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A micro-electrochemical sensor based on a nanostructured carbon paste electrode as an original tool for selective detection of ultra-traces of 17 α – ethinylestradiol in environmental water samples and in biologic fluids.

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Abstract

A new electrochemical micro-sensor has been designed for the sensitive detection of ultra-traces of 17 α – ethinylestradiol (EE₂). A carbon paste cavity micro-electrode modified by carbon black nanoparticles (GC-nCB)- μ CPE was used with an electrochemical preconcentration (EP) step of EE₂ on (GC-nCB)- μ CPE at 0.0 V during 300 s in a 0.01 mol.L⁻¹ phosphate buffer solution (pH = 7.4) before voltammetric determination. The results demonstrate that two line calibrations were obtained in the concentration ranges of 0.005 to 0.2 μ mol.L⁻¹ and 0.2 to 1 μ mol.L⁻¹. The detection and quantification limits are estimated at 1.4 nmol.L⁻¹ and 4.9 nmol.L⁻¹, respectively. Moreover, a high sensitivity of 4.16 μ A.mol⁻¹.L and good selectivity for the quantification of EE₂ in the presence of different interfering organic and inorganic compounds were observed. Based on the results (satisfactory recovery (less than 3.5%) and relationship standard deviation (less than 2.5%)), the designed micro-sensor can be successfully applied to the analysis of EE₂ in environmental water samples and in biologic fluids. The modified microsensor presented an efficient and inexpensive tool for the quantification of EE₂.

Keywords: Voltammetric micro-sensor, cavity micro-electrode, carbon paste, nanostructured materials, 17 α -éthinylestradiol.

1. Introduction

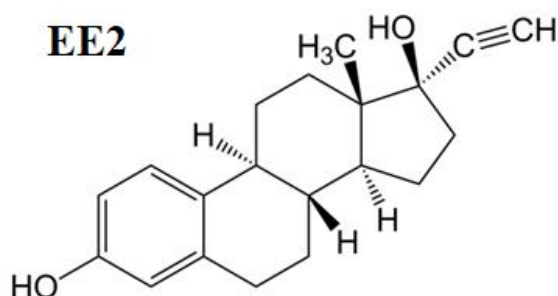
In this work, It was chosen to quantify a synthetic estrogen, 17 α -ethinylestradiol (EE₂) (Scheme 1), an active pharmaceutical compound introduced into aquatic environments by humans and animal excretions [1]. Due to its very high disruptive power, EE₂ is described as one of the most dangerous endocrine disruptors [2, 3]. This is why its quantification and effects on flora and fauna have been the subject of numerous studies in recent years [4-8]. Face with the necessity to dispose of quantification methods to assume the monitoring of water and wastewater, electrochemical sensors appear as reliable interesting detection technologies due to their capacity to be sensitive, selective, fast, inexpensive and suitable for on-site analysis. With conventional electrodes, it is difficult to quantify EE₂ at low concentrations by voltammetry due to the obtaining of a broad and poorly-defined irreversible EE₂ oxidation peak [9, 10]. Therefore, the design and manufacture of an efficient electrochemical microsensor for EE₂ trace analysis constitute a major challenge.

Recently, nanotechnology has become very popular in the sensor field. Indeed, the incorporation of nano-sized materials into the electrode matrix can have beneficial effects on the performance of sensors [11, 12]. It has been shown that nano-sized materials possess novel and very interesting physical and chemical properties. In this context, carbon black (nCB) is a nanostructured material widely used in several industrial processes [13]. This nanomaterial provides excellent properties, including a large specific surface area and high thermal and electrical conductivity [14]. Moreover, nCB is unique in its ability to intercalate ions between graphite layers [15], which affect the conduction between the particles and the surface reaction [16]. In particular, the structural defects formed during the synthesis or functionalization processes can create several functional groups useful for the immobilization of various species, such as enzymes [17], genetic materials and antibodies [18] and nanoparticles [19]. Thanks to its highly interesting structural and electrical/electronic characteristics, nCB is an economically attractive material for the design and development of electrochemical devices [20]. Moreover, due to these fascinating properties, carbon paste electrode (CPE) has gained a great theoretical and practical reputation in the electroanalytical field [21, 22]. CPE offers a number of unique and valuable electrochemical performances, providing: rich and biocompatible surfaces, high conductivity, low background current, low ohmic resistance, an extended potential window, long-term stability which can last up to four years storage, and cheap repair [23, 24]. Furthermore, carbon paste electrodes can be modified by adding various types of modifiers (such as multi-walled carbon nanotube, graphène,...) to increase sensitivity. [25-29]. We therefore focused our work on the development of a micro-sensor for the analysis of EE₂ using a carbon paste modified with carbon black nanoparticles (GC-nCB)-CP as electrode material.

In recent years, several studies presented the use of cavity microelectrode (CME) successfully as electrochemical sensors [30, 31]. Indeed, the CME is an original tool for the study of powdered materials, essentially characterized by its rapid and ease of implementation, without resorting to the use of binder. It offers the possibility of carrying out studies using very small quantities of materials, thus reducing the limitations of the ohmic drop and capacitive effects on voltammograms even at high potential scan rates. Moreover, comparative studies of the CME with the usual carbon paste electrodes (millimeter size) have shown for the CME better discrimination of the peaks on voltammograms with an increase in the sensitivity of detection [32, 33].

Thanks to these advantages linked to the properties of CME, the objective of this study was to design an innovative and ultra-sensitive electrochemical micro-sensor for the analysis of ultra-trace amounts of EE₂ in water samples and urine. The development of this microsensor is based on the carbon paste cavity microelectrode modified by carbon black nanoparticles ((GC-nCB)- μ CPE). The voltammetry technique was applied to evaluate the electroanalytical performance of the prepared micro-sensor. As far as is known, ((GC-nCB)- μ CPE) has never been described before for the determination of EE₂ and was applied to EE₂ determination in real wastewater sample and in synthetic urine.

Scheme 1: Chemical structure of EE₂



2. Materials and methods

2.1. Reagents

All chemical reagents used in this study were of analytical grade (the highest purity available). Graphite powder ($\leq 4 \mu\text{m}$; 99,99 %) from Aldrich, France, carbon black ($\leq 100 \text{ nm}$; 99,95%) from Cabot Corporation (Vulcan XC-72, average diameter 30 nm and SBET 254 m^2/g), France. Ethanol (99%), 17 α -ethinylestradiol (analytical standard; 99.9%) were purchased from Sigma-Aldrich, France, and phosphate buffer EMSURE for analysis (> 98 %) from Supelco, France. The pH of the solutions was adjusted using NaOH (98 %, Sigma-Aldrich, France) and sulfuric acid (98 %, for analysis EMSURE, France). All aqueous solutions were prepared with ultrapure water at 18.2 $\text{M}\Omega \text{ cm}$ (20 ° C) from the Millipore Direct-Q Water system. Stock solutions of EE₂ (0.01 M) were prepared with ethanol and subsequent dilutions were prepared daily with ultrapure water and stored at 4 ° C in the dark. All reagents were used as received, and all experiments in this study were performed at room temperature (20 \pm 1 ° C).

2.2. Apparatus

All electrochemical techniques were carried out using a PGSTAT 20 (Metrohm) potentiostat/galvanostat in combination with NOVA 2.1 software. The measurements were carried out using a classical electrochemical cell composed of three electrodes: a carbon paste cavity microelectrode (CME) (microcavity: 50 μm diameter; 32 μm depth) (WE), an auxiliary Pt wire electrode (AE) and an Ag/AgCl (3M KCl) reference electrode (RE). The pH of solutions was measured using a pH- meter: Mettler Toledo FE20/EL20. Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-7100F, EDS detector Oxford Instrument 50 mm^2 from SCANMAT (CMEBA platform) at University of Rennes, France.

2.3. Preparation of carbon paste micro-sensor

Carbon black modified carbon paste composite microelectrodes (GC-nCB)- μCPE were prepared by carefully mixing graphite carbon powder (GC) and carbon black nanoparticles (nCB). Indeed, in order to optimize the electrode matrix composition, various mass ratio of graphite and nCB have been used, respectively 40 to 100% and 0 to 60% for the preparation of the (GC-nCB)- μCPE . All pastes were prepared manually as described in the literature [34] and consisted of mixing the ingredients in an agate mortar with a pestle until a homogeneous paste was obtained (during 30 min). The duration of preparation plays an essential role in order to have reproducible pastes. These different pastes were introduced and compacted into CME is essential and can be done in an ultrasonic bath by immersing the CME for 5 minutes successively in the following solutions: a nitric acid solution (HNO_3 ; 2M); then, in a solution

of hydrogen peroxide (H_2O_2 ; 10%) and finally, in ultrapure water. Next, a control procedure was used to verify the emptying of the cavity that consists to plot cyclic voltammograms of the CME in an acidic medium (H_2SO_4 0.5 M) allowing the observation of typical compartment of platinum electrode. The steps for the preparation of (GC-nCB)- μCPE are illustrated in Figure 1.

Insert Fig 1

2.4. Real samples preparation

In this work, the aim was to study the micro-sensor developed in two local water samples (wastewater from WWTPs treated before being released into with a membrane process (WWTPs-M) and wastewater from WWTPs treated with a decantation process (WWTPs-D)) and in urine using synthetic urine. The synthetic urine used in this study was synthesized by using the method reported by N. Sarigul et al [35]. The individual samples were filtered through a syringe filter (0.22 μm) and diluted for 10 times in the case of urine samples and spiked with tens or hundreds of nanomoles of EE_2 concentration and stored in the dark at 4 $^\circ\text{C}$. The EE_2 concentration measurements were performed under optimal conditions using the standard addition method.

3. Results and discussion

3.1 Microscopic characterization of GC, nCB and (GC- nCB) –CP

The morphologies of the carbon graphite (CG) of 4 μm in size, the carbon black (nCB) and their mixtures as carbon paste (GC-nCB) -CP in the modified microelectrode were analyzed by scanning electron microscopy (SEM). Figure 2 shows the SEM images obtained for the GC (A), nCB (B) and (C) and (GC-nCB)–CP (D) mixtures used for the preparation of the modified cavity microelectrodes. We can see on the SEM image (A) the typical morphology of the graphite particles, which appear in the form of dispersed sheets of size less than 4 μm . (B) and (C) SEM images presents carbon black nanoparticles (nCB) that appears as spongy aggregates of small spherical particles with nanometric size (<100 nm). For the prepared mixture of GC and nCB particles (50/50, %w), the SEM image (D) shows that both materials are evenly dispersed in the GC sheets coated with nCB particles, avoiding graphite platelet stacking, promoting analyte accessibility in their surfaces and improving charge transfer in the paste. Globally, the (GC-nCB)–CP electrode material showed a uniform and nanostructured surface which could yield to large surface area for the microsensor.

Insert Fig 2

3.2. Electrochemical impedance spectroscopy (EIS) studies

EIS is a sensitive and efficient electrochemical technique to study and monitor the surface characteristics of modified electrodes which presents the electron transfer resistance (R_{ct}) at the electrode surface [36]. This procedure provides valuable information on the change in conductance or impedance of an electrode/electrolyte interface during its modification. For this purpose, the microelectrodes: bare (GC)- μCPE and modified (GC-nCB)- μCPE , were studied by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) in 5.0 mM $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ equimolar mixture with 0.1 M KCl as supporting electrolyte. As

shown in Figure 3 (A), it is evident that the CV current of (GC-nCB)- μ CPE is much higher than that of (GC)- μ CPE (2.5 times higher), this is due to the increase in the specific surface area due to the nanostructuring of the carbon paste electrode by the nCBs. In order to determine the electroactive surface area of bare and modified electrodes, it was calculated the effective electrochemical surface area of (GC)- μ CPE and (GC-nCB)- μ CPE, using the Randles–Sevcik equation (Eq. (1)) [37]:

$$I_{pa} = 2.69 \times 10^5 \times A \times D^{1/2} \times n^{3/2} \times v^{1/2} \times C \quad (1)$$

Where I_{pa} (A) refers to the anodic peak current, n is the electron transfer number ($n=1$), D is the diffusion coefficient ($6.70 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) [38], C is the concentration of $\text{Fe}(\text{CN})_6^{3-}$ in the solution (5 mmol.L^{-1}), v is the scan rate (0.05 V.s^{-1}) and A (cm^2) is the effective surface area, which is estimated for the electrodes. Based on the results presented in Figure 3 (A) the value of the active electrode area was found to be $2.1 \times 10^{-7} \text{ cm}^2$ and $1.4 \times 10^{-6} \text{ cm}^2$ for (GC)- μ CPE and (GC-nCB)- μ CPE, respectively, which was nearly 7 times greater.

Figure 3 (B) shows the Nyquist plot of (GC)- μ CPE and (GC-nCB)- μ CPE obtained by EIS measurements using an applied potential of 0.2 V, amplitude of 10 mV and in a frequency range of 10^{-2} to 10^5 Hz. As can be seen, in the (GC)- μ CPE, a semicircle was observed with resistance of charge transfer (R_{ct}) of 148.0 K Ω obtained from Randles circuit. Once the microelectrode was modified with nCB, a much smaller semicircle was obtained with R_{ct} of 60.6 K Ω , indicating that the subsequent nanostructuring with carbon black nanoparticles improved the charge transfer kinetics at the electrode-solution interface and reduced the R_{ct} value by a factor of 2.5.

Insert Fig 3

3.3. Cyclic voltammetry study of μ CPEs

3.3.1 Effect of paste composition on CV response of EE_2

In order to determine the best composition of the carbon paste in term of sensitivity in EE_2 CV response (highest oxidation peak current), we have prepared various mass compositions of the carbon paste, comprising 40 to 100% graphite and 0 to 60% nCB: (i) graphite powder (100%) (GC)-CP-A, (ii) graphite powder (85%) and carbon black (15%) (GC-nCB)-CP-B, (iii) graphite powder (80%) and carbon black (20%) (GC-nCB)-CP-C, (iv) powder graphite (70%) and carbon black (30%) (GC-nCB)-CP-D, (v) graphite powder (60%) and carbon black (40%) (GC-nCB)-CP-E, (vi) graphite powder (50%) and carbon black (50%) (GC-nCB)-CP-F and (vii) graphite powder (40%) and carbon black (60%) (GC-nCB)-CP-J.

Voltammograms for EE_2 oxidation were recorded in an aqueous solution containing 0.01 M phosphate buffer ($\text{pH} = 7.4$) as the supporting electrolyte, explored potential 0.0 to 1.0 V/Ag/AgCl, scan rate 50 mV.s^{-1} and a low EE_2 concentration of $1.0 \times 10^{-7} \text{ mol.L}^{-1}$ with an accumulation time of 5 minutes at 0.0 V/Ag/AgCl with stirring (300 rpm). By comparing the values of oxidation peak current obtained from these different (GC-nCB)- μ CPE (Figure 4), it is clear that the best current intensity was obtained with the compositions (vi) (GC-nCB)-CP-F and (vii) (GC-nCB)-CP-J of the modified microelectrode (GC-nCB)- μ CPE. This indicates that the addition of carbon black has significantly improved the electrochemical signal of EE_2 thanks to the exceptional properties of carbon black (nCB), such as its large surface area, its high thermal and electrical conductivity and its very low cost. Several studies have shown

that the charge transfer and electroactive surface of modified nCB-based electrodes are comparable to those of carbon and graphene nanotubes [39]. These characteristics make nCB a promising candidate for the design of electrochemical sensors and biosensors [14, 40, 41]. For the two compositions (vi) and (vii) containing 50% and 60% nCB respectively, it was possible to record the same current intensity value as the best response (see figure 4). Consequently, using the composition containing 50%-50% as the optimum composition for the remainder of the experiments.

Insert Fig 4

3.3. 2 Effect of preconcentration time

The influence of the preconcentration time for the EP step at 0V/Ag/AgCl on the response of micro-sensor in linear sweep voltammetry was examined under the following operating conditions: 0.01 M phosphate buffer solution (pH = 7.4) in the presence of 2.5×10^{-7} mol.L⁻¹ of EE₂, scan rate 50 mV.s⁻¹, explored potential 0.0 to 1.0 V/Ag/AgCl and ET at 0V under stirring (300 rpm). As can be seen in insert Figure 5, the voltammograms present an oxidation peak of EE₂ at 0.48 V with a Gaussian shape that is characteristic of a diffusion less electrochemical behavior [42] corresponding to the oxidation of adsorbed EE₂. The adsorption of EE₂ occur for EP step with the polarization of (GC-nCB)- μ CPE at the potential 0V/Ag/AgCl under stirring. The oxidation current peak intensity of adsorbed EE₂ increases with the accumulation time and reaches a maximum at 5 minutes. At longer preconcentration times up to 8 minutes, no change was observed, indicating that EE₂ absorption equilibrium is reached on (GC-nCB) - μ CPE. Therefore, the accumulation time of 5 minutes was chosen as optimal condition for the subsequent experiments.

Insert Fig. 5

3.3. 3 Effect of sweep rate

In order to obtain information about the oxidation process of EE₂ on the (CG-nCB) - μ CPE electrode, the electrochemical response has been investigated for various scan rates 10 - 80 mV.s⁻¹. Voltammograms were recorded in 0.01 M phosphate buffer (pH = 7.4), in the presence of 1.5×10^{-7} mol.L⁻¹ of EE₂, an accumulation time of 5 minutes at 0.0 V/Ag/AgCl with stirring (300 rpm), and explored potential 0.0 to 1.0 V/Ag/AgCl (Figure 6 (A)). The results obtained show that the oxidation peak current of EE₂ increases with the potential scan rate and with a linear variation (correlation coefficient R² 0.999). This demonstrates diffusion less electrochemical process controlled by adsorbed electroactive species (EE₂) in the nanostructured carbon paste (GC-nCB-CP) in the cavity microelectrode [41, 42].

Additionally, the plot of oxidation peak potentials versus the logarithm of the scan rate allows us to determine the number of electrons involved in the electrochemical reduction of EE₂. The results in Figure 6 (B) show that E_{pa} as a function of $\log(v)$ is a straight line with a regression equation as follows:

$$E_{pa} (V) = 0.0648 \log (v / mV. s^{-1}) + 0.397 (R^2 = 0.998) \quad (2).$$

As indicated by Laviron's theory [42], the slope value obtained corresponds to $(- 2.3RT/\alpha nF)$; where R is the gas constant (8.314 J.mol⁻¹.K⁻¹), T is the ambient temperature; α is the transfer coefficient which in the case of a non-reversible process is equal to 0.5, n is the number of electrons transferred and F is the Faraday constant. Using the slope value, the number of

electrons transferred during the reaction of EE₂ was calculated to be equal to $1.822 \approx 2$. Thus, it can be assumed that the global process of electro-oxidation of EE₂ on the (CG- nCB) - μ CPE involves two electrons.

Insert Fig. 6

3.3. 4 Effect of pH

The influence of pH on the oxidation of EE₂ with the micro-sensor (GC-nCB) - μ CPE was studied by voltammetry in the pH range from 3.0 to 10.0. Operating parameters include a supporting electrolyte consisting of 0.01 M phosphate buffer solution with 1.0×10^{-7} mol.L⁻¹ EE₂, a scan rate of 50 mV.s⁻¹, a potential studied from 0.0 to 1.0 V/Ag/AgCl, an accumulation time of 5 minutes at 0.0 V/Ag/AgCl with stirring (300 rpm) and ET at 0 V. From the recorded voltammograms at different pH to the plots of Ip (A) and Ep (V) as a function of pH are presented respectively Figure 7 (A) and Figure 7 (B).

Insert Fig. 7

The pH has a direct influence on the peak current intensity of the electro-oxidation of EE₂, as can be seen in Figure 7 (A). From pH 3 to pH 8, the EE₂ oxidation peak intensity (Ip) increases linearly with the pH (slope of 0.07 μ A/pH unit), reaches a maximum and decreases for pH upper to 8. This decreasing is justified by the formation of the ionized form of EE₂ molecule (phenolate), which is anionic, since its pKa value is of about 10 [5]. The adsorption of this anionic form of EE₂ is not favorable during the electrochemical deposition step at 0V. In order to optimize the current intensity, a phosphate buffer at pH 7.4 was chosen for recording the voltammograms. By plotting the variation of the anodic peak potential (Epa) of EE₂ as a function of pH (Figure 7 (B)), it was observed that Ep shift to more negative values with increasing pH of the electrolyte. This demonstrates the involvement of proton in the electro-oxidation reaction of EE₂. Indeed, Epa for the oxidation of EE₂ on the electrode (GC-nCB) - μ CPE showed a linear relationship with the pH of the solution, according to the following equation:

$$E_{pa} = -0.0592 \text{ pH} + 0.918 \quad (R^2 = 0.997) \quad (3).$$

The value of the slope is equal to the theoretical one 0.059 V/pH (Nernstian behavior) [43], according to the Nernst equation; $E_p \text{ (V)} = 0.059 \text{ (m/n)} / \text{pH}$ (n is the number of electrons while m is the number of protons). This suggests the participation of the same number of protons and electrons in the electrochemical EE₂ oxidation mechanism (n=m= 2) [44] which is consistent to the proposed reaction mechanism for electro-oxidation of EE₂ on (CG-nCB) - μ CPE presented in the scheme of Figure 8 [45].

Insert Fig. 8

3.4. Validation of the proposed analytical method with GC-nCB) - μ CPE

3.4.1 Micro-sensor performance

The calibration curve was plotted under the optimal conditions previously determined: 0.01 M phosphate buffer solution (pH = 7.4) as supporting electrolyte, a potential range of 0.0 – 1.0 V/Ag/AgCl, a scan rate of 50 mV. s⁻¹, a preconcentration time of 5 min at 0V. Voltammograms were recorded with (GC-nCB)- μ CPE by varying the EE₂ concentrations in the cell. The intensity of the EE₂ oxidation current peak increases with increasing

concentration (Figure 9). Two calibration lines were obtained for the lowest concentrations, in the range 5 -200 nmol.L⁻¹, and for highest concentrations from 0.2 to 1 μmol.L⁻¹ (Figure 9); with respectively the following regression equations:

$$I_{pa} (\mu A) = 4.16 C + 4.47 \times 10^{-3} (\text{mol.L}^{-1}) \quad (R^2 = 0.999) \quad (4).$$

$$\text{And } I_{pa} (\mu A) = 1.232 C + 0.752 (\text{mol.L}^{-1}) \quad (R^2 = 0.993) \quad (5).$$

Moreover, the sensitivity of the micro-sensor is 3.4 times larger for the lowest concentrations than those above 0.2 μmol.L⁻¹. Furthermore, the LOD and LOQ were calculated, considering the linearity for the lowest concentrations, using the following equations: LOD = 3S/m and LOQ = 10S/m respectively [46, 47], where: S is the standard deviation of the blank and m is the slope of the calibration line. The calculated detection LOD and LOQ are 1.4 nmol.L⁻¹ and 4.9 nmol.L⁻¹, respectively.

Insert Fig 9

3.4.2. Repeatability and reproducibility of the proposed method

For the purpose of estimating the accuracy of the proposed analytical method, studies of reproducibility and repeatability were conducted and evaluated for three standard solutions with EE₂ concentrations: 5.0, 7.5 and 10.0 × 10⁻⁸ mol.L⁻¹. The voltammogrammes were recorded on (CG-nCB)-μCPE under optimal operating conditions (0.01 M phosphate buffer (pH = 7.4), an accumulation time of 5 minutes at 0.0 V/Ag/AgCl with stirring (300 rpm), a scan rate of 50 mV.s⁻¹, and explored potential 0.0 to 1.0 V/Ag/AgCl).

Indeed, the repeatability of (CG-nCB) -μCPE was evaluated using the same microsensor and the reproducibility was studied using four micro-sensors prepared independently by the same procedure under the same operating conditions. The results obtained from the measured oxidation peak currents are collected in Table 1. For the different concentrations, the calculated relative standard deviations (RSD) do not exceed 3.8% for repeatability and 4.8% for reproducibility. These low RSD values show that the modified microelectrode (CG-nCB) -μCPE present a good repeatability and reproducibility indicating that the filling of the microcavity by the (CG-nCB) carbon paste was very reproducible.

Insert Tab. 1

3.4.3 Effect of interference

The influence of various substances as compounds that could potentially interfere with the determination of EE₂ at (GC-nCB) -μCPE in water or urine was investigated under the optimal conditions previously described. Voltammetry measurements were carried out on solutions containing EE₂ 1.0 × 10⁻⁷ mol.L⁻¹ in the presence of 1.0 × 10⁻⁷ mol.L⁻¹ of molecules commonly found in water and/or urine (sulfamethazine, amoxicillin, metronidazole, danofloxacin, ofloxacin, atrazine, ketoprofen, propazine, glyphosate, ampicillin, uric acid, urea and glucose). Some cations and anions species were also tested as potential interfering compounds (SO₄²⁻, K⁺, Cl⁻, CH₃COO⁻, HPO₄²⁻) but the results indicated that no modification of the EE₂ oxidation peak current occurred for these ionic species.

By exploiting the intensities of the oxidation peaks in the voltammograms recorded with (CG-nCB) -μCPE, in the absence and in the presence of the organic compounds tested (Figure 10),

no Ipa variation was recorded for the majority of compounds. Only a few compounds (metronidazole, ketoprofen, propazine and ampicillin) led to, slight variations of less than 7.0 % of the initial oxidation current.

These results reveal that the micro-sensor (CG-nCB) μ CPE with EP step at 0V provides a sufficient high selectivity for the EE₂ voltammetric detection in presence of interfering organic and inorganic compounds.

Insert Fig. 10

3.4.4 Analysis of real samples

To evaluate the validity of the proposed method, the (CG-nCB) μ CPE was applied for the determination of the EE₂ in two local water samples collected after two different tertiary treatments before returning to environment (WWTPs -M and WWTPs -D) and in synthetic urine. Voltammetry measurements were carried out in an aqueous solution containing 0.01 M phosphate buffer (pH = 7.4) as supporting electrolyte, the potential range is 0 to 1 V versus Ag/AgCl, a scan rate of 50 mV.s⁻¹, and a preconcentration time of 5 min with stirring (300 rpm). In all the three samples, no trace of EE₂ was detected with the modified electrode (less than LOD 1.4 nmol.L⁻¹). Consequently, the analyses were carried out on water and urine samples enriched respectively with tens and hundreds of nanomolar of EE₂ concentration. The EE₂ concentration measurements were obtained by the standard addition method under the optimal operating conditions for the voltammetric method at (CG-nCB) μ CPE.

The obtained results are summarized in Table 2. The values of the recoveries obtained for 4 replicates for the samples (WWTPs -M), (WWTPs -D) and urine are respectively of 101.3%, 101.1% and 103.3%, with RSD of 1.4%, 1.3% and 2.3%, indicating that there were no significant matrix interferences on the response of the (CG-nCB) μ CPE. Therefore,

the CG-nCB) μ CPE appears as a precise sensor for the EE₂ analysis in environmental water and urine samples.

Insert Tab. 2

3.4.5 Comparison with subsequent work

Table 3 lists some examples of electrochemical sensors intended for the analysis of the hormone 17 α – ethinylestradiol (EE₂) and the comparison of analytical performances of the micro-sensor presented in this paper (the linearity range and the detection limit) with those of other electroanalytical methods reported recently in the literature. In this work, it was achieved some of the lowest LOD values (in the nanomolar range), similar to those obtained in AdSV at mercury electrodes (HMDE) [52, 53], with a large range of analyzable EE₂ concentrations. Therefore, these results confirm that the proposed micro-sensor (CG-nCB) μ CPE could be a good alternative method for the quantification of ultra-traces of EE₂ in the environment and urine.

Insert Table.3

4. Conclusion

For the analysis of the hormone 17 α -ethinylestradiol, it has been proposed a simple, easy-to-prepare and economical voltammetric method by using a novel micro-sensor (GC-nCB)- μ CPE) based on microcavity electrode and nanostructured carbon paste. In fact, the combination of a carbon paste microsensor with nCB guarantees interesting sensitivity and selectivity of the method developed. Under optimum operating conditions (0.01 M phosphate buffer (pH = 7.4), scan rate of 50 mV. s⁻¹ and preconcentration time of 5 min at 0V under stirring), the proposed micro-sensor exhibits fast and precise response, and exceptional selectivity. In addition, (GC-nCB)- μ CPE has a very low detection limit of around 1.5 nmol.L⁻¹, a wide linear concentration range (3 decades), good repeatability and reproducibility (overall the RSDs are less than 4.8%). (GC-nCB)- μ CPE) was successfully applied, without interference, to the quantification of ultra-trace EE₂ in environmental water and urine samples (the recovery percentages for the spiked samples being very close to 100% with a maximum deviation of 3.3%). This method is also more sensitive than many other analytical techniques reported in the literature.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to impact the work reported in this paper.

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Journal Pre-proofs

Figures

Fig 1

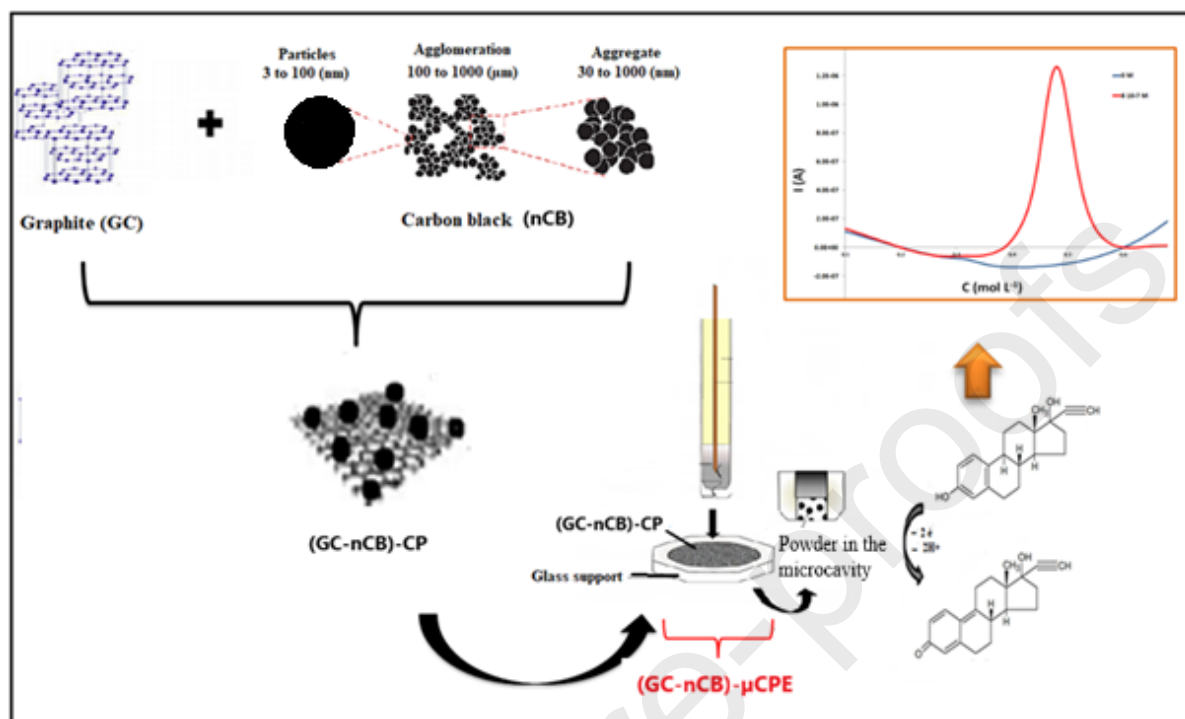


Fig 2

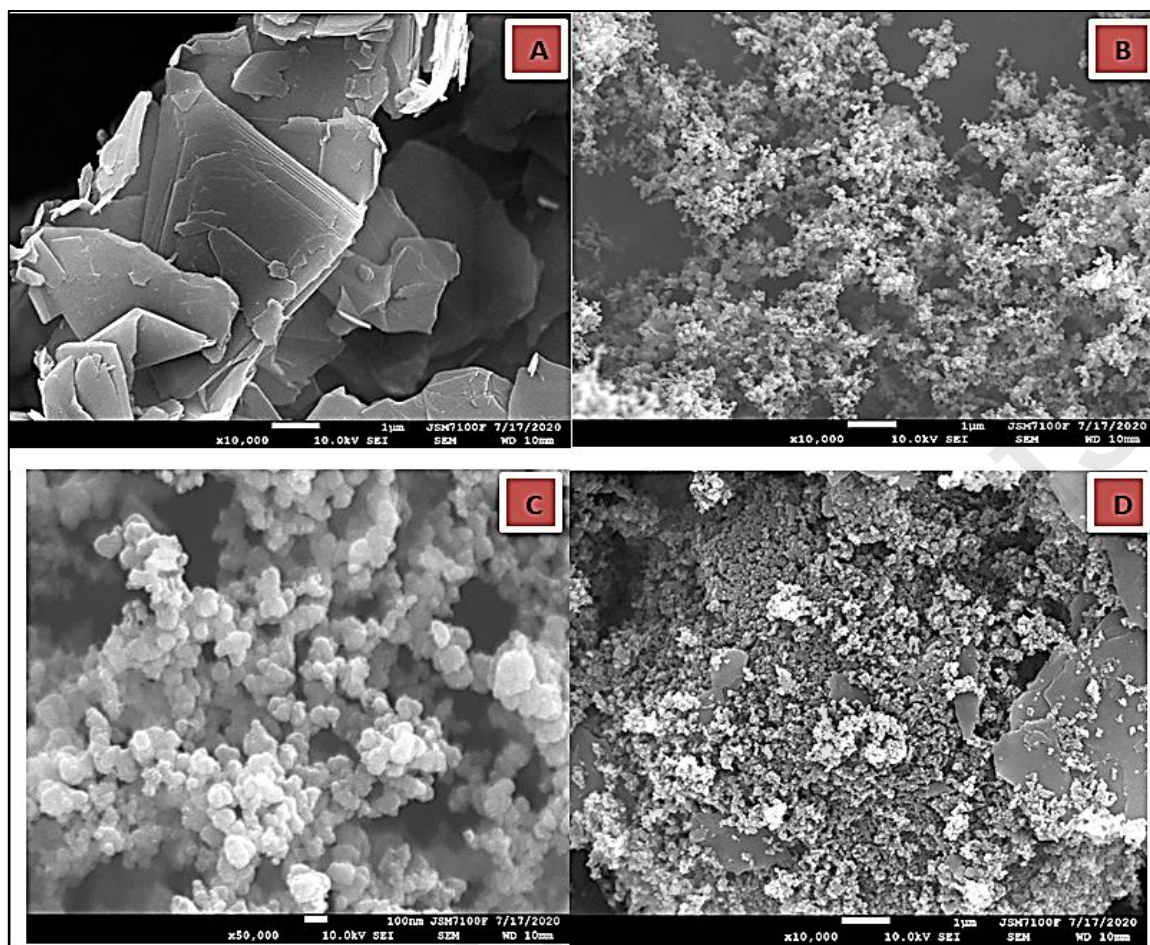


Fig 3

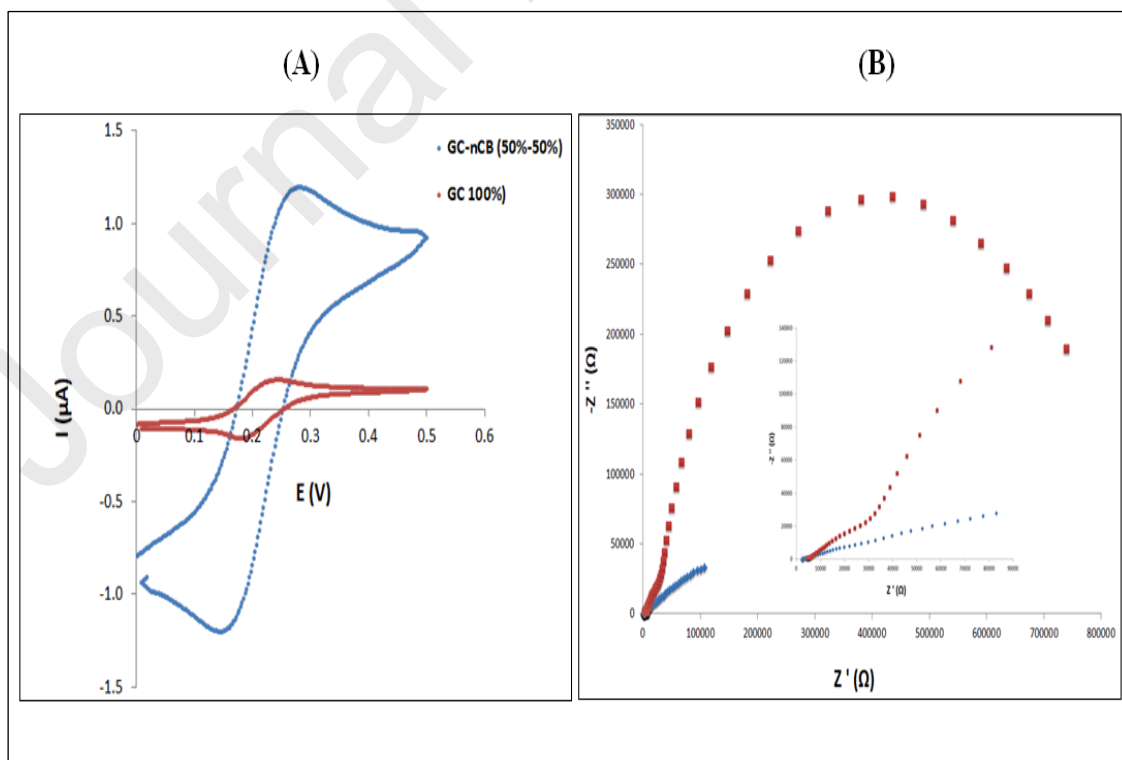


Fig 4

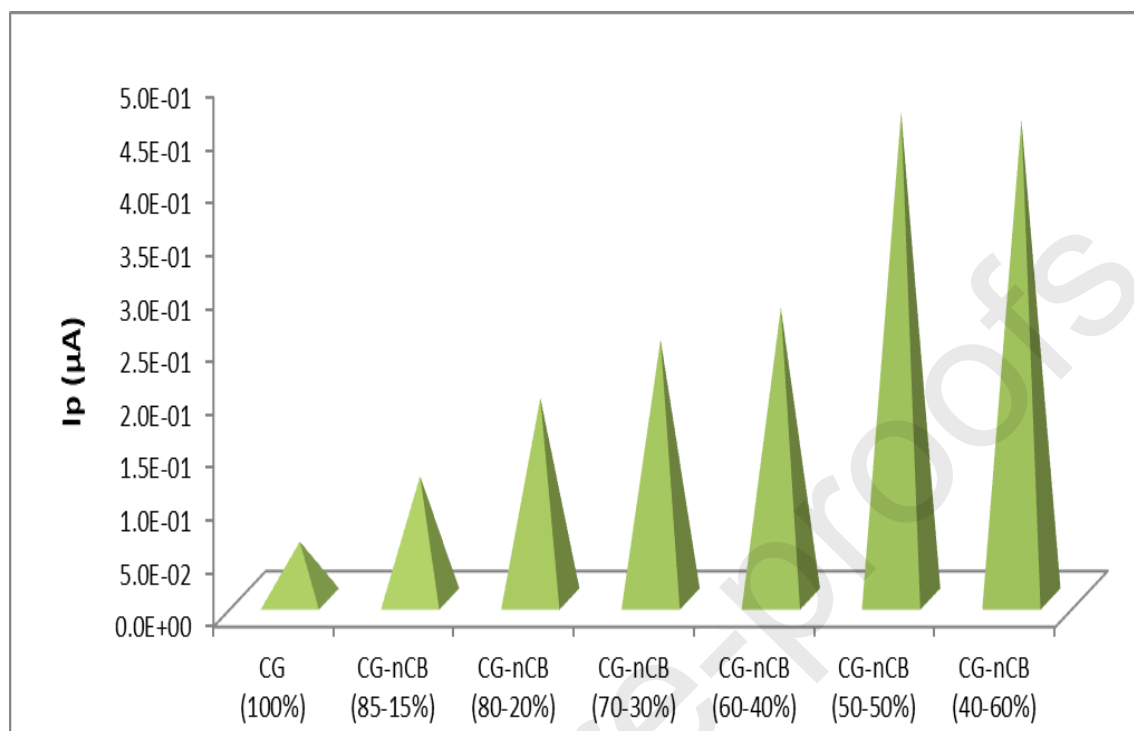


Fig 5

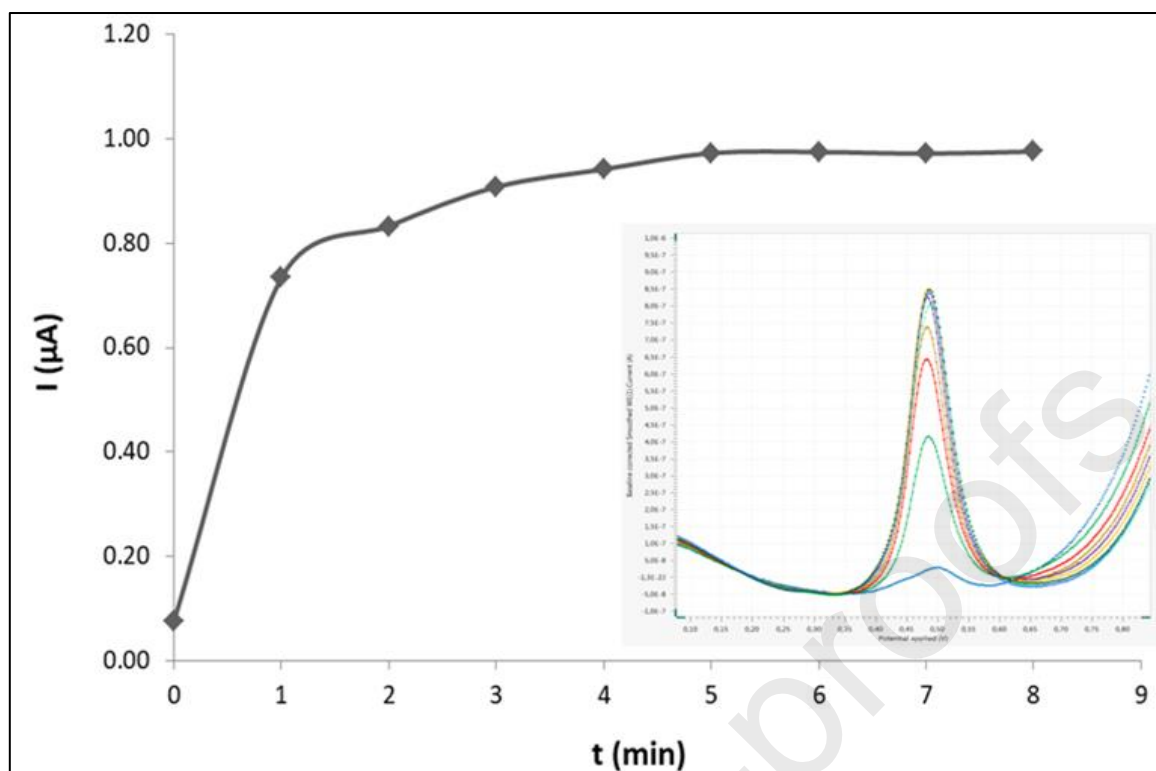
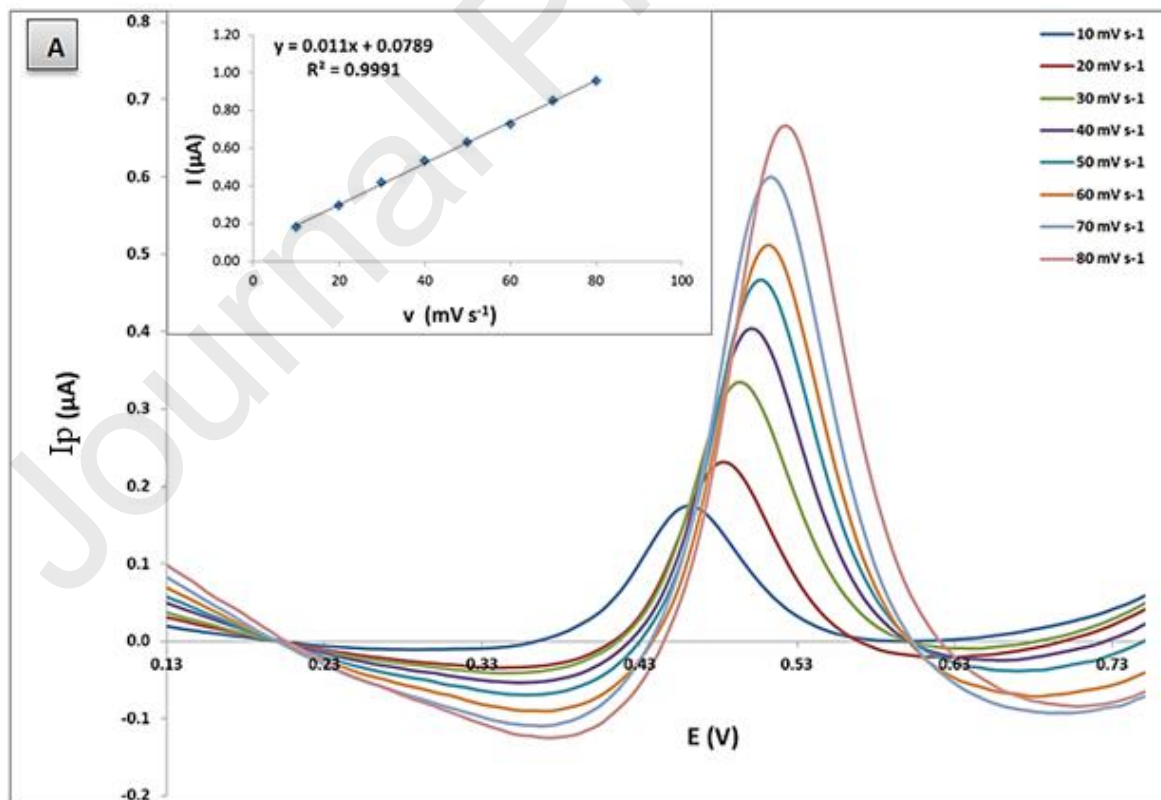


Fig 6



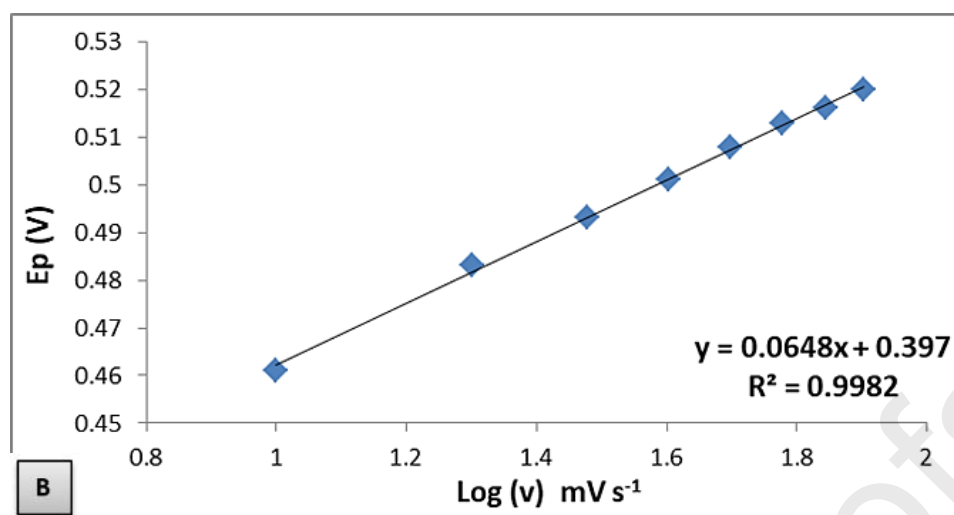


Fig 7

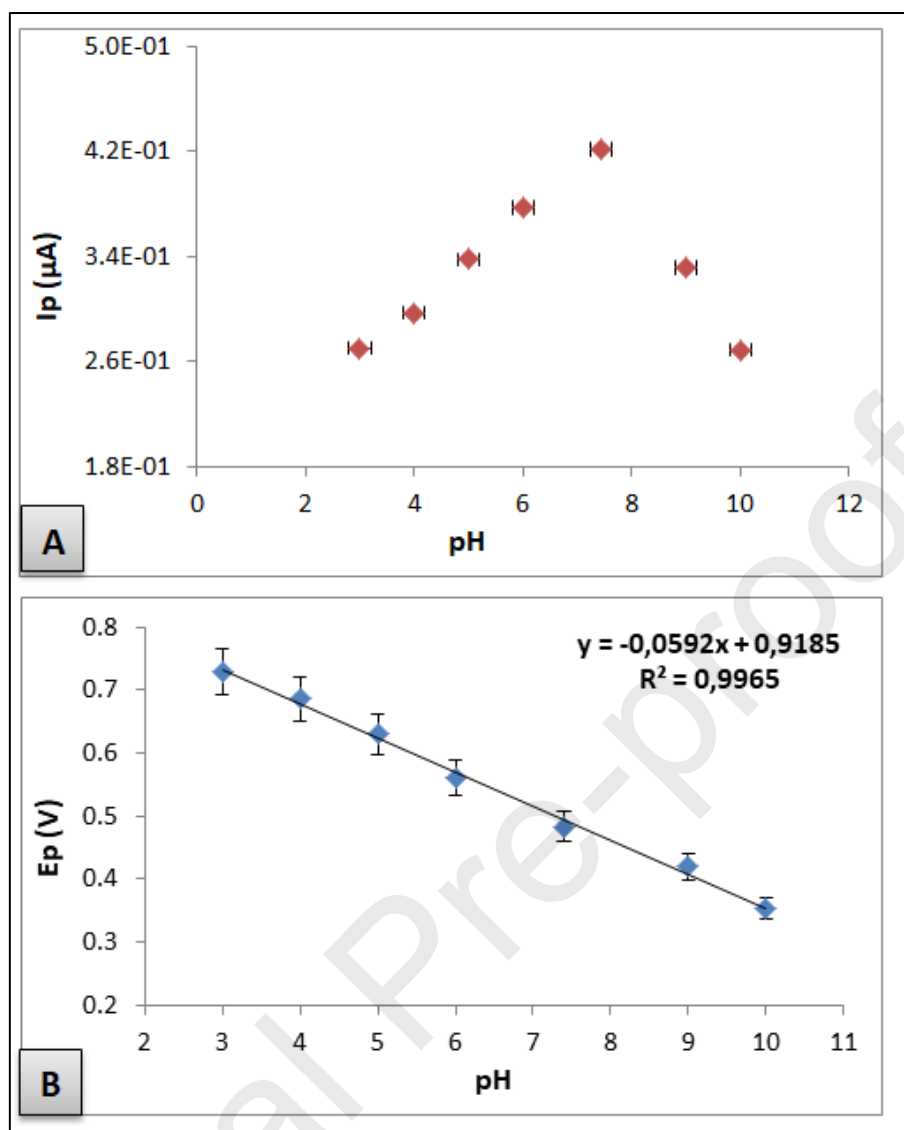


Fig 8

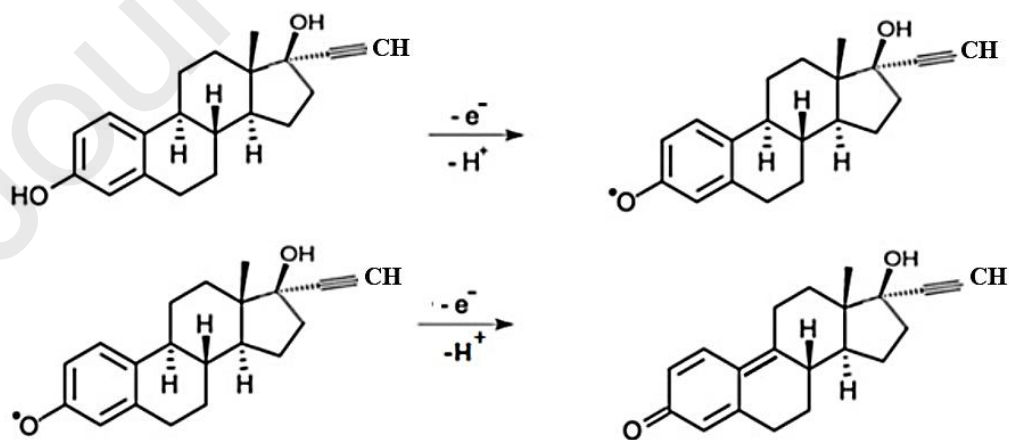


Fig 9

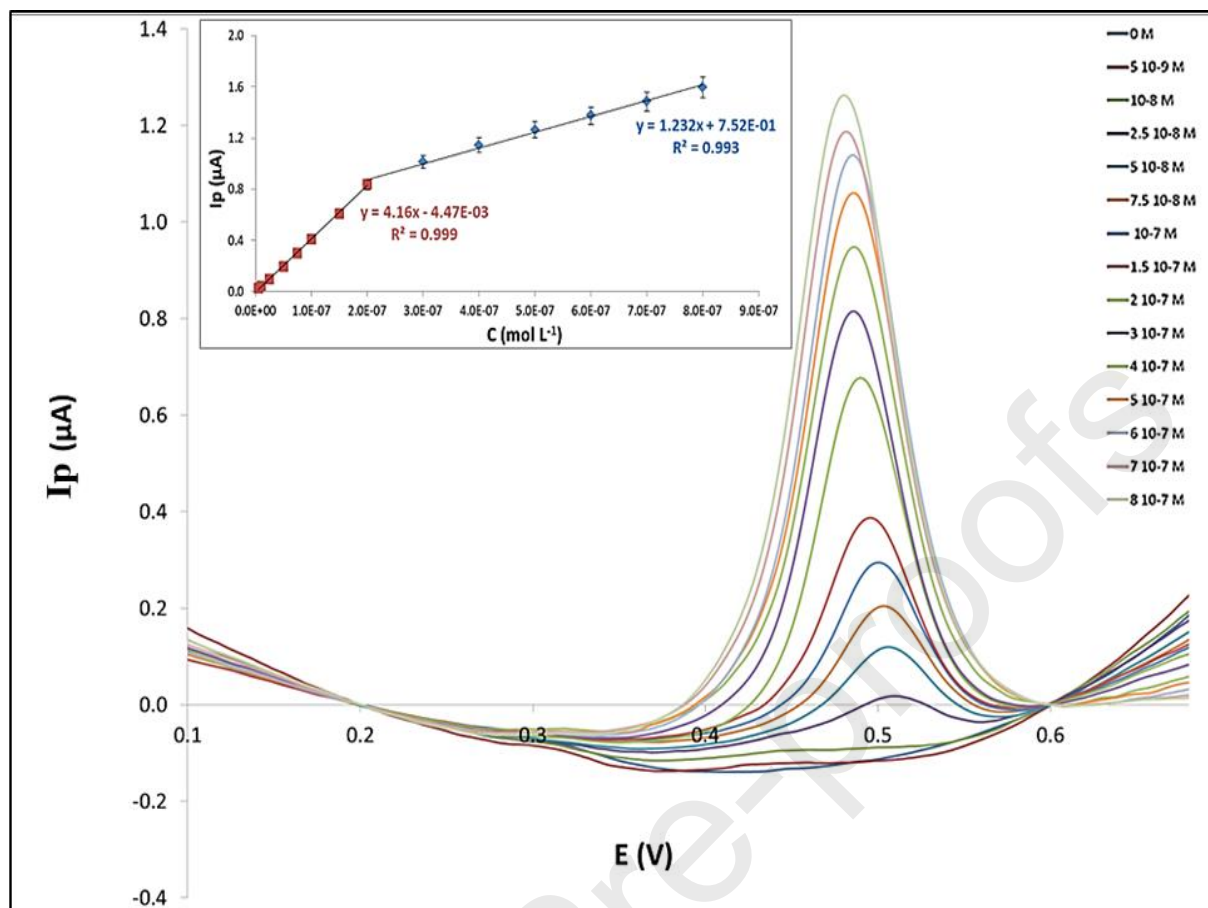


Fig 10

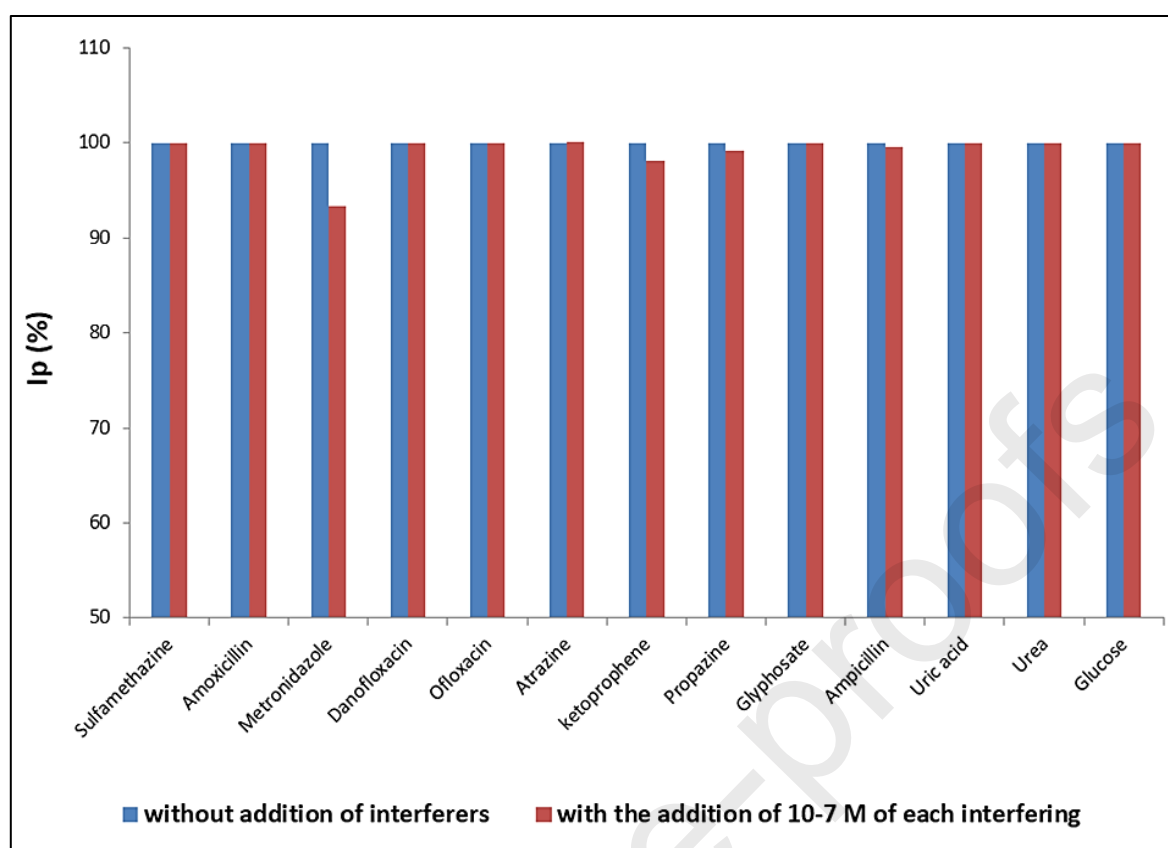


Figure captions

Figure 1. Graphical presentation of (GC-nCB)-CP micro-sensor fabrication.

Figure 2. SEM images of (A) graphite carbon (GC), (B) and (C) carbon black. (nCB) and (D) the (GC- nCB)-CP mixture (graphite powder (50%) and carbon black (50%) –CP).

Figure 3. (A) CVs and (B) Nyquist plots of different electrodes for the unmodified (GC)- μ CPE and modified (GC-nCB)- μ CPE carbon paste microelectrode in a 0.1 M KCl solution containing 5 mmol L⁻¹ Fe (CN)₆^{3-/4-}.

Figure 4. Histogram illustrating the influence of CPE composition on micro-sensor voltammetric response, in 0.01 M phosphate buffer (pH = 7.4), EE₂ concentration of 1.0 \times 10⁻⁷ mol L⁻¹, scan rate of 50 mV. s⁻¹, explored potential 0.0 to 1.0 V/Ag/AgCl.

Figure 5. Effect of preconcentration time on the EE₂ peak oxidation current intensity in voltammetry with (GC-nCB)- μ CPE; insert the corresponding voltammograms in 0.01 M phosphate buffer solution (pH = 7.4) in the presence of 2.5 \times 10⁻⁷ mol.L⁻¹ of EE₂, scan rate 50 mV.s⁻¹ and ET at 0V under stirring (300 rpm).

Figure 6. Study of the effect of scan rate on (GC-NC)- μ CPE in 0.01 M phosphate buffer (pH = 7.4) containing 1.5 \times 10⁻⁷ mol L⁻¹ of EE₂, with 5 min accumulation time at 0.0 V / Ag/AgCl for various scan rates 10 - 80 mV. s⁻¹: voltammograms and curve of variation of anodic peak currents as a function of scan rate (A) and variation of anodic peak potentials as a function of logarithms of scan rates (B).

Figure 7. Influence of pH (from 3.0 to 10.0) on the voltammetric response obtained for 1.0×10^{-7} mol.L⁻¹ of EE₂, with 5 min accumulation time at 0.0 V / Ag/AgCl, and a scan rate of 50 mV.s⁻¹: the variation of the anodic peak current intensity, *I*_p, (A) and the variation of the anodic peak potential, *E*_p, (B).

Figure 8. Probable mechanism of EE₂ electrooxidation at (GC-nCB)-μCPE.

Figure 9. Voltammograms and curve of variation of anodic peak currents as a function of EE₂ concentration, obtained at the (GC-nCB)-μCPE electrode with 0.01 phosphate buffer (pH = 7.4) for different EE₂ concentrations (5 to 800 nmol L⁻¹), with 5 min accumulation time at 0.0 V / Ag/AgCl, ; *v* = 50 mV. s⁻¹.

Figure 10. Influence of potential interfering compounds on the EE₂ oxidation peak current in voltammetry at (GC-nCB)-μCPE, the voltammetric response obtained for 1.0×10^{-7} mol.L⁻¹ of EE₂, with 5 min accumulation time at 0.0 V / Ag/AgCl, and a scan rate of 50 mV.s⁻¹

Tables

Tab 1

Concentration of EE ₂ ($\times 10^{-8}$ mol L ⁻¹)	Repeatability of <i>I</i> _p	Reproducibility of <i>I</i> _p
	RSD* (%)	RSD* (%)
5.0	3.8	4.8
7.5	1.1	2.9
10.0	1.3	3.7

* n= (4)

Tab 2

Samples	Added	Found	RSD*	Recovery
	[EE ₂] (μM)	[EE ₂] (μM)	(%)	(%)
(WWTPs -M)	non-doped	< LOD	-	-
	0.050	0.051 ± 0.001**	1.4	101.3

(WWTPs -D)	non-doped	< LOD	-	-
	0.050	0.050 ±0.001**	1.3	101.1
urine	non-doped	< LOD	-	-
	0.100	0.103 ±0.003**	2.3	103.3

* n= (3) ** Confidence interval for $\alpha= 0.05$.

Tab 3

Technique / Electrode	Linear range ($\mu\text{mol L}^{-1}$)	LOD ($\mu\text{mol L}^{-1}$)	Ref
SWV/ (Chi/CNTs) ¹	0.05 - 20	0.09	[46]
SWV/ (BDD) ²	0.99 – 5.2	0.24	[47]
LSV /(CPE) ³	0.05 - 20	0.03	[48]
DPV/ (CBGC) ⁴	0.25 -3.0	0.13	[49]
DPV/ (CB- immobilized palladium) ⁵	0. 5 - 115	0.081	[50]
SCV /(AgNP-SAE) ⁶	0.64 - 7.8	0.103	[51]
DPAdSV /(HMDE) ⁷	0.0054 – 0.202	0.0016	[52]
SWAdSV /(HMDE) ⁸	0.0019 – 0.6	0.0006	[53]
CV / (CoFe2O4/RGO) ⁹	0.05 – 100.0	0.042	[7]
DPV/ (ErGO) ¹⁰	0.04 – 8. 28	0.0068	[6]
DPV /(NTC/ aptamére) ¹¹	0.05 – 1.6	0.05	[9]

DPV/ (MIP) ¹²	0.1 – 23.0	0.03	[20]
CV/ ((CG-nCB)- μ CPE)	0.005 – 0.8	0.0014	This work

(1) Square wave voltammetry with chitosan / Carbon Nanotube; (2) Square wave voltammetry/boron doped diamond electrode; (3) Linear sweep voltammetry/carbon paste electrode; (4) Differential pulse voltammetry/ glassy carbon black electrode; (5) Differential pulse voltammetry / carbon black containing immobilized palladium nanoparticles; (6) Staircase voltammetry / solid amalgam electrode made with silver nanoparticles; (7) Differential pulse adsorption stripping voltammetry / mercury drop suspended electrode; (8) Square wave adsorption denudation voltammetry/ suspended mercury drop electrode; (9) Cyclic voltammetry/ cobalt ferric oxide with anti-spinel structure and reduced graphene oxide composite; (10) Differential pulse voltammetry / modified electrode with reduced graphene oxide; (11) Differential pulse voltammetry/ Carbon Nanotube aptasensor and (12) Differential pulse voltammetry / molecularly imprinted polymer.

Table caption

Table 1. Repeatability and reproducibility study of (GC-nCB)- μ CPE for different EE₂ concentrations (50, 75 and 100 nmol L⁻¹) in 0.01 mol L⁻¹ phosphate buffer (pH = 7.4), $v = 50$ mV. s⁻¹.

Table 2. Analysis of WWTPs samples and urine enriched with EE₂ (respectively 0.05 and 0.1 μ mol.L⁻¹). Concentration determination at (GC-nCB) - μ CPE with standard addition method and recovery for spiked samples.

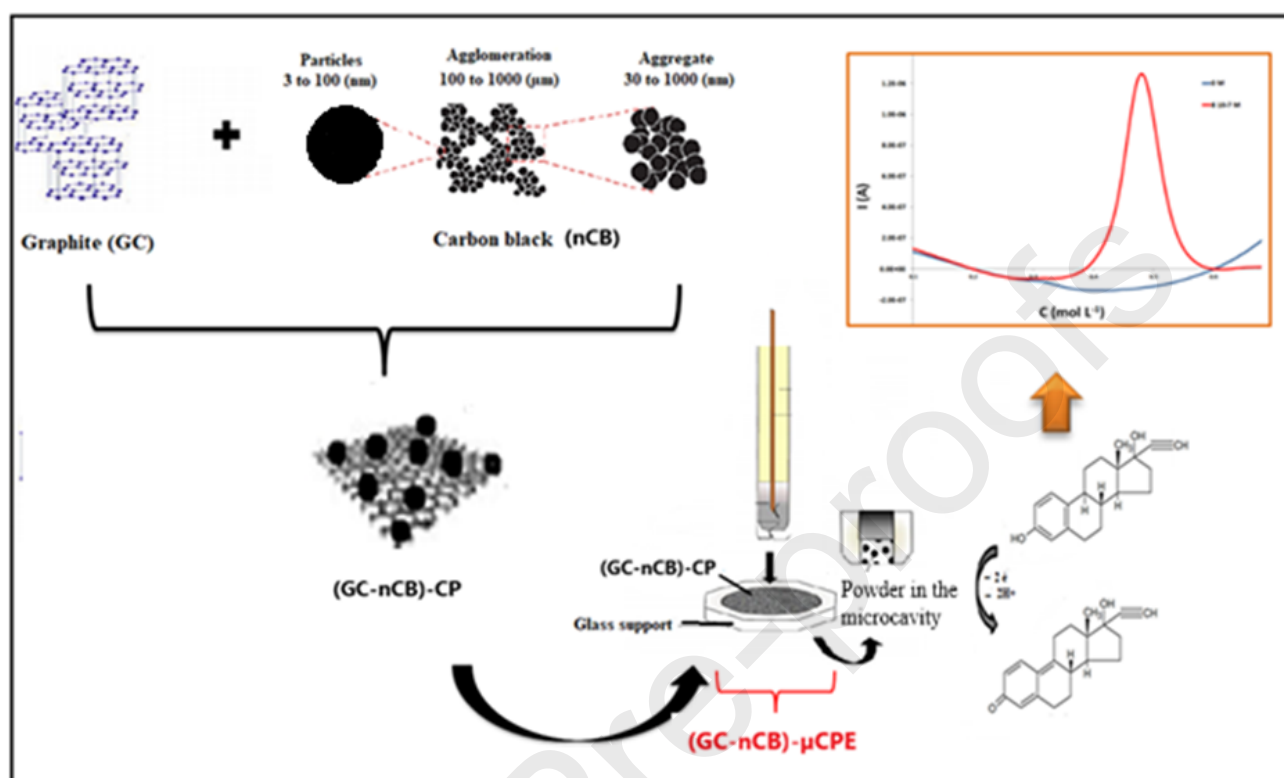
Table 3. Comparison of the analytical performances of different electrochemical sensors from literature for EE₂ analysis.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



HIGHLIGHTS

- Electrochemical micro-sensor for direct analysis of 17α – ethinylestradiol.
- A carbon paste cavity micro-electrode modified and nanostructured by nCB (GC-nCB)-μCPE was used.
- Electrochemical preconcentration (EP) step was used to enhance the sensitivity of EE₂ detection.
- The micro-sensor displayed a detection limit of 1.4 nmol.L⁻¹.
- The method developed was applied in environmental water and urine samples.