Talanta 111 (2013) 76–84

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A novel amperometric sensor for ascorbic acid based on poly(Nile blue A) and functionalised multi-walled carbon nanotube modified electrodes

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ARTICLE INFO

Article history:
Received 8 October 2012
Received in revised form
14 February 2013
Accepted 17 February 2013
Available online 24 February 2013

Keywords:
Poly(Nile blue A)
Multi-walled carbon nanotubes
Electropolymerisation
Ascorbic acid determination
Electrochemical impedance spectroscopy

ABSTRACT

A new type of modified electrode sensor for ascorbic acid has been prepared by deposition of multi-walled carbon nanotubes (MWCNT) and poly(Nile blue A) on the surface of glassy carbon electrodes. Nile blue A was electropolymerised either beneath (directly on glassy carbon) or onto the MWCNT layer by potential cycling in phosphate buffer solution at pH 6.0. Characterisation of the modified electrodes was carried out by cyclic voltammetry and electrochemical impedance spectroscopy. Quantitative determination of ascorbate was achieved by cyclic voltammetry and fixed potential amperometry in phosphate buffer solution at pH 5.3. The modified electrodes exhibited good sensitivity, wide linear range, a detection limit of 1.6 μM and good stability, showing that they can be used as sensors for ascorbic acid. There is no interference from compounds commonly found in clinical and pharmaceutical samples and the determination of ascorbic acid in commercial tablet samples was successfully performed.

1. Introduction

Carbon nanotubes (CNT) have been used for a wide range of applications last decade due to their high electrical conductivity, high surface area, and electronic properties [1–4]. CNTs, single-wall (SWCNT) and multi-walled carbon nanotubes (MWCNT) showed a good chemical stability and affinity to biomolecules, since they have a unique structure. Thus, they have often been used in electrochemical sensors and biosensors [4,5–7] as enzyme [8–9] and protein (myoglobin and haemoglobin) [10–11] biosensors, immunosensors [12], and DNA sensors [13–14]. The insolubility of CNTs in all solvents is their principal drawback. Functionalisation of CNTs improves the solubility and processability; thus, new types of nanotube-based materials can be produced [15]. Various electrochemical biosensors based on CNTs can be prepared modifying them chemically or physically with redox mediators to improve the electrical and mechanical properties of the sensors [3,16–20].

Nile blue A (NB) is one of the electroactive phenoxazine dyes [21] recently used also as a redox mediator, and it can be easily adsorbed or electropolymerised from aqueous solution on the surface of solid electrodes [22,23]. Electrodes modified with Nile blue have been recently used as sensors and biosensors, especially for the electrocatalytic oxidation of the nicotinamide-based coenzymes, NADH and NADPH [24].

Ascorbic acid (or ascorbate at higher pH) (AA), vitamin C, is a well-known water-soluble pharmaceutical compound. It is an effective reducing agent and biological antioxidant which is commonly used in foods and drugs. It is also important for industrial applications, since it prevents the colour and aroma changes of the products, thus extending their storage life. Electrochemical techniques have been widely used for the determination of AA in drugs and foods [25–28]. Various sensitive films on electrode substrates have been used for electrochemical AA determination such as Prussian Blue [29], copper hexacyanoferrate [30], tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCNQ) organic salt [31], a PVC/TTF-TCNQ composite [32], and an organoclay film [33]. Most recently carbon nanotubes modified electrodes were also employed: single-walled with ionic liquid, IL-SWCNT [34] or multi-walled together with ruthenium oxide hexacyanoferrate, MWCNT/RuOHCF [35] or polyaniline/polyacrylic acid, PANI/PAA/MWCNT [36].

Electrochemical impedance spectroscopy (EIS), a method which has been recently applied in the characterisation of electrochemical sensors and biosensors [37–40], has been used to gain valuable information about the electrochemical characteristics of the polyphenazine films, which is not possible by voltammetric techniques.

In this work, modification of glassy carbon electrodes (GCE) with functionalised-MWCNT and poly(Nile blue) (PNB) is

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http://dx.doi.org/10.1016/j.talanta.2013.02.043

reported. These modified electrodes were varied by changing the MWCNT loadings, the thickness of the PNB film, and the PNB film being formed either beneath or on the top of the MWCNT coatings. Their characterisation was achieved by cyclic voltammetry (CV) and EIS. The prepared electrodes were also used for determination of ascorbic acid by CV and fixed-potential amperometry. The results were optimised to obtain the best modification of GCEs with MWCNT coatings and PNB films in order to achieve a good sensor performance for AA. Different compounds were tested as possible interferents and finally, ascorbic acid was determined in two different pharmaceutical drugs.

2. Experimental

2.1. Materials

Multi-walled carbon nanotubes were from NanoLab, U.S.A., with ~95% purity, 30 ± 10 nm diameter and 1–5 μm length.

Nile blue A and N,N-dimethylformamide (DMF) were purchased from Fluka (Switzerland) and used without further purification. L-Ascorbic acid was obtained from Sigma (Germany) and used as received.

Phosphate buffer solutions (PB), 0.1 M pH 5.3 and 6.0, were prepared from sodium di-hydrogenphosphate and di-sodium hydrogenphosphate (Riedel-de-Haën, Germany), adjusting the pH if necessary with 5 M NaOH (Riedel-de Haën, Germany) solution. All chemicals were of analytical grade.

Millipore Milli-Q nanopure water (resistivity ≥ 18 MΩ cm), and analytical grade reagents were used for preparation of all solutions. Experiments were performed at room temperature, 25 ± 1 °C.

2.2. Instrumentation

A three-electrode electrochemical cell was used for CV, chronoamperometry, and EIS measurements. It contained a MWCNT- and PNB- modified glassy carbon working electrode of diameter 5.5 and 1.5 mm, a platinum foil as counter electrode, and a saturated calomel electrode (SCE) served as reference. All CV and chronoamperometry measurements were performed using a computer-controlled μ-Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm-Autolab, The Netherlands).

EIS measurements were carried out with a PC-controlled Solartron 1250 Frequency Response Analyser coupled to Solartron 1286 Electrochemical Interface using ZPlot 3.1 software (Scribner Associates, USA). A sinusoidal voltage perturbation of root-mean-square amplitude 10 mV was applied in the frequency range between 65 kHz and 0.1 Hz with 10 frequency steps per decade, integration time 60 s. Fitting to equivalent circuits was performed with ZView 3.1 software (Scribner Associates, USA).

The pH measurements were carried out with a CRISON 2001 micro-pH-meter (Crison Instruments, Spain) at room temperature.

2.3. Electrode preparation

MWCNT (120 mg) were stirred in 10 mL of a 3 M nitric acid solution for 24 h at room temperature. The solid product was filtered and washed several times with nanopure water until the filtrate became close to neutral (pH ≈ 7). The functionalised MWCNTs were then dried in an oven at 80 °C for 24 h [2].

The dried MWCNTs were dispersed in DMF, 0.2 and 1.0%, and sonicated for 4 h to ensure a homogeneous mixture. Before the coating, GC electrode (with a diameter of 5.5 mm) was cleaned by polishing with diamond spray (Kemet International Ltd., U.K.), then sonicated in Milli-Q nanopure water and finally rinsed with nanopure water. Coating of GC electrode was performed using three different loadings of MWCNT in DMF: 20 μL/0.2% (MWCNT-1), 10 μL/1.0% (MWCNT-2), and 20 μL/1.0% (MWCNT-3). Then, 10 or 20 μL of the MWCNT-DMF dispersion was dropped by using a micropipette on the surface of the GC electrode and allowed to dry at room temperature [41].

Table 1
Modification of GC electrodes by MWCNT and PNB.

Modified electrode	MWCNT-DMF dispersion	Γ (MWCNT)/ mg cm^{-2}	NB electro-polymerisation cycles
PNB/MWCNT-1	20 μL, 0.2%	0.17	5
MWCNT/PNB-1	20 μL, 0.2%	0.17	17
PNB/MWCNT-2	10 μL, 1.0%	0.42	5
MWCNT/PNB-2	10 μL, 1.0%	0.42	17
PNB/MWCNT-3	20 μL, 1.0%	0.84	5
MWCNT/PNB-3	20 μL, 1.0%	0.84	17

Electropolymerisation of NB was performed either prior to MWCNT coating (denoted as MWCNT/PNB) or on the top of MWCNT (denoted as PNB/MWCNT). It was carried out by cycling the potential in the region from -0.6 to $+1.2$ V vs SCE at a scan rate of 50 mV s^{-1} for 5 or 17 cycles in 0.1 M PB at pH 6.0 containing 0.5 mM NB monomer. Then, it was left to dry overnight in air at room temperature. The modification conditions of the GCE with MWCNT and PNB are listed in Table 1. The surface concentration of MWCNT cast on the electrode was calculated from its dispersion in DMF dividing it by electrode geometric area.

2.4. Sample preparation

Pharmaceutical samples of aspirin and paracetamol with vitamin C, containing 240 mg and 250 mg ascorbic acid, respectively, were purchased from local pharmacy. Before testing, the samples were prepared by simply dissolving in water. The determination of ascorbic acid was performed by amperometry at fixed potential using standard addition method.

3. Results and discussion

3.1. Preparation and characterisation of MWCNT- and PNB-modified GC electrodes

Various MWCNT- and PNB-modified GC electrodes were prepared by changing (i) the loadings of MWCNT, (ii) the number of cycles for electropolymerisation of NB, and (iii) the position of the PNB film, either beneath or on the top of MWCNT coatings, Table 1. Electropolymerisation of NB was carried out by potential cycling in 0.1 M PB at pH 6.0 containing 0.5 mM of NB monomer. These polymerisation conditions were chosen in agreement with the detailed study between pH 5 and 8 in previous work [42], which showed that these settings lead to the formation of polymer films with the most well-defined voltammetric peaks and the most stable PNB film.

Cyclic voltammograms of NB electropolymerisation on the surface of GCE and on the top of MWCNT of 20 μL of 1.0% MWCNT-DMF loading are presented in Fig. 1. The irreversible oxidation peak at ~0.9 V, formation of the monomer radical, decreased with the number of cycles. The CVs of NB show two redox couples. The first redox couple at around -0.4 V is due to oxidation/reduction of the NB monomer. The other redox couple at around -0.1 V corresponds to oxidation/reduction of PNB and the peaks increase linearly in height with the number of cycles as PNB forms a film. Comparing the CVs of PNB on bare GCE and on the MWCNT coatings, the polymer redox couple peaks obtained

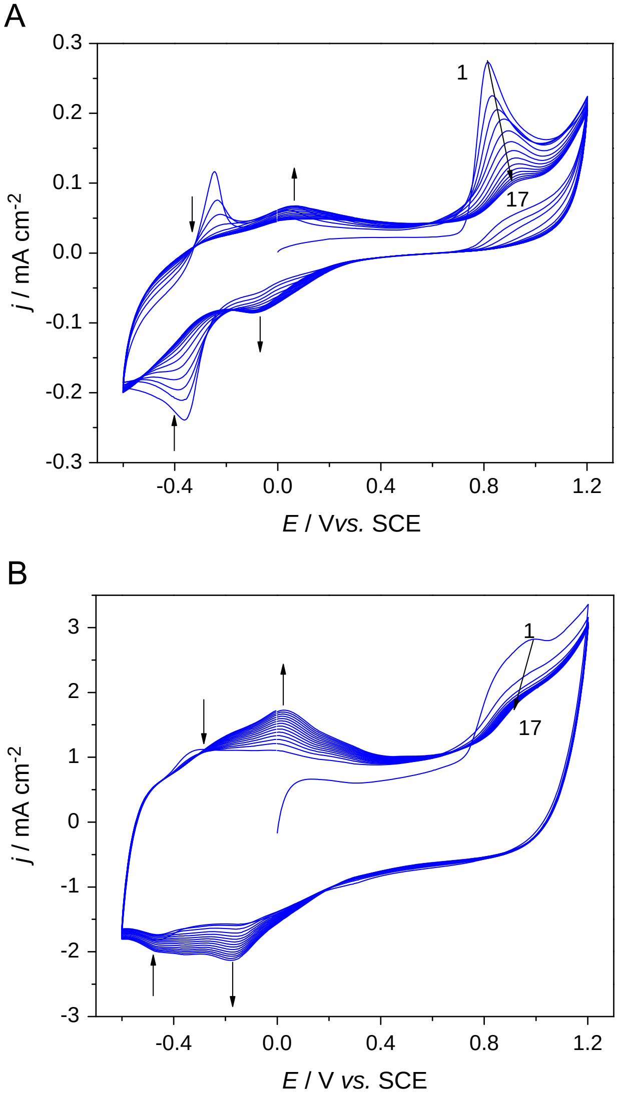

Fig. 1. Cyclic voltammograms in 0.5 mM NB in 0.1 M PB at pH 6.0 (A) on bare GCE and (B) on GCE modified with 20 μL of 1.0% MWCNT-DMF loadings. Scan rate: $50\ \mathrm{mV\ s^{-1}}$.

with MWCNT modified electrodes are much higher than on bare GCE, owing to the larger electroactive surface area. Additionally, the current of the monomer redox couple decreased faster during polymerisation of NB on MWCNT coatings than on bare GCE, showing that the MWCNT surface was covered with PNB, in this way impeding access and thence nucleation [42,43].

Different numbers of cycles for the electropolymerisation of NB were used to obtain MWCNT/PNB and PNB/MWCNT modified electrodes, 17 and 5, respectively. These numbers of cycles were chosen according to cyclic voltammetric data: film growth stops after 17 cycles for MWCNT/PNB and 5 cycles for PNB/MWCNT modified electrodes (Fig. 1). A thicker PNB underlying film in the MWCNT/PNB electrodes was more suitable for electrochemical sensing, since it gave a stable signal. However, in the case of PNB/MWCNT modification, a thick PNB film on the top of MWCNT caused mechanical instability of the modifying layers; therefore, a thinner PNB film was deposited which gave a much better stability and electrochemical reactivity for the PNB/MWCNT electrodes.

Characterisation of the modified electrodes was done by cyclic voltammetry mainly in 0.1 M PB at pH 5.3, since the best results were obtained under these conditions [37]. Fig. 2 shows CVs at MWCNT, MWCNT/PNB and PNB/MWCNT, modified electrodes prepared using 20 μL/0.2%, 10 μL/1.0%, and 20 μL/1.0% MWCNT loadings in DMF. Dashed lines in Fig. 2 show the CVs at the MWCNT electrodes without PNB. For both types of modified electrode, the

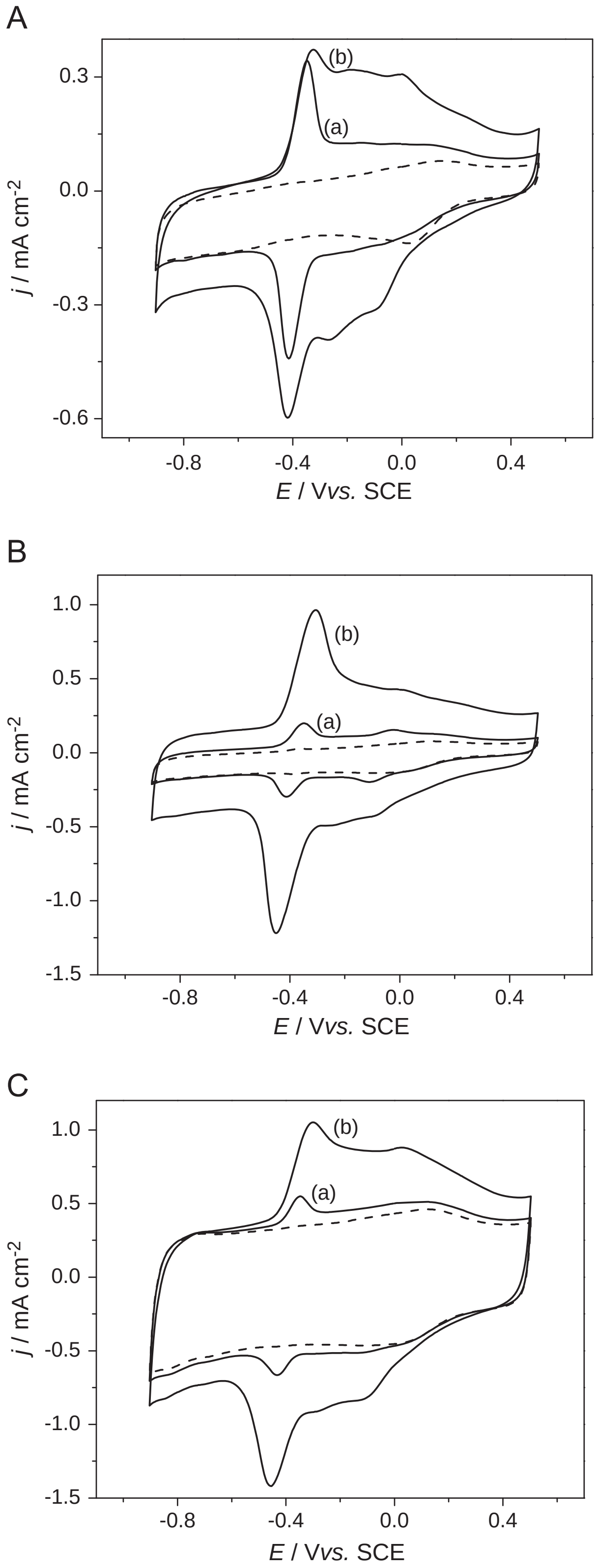

Fig. 2. Cyclic voltammograms at MWCNT (dashed lines), (a) MWCNT/PNB and (b) PNB/MWCNT modified electrodes in 0.1 M PB at pH 5.3. The electrodes were prepared by using (A) 20 μL of 0.2%, (B) 10 μL of 1.0%, and (C) 20 μL of 1.0% MWCNT-DMF loading. Scan rate $25\ \mathrm{mV\ s^{-1}}$.

redox couple at around −0.4 V (attributed to NB monomer, the redox properties of which are retained on polymerisation of phenazine dyes [21]) and the other couples at around −0.1 and 0.0 V are attributed to PNB and MWCNT, respectively [3,42].

The redox peak currents for all MWCNT/PNB and PNB/MWCNT modified electrodes increased linearly with the square root of the scan rate ($v^{1/2}$), demonstrating a diffusion-controlled electrochemical process. However, the process for PNB on the surface of GCE was adsorption-controlled, as seen in previous work [42]. The presence of MWCNT changed this process of PNB to diffusion control no matter it is polymerised beneath or on the top of MWCNT.

3.2. Characterisation of the modified electrodes by electrochemical impedance spectroscopy

Electrochemical characterisation of MWCNT/PNB and PNB/MWCNT modified electrodes was also performed by EIS in 0.1 M PB at pH 5.3 at applied potential values of 0.0 and +0.45 V; at 0.0 V

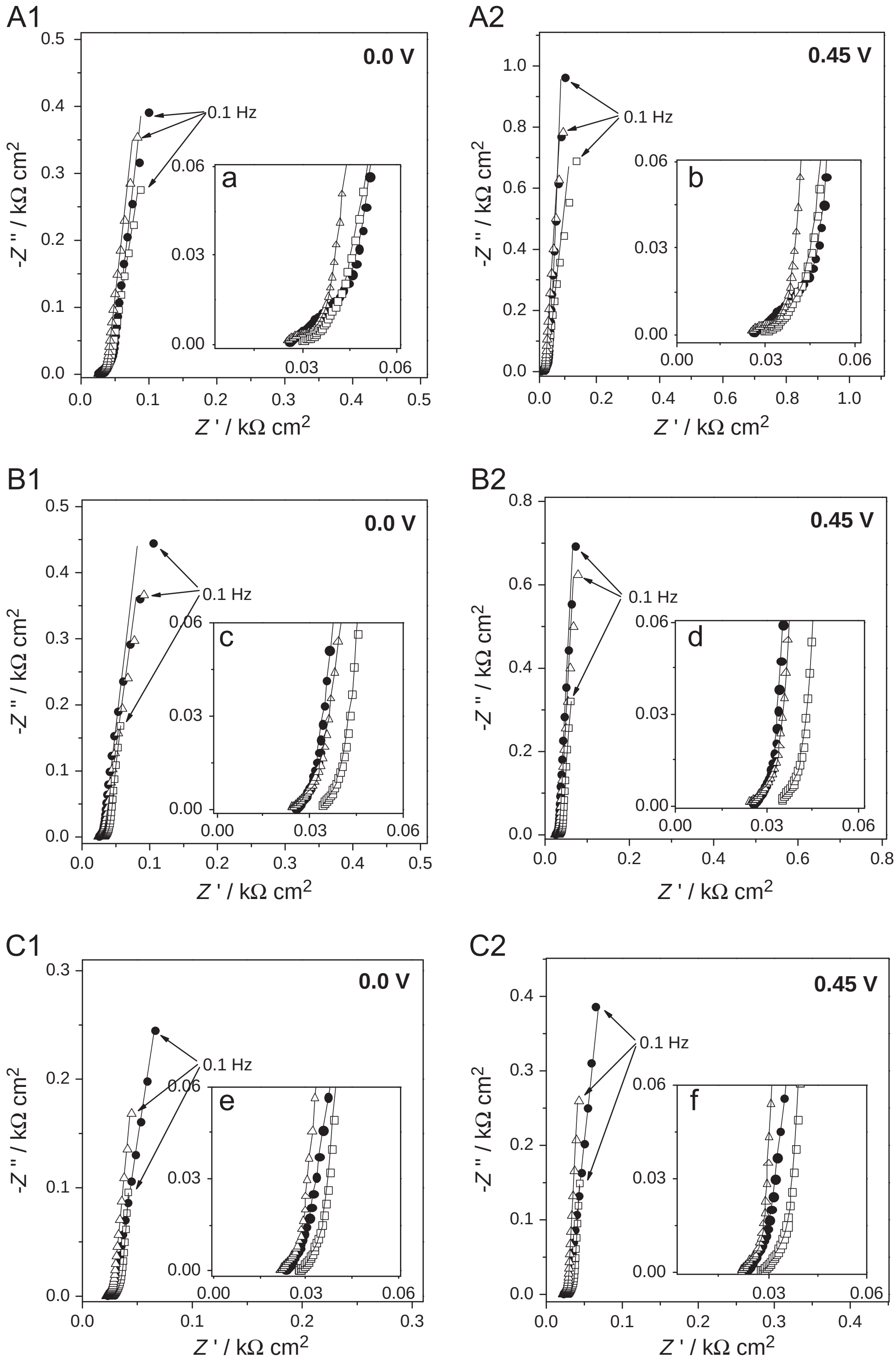

Fig. 3. Complex plane impedance spectra for modified electrodes prepared with (A) 20 μL of 0.2%; (B) 10 μL of 1.0%; (C) 20 μL of 1.0% MWCNT-DMF loadings in 0.1 M PB at pH 5.3. ●—MWCNT; △—MWCNT/PNB, and □—PNB/MWCNT. Applied potentials: 0.00 V for (A1), (B1), and (C1); +0.45 V vs. SCE for (A2), (B2), and (C2). Insets magnify the high frequency portion. Lines indicate equivalent circuit fitting.

since there was a small oxidation wave of the PNB and the potential value of +0.45 V was more positive than all redox peaks.

Complex plane spectra are shown in Fig. 3. The impedance values for the modified electrodes are in the sequence: MWCNT > MWCNT/PNB > PNB/MWCNT at both potentials for all MWCNT-DMF loadings.

All spectra showed a typical shape usually obtained for CNTs: lines with two different angles, i.e. at high frequencies around 45° and at low frequencies close to 90°. The imaginary impedance value was higher at +0.45 V than at 0.00 V, indicating easier charge separation at +0.45 V. In all cases, MWCNT by itself had the highest imaginary impedance value as well as the lowest when PNB was deposited on the top of CNTs, as expected.

The spectra in Fig. 3 were analysed by fitting to electrical equivalent circuit models usually applied for CNT-based electrodes, including a Warburg element, as seen in Fig. 4. The models include a cell resistance, R_s, a constant phase element, CPE, where CPE_1 reflects a double layer capacitance (C_{dl}) and CPE_2 gives a PNB film capacitance (C_f), R_1 is a charge transfer resistance (R_{ct}), and W_o is an open Warburg element. The CPE is modelled as a non-ideal capacitor and given by $CPE = -1/(i\omega C)^{\alpha}$, where C is the capacitance in F, ω is the frequency in rad s^{-1}, and the exponent α reflects the surface non-uniformity and porosity of the polymer film, where $\alpha = 1$ corresponds to a perfectly uniform and smooth surface [44]. The calculated values of the parameters for all modified electrodes in 0.1 M PBS (pH 5.3) at +0.45 and 0.00 V are shown in Table 2. Results obtained from equivalent circuit fitting showed that cell resistance was ca. 22, 24 and 26 Ω cm^2 at electrodes modified with 20 μL/0.2%, 10 μL/1.0%, and 20 μL/1.0% MWCNT-DMF, respectively. The charge transfer is easy in all cases since CNTs lowered charge transfer resistance of the electrode. A higher R_{ct} from all cases was found, as expected, when the lowest loading of CNTs was used. The double layer capacitance was rather high, as happens at electrodes modified with CNT, and increased when PNB was deposited on the top of the CNT layer, showing that charge separation at the polymer film was faster. The value of C_{dl} depended on the electrode modification but almost did not depend on potential applied (in the studied range). Surprisingly, MWCNT modification with PNB caused a less uniform structure at the electrode-solution interface as revealed by values of the roughness exponent. This showed that the polymer film is rather porous. The diffusion resistance calculated from the Warburg element decreased with MWCNT modification and with PNB film and in most cases it was lowest when PNB was on top of MWCNT. However, the diffusion time calculated as factor τ from the same Warburg element [45] showed that diffusion was rather slow and after modification with the PNB film it was the slowest, except for the highest loading of MWCNT (20 μL/1.0% MWCNT-DMF). The exponent α calculated from the open Warburg element was close to 0.45 showing an infinite diffusion which is usually found in thin films [45].

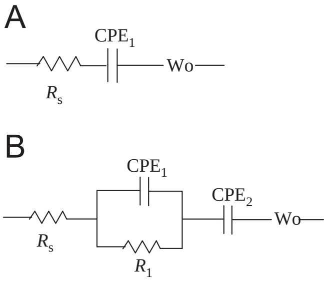

Fig. 4. Equivalent circuits used for fitting to electrochemical impedance spectra in Fig. 3. R_s is cell resistance; CPE_1 is as double layer capacitance (C_{dl}); R_1 is charge transfer resistance (R_{ct}); CPE_2 is as PNB film capacitance (C_f); R_2 is as PNB film resistance (R_f); W_o is Warburg-open element.

3.3. Electrochemical determination of ascorbate at MWCNT/PNB and PNB/MWCNT modified electrodes by cyclic voltammetry

The electrochemical measurement of AA was carried out with MWCNT/PNB and PNB/MWCNT modified electrodes using CV in 0.1 M PB at pH 5.3. For both modifications, an irreversible oxidation peak for AA was observed at around 0.0 V. CV responses to AA at MWCNT/PNB and PNB/MWCNT modified electrodes prepared with 20 μL/0.2%, 10 μL/1.0%, and 20 μL/1.0% loadings, can be seen in Fig. 5. Fig. 5(A) shows that the peak current of AA increased faster at MWCNT/PNB-1 and PNB/MWCNT-1 modified electrodes than that modified only with MWCNT. Fig. 5(B) and (C) demonstrate that the peak current of AA did not increase at MWCNT/PNB and

Table 2
Data obtained from analysis of the impedance spectra for the modified electrodes in 0.1 M PB at pH 5.3 using equivalent circuit fitting.

Modified Electrode	E/V vs SCE	R_{ct}/Ω cm^2	C_{dl}/mF cm^{-2} $s^{\alpha-1}$	α_1	C_f/mF cm^{-2} $s^{\alpha-1}$	α_2	R_{dif} (W_o)/Ω cm^2	τ (W_o)/s	α_3 (W_o)
MWCNT: loading 20 μL, 0.2%									
MWCNT-1	0.00	–	–	–	14.6	0.94	60.1	0.16	0.46
MWCNT/PNB-1	0.00	6.9	0.94	0.69	4.82	0.93	17.1	0.19	0.47
PNB/MWCNT-1	0.00	6.7	5.61	0.74	3.50	0.89	12.9	0.21	0.46
MWCNT-1	0.45	–	–	–	4.91	1.00	65.9	0.13	0.46
MWCNT/PNB-1	0.45	7.5	0.35	0.75	2.57	0.96	19.1	0.15	0.49
PNB/MWCNT-1	0.45	7.7	2.30	0.75	1.28	0.94	12.4	0.20	0.47
MWCNT: loading 10 μL, 1.0%									
MWCNT-2	0.00	–	–	–	6.01	0.92	17.6	0.13	0.47
MWCNT/PNB-2	0.00	–	–	–	9.11	0.95	25.4	0.15	0.45
PNB/MWCNT-2	0.00	13.2	9.78	0.74	9.12	0.98	20.6	0.16	0.47
MWCNT-2	0.45	–	–	–	3.40	0.98	21.9	0.13	0.47
MWCNT/PNB-2	0.45	–	–	–	4.52	1.00	28.8	0.13	0.45
PNB/MWCNT-2	0.45	12.4	9.80	0.79	4.79	0.99	25.4	0.15	0.45
MWCNT: loading 20 μL, 1.0%									
MWCNT-3	0.00	–	–	–	8.96	0.90	18.2	0.25	0.46
MWCNT/PNB-3	0.00	–	–	–	8.28	0.92	16.4	0.23	0.47
PNB/MWCNT-3	0.00	1.0	3.05	0.75	8.42	0.98	23.0	0.15	0.45
MWCNT-3	0.45	–	–	–	5.56	0.94	19.9	0.23	0.46
MWCNT/PNB-3	0.45	–	–	–	5.88	0.99	15.8	0.18	0.48
PNB/MWCNT-3	0.45	1.6	5.21	0.73	5.37	0.97	23.2	0.17	0.48

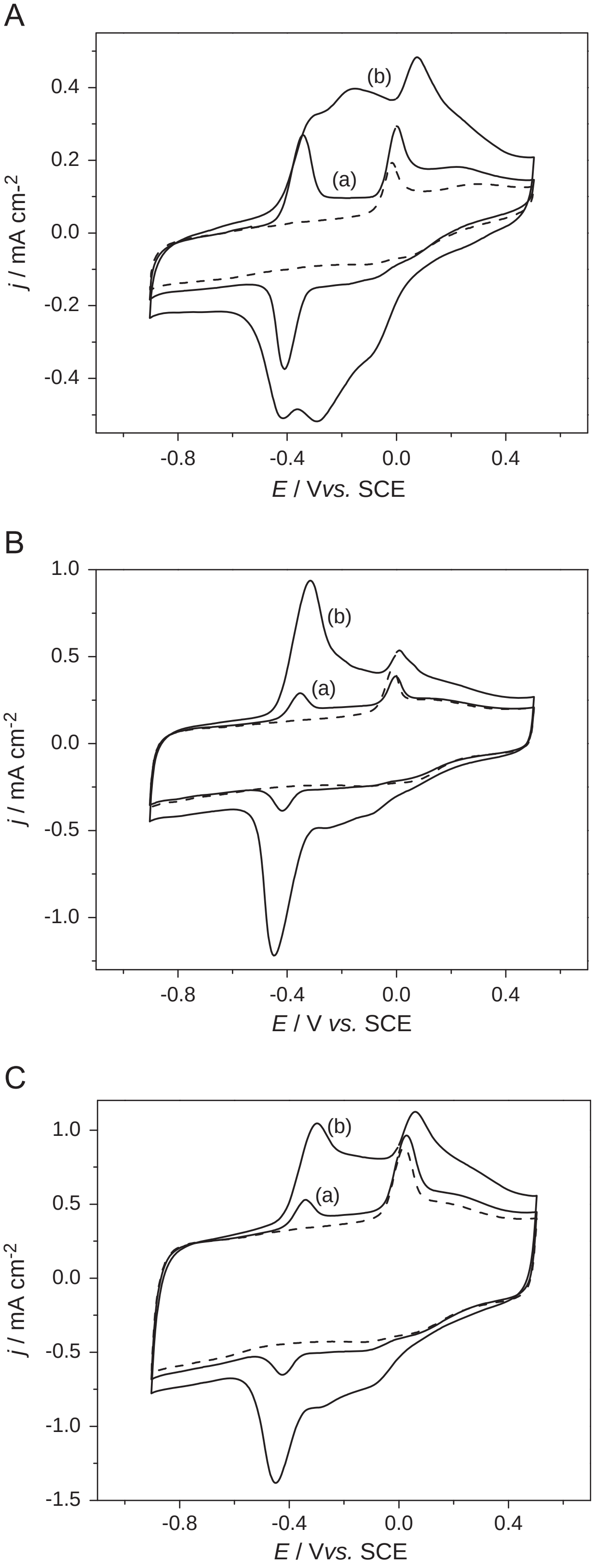

Fig. 5. Cyclic voltammetric responses of MWCNT (dashed lines), (a), MWCNT/PNB and (b) PNB/MWCNT modified electrodes in 0.1 M PB at pH 5.3 containing 0.5 mM AA. The electrodes were prepared by using (A) 20 μL of 0.2%, (B) 10 μL of 1.0%, and (C) 20 μL of 1.0% MWCNT-DMF dispersions. Scan rate 25 mV s^{-1}.

PNB/MWCNT modified electrodes prepared with 10 μL/1.0% and 20 μL/1.0% loadings. Only modified electrodes prepared with 20 μL/0.2% loading gave a good response to AA.

Table 3
Analytical parameters from the calibration plots for quantitative determination of AA in 0.1 M PB at pH 5.3 by cyclic voltammetry (scan rate 25 mV s^{-1}).

Modified electrode	Linear range/mM	Sensitivity/μA cm^{-2} mM^{-1}	LOD/μM
MWCNT: loading 20 μL, 0.2%			
MWCNT-1	0.05–2.0	105 ± 2	12.1
PNB/MWCNT-1	0.05–2.0	125 ± 3	11.9
MWCNT/PNB-1	0.05–1.0	117 ± 6	9.16
MWCNT: loading 10 μL, 1.0%			
MWCNT-2	0.05–1.0	250 ± 5	13.9
PNB/MWCNT-2	0.05–1.0	246 ± 3	14.7
MWCNT/PNB-2	0.05–1.0	208 ± 1	10.6
MWCNT: loading 20 μL, 1.0%			
MWCNT-3	0.05–1.0	449 ± 11	9.60
PNB/MWCNT-3	0.05–0.5	439 ± 2	14.8
MWCNT/PNB-3	0.05–1.0	461 ± 18	10.8

Calibration via quantitative determination of AA was carried out in 0.1 M PB at pH 5.3. The results obtained are listed in Table 3. The linear ranges for 20 μL/0.2% loadings of MWCNT were greater than those of the other loadings. The slopes of MWCNT/PNB-1 and PNB/MWCNT-1 modified electrode plots were 159 and 125 μA cm^{-2} μM^{-1}, respectively. When the amount of MWCNT was increased to 10 μL/1.0%, the slopes of the MWCNT/PNB-2 and PNB/MWCNT-2 modified electrodes were 208 and 246 μA cm^{-2} μM^{-1}, respectively, showing that the slopes did not increase proportionally with the amount of MWCNT. Similar results were obtained for MWCNT/PNB-3 and PNB/MWCNT-3 modified electrodes; the amount of MWCNT was 5 times greater than those of MWCNT/PNB-1 and PNB/MWCNT-1 modified electrodes, whereas the slopes of the calibration lines also did not increase proportionally. These results showed that 20 μL/0.2% MWCNT loadings gave the better sensitivity to AA.

According to the peak shapes of AA in Fig. 5(A) and the slopes and LOD, for 20 μL/0.2% MWCNT loadings, it can be deduced that MWCNT/PNB-1 modified electrodes are more suitable for AA determination than the others.

3.4. *Determination of ascorbate by fixed potential amperometry at MWCNT/PNB and PNB/MWCNT modified electrodes*

The quantitative amperometric determination of AA using MWCNT/PNB and PNB/MWCNT modified electrodes was performed using the standard addition method. The amperometric response due to oxidation of AA was measured in 0.1 M PB at pH 5.3 at the potentials of 0.00 V and 0.10 V under constant solution stirring. The currents plotted against the concentration of AA after background subtraction showed a linear relationship between AA oxidation current and concentration at both MWCNT/PNB and PNB/MWCNT modified electrodes for all MWCNT loadings, see Fig. 6.

The analytical parameters obtained from the amperometric response to AA are given in Table 4 for all modified electrodes, at potentials of 0.00 V and 0.10 V. As a typical example, the linear equations obtained for PNB/MWCNT-1 modified electrode are given below

$$\Delta I(\mu\text{A}) = 72.7c(\text{mM}) + 0.60,\ \text{R} = 0.997\ (\text{at } 0.0\ \text{V}) \tag{1}$$

$$\Delta I(\mu\text{A}) = 65.8c(\text{mM}) + 0.57,\ \text{R} = 0.998\ (\text{at } 0.1\ \text{V}) \tag{2}$$

The sensitivities for MWCNT/PNB-1 modified electrodes were greater than PNB/MWCNT-1 and MWCNT-modified electrodes for 20 μL/0.2% loading at both potentials (Fig. 6(A) and Table 4). However, for 10 μL/1.0% loading the sensitivities for MWCNT/PNB and PNB/MWCNT modified electrodes were lower than MWCNT alone or greater only at 0.10 V for 20 μL/1.0% loadings.

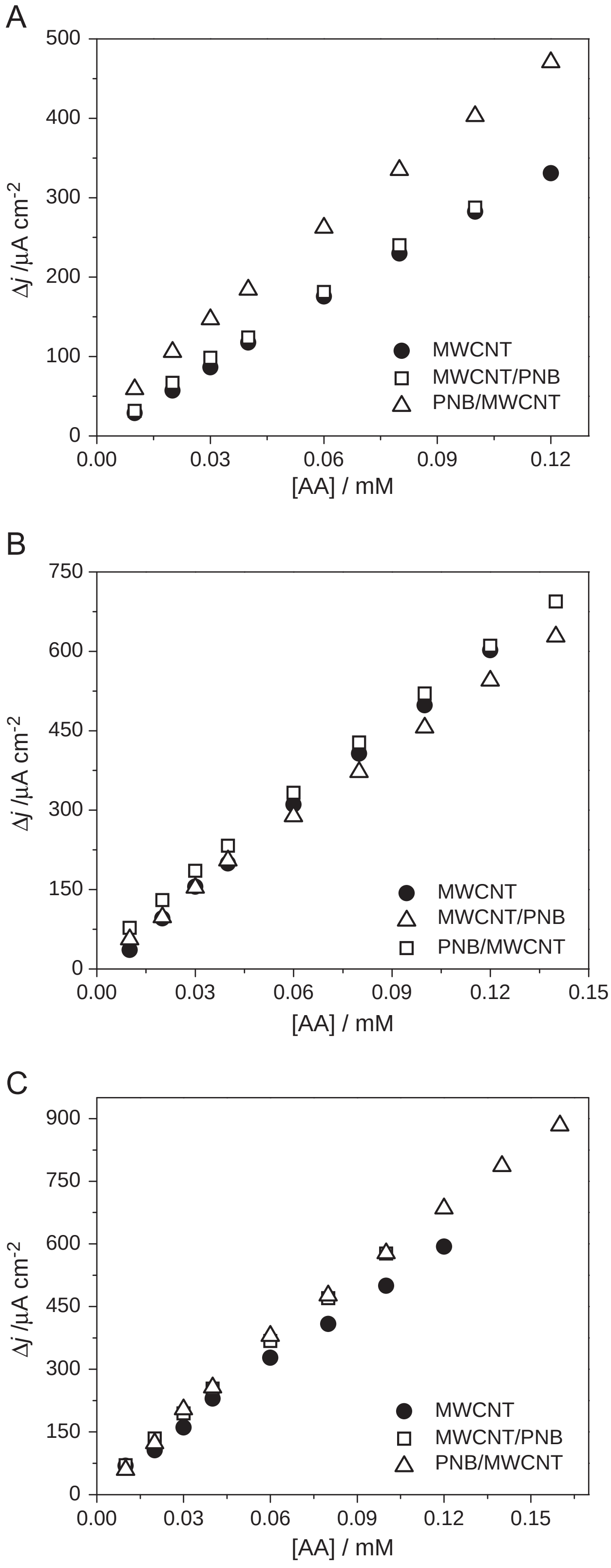

Fig. 6. Calibration curves of (●) MWCNT, (△) MWCNT/PNB and (□) PNB/MWCNT modified electrodes from the determination of AA in 0.1 M PB at pH 5.3 by fixed potential amperometry. Electrodes prepared with (A) 20 µL of 0.2%, (B) 10 µL of 1.0%, and (C) 20 µL of 1.0% MWCNT-DMF dispersions. Applied potential 0.10 V.

The detection limit was lowest for MWCNT/PNB-1 modified electrodes, at 1.6 µM, in agreement with the determination by CV described in Section 3.3.

Table 4
Analytical parameters from the calibration plots for quantitative determination of AA in 0.1 M PB at pH 5.3 by fixed potential amperometry.

Modified electrode	*E*/V vs SCE	Linear range/mM	Sensitivity/ µA cm^{-2} mM^{-1}	LOD/ µM
MWCNT: loading 20 µL, 0.2%				
MWCNT-1	0.00	0.02–0.16	225 ± 3	3.9
	0.10	0.01–0.12	207 ± 3	2.2
PNB/MWCNT-1	0.00	0.01–0.10	154 ± 4	3.0
	0.10	0.01–0.10	212 ± 4	2.2
MWCNT/PNB-1	0.00	0.01–0.12	307 ± 7	1.7
	0.10	0.01–0.12	278 ± 5	1.6
MWCNT: loading 10 µL, 1.0%				
MWCNT-2	0.00	0.01–0.10	460 ± 5	2.6
	0.10	0.01–0.12	380 ± 5	2.9
PNB/MWCNT-2	0.00	0.01–0.14	300 ± 3	2.8
	0.10	0.01–0.14	355 ± 4	2.1
MWCNT/PNB-2	0.00	0.01–0.14	302 ± 6	2.1
	0.10	0.01–0.14	327 ± 4	2.0
MWCNT: loading 20 µL, 1.0%				
MWCNT-3	0.00	0.01–0.10	435 ± 6	2.6
	0.10	0.01–0.12	359 ± 8	3.1
PNB/MWCNT-3	0.00	0.01–0.10	383 ± 6	2.8
	0.10	0.01–0.10	418 ± 6	2.5
MWCNT/PNB-3	0.00	0.01–0.14	857 ± 16	2.4
	0.10	0.01–0.16	406 ± 8	2.2

Experiments were also conducted by differential pulse voltammetry, but the results indicated a lower sensitivity than with fixed potential amperometry. This could be due to kinetic limitations together with analyte access to within the PNB/MWCNT structure.

AA can be oxidised at a variety of modified electrodes, so their performance was compared to the sensor obtained in this work and the data are presented in Table 5. Hexacyanoferrate salt based electrodes such as Prussian Blue deposited on GCE [29], and copper hexacyanoferrate modified carbon film electrodes [30] were used as amperometric sensors for AA. Conducting polymers are often involved in sensor preparation to monitor AA. Screen printed electrodes modified with dodecylbenzene sulphonate-doped polyaniline nanoparticles were used for AA determination by fixed potential amperometry at 0.0 V vs. Ag/AgCl in PBS, pH 6.8 [46]. The linear range in that case was rather large with a quite low sensitivity and an LOD that is higher than in this work. Also, various phenazine dye modified electrodes were applied for determination of AA in different media. For example, an electrode prepared with methylene blue adsorbed on phosphorylated zirconia–silica mixed oxide particles, for simultaneous determination of dopamine, uric acid and ascorbic acid using differential pulse voltammetry [47] gave an LOD value similar to the sensor in this work obtained in CV mode, but the sensitivity is 10 times lower. In this work, PNB/MWCNT and MWCNT/PNB modified electrodes gave a higher sensitivity and lower LOD than most of the electrodes reported. Moreover, a similar sensor MWCNT/poly(neutral red) modified glassy carbon electrodes showed a lower sensitivity (384 µA cm^{-2} mM^{-1}) under similar conditions as here, i.e. PBS, pH 5.5, the sensitivity and LOD depending on the MWCNT brand [41].

In [34–36], all modified electrodes with CNT as one of the modifier components but of more complex architecture, and together with differential pulse voltammetry, the detection limits for ascorbic acid were lower than here. However, for the MWCNT/RuOHCF electrode [35] the linear range was much less wide, whilst the other sensors: PANI/PAA/MWCNT [36] and IL-SWCNT [34] exhibited longer linear ranges. The sensitivity of the ionic liquid carbon nanotubes modified electrode [34] was lower than here

Table 5
Comparison of analytical parameters for AA at different sensors under various conditions.

Electrode	Method	Medium	Linear range/mM	Sensitivity /mA cm^{-2} mM^{-1}	LOD/μM	Ref.
PB[a]/GCE	Amp 0.27 V (Ag/AgCl)	1.0 M KCl (pH 3.7)	0.005–1.00	1.5	2.5	[29]
CuHCF[b]/CFE[c]	Amp 0.05 V (SCE)	0.1 M PBS (pH 7.0)	0.011–5.00	0.011	1.3	[30]
PVC/TTF-TCNQ[d]	Amp 0.15 V (Ag/AgCl)	0.15 M PB (pH 8.0)	0.4400–10.00	0.066	120	[32]
TMP[e]FCs/GCE	Amp 0.4 V (Ag/AgCl)	0.1 M PBS (pH 7.0)	0.0770–11.00	0.45	10	[33]
	CV		0.110–5.00	0.11	44	
IL[f]SWCNT	DPV	0.1 M PBS (pH 7.0)	0.0033–4.200	0.81	1.0	[34]
MWCNT/RuOHCF[g]/GCE	DPV	0.1 M PB (pH 7.0)	0.2–15	132	0.087	[35]
PANI/PAA[h]MWCNT-Nafion/Pt	DPV	0.1 M PB (pH 5.0)	0.001–1.00	9.6	0.25	[36]
MWCNT/GCE	Amp 0.05 V (SCE)	0.1 M KPBS (pH 5.5)	Up to 0.8800	0.38	4.3	[41]
DBSA[i]PANI-NP[j]/SPCPE[k]	Amp 0.0 V (Ag/AgCl)	0.1 M PBS (pH 6.8)	0.5–8.0	0.010	8.3	[46]
MB[l]/SZP[m]	DPV	0.35 M TrisHCl+0.5 M KCl (pH 7.4)	0.1–1.6	0.076	8.3	[47]
MWCNT/PNB-3/GCE	CV	0.1 M PB (pH 5.3)	0.05–1.0	0.46	10.8	This work
	Amp 0.0 V (SCE)		0.01–0.14	0.86	2.4	

[a] PB—Prussian blue.
[b] CuHCF—copper hexacyanoferrate.
[c] CFE—carbon film electrode.
[d] TTF-TCNQ—tetrathiafulvalene-tetracyanoquinodimethane.
[e] TMP/FCs—trimethylpropylammonium/ferrocene.
[f] IL—ionic liquid.
[g] RuOHCF—ruthenium oxide hexacyanoferrate.
[h] PANI/PAA— poly(aniline)/polyacrylic acid.
[i] DBSA—dodecylbenzene sulphonic acid.
[j] PANI-NP—polyaniline nanoparticles.
[k] SPCPE—screen printed carbon paste electrode.
[l] MB—methylene blue.
[m] SZP—silica–zirconia oxide particles.

(Table 5), but for the other two sensors the sensitivities were higher: 132 mA cm^{-2} mM^{-1} [35] and 9.6 mA cm^{-2} mM^{-1} [36].

The PNB/MWCNT modified electrodes exhibited rapid response to changes in the concentration of AA, producing steady-state signals in less than 5 s. The relative standard deviations of PNB and MWCNT-modified electrode sensitivities are less than 5%, shelf stability is at least 2 months, and operational stability is around 2–3 weeks. These factors indicate that MWCNT/PNB and PNB/MWCNT modified electrodes can be successfully and reliably used as amperometric sensors for AA.

3.5. Interferences and determination in pharmaceutical samples

Since by amperometry, a higher sensitivity was obtained than by CV, this method was chosen to perform an interference study, as well as to determine ascorbate in pharmaceutical samples. The electrode selected for these investigations was MWCNT/PNB-3, as it was the one which exhibited the highest sensitivity by both CV and amperometry. The applied potential was 0.00 V, since under these circumstances the sensor showed a higher sensitivity than at 0.10 V, and is also better to avoid interferences.

For the interference study several compounds that could be oxidised at the same potential as AA were tested, namely salicylic acid (SA), uric acid (UA), aspirin (Asp), dopamine (DA) and paracetamol (Par). The concentration of interferents was twice that of ascorbic acid. Fixed potential amperometry at 0.00 V was used and ascorbic acid, followed by interferents was added and the responses measured; the results of interference studies are presented in Fig. 7. Under these circumstances, the only one that exhibited any response was uric acid; the increase in response being 29% relative to ascorbic acid. However, this should not represent a problem for the determination of ascorbic acid in pharmaceutical samples, since this compound is not present.

The sensor was further used for the determination of ascorbic acid in tablets of aspirin and paracetamol containing vitamin C by using the standard addition method. The aspirin tablets were dissolved in 10 mL of water and paracetamol in 25 mL of water, and then diluted 10 and 5 times, respectively, in order to fit in the concentration range of the sensor. After baseline stabilisation in phosphate buffer solution the sample was added, followed by three additions of 20 μM standard ascorbate solution. Each sample was analysed in triplicate and the mean value calculated.

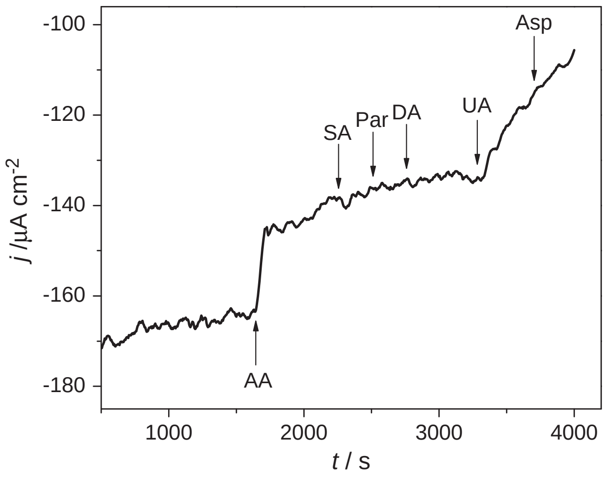

Fig. 7. Current-time response curve showing the response of ascorbic acid (AA) followed by addition of interferents: salicylic acid (SA), paracetamol (Par), dopamine (DA), uric acid (UA) and aspirin (Asp) in 0.1 M PB at pH 5.3 by fixed potential amperometry at 0.0 V.

The content of ascorbic acid was determined to be 246.0 ± 8.0 mg in aspirin with vitamin C and 245.2 ± 1.4 mg in paracetamol with vitamin C per tablet. The declared content of ascorbic acid was 240 mg in aspirin and 250 mg in paracetamol, respectively. The recoveries were 103 ± 2% and 98 ± 0.6%, indicating that the method is suitable for the determination of ascorbate in pharmaceutical samples.

4. Conclusions

A new modified electrode has been prepared by electropolymerisation of Nile blue A on GC electrodes or on functionalised MWCNT by potential cycling in 0.1 M PBS at pH 6.0. Different modifier layers were prepared by casting various MWCNT concentrations on the electrode surface or on the PNB film, and varying the number of NB electropolymerisation cycles. Cyclic voltammetry of the resulting modified electrodes showed a diffusion-controlled process. Impedance spectra revealed that an increase in MWCNT loading facilitated electron transfer and that the double layer capacitance increased with PNB deposition on the MWCNT layer without any dependence on applied potential, in the potential range studied. The film diffusion resistance decreased with MWCNT modification and with the PNB film and was lowest when PNB was formed on top of MWCNT.

Quantitative determination of ascorbate was successfully performed at MWCNT and PNB modified electrodes by cyclic voltammetry and fixed potential amperometry in 0.1 M PB at pH 5.3. The best results were obtained at the modified electrodes prepared with PNB films beneath the thinnest MWCNT layer at 0.10 V, without interferences from substances present in natural samples. The content of ascorbate determined in tablet samples was in agreement with that declared, showing that the modified electrodes, which are simple to prepare and mechanically robust, can be successfully used as ascorbate sensors.

Acknowledgements

Financial support from the Fundação para a Ciência e a Tecnologia (FCT), Portugal, PTDC/QUI-QUI/116091/2009, POCH, POFC-QREN (co-financed by the FSE and European Community Fund FEDER/COMPETE) and FCT project PEst-C/EME/UI0285/2011 (CEMUC®—Research Unit 285), Portugal, is gratefully acknowledged. D.K. thanks T.C., The Council of Higher Education (YÖK, Turkey) for a Postdoctoral research fellowship and Karadeniz Technical University (Turkey). R.P. and M.E.G. thank FCT for postdoctoral fellowships (SFRH/BPD/27075/2006 and SFRH/BPD/36930/2007).

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