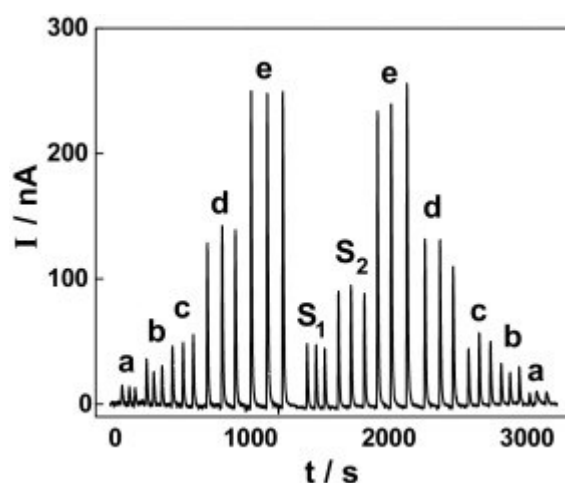


Fig. 6. (a) Successive amperometric responses in μ TED obtained for injections of 2.0 μ L of estriol standard solutions aliquots (a–e) and two pharmaceutical preparations (S1 and S2), varying over a range (a) 10.0; (b) 50.0; (c) 100.0; (d) 200.0; (e) 400.0, with a flow rate of 0.50 μ L s⁻¹. Applied potential: 0.75 V.

As recommended in European Pharmacopeia [38], a spectrophotometric method was realized as a comparative method for determination of estriol. The spectrophotometric measurements were realized in methanol at wavelength 281 nm. The analytical curve presented good linearity ($R^2 = 0.999$) and the estriol determination was realized in a pharmaceutical sample, which resulted in an estriol concentration of 1.10 mg/tablet. The proposed method provided estriol concentration of 1.11 ± 0.04 mg/tablet which presented a relative error of 0.8% compared to spectrophotometric method indicating a statistical concordance between the results obtained using the μ TED equipped with SPCE/CNT and comparative methods for a confidence level of 95% (t -test). The similarity of estriol concentration determined through the reference and the proposed method indicates the great reliability of the method studied in this work.

Conclusions

The described results have shown for the first time that the SPCE modified can be an efficient sensor for amperometric determination of estriol when combined with μ TED system. The adopted strategy is an interesting alternative to demonstrate the versatility of other electrodes as detector in microfluidic device which allows significative minimization of the amount of reagents and solutions.

Additionally, the system is promising tool for estriol determination and can be used for direct applications in pharmaceutical samples without any pretreatment. Low detection limit, good sensitivity and high stability of the sensor have demonstrated an excellent analytical performance. The procedure proposed has shown better results than those based on traditional flow injection systems and could be an alternative for development of microfluidic devices based on thread.

References

- [1] D.R. Reyes, D. Iossifidis, P. Auroux, A. Manz; Micro total analysis systems. 1. Introduction, theory, and technology; *Anal. Chem.*, 74 (2002), pp. 2623–2636
- [2] K. Ren, J. Zhou, H. Wu; Materials for microfluidic chip fabrication; *Acc. Chem. Res.*, 46 (2013), pp. 2396–2406
- [3] G.M. Whitesides; The origins and the future of microfluidics; *Nature*, 442 (2006), pp. 368–373
- [4] P. Lisowski, P.K. Zarzycki; Microfluidic paper-based analytical devices (μ PADs) and micro total analysis systems (μ TAS): development, applications and future trends; *Chromatographia*, 76 (2013), pp. 1201–1214
- [5] A.W. Martinez, S.T. Phillips, G.M. Whitesides, E. Carrilho; Diagnostics for the developing world: microfluidic paper-based analytical devices; *Anal. Chem.*, 82 (2010), pp. 3–10
- [6] K. Yamada, T.G. Henares, K. Suzuki, D. Citterio; Paper-based inkjet-printed microfluidic analytical devices; *Angew. Chem. Int. Ed. Engl.*, 54 (2015), pp. 5294–5310
- [7] D.R. Ballerini, X. Li, W. Shen; Patterned paper and alternative materials as substrates for low-cost microfluidic diagnostics; *Microfluid. Nanofluid.*, 13 (2012), pp. 769–787
- [8] D. Agustini, M.F. Bergamini, L.H. Marcolino-Junior; Low cost microfluidic device based on cotton threads for electroanalytical application; *Lab Chip*, 16 (2016), pp. 345–352
- [9] Y.C. Wei, L.M. Fu, C.H. Lin; Electrophoresis separation and electrochemical detection on a novel thread-based microfluidic device; *Microfluid. Nanofluid.*, 14 (2013), pp. 583–590

- [10] M.J.A. Shiddiky, Y. Shim; Trace analysis of DNA: preconcentration, separation, and electrochemical detection in microchip electrophoresis using Au nanoparticles; *Anal. Chem.*, 79 (2007), pp. 3724–3733
- [11] R.S. Martin, A.J. Gawron, S.M. Lunte, C.S. Henry; Dual-electrode electrochemical detection for poly(dimethylsiloxane)-fabricated capillary electrophoresis microchips; *Anal. Chem.*, 72 (2000), pp. 3196–3202
- [12] J. Wang; Electrochemical detection for microscale analytical systems: a review; *Talanta*, 56 (2002), pp. 223–231
- [13] O.D. Renedo, M.A. Alonso-Lomillo, M.J.A. Martínez; Recent developments in the field of screen-printed electrodes and their related applications; *Talanta*, 73 (2007), pp. 202–219
- [14] H.M. Mohamed; Screen-printed disposable electrodes: pharmaceutical applications and recent developments; *TrAC-Trends Anal. Chem.*, 82 (2016), pp. 1–11
- [15] F. Arduini, L. Micheli, D. Moscone, G. Palleschi, S. Piermarini, F. Ricci, G. Volpe; Electrochemical biosensors based on nanomodified screen-printed electrodes: recent applications in clinical analysis; *TrAC-Trends Anal. Chem.*, 79 (2016), pp. 114–126
- [16] N. Soltani, H. Haddadi, M. Asgari, N. Rajabzadeh; Adsorptive stripping voltammetric detection of thorium on the multi-walled carbon nanotube modified screen printed electrode; *Sens. Actuators B-Chem.*, 220 (2015), pp. 1212–1216
- [17] J. Upan, P. Reanpang, O. Chailapakul, J. Jakmunee; Flow injection amperometric sensor with a carbon nanotube modified screen printed electrode for determination of hydroquinone; *Talanta*, 146 (2016), pp. 766–771
- [18] Q. Zheng, P. Yang, H. Xu, J. Liu, L. Jin; A simple and sensitive method for the determination of 4-n-octylphenol based on multi-walled carbon nanotubes modified glassy carbon electrode; *J. Environ. Sci.*, 24 (2012), pp. 1717–1722
- [19] T. Blaudeck, D. Adner, S. Hermann, H. Lang, T. Gessner, S.E. Schulz; Wafer-level decoration of carbon nanotubes in field-effect transistor geometry with preformed gold nanoparticles using a microfluidic approach; *Microelectron. Eng.*, 137 (2015), pp. 135–140
- [20] J. Kim, J. Elsnab, C. Gehrke, J. Li, B.K. Gale; Microfluidic integrated multi-walled carbon nanotube (MWCNT) sensor for electrochemical nucleic acid concentration measurement; *Sens. Actuators B: Chem.*, 185 (2013), pp. 370–376

- [21]; K.F. Lei, K.F. Lee, S.I. Yang; Fabrication of carbon nanotube-based pH sensor for paper-based microfluidics; *Microelectron. Eng.*, 100 (2012), pp. 1–5
- [22] K.D. Santos, O.C. Braga, I.C. Vieira, A. Spinelli; Electroanalytical determination of estriol hormone using a boron-doped diamond electrode; *Talanta*, 80 (2010), pp. 1999–2006
- [23] R. Suri, G. Helleman, L. Cohen, A. Aquino, L. Altshuler; Saliva estriol levels in women with and without prenatal antidepressant treatment; *Biol. Psychiatry*, 64 (2008), pp. 533–537
- [24] M. Taylor; Unconventional estrogens: estriol, biest, and triest; *Clin. Obstet. Gynecol.*, 44 (2001), pp. 864–879
- [25] I. Cesarino, F.H. Cincotto, S.A.S. Machado; A synergistic combination of reduced graphene oxide and antimony nanoparticles for estriol hormone detection; *Sens. Actuators B-Chem.*, 210 (2015), pp. 453–459
- [26] P. Gan, R.G. Compton, J.S. Foord; The voltammetry and electroanalysis of some estrogenic compounds at modified diamond electrodes; *Electroanalysis*, 25 (2013), pp. 2423–2434
- [27] T.S. Chen, K.L. Huang; Effect of operating parameters on electrochemical degradation of estriol (E3); *Int. J. Electrochem. Sci.*, 8 (2013), pp. 6343–6353
- [28] I.C. Beck, R. Bruhn, J. Gandrass, W. Ruck; Liquid chromatography–tandem mass spectrometry analysis of estrogenic compounds in coastal surface water of the Baltic Sea; *J. Chromatogr. A*, 1090 (2005), pp. 98–106
- [29] D. Matějček, P. Houserová, V. Kubáň; Combined isolation and purification procedures prior to the high-performance liquid chromatographic-ion-trap tandem mass spectrometric determination of estrogens and their conjugates in river sediments; *J. Chromatogr. A*, 1171 (2007), pp. 80–89
- [30] Y. Tang, S. Zhao, Y. Wu, J. Zhou, M. Li; A direct competitive inhibition time-resolved fluoroimmunoassay for the detection of unconjugated estriol in serum of pregnant women; *Anal. Methods*, 5 (2013), pp. 4068–4073

- [31] Z. Li, S. Wang, N.A. Lee, R.D. Allan, I.R. Kennedy; Development of a solid-phase extraction—enzyme-linked immunosorbent assay method for the determination of estrone in water; *Anal. Chim. Acta*, 503 (2004), pp. 171–177
- [32] P. Su, X. Zhang, Y. Wang, W. Chang; Direct immunoassay of estriol in pregnancy serum by capillary electrophoresis with laser-induced fluorescence detector; *Talanta*, 60 (2003), pp. 969–975
- [33] A.P. Fonseca, D.L.D. Lima, V.I. Esteves; Degradation by solar radiation of estrogenic hormones monitored by UV-visible spectroscopy and capillary electrophoresis; *Water Air Soil Pollut.*, 215 (2011), pp. 441–447
- [34] S. Flor, S. Lucangioli, M. Contin, V. Tripodi; Simultaneous determination of nine endogenous steroids in human urine by polymeric-mixed micelle capillary electrophoresis; *Electrophoresis*, 31 (2010), pp. 3305–3313
- [35] X. Lin, Y. Li; A sensitive determination of estrogens with a Pt nano-clusters/multi-walled carbon nanotubes modified glassy carbon electrode; *Biosens. Bioelectron.*, 22 (2006), pp. 253–259
- [36] R.F. Brocenschi, R.C. Rocha-Filho, B. Duran, G.M. Swain; The analysis of estrogenic compounds by flow injection analysis with amperometric detection using a boron-doped diamond electrode; *Talanta*, 126 (2014), pp. 12–19
- [37] N. Terui, B. Fugetsu, S. Tanaka; Voltammetric behavior and determination of 17 beta-estradiol at multi-wall carbon nanotube-Nafion modified glassy carbon electrode; *Anal. Sci.*, 22 (2006), pp. 895–898
- [38] European Pharmacopoeia, EDQM – European Directorate for the Quality of Medicines and HealthCare, 5th ed., 2004.
- [39] L. Papouchado, R.W. Sandford, G. Petrie, R.N. Adams; Anodic oxidation pathways of phenolic compounds Part 2. Stepwise electron transfers and coupled hydroxylations; *J. Electroanal. Chem. Interfacial Electrochem.*, 65 (1975), pp. 275–284
- [40] M. Gattrell, D.W. Kirk; A study of electrode passivation during aqueous phenol electrolysis; *Electrochem. Soc.*, 140 (1993), p. 903

- [41] I.V. Batista, M.R. Lanza, I.L. Dias, S.M. Tanaka, A.A. Tanaka, M.D. Sotomayor; Electrochemical sensor highly selective for estradiol valerate determination based on a modified carbon paste with iron tetrapyrroline; *Analyst*, 133 (2008), pp. 1692–1699
- [42] D. Vega, L. Agüí, A. González-Cortés, P. Yáñez-Sedeño, J.M. Pingarrón; Electrochemical detection of phenolic estrogenic compounds at carbon nanotube-modified electrodes; *Talanta*, 71 (2007), pp. 1031–1038
- [43] M. Musameh, J. Wang, A. Merkoci, Y. Lin; Low-potential stable {NADH} detection at carbon-nanotube-modified glassy carbon electrodes; *Electrochem. Commun.*, 4 (2002), pp. 743–746
- [44] J. Wang, R.P. Deo, M. Musameh; Stable and sensitive electrochemical detection of phenolic compounds at carbon nanotube modified glassy carbon electrodes *Electroanalysis*, 15 (2003), pp. 1830–1834
- [45] J. Wang, S.B. Hocevar, B. Ogorevc; Carbon nanotube-modified glassy carbon electrode for adsorptive stripping voltammetric detection of ultratrace levels of 2,4,6-trinitrotoluene; *Electrochem. Commun.*, 6 (2004), pp. 176–179
- [46] F.H. Cincotto, T.C. Canevari, S.A.S. Machado, A. Sánchez, M.A.R. Barrio, R. Villalonga, J.M. Pingarrón; Reduced graphene oxide-Sb₂O₅ hybrid nanomaterial for the design of a laccase-based amperometric biosensor for estradiol; *Electrochim. Acta*, 174 (2015), pp. 332–339