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ORIGINAL PAPER

Poly(thionine)-carbon nanotube modified carbon film electrodes and application to the simultaneous determination of acetaminophen and dipyrone

M. Emilia Ghica[1] · **Grasyelle M. Ferreira**[1,2] · **Christopher M. A. Brett**[1]

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Abstract Multi-walled carbon nanotube (MWCNT)-poly(thionine) (PTH) nanostructures were formed on carbon film electrodes (CFE), the polymer film being formed beneath or on top of MWCNT, to give two different sensor architectures, PTH/MWCNT/CFE or MWCNT/PTH/CFE. Characterization of the novel nanostructures was carried out by cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy. The modified electrodes were then used for sensing acetaminophen (ACOP) and dipyrone by differential pulse voltammetry and fixed-potential amperometry independently and simultaneously. Analytical parameters were determined and the results compared with similarly modified electrodes reported in the literature. An interference study as well as recovery measurements in pharmaceutical samples was successfully performed.

Keywords Carbon nanotubes · Poly(thionine) · Electrochemical sensors · Acetaminophen · Dipyrone

Introduction

Electroactive polymer-modified electrodes are currently finding extensive application in electrochemical sensing, particularly modified electrodes based on poly(phenazine) or poly(triphenylmethane) polymers together with carbon nanotubes (CNT) [1]. The combination of polymer with CNT leads to a large enhancement of the double layer capacitance, as exemplified by poly(thionine) [2], and synergistic effects in terms of sensitivity, e.g. [3] using poly(methylene blue), and can improve the access of analyte to the electrode surface as well as leading to electrocatalytic effects. Electropolymerisation of the phenazine on a bare carbon electrode surface or on multi-walled CNT (MWCNT), previously deposited on the electrode, leads to different polymer growth rates and mechanisms. The combinations of polymer and CNT modifiers with polymer on top of or underneath the MWCNT can give different responses towards electroactive species, which depends on the size of the monomer molecule and ease of nucleation [1]. For example, polymerization of thionine, Scheme 1a, on MWCNT-modified glassy carbon electrodes, was found to increase the sensitivity and reduce the detection limit for nitrite determination [4]. Other composite electrodes with two different polymers, poly(brilliant cresyl blue) and poly 5-amino-2-naphthalenesulfonic acid [5] show also improved response for the determination of hydrogen peroxide.

Acetaminophen and dipyrone form the main active constituent of a number of widely used pharmaceutical preparations, and therefore, the development of fast, low-cost, sensitive and selective methods for routine analysis of these compounds is required. Modern multimodal analgesia should be based on a good combination of analgesics, and non-opioid agents are recommended wherever possible for the treatment of acute pain [6]. Acetaminophen (N-(4-hydroxyphenyl)ethanamide, paracetamol, Scheme 1b), and dipyrone (sodium [2,3-dihydro-1,5-dimethyl-3-oxo-2-phenyl-1H-pyrazol-4-yl)methylamino]methanesulfonate, metamizol, Scheme 1c), are analgesic and antipyretic drugs mainly used to reduce fever and relieve moderate pain. Recent studies have shown that both medicines provide effective postoperative pain control,

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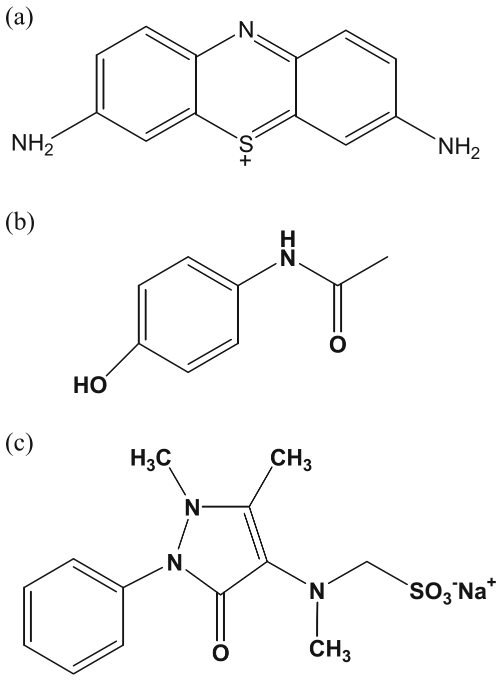
✉ Christopher M. A. Brett
cbrett@ci.uc.pt

[1] Department of Chemistry, Faculty of Sciences and Technology, University of Coimbra, 3004-535 Coimbra, Portugal

[2] Instituto de Ciência e Tecnologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil



Scheme 1 Chemical structures of (**a**) thionine, (**b**) acetaminophen and (**c**) dipyrone

indicating a similar or superior effectiveness of dipyrone [7]. Acetaminophen is likely to cause hepatocyte damage, renal necrosis, as well as vomiting, diarrhoea and skin reactions [6], whereas dipyrone is associated with complications such as bone marrow damage (agranulocytosis, aplastic anaemia), chronic interstitial nephritis and gastro-intestinal disturbances. Nevertheless, the safety of both drugs is several times higher than that of other commonly used anti-inflammatory drugs.

The most common techniques for acetaminophen and dipyrone determination, as reported in [8, 9], include spectrophotometry, titrimetry, chromatography, chemiluminescence, capillary electrophoresis and spectrofluorimetry. Although sensitive, these techniques are not suitable for routine analysis being time consuming or involving sample preparation before detection. Both acetaminophen and dipyrone are electroactive, so that different electrochemical methods have been used for their detection [10–13]. In relation to determination at electrodes modified with CNT, there is only one study on the determination of dipyrone at a CNT-modified graphite-epoxy resin composite electrode, GrCE [14] and for acetaminophen; most of the recent work is based on carbon nanotube paste electrodes (CNTPE) [15–20]. In other studies, CNT were used together with metallic nanoparticles [20–22], magnetic microparticles [11] or graphene [23]; ordered mesoporous carbon [24] has been also used as electrocatalyst. Only a few reports concern incorporation of redox mediators with CNT [10, 16, 18, 19, 21]. Poly(thionine) together with carbon nanotubes has been used in sensors for other biological compounds [25–27], although, to our knowledge, it has not yet been used in the determination of either acetaminophen or dipyrone.

The present work deals with development and characterization of multi-walled carbon nanotube (MWCNT)-poly(thionine) (PTH) electrode architectures obtained by electropolymerisation of thionine above or beneath MWCNT deposited on carbon film electrodes (CFE). CFE present similar properties to glassy carbon, such as excellent electrical and mechanical properties, wide potential range, chemical inertness, robustness and reproducible performance [28]. CFE are easy to prepare, inexpensive, disposable and small [29], which facilitates their incorporation in portable devices. The modified electrodes were characterized by cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy and then used for sensing acetaminophen (ACOP) and dipyrone by differential pulse voltammetry and fixed-potential amperometry. The highest sensitivity for both acetaminophen and dipyrone was obtained when PTH was formed on top of MWCNT. The two drugs were measured simultaneously by differential pulse voltammetry. Selectivity as well as recovery in pharmaceutical formulation is discussed.

Experimental

Reagents and solutions

All reagents were of analytical grade and were used without further purification. Acetaminophen (ACOP), L-ascorbic acid (AA) and dipyrone were purchased from Sigma. Multi-walled carbon nanotubes (MWCNT) were from NanoLab, USA, with ~95 % purity, 30 ± 10 nm diameter and 1–5 μm length. Chitosan (Chit) of low molecular weight with a degree of deacetylation of 80 %, Nafion® (5 % in ethanol, *v*/*v*) and thionine (TH, dye content 90 %) were obtained from Aldrich.

All solutions were prepared using Millipore Milli-Q nanopure water (resistivity >18 MΩ cm). The supporting electrolyte for sensors evaluation was sodium phosphate buffer (NaPB) (0.1 M NaH_2PO_4/Na_2HPO_4), pH 7.0. For TH polymerization, the buffer was sodium tetraborate (0.025 M $Na_2B_4O_7$) + 0.1 M KNO_3, pH 9.0.

Methods, instruments and cell

A one-compartment three-electrode system was employed, which consisted of a (modified) carbon film electrode (CFE) as working electrode, a platinum wire as counter electrode and a saturated calomel electrode (SCE) as reference.

Voltammetric and amperometric experiments were carried out using a PGSTAT30 potentiostat/galvanostat with GPES v4.9 software (Metrohm Autolab, Netherlands). Differential pulse voltammetric measurements were carried out, under optimized conditions, using a pulse amplitude of 50 mV, pulse width 70 ms and potential increment of 2 mV every 0.40 s, corresponding to an effective scan rate of 5 mV s^{-1}.

Electrochemical impedance spectroscopy (EIS) measurements were performed using a Solartron 1250 Frequency Response Analyser, coupled to a Solartron 1286 Electrochemical Interface controlled by ZPlot software (Solartron Analytical, UK). The frequency range used was 65 kHz to 0.1 Hz with 10 frequencies per decade, and integration time 60 s, with an rms perturbation voltage of 10 mV. Fitting to equivalent circuits was performed with ZView 3.1 software.

Scanning electron microscopy (SEM) images were obtained using a Zeiss Merlin field emission scanning electron microscope with Gemini 2 column (Carl Zeiss, Germany). A secondary electron (SE) detector was used, the working distance was 5 mm and the electron acceleration voltage was 2.0 kV.

Measurements of pH were performed with a CRISON 2001 micro pH-meter. All experiments were performed at room temperature, 25 ± 1 °C.

Carbon film electrode preparation and pre-treatment

The working electrodes were made from carbon film resistors (2 Ω nominal resistance, 15 μm film thickness) of length 6 and 1.5 mm in diameter; the detailed preparation is described elsewhere [30]. The resistance is protected with two tight-fitting metal caps, linked to an external contact wire. In order to make the electrode, one contact wire was removed and the other one covered in plastic and protected by normal epoxy resin. The exposed geometric area of the electrodes is 0.20 cm^2.

Since carbon film electrode surfaces cannot be renewed by polishing or other mechanical methods, electrochemical pre-treatment was always performed before electrode use to achieve a reproducible electrode response. This consisted in potential cycling between −1.0 and +1.0 V vs. SCE, at 100 mVs^{-1}, until a stable voltammogram was obtained.

Carbon nanotube functionalisation and deposition

Multi-walled carbon nanotubes (MWCNT) were purified and functionalised as previously described [31]. A mass of 120 mg of MWCNT was stirred in 10 mL of a 5 M nitric acid solution for 24 h, in order to cause partial destruction of the CNT and introduce –COOH groups at the ends and sidewall defects of the CNT [32]. The solid product was collected on a filter paper and washed several times with nanopure water until the filtrate solution became neutral (pH ≅ 6). The functionalised MWCNT were then dried in an oven at 80 °C for 24 h.

In order to prepare a 1.0 % *w*/*v* chitosan solution, 100 mg of Chit powder was dissolved in 10 mL of 1.0 % *v*/*v* acetic acid solution and stirred for 3 h at room temperature to ensure complete dissolution. The chitosan solution was stored at 4 °C.

A 1.0 % *w*/*v* MWCNT solution was prepared by dispersing 3 mg of functionalised MWCNT in 300 μL of 1.0 % *w*/*v* Chit in 1.0 % *v*/*v* acetic acid solution and sonicating for 3 h. For CNT deposition, a 10 μL drop of the 1 % *w*/*v* MWCNT solution was placed on the surface of a CFE, or poly(thionine) modified CFE (see below), to give MWCNT/CFE or MWCNT/PTH/CFE, respectively, left to dry in air at room temperature and this step was then repeated.

Thionine polymerization

Poly(thionine) (PTH) films were obtained on bare CFE (PTH/CFE) and on CFE modified with carbon nanotubes (PTH/MWCNT/CFE) by electropolymerisation using potential cycling in an aqueous solution containing 1 mM thionine in 0.025 M $Na_2B_4O_7$ + 0.1 M KNO_3, pH 9.0 buffer. The applied potential was cycled between −1.0 and +1.0 V at a scan rate of 50 mV s^{-1} during 30 cycles for bare electrodes and 40 cycles for CNT-modified carbon film electrodes. The PTH/CFE was also covered with carbon nanotubes, according to the procedure described in the previous subsection, to give MWCNT/PTH/CFE.

For protection of the modified electrodes by a Nafion® layer to avoid interferences from ascorbate, a solution of 0.25 % *w*/*v* Nafion® was prepared by dissolving the required volume of Nafion®(5 % *w*/*v*) in ethanol. A volume of 5 μL of this solution was dropped on top of the modified electrode and allowed to dry for at least 2 h.

Sample preparation

Pharmaceutical samples, containing acetaminophen and dipyrone, namely Ben-U-Ron, Paramolan® C, Metamizol CINFA and Nolotil were purchased from a local pharmacy. Before testing, the samples were prepared by powdering the tablets in a mortar, then weighing an appropriate amount and dissolving in 0.1 M NaPB buffer to get final concentrations of 33.0 mM acetaminophen and 34.5 mM dipyrone, according to the labelled amounts. Aliquots of the required volume of these stock solutions were added to the electrolytes. The determination of acetaminophen and dipyrone was performed using the standard addition method, as described below.

Results and discussion

Poly(thionine) deposition on CFE and MWCNT/CFE

The electropolymerisation of thionine on carbon film electrodes, PTH/CFE, and CFE modified with carbon nanotubes, PTH/MWCNT/CFE, by potential cycling is shown in Fig. 1.

A cyclic voltammogram in the absence of thionine is also included in the right inset of Fig. 1 in order to show the capacitive current at bare CFE. No peaks appeared in the absence of thionine. In the presence of thionine, at around + 0.8 V, irreversible oxidation of monomer occurs, generating a radical cation, which initiates polymerization. Polymerization of TH at CFE and MWCNT/CFE has been previously performed [33] and two redox couples, B_1/B_2, ascribed to monomer oxidation/reduction and A_1/A_2, attributed to oxidation and reduction of the polymer are observed. Poly(thionine) film deposition is shown by the increase in the polymer current peaks A_1/A_2. The peak currents increase linearly with cycling, tending to a maximum after 40 cycles on MWCNT/CFE and earlier, after 30 cycles, on bare CFE. Polymer film growth is better, estimated from the increase in the anodic peak current from the first to the last scan, on bare carbon film electrodes (Fig. 1, left inset) than at CNT-modified carbon film electrodes, being 170 % at CFE and only 74 % at MWCNT/CFE. At CFE, the polymer anodic/cathodic peak separation at scan rate 50 mV s^{-1} is ΔE_p = 58 ± 2 mV corresponding to a reversible system, whilst at MWCNT/CFE, the process is no longer reversible, with ΔE_p = 130 ± 5 mV. Similar behaviour, with less good polymerization at a MWCNT-modified electrode, was previously observed for poly(brilliant cresyl blue) (PBCB) [34]. An explanation for this behaviour can be due to lack of flexibility of the monomer molecule (Scheme 1c), which can impede its entry into the CNT bamboo network. However, the fact that the polymer grows slower at MWCNT/CFE compared to CFE leads to a more compact and robust film, as will be discussed below.

Characterization of the nanostructured modified electrodes

Electrochemical and morphological characterization of the modified electrodes PTH/CFE, PTH/MWCNT/CFE and MWCNT/PTH/CFE was assessed by cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and scanning electron microscopy (SEM).

Cyclic voltammetry

The modified electrodes were characterized by cyclic voltammetry at different scan rates, Fig. 2. In all cases, two redox couples were observed, the more negative one with midpoint potential, measured at 50 mV s^{-1}, E_m = −387 ± 9 mV (PTH/CFE), −397 ± 9 mV (PTH/MWCNT/CFE) and −252 ± 6 mV (MWCNT/PTH/CFE), is ascribed to monomer. The other couple, with midpoint potential −133 ± 4 mV (PTH/CFE), −219 ± 5 mV (PTH/MWCNT/CFE) and −74 ± 3 mV (MWCNT/PTH/CFE), is due to polymer. The peaks corresponding to polymer are better defined when thionine is polymerized on top of nanotubes (PTH/MWCNT/CFE) and the polymer redox reaction is more reversible at this electrode, ΔE_p = 75 ± 3 mV (at 50 mV s^{-1}), compared to ΔE_p = 118 ± 4 mV when thionine was beneath the nanotubes (MWCNT/PTH/CFE, Fig. 2b1) and ΔE_p = 212 ± 5 mV (Fig. 2a1) in the absence of nanotubes (PTH/CFE). The

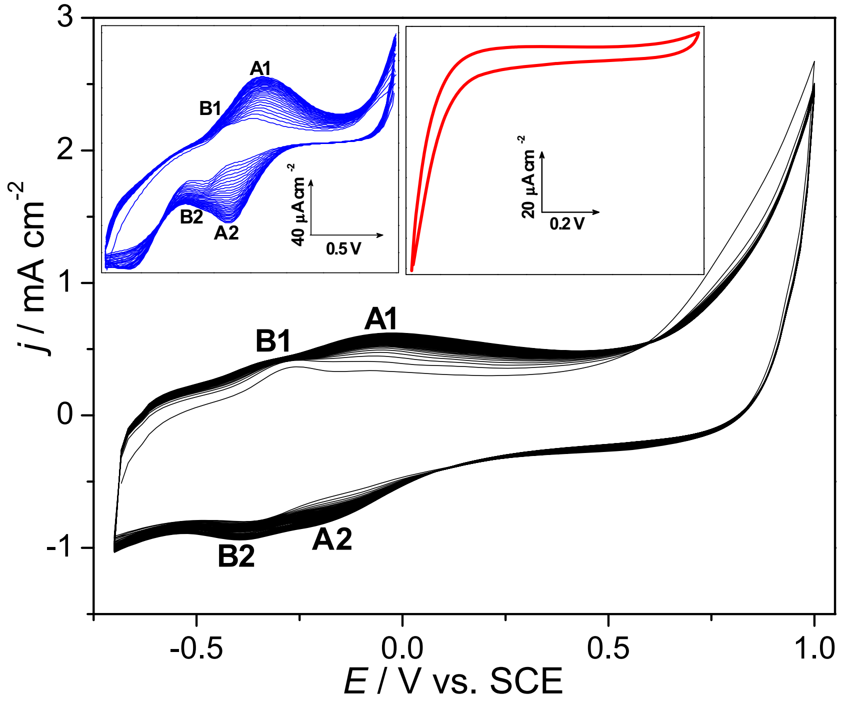


Fig. 1 Cyclic voltammograms obtained during thionine polymerization in 1 mM TH, 0.025 M $Na_2B_4O_7$ + 0.1 M KNO_3, pH 9.0 at MWCNT/CFE. *Left inset* shows polymerization under the same conditions at CFE. *Right inset* is the CV at CFE in the absence of TH. Scan rate 50 mV s^{-1}

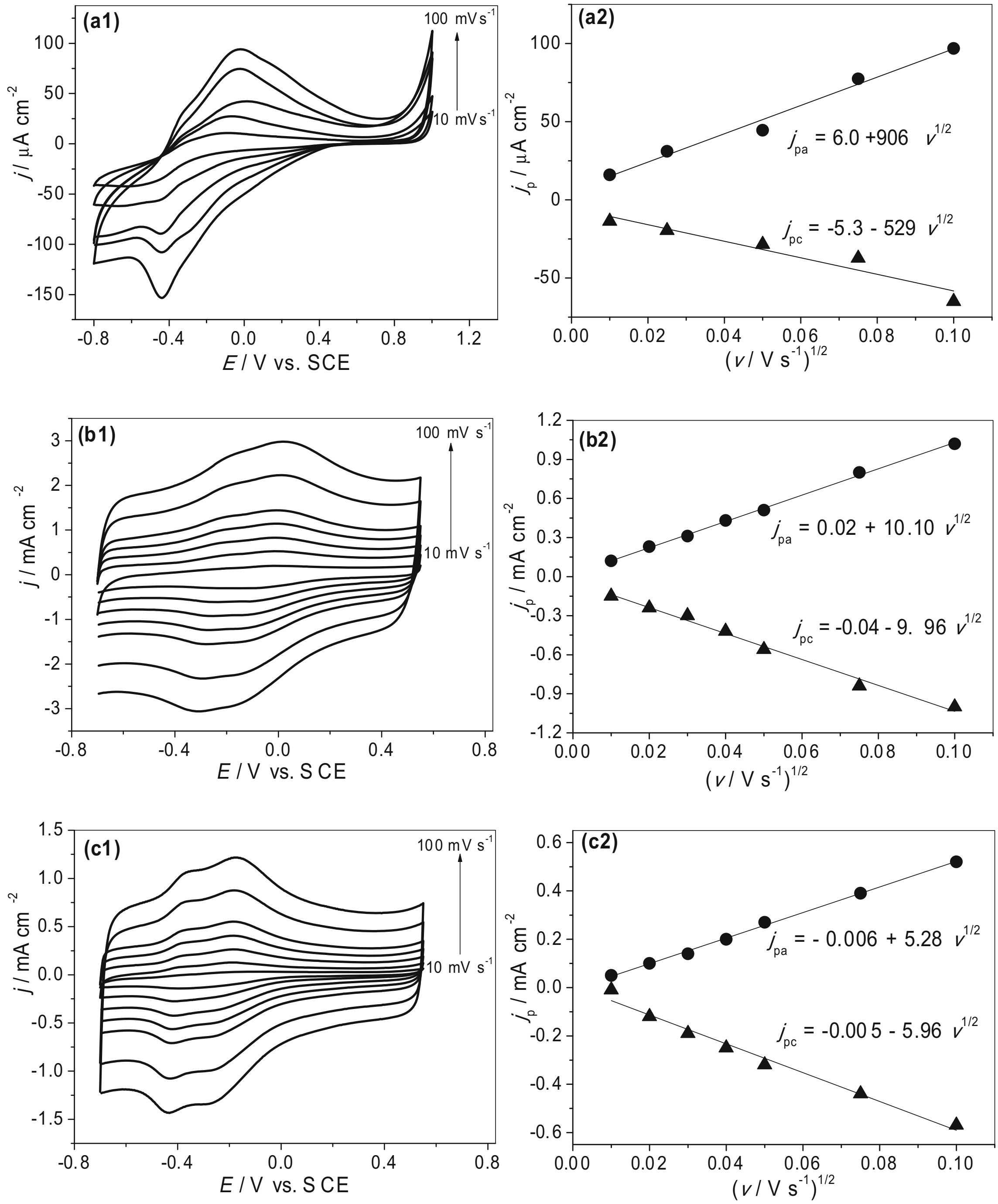


Fig. 2 Cyclic voltammetry at different scan rates for (**a1**) PTH/CFE, (**b1**) MWCNT/PTH/CFE and (**c1**) PTH/MWCNT/CFE in 0.1 M KCl and (**a2**, **b2**, **c2**) corresponding current peak dependence on square root of scan rate for (*black circle*) anodic and (*black up-pointing triangle*) cathodic peak

smaller the peak separation, the easier should be the electron transfer reaction in mediated bio(sensors). It is also noticeable that at MWCNT/PTH/CFE, the polymer peaks are less well defined than at PTH/CFE and PTH/MWCNT/CFE, due to the fact that CNT cover the polymer. In all cases, the anodic and cathodic peak current density increases linearly with the square root of scan rate, indicating a diffusion-controlled process, attributed to the rate determining process being insertion or extraction of counterions from solution into the polymer structures, as previously observed with other poly(phenazines) [35], as well as with CNT/polyphenazines [36].

For PTH/CFE (Fig. 2a2), the slope of the plot of anodic peak current vs. square root of scan rate is 1.7 times higher than that of the cathodic peak, demonstrating that expulsion of the counter-ion during oxidation is quicker than its diffusion into the polymer film. This behaviour is not observed in the combination of the polymer with CNT, the ratio of slopes being almost equal (Fig. 2b2, c2). On the other hand, the slopes of both anodic and cathodic peak exhibited higher values for MWCNT/PTH/CFE (Fig. 2b2) than with PTH/MWCNT/CFE (Fig. 2c2), which means that, for the latter modified electrode, diffusion is slower.

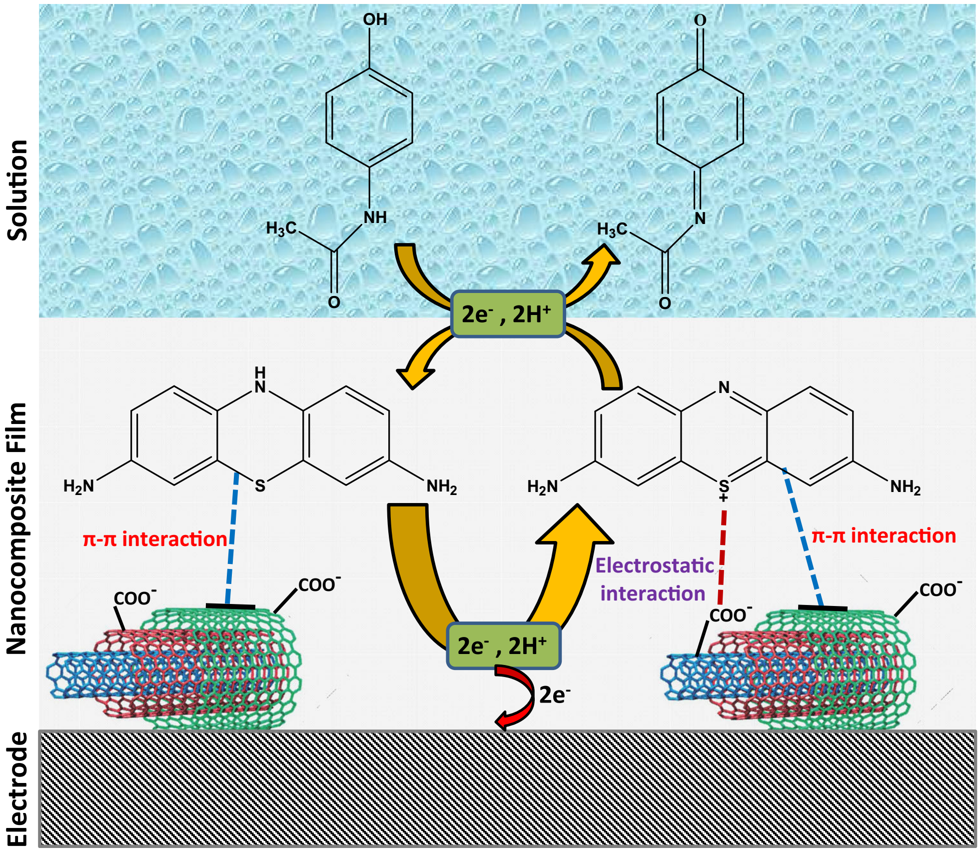
Carbon nanotubes also decrease the rate of degradation of the poly(thionine) film: after 50 consecutive cycles, only 4 % loss was observed, compared with 12 % in their absence (not shown). The increased stability in the presence of MWCNT can be attributed to strong interactions that occur between CNT and PTH that can be electrostatic, between negatively charged carboxylate groups on the CNT and positively charged sulphur in PTH and/or π-π interaction, Scheme 2.

Electrochemical impedance spectroscopy

Electrochemical impedance spectra were recorded in 0.1 M KCl at different applied potentials. A potential of −0.20 V vs. SCE is discussed here, since it is in the electroactive potential range of PTH for all the configurations containing the polymer. Impedance spectra obtained at bare carbon film electrode, CNT, polymer, polymer/CNT and CNT/polymer-modified electrodes are shown in Fig. 3a, b. A large decrease in the absolute value of the impedance was observed from the unmodified electrode to the modified ones, especially with the addition of carbon nanotubes. Similarities in spectra were observed for all the electrodes containing PTH, which exhibited a small semicircle in the high frequency region, due to charge transfer reaction of the polymer. On the other hand, all the electrodes containing MWCNT present capacitive lines in the low-frequency region. The spectrum at CFE is represented by a single process, at the electrode/electrolyte interface.

The general equivalent electrical circuit used to fit the spectra is shown in Fig. 3c, with some elements removed for the less complex modified electrode architectures. At bare CFE, it comprises the cell resistance, R_Ω, in series with an RCPE parallel combination, where CPE is a constant phase element, representing the double layer capacitance, $\mathrm{CPE}_{\mathrm{dl}}$, and a charge transfer resistance, R_{ct}. The constant phase element is modelled as non-ideal capacitor and is described by $\mathrm{CPE} = -(\mathrm{i}\omega C)^{-\alpha}$, where ω is the angular frequency and α the CPE exponent, reflecting a non-uniform surface. For the electrode modified with PTH, an additional $R_{\mathrm{f}}\mathrm{CPE}_{\mathrm{f}}$ parallel combination in series is needed, where R_{f} is the film resistance and $\mathrm{CPE}_{\mathrm{f}}$ is the charge accumulated in the modifier film. The spectra of the electrodes modified with carbon nanotubes, MWCNT/CFE, show a straight line at low frequencies, which was modelled with a pure capacitor, C_{f}, and, in the middle

Scheme 2 Representation of the processes occurring at the electrode/electrolyte interface and within the nanocomposite film at PTH/MWCNT/CFE for oxidation of acetaminophen



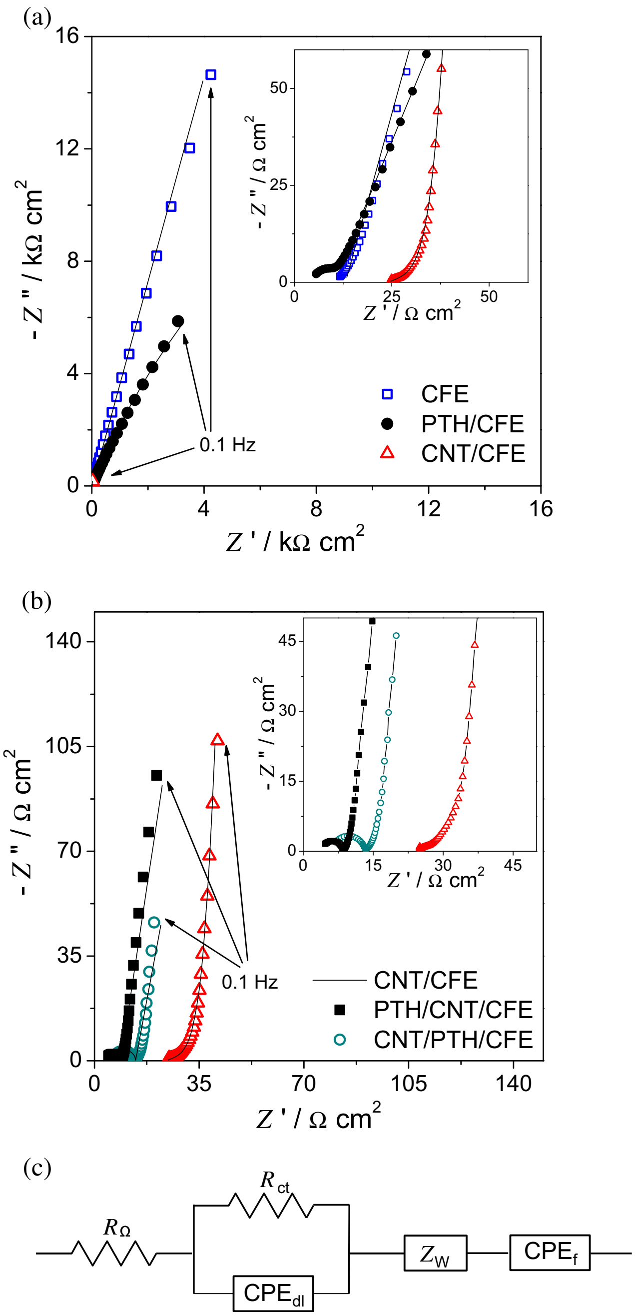


Fig. 3 Electrochemical impedance spectroscopy. Complex plane spectra for (**a**) (*white square*) CFE, (*black circle*) PTH/CFE, (*white triangle*) MWCNT/CFE; (**b**) (*white triangle*) MWCNT/CFE, (*black square*) PTH/MWCNT/CFE, (*white circle*) MWCNT/PTH/CFE at −0.20 V vs. SCE in 0.1 M KCl. Insets are magnifications of the high frequency portion. The *lines* show equivalent circuit fitting. (**c**) Equivalent circuit used to fit the spectra

frequencies region an open Warburg element, Z_W, was included, due to diffusion through the nanotube layer, no R_fCPE$_f$ parallel combination being necessary. At electrodes modified with both CNT and PTH, the equivalent circuit required both Z_W and R_fCPE$_f$, Fig. 3c. The open Warburg element is expressed by $Z_w = R_w\text{cth}[(\tau i\omega)^{\alpha}]/(\tau i\omega)^{\alpha}$, where $\alpha < 0.5$, τ is the diffusional time constant and R_w the diffusional resistance.

The data obtained from fitting the spectra are shown in Table 1. A large decrease of the values of R_{ct} was observed when the electrodes were modified by polymer or by polymer and carbon nanotubes, indicating easier electron transfer. Small variations between the values of the charge transfer resistance of the modified electrodes were observed (Table 1), from 5.0 (PTH/CFE and PTH/MWCNT/CFE) to 7.0 (MWCNT/PTH/CFE) Ω cm^2, showing that the electrons transferred are due to the polymer redox reaction. The slightly higher value for MWCNT/PTH/CFE is because CNT cover the polymer, hindering electron transfer. The values of the CPE$_{dl}$ capacitance are higher when MWCNT and PTH are present together, compared with those of only polymer, most probably due to an increase in surface area. The α_{dl} values decreased for MWCNT/PTH/CFE and PTH/MWCNT/CFE compared with PTH/CFE, attributable to an increase in roughness and non-uniformity when CNT were added. The value of CPE$_f$ increases with each layer of modifier, being the highest for the MWCNT/PTH/CFE. Regarding the Warburg element, there are small differences in the diffusional resistance; however, the diffusional time, τ, is increasing with addition of modifiers, as expected. The EIS spectra were similar for the electrodes modified with both CNT and PTH, as previously observed for other poly(phenazine)/CNT combinations [34, 36]; the results obtained here indicate PTH/MWCNT/CFE as better electrocatalyst, since lower values of charge transfer resistance and diffusional time were obtained, compared with MWCNT/PTH/CFE.

Scanning electron microscopy

Scanning electron micrographs of bare CFE and modified PTH/CFE, MWCNT/CFE, MWCNT/PTH/CFE and PTH/MWCNT/CFE are illustrated in Fig. 4. A typical rough surface was observed for the unmodified carbon film electrode, as previously observed by AFM [37], owing to the ceramic cylinder on which the carbon films are pyrolytically deposited. The roughness increased when CFE was modified with poly(thionine), Fig. 4b. The typical carbon nanotube bamboo structure is visible in Fig. 4c, the nanotubes completely covering the electrode surface. Figure 4d, e displays the images of MWCNT/PTH/CFE and PTH/MWCNT/CFE. Two distinct structures are easily observed in both cases, which distinguish the two layers of nanotubes and polymer. In both cases, it is easy to see the structure beneath, meaning that the outer layer does not completely cover it. When poly(thionine) is deposited on carbon nanotubes, the formation of high-aspect ratio structures, higher that 1 μm is seen, evidence of the greater polymer growth on top of nanotubes rather than CFE. The formation of an interpenetrating composite is also possible,

Table 1 Data obtained from analysis of the impedance spectra for the modified electrodes in 0.1 M KCl at −0.20 V vs. SCE using equivalent circuit fitting

Electrode	$R_{ct}/\Omega\ cm^2$	$C_{dl}/\mu F\ cm^{-2}\ s^{\alpha-1}$	α_{dl}	$R_W/\Omega\ cm^2$	τ/ms	α_W	$C_f/mF\ cm^{-2}\ s^{\alpha-1}$	α_f
CFE	894,000	86.3	0.84	–	–	–	–	–
PTH/CFE	4.9	2.0	1.00	–	–	–	0.18	0.76
MWCNT/CFE	–	–	–	4.1	10	0.23	15.9	1.00
PTH/MWCNT/CFE	5.0	7.4	0.87	2.9	52	0.39	14.6	1.00
MWCNT/PTH/CFE	7.1	4.1	0.90	6.8	460	0.38	28.2	1.00

especially in the case of PTH/MWCNT/CFE, due to the high porosity of the surface created by nanotubes; polymer easily can get inside this matrix.

Acetaminophen and dipyrone determination

Acetaminophen determination

It has been previously shown that the oxidation of acetaminophen (ACOP) is pH dependent [24, 38] with slope values close to the Nernstian value, 59 mV pH^{-1}, indicating an equal number of electrons and protons participating in the electrochemical process. The number of electrons has been calculated [8] and assumed [15, 24] to be 2, and according to this, the proposed reaction mechanism occurring at the modified electrode is presented in Scheme 2.

The analytical determination of ACOP was investigated by oxidation in 0.1 M NaPB pH 7.0, which has been chosen as optimum in other studies [23, 38], and differential pulse

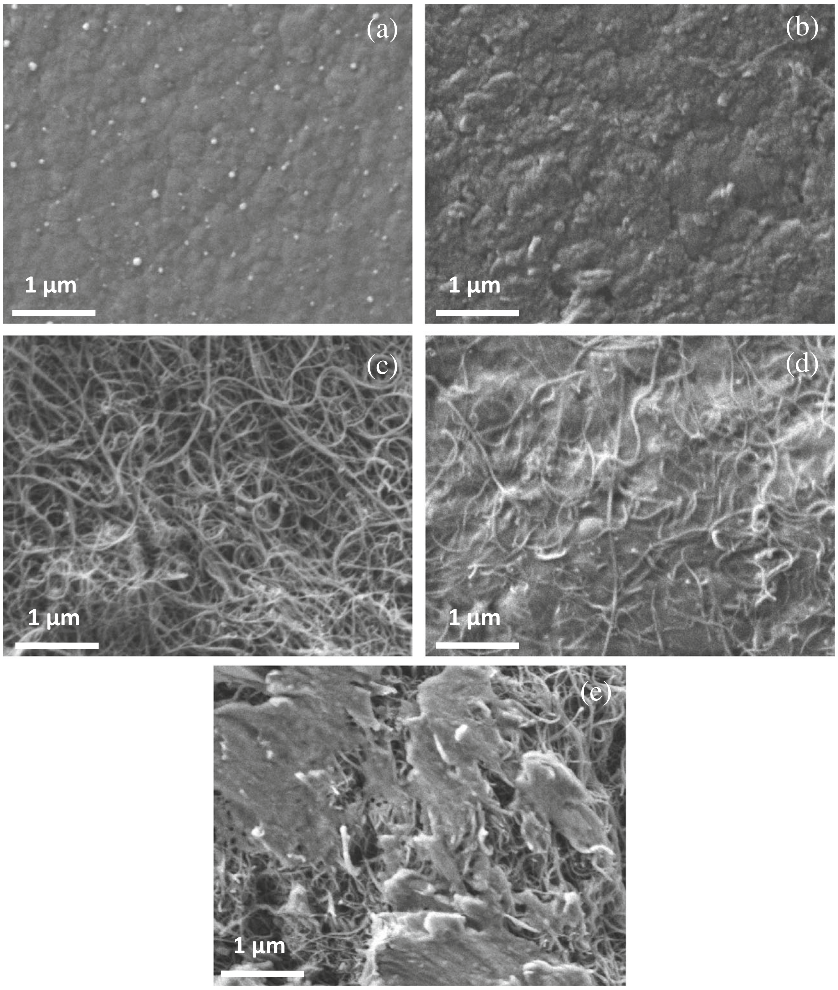


Fig. 4 Scanning electron micrographs of (**a**) CFE, (**b**) PTH/CFE, (**c**) MWCNT/CFE, (**d**) MWCNT/PTH/CFE and (**e**) PTH/MWCNT/CFE

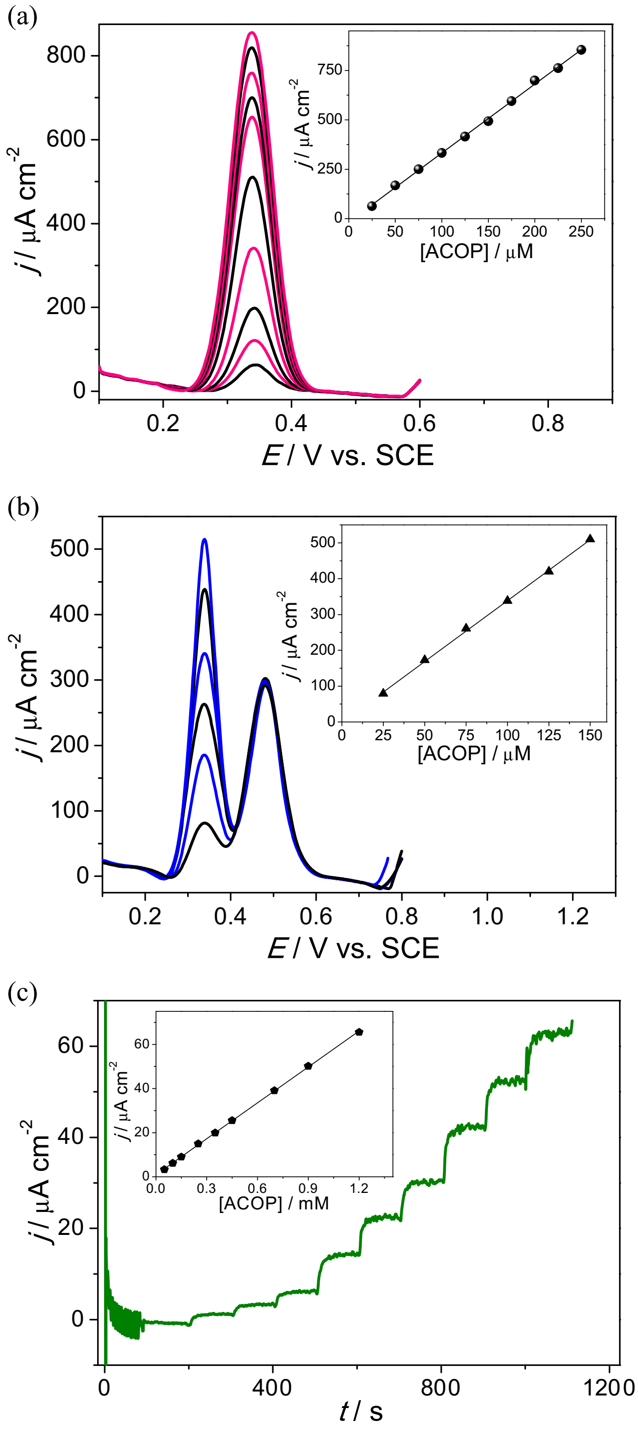


Fig. 5 (**a**, **b**) DPV of acetaminophen from (**a**) 25 to 250 μM and (**b**) 25 to 150 μM at MWCNT/PTH/CFE, scan rate 5 mV s^{-1}, amplitude 50 mV, in the absence (**a**) and presence (**b**) of 2.5 mM dipyrone. (**c**) Typical amperometric response for ACOP at PTH/MWCNT/CFE at +0.30 V vs. SCE. *Insets* are the calibration curves

voltammetry (DPV) was applied at the different modified electrodes, see Fig. 5a.

It has been previously remarked that acetaminophen detection at graphene- and CNT-modified electrodes is partly a surface-confined process [24] and that determination depends on the accumulation time but is independent of the applied potential in the potential range tested [39–41]. In [11], it appeared that electrode polarization during the measurement also plays a role in increasing the concentration near the electrode surface and leads to signal enhancement, as well as ACOP adsorption. However, with our sensor architectures, pre-concentration during different times and at different potentials did not lead to any increase in response. Some adsorption of species in the modifier film was found after measurements, manifested as residual current peaks, but this was totally removed by performing several potential cycles in buffer solution.

DPV at MWCNT/PTH/CFE and PTH/MWCNT/CFE both exhibited one peak, corresponding to oxidation of acetaminophen to N-acetyl-p-quinoneimine [38], which appears at 0.337 V vs. SCE. The peak current increased linearly with increase of acetaminophen concentration, over the concentration range from 25 to 250 μM. The analytical parameters obtained are shown in Table 2 together with those of some recent similar sensors in the literature. The detection limits, calculated as 3*standard deviation/slope, were similar at the two modified electrodes, 4.7 ± 0.4 μM (RSD = 8.5 %, n = 3) and 6.0 ± 0.5 μM (RSD = 8.3 %, n = 3); however, a higher sensitivity was obtained at PTH/MWCNT/CFE, 4.3 ± 0.2 mA cm^{-2} μM^{-1} (RSD = 4.9 %, n = 3).

Fixed-potential amperometry (Fig. 5c) was also performed at both modified electrodes, at a potential of +0.30 V vs. SCE, close to where the acetaminophen peak appears in differential pulse voltammetry. The linear range was wider than in DPV, 50 μM to 1.2 mM, but the limits of detection were much higher by an order of magnitude, 15.2 ± 1.0 μM (RSD = 6.6 %, n = 3) at MWCNT/PTH/CFE and 19.1 ± 1.3 μM (RSD = 6.8 %, n = 3) at PTH/MWCNT/CFE. For this modified electrode architecture, the highest sensitivity was achieved at MWCNT/PTH/CFE (Table 2). This behaviour was also observed in [36], with CNT and poly(methylene green) modified electrodes. The explanation is that considering that the oxidation of ACOP occurs at the surface of the polymer redox mediator, in fixed-potential amperometry, there is enough time for substrate to diffuse through CNT and reach PTH, some accumulation also occurring within the CNT layer. In DPV, the timescale is too short for this to happen, unless pre-concentration can be successfully used, which was not the case here. Thus, the sensitivity is higher in DPV with PTH on top of CNT, and in amperometry, it is the opposite.

In the literature, the DPV technique is generally more sensitive for ACOP determination, as expected, with lower detection limits. In the recent literature, since 2012, Table 2, there are only two sensors more sensitive than the sensor proposed in this work, both using single-walled CNT, and adding magnetic nanoparticles [11] or graphene [23], which greatly improve the response, but require more complex sensor

Table 2 Comparison of analytical parameters for acetaminophen determination at different modified electrode assemblies

Modified electrode	Method	Linear range/μM	Sensitivity/mA cm^{-2} μM^{-1}	LOD/μM	Reference
(CMWCNT-NHCH$_2$CH$_2$NH)$_6$/GCE	DPV	1.0–200	2.29	0.092	[8]
5ADBCNTPE	SWV	40–950	0.34	–[a]	[10]
SWCNT(magnetic entrapped)/AuSPE	CV	0.2–5.0	15.0	0.2	[11]
CNTPE	DPV	50–92.6	4.21	0.57	[15]
MC-CNTPE	DPV	12–550	0.49	–[a]	[18]
nPt-MWCNTPE	DPV	0.5–100	3.06	0.17	[19]
SWCNT-GNS/GCE	DPV	0.05–64.5	15.5	0.038	[23]
PMG/*f*MWCNT/GrCE	DPV	25–200	3.64	4.3	[36]
PTH/MWCNT/CFE	DPV	25–250	4.28	6.0	This work
MWCNT/PTH/CFE	DPV	25–250	3.48	4.7	This work
PTH/MWCNT/CFE	Amp	50–1200	0.054	19.1	This work
MWCNT/PTH/CFE	Amp	50–1200	0.068	15.2	This work

DPV differential pulse voltammetry, *SWV* square wave voltammetry, *CV* cyclic voltammetry, *Amp* fixed-potential amperometry, *CMWCNT* carboxylic acid functionalized multi-wall carbon nanotubes, *5ADB* 5-amino-3′,4′-dimethyl-biphenyl-2-ol, *AuSPE* Au screen printed electrode, *CNTPE* CNT paste electrode, *MC-CNTPE* molybdenum complex-carbon nanotube paste electrode, *GNS* graphene nanosheet, *PMG* poly(methylene green)

[a] Not specified

architecture. Furthermore, the CNT paste electrode sensor with a similar sensitivity to the PTH/MWCNT/CFE sensor developed here exhibited a narrower linear range [15].

To test stability, two calibration curves were recorded daily, consisting of 10 additions of ACOP. After 15 days, the response to ACOP decreased to 77 % at PTH/MWCNT/CFE and 73 % of the original value at MWCNT/PTH/CFE. The repeatability was also assessed from the slope of the response to ACOP for three consecutive calibration curves, and the standard deviations were 5.4 and 6.0 %. These changes can be minimized in real samples by use of the standard addition method.

Dipyrone determination

The literature has very few reports on the use of CNT-modified electrodes for dipyrone determination, and no reports were found on the use of electrodes modified with both CNT and redox polymers for dipyrone measurement. It was previously found [14] that electrodes modified with MWCNT exhibited an electrocatalytic effect for dipyrone determination compared to unmodified electrodes; the peak around 0.5 V is the most pronounced and that in pH 7.0, weaker adsorption occurs. Based on these results, and considering also the determination under similar conditions as for ACOP, DPV was applied for the determination of dipyrone at 0.50 V in pH 7.0 supporting electrolyte.

Here, both types of electrode modified with CNT and PTH were tested using DPV, which was more sensitive (130 ± 8 μA cm^{-2} mM^{-1}) than fixed-potential amperometry (6.6 ± 0.3 μA cm^{-2} mM^{-1}) at PTH/MWCNT/CFE electrode. Similar linear ranges were obtained by both methods, but the detection limit was much lower by DPV, 13.4 ± 1.1 μM (RSD = 8.4 %, *n* = 3) compared with amperometry, 91.2 ± 7.5 μM (RSD = 8.2 %, *n* = 3). Over the potential range studied, the DP voltammograms for both PTH/MWCNT/CFE and MWCNT/PTH/CFE exhibited one oxidation peak at 0.482 V, Fig. 6. As for acetaminophen, a higher response was obtained when polymer was deposited on top of the nanotubes. The analytical parameters obtained are presented in Table 3, as well as of other sensors recently published in the literature. Higher sensitivities were obtained in [12, 14], but with narrower linear ranges. Detection limits were similar to those in [12] and much lower than in [9]; although they were higher than those in [13, 42, 43] ([13, 43] using stripping voltammetric techniques), in these cases, the upper linear range limits were only 2.6 μM [13, 43] and 16 μM [42].

Simultaneous measurement of acetaminophen and dipyrone

The simultaneous determination of acetaminophen and dipyrone was investigated, keeping the concentration of one of them constant and changing the concentration of the other (Figs. 5b and 6b). Two well-defined voltammetric peaks with a difference of peak potential of 150 mV were observed, which is large enough to determine acetaminophen and dipyrone simultaneously. The sensitivities for ACOP determination in the presence of 2.5 mM dipyrone were very similar to those in its absence (given in brackets), 4.2 ± 0.2 (4.3 ± 0.2) mA cm^{-2} μM^{-1} at PTH/MWCNT/CFE and 3.4 ± 0.2 (3.5 ± 0.2) mA cm^{-2} μM^{-1} at MWCNT/PTH/CFE. For dipyrone in the presence of 50 μM ACOP (in its absence in brackets), the sensitivities were 129 ± 8 (130 ± 8) μA cm^{-2} mM^{-1} at PTH/MWCNT/CFE and 96 ± 4 (100 ± 5) μA

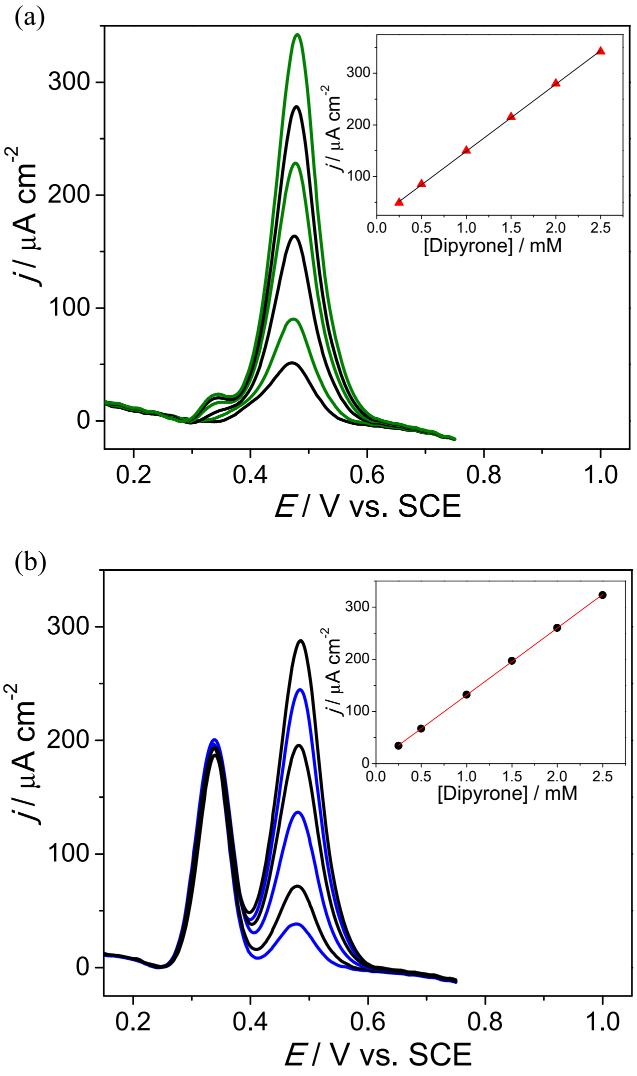


Fig. 6 DPV of dipyrone from 0.25 to 2.5 mM in the absence (**a**) and presence (**b**) of 50 µM acetaminophen at PTH/MWCNT/CFE. Effective scan rate 5 mV s^{-1}, amplitude 50 mV. *Insets* are the calibration curves

cm^{-2} mM^{-1} at MWCNT/PTH/CFE. These data demonstrate that the oxidation processes of acetaminophen and dipyrone at the CNT-poly(thionine) modified electrodes are independent, and therefore, simultaneous determination of the two analytes is possible.

Interferences and determination in pharmaceutical samples

The common presence of ascorbic acid (AA) with acetaminophen in pharmaceuticals leads to intensified positive effects of acetaminophen and reduced toxicity [19]. Thus, ascorbic acid was tested as interferent. The response to ACOP was increased by 15 % at both PTH/MWCNT/CFE and MWCNT/PTH/CFE when equal concentrations of ACOP and AA were present in solution; normally, the amount of AA, if it exists in the formulation, is half that of ACOP. The interference can be minimized by protecting the sensor with a cation-exchange membrane. At the pH 7.0 used in this work, ACOP (p$K_a \approx 9.4$) is neutral, whilst AA (p$K_a \approx 4$) is negatively charged. Hence, covering the electrodes with the cation-exchange polymer Nafion®, a highly efficient perfluorosulfonate membrane should reduce AA interference. Amperometric measurements performed under these conditions, with equal concentrations of 50 µM analyte showed a decrease of the AA response to one third of that obtained without the Nafion layer, that of ACOP being 83 % of that without the membrane layer. Using DPV, the interference was reduced to only 4 % when equal amounts of ACOP and AA were present.

The amperometric determination of acetaminophen and dipyrone in tablets was carried out by the standard addition method, first adding the sample, followed by four additions of standard analyte. Both Ben-U-Ron and Paramolan C tablets

Table 3 Comparison of analytical parameters for dipyrone determination at different modified electrode assemblies

Modified electrode	Method	Linear range/µM	Sensitivity/µA cm^{-2} mM^{-1}	Detection limit/µM	Reference
PPy-DP/GPE	Potentiometry	100–40,000	NC[a]	86.2	[9]
RB4-APFSi/CPE	CV	50–400	221.3	22.0	[12]
PEDOT/GCE	DPSV	0.1–2.6	–[b]	0.066	[13]
CNT/GrCE	DPV	50–1500	194.0	1.4	[14]
GPE	DPV	1.0–16	–[b]	0.046	[42]
RF/GCE	SWSV	0.13–2.64	–[b]	0.106	[43]
PTH/MWCNT/CFE	DPV	250–2500	130.0	13.4	This work
MWCNT/PTH/CFE	DPV	250–1500	100.2	17.8	This work

FIA flow injection analysis, *CV* cyclic voltammetry, *DPSV* differential pulse stripping voltammetry, *DPV* differential pulse voltammetry, *SWSV* square wave stripping voltammetry, *PPy-DP* polypyrrole doped dipyrone, *GPE* graphite pencil electrode, *CoPc* cobalt phthalocyanine, *CPE* carbon paste electrode, *RB4-APFSi* Reactive Blue 4–3 aminopropyl-functionalized silica, *PEDOT* poly(3,4-ethylenedioxythiophene, *GrCE* graphite-composite electrode, *RF* riboflavin

[a] *NC* not comparable

[b] Not specified

contain 500 mg of acetaminophen, Paramolan C additionally having 250 mg AA. Tablets of Metamizol CINFA and Nolotil contain 575 mg dipyrone.

With the sensors developed in this article, the amounts of acetaminophen found were 504 ± 2 mg in Ben-U-Ron and 507 ± 3 mg in Paramolan C using PTH/MWCNT/CFE and 505 ± 3 mg in Ben-U-Ron and 508 ± 3 mg in Paramolan C at MWCNT/PTH/CFE using the Nafion® protective layer for analysis of Paramolan C. For dipyrone, values of 578 ± 3 mg and 560 ± 2 mg in Metamizol CINFA at PTH/MWCNT/CFE and MWCNT/PTH/CFE were found and in Nolotil, the amounts were 573 ± 4 mg at PTH/MWCNT/CFE and 572 ± 3 mg at MWCNT/PTH/CFE, so that PTH/MWCNT/CFE is to be preferred as it gives results statistically equal to the labelled amount of dipyrone for both formulations.

Recovery measurements were also carried out by adding three different concentrations of acetaminophen and dipyrone, 50, 100 and 200 μM to solutions of the corresponding tablets. Each sample was analysed in triplicate and the mean value was calculated. The relative standard deviations for acetaminophen were between 98 and 101 % in Ben-U-Ron and 95–104 % in Paramolan, and for dipyrone, they were from 97 to 102 % in Metamizol CINFA and Nolotil, indicating good agreement and suitability for determination in pharmaceutical samples.

Conclusions

Novel electrode nanostructured sensing platforms have been developed on carbon film electrodes using multi-walled carbon nanotubes and poly(thionine). The monomer polymerized better on the bare electrode than on the CNT-modified electrode, probably due to the lack of flexibility of the monomer to enable it to diffuse easily within the CNT structure. In cyclic voltammetry experiments, faster diffusion was observed at PTH/MWCNT/CFE and electrochemical impedance spectroscopy showed that it exhibits easier electron transfer, which is in agreement with differential pulse voltammetry results for sensing acetaminophen and dipyrone. The proposed method is sensitive and sufficiently selective to measure acetaminophen and dipyrone individually and simultaneously. Interferences from AA can be reduced by use of a Nafion® protective layer. The applicability of the new electrode architectures for the determination of ACOP and dipyrone in pharmaceutical samples was successful with good accuracy and reproducibility, auguring well for their future application in electrochemical sensing.

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