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Selective detection of dopamine in the presence of ascorbic acid using carbon nanotube modified screen-printed electrodes

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ABSTRACT

This work reports on the performance of carbon nanotube modified screen-printed electrodes (SPE-MWCNT) for the selective determination of dopamine (DA) in the presence of ascorbic acid (AA) by adsorptive stripping voltammetry (AdSV). Several operating conditions and parameters were examined including the electrochemical pre-treatment and the previous AA interaction and DA accumulation in the presence AA at physiological conditions. Under the chosen conditions, DA peak current of differential pulse voltammograms increases linearly with DA concentration in the range of 5.0×10^{-8} to 1.0×10^{-6} mol L^{-1} with a limit of detection of 1.5×10^{-8} mol L^{-1} in connection with 600 s accumulation time. The sensitivity obtained for DA was independent from the presence or absence of AA; therefore, the proposed method can be readily applied to detect DA in real samples. The proposed methodology was successfully used for the quantification of DA in urine samples.

1. Introduction

Dopamine (DA) is one of the most important and representative catecholamine which plays an important role in the function of the central nervous, renal, hormonal and cardiovascular systems. The determination of DA is a subject of great significance in the investigation of its physiological functions and the diagnosis of nervous diseases resulting from DA abnormal metabolism, such as epilepsy, Parkinsonism and senile dementia [1]. Since DA (and the like catecholamines) is an easily oxidizable compound, electrochemical methods based on its anodic oxidation are an ideal choice for its quantitative determination [2]. The electrochemical determination of dopamine allows reaching low detention limit but it is often complicated by many coexisting compounds. Among them, ascorbic acid (AA) is of particular importance because it accompanies dopamine in biological samples. Usually, the concentration of DA is 10^{-8} to 10^{-6} mol L^{-1} while AA is as high as 10^{-4} mol L^{-1} in these samples (in the extracellular fluid of the central nervous system) [3,4]. Furthermore, DA and AA are oxidized at nearly the same potential at almost all carbon-based or metal electrodes; hence an overlapping voltammetric response for the oxidation of a mixture of DA and AA is usually obtained. Moreover, the solid electrodes very often suffer from the fouling effect due to the accumulation of oxidized products on the electrode surface which results in rather poor selectivity and sensitivity [2].

Several examples are reported in the literature regarding on the detection of DA using chemically modified electrodes including electrochemically pre-treated electrodes [5], self-assembled monolayer modified electrodes [6,7], covalent modification [8–10], polymer film [11,12], composite electrodes [13–15], among other. The different properties of each type of electrode define its own associated advantages and limitations. The detection of DA in the presence of excess of AA is still a challenging task in electroanalytical research where both sensitivity and selectivity are equally important in the development of an appropriate sensor system. Screen-printing technique is a well-established and simple process for the mass production of single use electrodes and biosensors. These electroanalytical tools combine ease of use, portability and inexpensive manufacture procedure [16] with minimum analysis volume, low reagent and sample consumption or minor generation of contaminant residuals as well as their usefulness as disposable devices. Their inherent miniaturization promotes and facilitates the possibility of rapid in situ analysis even in a small scale environment. The incorporation of nanomaterials to these screen-printed devices is of great interest for the further development of electrochemical sensors. Among these materials, carbon nanotubes present a singular structure and dimensions together with unique electronic, chemical and mechanical properties [17,18].

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These remarkable features open new venues for the construction of sensors and the development of novel electrochemical bioassays [19–22]. As electrode material carbon nanotubes can facilitate electron transfer between the electroactive species and the electrode. In fact, different papers can be found in the literature where the enhancement of the rate constants for the electron transfer or electrocatalysis has been already demonstrated [23] and this was also observed when carbon nanotubes were employed in screen-printed electrodes [24]. The goal of the present paper is to investigate the voltammetric behavior of DA on screen-printed carbon nanotube electrode with the aim to propose a new methodology for its determination at low levels in the presence of ascorbic acid using a previous preconcentration step.

2. Experimental

2.1. Reagents

L-(+)-Ascorbic acid, $K_3Fe(CN)_6$ and $FeSO_4{\cdot}7H_2O$ were purchased from Panreac (Madrid, Spain) and 3-hydroxytyramine (dopamine) chloride, glucose, uric acid, 3,4 dihydroxyphenylacetic acid (dopac) and $Ru(NH_3)_6Cl_3$ were from Sigma (Madrid, Spain). All other chemicals were analytical grade and were used as received without further purification. Ultrapure water ($\rho > 18\,M\Omega\,cm$) from a Millipore-MilliQ system was used for preparing all solutions.

2.2. Apparatus and electrodes

The planar screen-printed electrode (DropSens, Oviedo, Spain) 3.4 cm × 1.0 cm × 0.05 cm (length × width × height) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode, unmodified (SPE, ref. 110) or modified with multiwalled carbon nanotubes (10 nm diameter and 1–2 μm length) containing approximately 5% ratio of carboxylic groups (SPE-MWCNT, ref. 110CNT). The graphite working surface is 4 mm in diameter. Further details about them can be supplied from the following web site: www.dropsens.com.

Electrochemical measurements were performed by placing a 50 μL drop of the corresponding solution on the working area. Electrochemical measurements were conducted using a BAS 100B (BAS, West Lafayette, USA) electrochemical analyzer controlled by BAS W 2.3 software (BAS, West Lafayette, USA) through a Pentium IV PC computer or a μStat 100 potentiostat (DropSens, Oviedo, Spain) controlled by PSLite 1.6 software. A specific DropSens connector (ref. DSC) allowed the connection of the SPEs to the potentiostat. All experiments were carried out at room temperature (20–23 °C) using a new electrode for each assay.

2.3. Procedures

2.3.1. Electrode pre-treatment

The usual electrochemical pre-treatment of SPEs was carried out by applying +1.4 V for 300 s in 0.050 M phosphate buffer solution of pH 7.4.

2.3.2. Adsorptive stripping voltammetry

A 50 μL drop of 5.0×10^{-4} M AA in 0.050 M phosphate buffer (pH 7.4) was placed onto the working electrode area of a pre-treated SPE for 200 s, then rinsed with water and a volume of 50 μL of the given accumulation solution was placed for 10 min. Finally, after washing the electrode with water again, the differential pulse voltammetry (DPV) signal was recorded in fresh 0.050 M phosphate buffer (pH 7.4). The DPV parameters are the following: scan rate: 20 mV s^{-1}; pulse amplitude: 50 mV; sample width: 17 ms; pulse width: 50 ms; pulse period: 200 ms and quiet time: 2 s.

2.4. Sample preparation

Dopamine hydrochloride injection (CLORHIDRATO DOPAMINA GRIFOLS Amp. 200 mg/5 mL) was from GRIFOLS LABORATORY (Barcelona, Spain). Saline serum (Lactato de Ringer Hartmann Braun Sol. Iny) was from B. BRAUN MEDICAL, S.A. (Barcelona, Spain). Fresh urine samples from healthy donors were collected over a period of 24 h and they were acidified with HCl (to yield a final concentration of 1% of acid) and centrifuged to precipitate proteins. Then fractions of 10.0 mL of filtered urine were stored under refrigeration.

Dopamine hydrochloride injection samples were suitably diluted with 0.050 M phosphate buffer (pH 7.4) and doped with AA to obtain the desired concentration while saline serum was directly doped with AA and DA to obtain the desired concentrations. Urine samples were 10-fold diluted with 0.050 M phosphate buffer (pH 7.4) and spiked with DA and AA to obtain the desired concentrations.

3. Results and discussion

3.1. Electrode pre-treatment

Carbon electrodes are often preconditioned by electrochemical pre-treatments (EP) to enhance their electroactivity. The main effect of EP is the increase in the density of oxygenated groups on the electrode surface and the consequent improvement on the electron transfer [25,26]. In the case of SPE, the polymers present in the ink are responsible for slow charge transfer kinetics. Therefore, the activation of SPEs is often necessary for an optimum electrochemical performance *via* electrochemical pre-treatment [27].

To find an appropriate electrochemical pre-treatment for SPE and SPE-MWCNT, different electrolytes and conditions were studied using 1,4-benzoquinone in 0.050 M phosphate buffer solution of pH 7.4 as benchmark redox system. The electrochemical reduction of this marker in aqueous buffer to 1,4-hydroquinone is a well-known diffusion-controlled process involving 2 electrons and 2 protons [28].

Initially, the EP of SPE and SPE-MWCNT was carried out by applying anodic potentials ranging from +1.0 to +1.6 V in two different electrolyte solutions, 0.10 M H_2SO_4 and 0.050 M phosphate buffer solution of pH 7.4 for 60 s. The anodic-to-cathodic peak potential separation (ΔE_p), and the peak currents ratio (i_{pa}/i_{pc}) obtained from the cyclic voltammograms of 1,4-benzoquinone at untreated and pretreated SPE and SPE-MWCNT are summarized in Table 1. Regarding the untreated electrodes, the decrease in ΔE_p and the proximity of the obtained current ratio values to 1 at SPE-MWCNT, clearly demonstrate that the presence of MWCNTs at the SPE largely improved the electrochemical response of 1,4-benzoquinone. Considering pretreated electrodes, the EP mainly improves the electrochemical response of SPE. In general, as the EP applied potential increases, the reversibility of the redox couple improves at both electrodes, although in the case of SPE-MWCNT potentials as high as +1.6 V produce a negative effect on the response towards 1,4-benzoquinone. An EP is also necessary to eliminate small current contributions due to impurities present at SPE and SPE-MWCNT. Thus, a potential of +1.4 V was selected for further work.

The effect of the pre-treatment time at +1.4 V (0–300 s) in sulfuric acid and phosphate buffer solutions was also studied at SPE and SPE-MWCNT (not shown). As the pre-treatment time increased, up to 300 s, the reversibility of 1,4-benzoquinone improved. This EP time was also enough to eliminate the small contributions of fabrication impurities. A pre-treatment procedure consisting of

Table 1
Summary of cyclic voltammetry data for 5.0×10^{-4} M 1,4-benzoquinone before and after the pre-treatment[a].

Anodic potential (V)	SPE				SPE-MWCNT			
	0.10 M H_2SO_4		0.050 M phosphate buffer (pH = 7.4)		0.10 M H_2SO_4		0.050 M phosphate buffer (pH = 7.4)	
	ΔE_p (mV)	i_{pa}/i_{pc}	ΔE_p (mV)	i_{pa}/i_{pc}	ΔE_p (mV)	i_{pa}/i_{pc}	ΔE_p (mV)	i_{pa}/i_{pc}
Untreated	242	0.62	242	0.62	192	0.79	145	0.79
1.0	230	0.65	230	0.64	83	0.88	93	0.90
1.2	185	0.71	247	0.62	78	0.94	71	0.96
1.4	126	0.84	121	0.79	92	0.90	73	0.97
1.6	86	0.91	102	0.80	111	0.75	92	0.80

[a] Scan rate: 50 mV s^{-1}.

applying +1.4 V for 300 s in 0.050 M phosphate buffer solution of pH 7.4 was selected as the most advantageous and reproducible.

3.2. Electrochemical behavior of dopamine and ascorbic acid at SPE and SPE-MWCNT

Fig. 1 shows the cyclic voltammograms obtained for 1.0×10^{-3} M AA (A and D), 1.0×10^{-4} M DA (B and E) and a mixture of 1.0×10^{-3} M AA and 1.0×10^{-4} M DA (C and F) in 0.050 M phosphate buffer (pH 7.4) solution, on untreated SPE and SPE-MWCNT (a) and pre-treated ones (b). A decrease in the oxidation overvoltage for AA and a significant improvement in the reversibility for DA, compound that displays a quasi-reversible behavior at SPE (ΔE_p decreases 62 mV at SPE-MWCNT), was found at untreated SPE-MWCNT, demonstrating that the carbon nanotubes present at SPE-MWCNT make possible an important improvement in the voltammetric behavior of both compounds. At pre-treated SPE and SPE-MWCNT, the oxidation potential peak of AA shifts to more negative potentials at SPE and SPE-MWCNT (244 and 180 mV shifting, respectively); the ΔE_p for DA decreases (85 and 16 mV, respectively) while the anodic-to-cathodic peak currents ratio decreases from 2.5 to 2.3 at pre-treated SPE and from 3.1 to 1.7 at pre-treated SPE-MWCNT. Therefore, the EP improves the electrochemical response of AA and the reversibility of electron transfer processes for DA at both electrodes, although the most significant decrease in the oxidation overvoltages is obtained at pre-treated SPE. Nevertheless, as could be seen in panels C and F, these good features are not enough to allow the voltammetric quantification of a mixture of DA and AA, since unresolved signals were obtained either at untreated or pre-treated SPE and SPE-MWCNT. Indeed, DA oxidation signal was shifted to more positive potentials and overlapped with AA peak, probably as a consequence of crossing reactions of electrochemically generated DA oxidized forms with AA [2].

In order to evaluate the influence of adsorption processes on the electrochemical oxidation of AA and DA at pre-treated SPE and SPE-MWCNT, the effect of the scan rate on their voltammetric signals was studied from 5 to 200 mV s^{-1} (not shown). The anodic peak current, i_p, of AA (1.0×10^{-4} M) increased linearly with the square root of scan rate, $v^{1/2}$, at both electrodes (i_p (μA) = (0.26 ± 0.01) $v^{1/2}$ (mV s^{-1})$^{1/2}$, r = 0.990, n = 8 for SPE; i_p (μA) = (0.18 ± 0.01) $v^{1/2}$ (mV s^{-1})$^{1/2}$, r = 0.991, n = 7 for SPE-MWCNT), indicating that the AA electrode reaction is a diffusion-controlled process in both cases. On the other hand, the results obtained for DA (1.0×10^{-4} M) showed that, at variance with AA, the anodic (i_{pa}) and cathodic (i_{pc}) peak currents are proportional to the scan rate at SPE (i_{pa} (μA) = (0.073 ± 0.004) v (mV s^{-1}), r = 0.9997, n = 5; i_{pc} (μA) = (−0.047 ± 0.001) v (mV s^{-1}), r = −0.9997, n = 7) and at SPE-MWCNT (i_{pa} (μA) = (0.12 ± 0.01) v (mV s^{-1}), r = 0.997, n = 5; i_{pc} (μA) = (−0.073 ± 0.001) v (mV s^{-1}), r = −0.996, n = 7) suggesting that the electron transfer reaction is a surface (adsorption) controlled process [29].

For further demonstration, a preconcentration experiment was made by placing of AA (1.0×10^{-3} M) or DA (1.0×10^{-4} M) solutions on the working area of pre-treated SPE and SPE-MWCNT for 10 min and, after rising with water, recording the cyclic voltammogram in fresh buffer solution. No signal was obtained for AA, while DA peaks were clearly observed at SPE-MWCNT and weakly at SPE, pointing that DA is significantly adsorbed onto the surface of the SPE-MWCNT whereas AA was not.

3.3. Determination of DA in the presence of AA

The adsorption of DA at pre-treated SPE-MWCNT offers good possibilities for selective voltammetric detection of DA after a preconcentration in the presence of AA under physiological conditions.

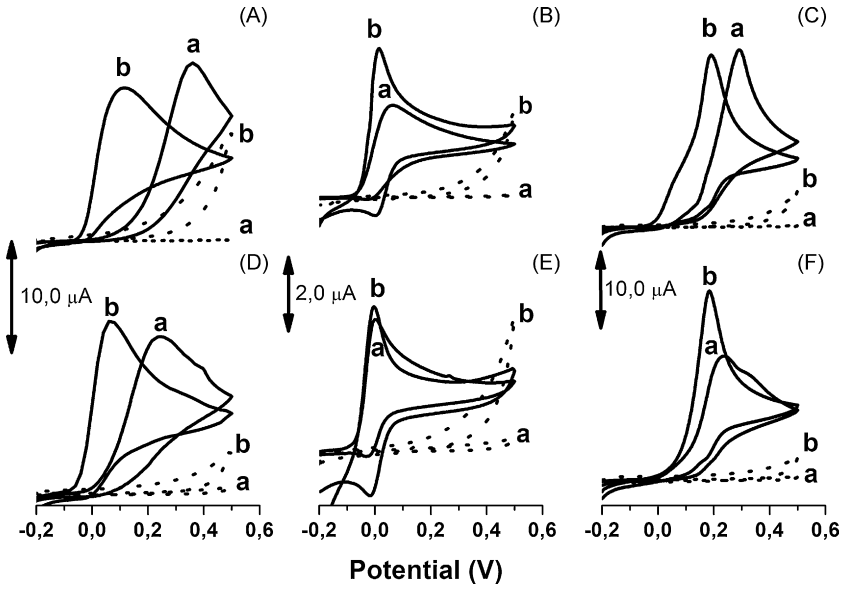

Fig. 1. Cyclic voltammograms for 1.0×10^{-3} M AA (A and D) and 1.0×10^{-4} M DA (B and E) and a mixture of both analytes in the same concentration as in individual solutions (C and F) in 0.050 M phosphate buffer (pH 7.4) solutions on SPE (A–C) and SPE-MWCNT (D–F) before (a) and after (b) the EP. The corresponding responses for the blank are also shown (dotted lines). Electrode pre-treatment: applying +1.4 V during 300 s in 0.050 M phosphate buffer (pH 7.4) solution. Scan rate: 25 mV s^{-1}.

In this sense, the selection of the experimental parameters was optimized for the development of a methodology employed differential pulse voltammetry (DPV), which allows the sensitive and selective determination of DA in the presence of a large excess of AA.

3.3.1. Effect of AA on the voltammetric response of DA

Fig. 2A displays differential pulse voltammograms obtained at pre-treated SPE-MWCNTs for 1.0×10^{-3} M AA, 1.0×10^{-5} M DA and a mixture of both analytes in the same concentration as in the individual solutions. The results are consistent with those obtained in the study by CV. The direct voltammetric determination of DA in the presence of AA is severely interfered due to the co-oxidation of ascorbic acid and an overlapping DPV response was obtained from a mixture of DA and AA. Furthermore, the results shown that the electrochemical process of DA is strongly influenced by the presence of AA (the oxidation peak of DA shifts to more positive potential), so different experiments were performed with the aim to acquire more insights about the influence of AA in the final DA electrochemical response. Fig. 2B displays a DPV obtained in 0.050 M phosphate buffer (pH 7.4) solution at a pre-treated SPE-MWCNT previously exposed for 5.0 min to 1.0×10^{-3} M AA and rinsed with ultrapure water (a). As expected, according to previous results, no oxidation current was obtained for AA after 5.0 min in the AA solution. Panel b shows a DPV obtained in 1.0×10^{-5} M DA solution at a pre-treated SPE-MWCNT previously exposed for 5.0 min to 1.0×10^{-3} M AA and rinsed with water, the DA oxidation peak shifts to more positive potentials (about 200 mV shifting) and the corresponding currents increases (2.0-fold) compared to DA signal obtained at an electrode previously exposed for 5.0 min to the phosphate buffer solution and rinsed with water (panel c). In this case, the oxidation peak potential and current for DA are the same as the ones obtained in the solution of DA (Fig. 2A). These results suggest that, although there is no electro-oxidation signal indicating the adsorption of AA, the interaction of AA with the electrode surface produces some modification that generates important changes in DA electrochemical process.

In order to take advantage of the improvement in DA sensitivity after AA exposure, the effect of the interaction time (from 0 to 10 min) of pre-treated SPE-MWCNT with 1.0×10^{-3} M AA on the differential pulse voltammetric peak of 1.0×10^{-5} M DA was evaluated and the corresponding results are shown in Fig. 3A. The peak current increases with the AA interaction time up to 150 s (2.0-fold) and then remains constant while the peak potential shifts to more positive potential up to 100 s (208 mV shifting). Hence, an interaction time of 200 s was selected for subsequent measurements.

The effect of AA concentration (from 5.0×10^{-6} to 1.0×10^{-3} M) on the differential pulse voltammetry peak of 1.0×10^{-5} M DA was also investigated. The experiments were carried out setting

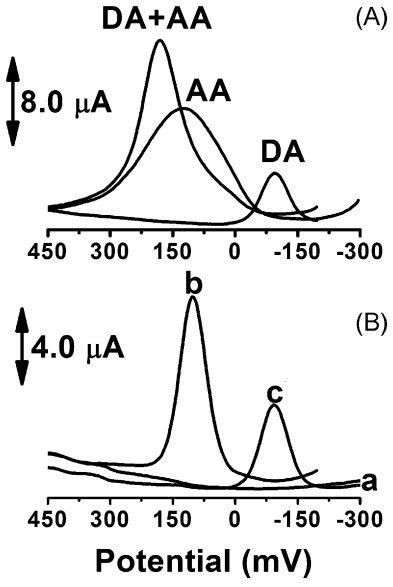

Fig. 2. DPV signals obtained in 0.050 M phosphate buffer (pH 7.4) with a pre-treated SPE-MWCNT. (A) Direct measurement of DA (1.0×10^{-5} M), AA (1.0×10^{-3} M) and a mixture of DA and AA (1.0×10^{-5} and 1.0×10^{-3} M, respectively). (B) DPV signals of blank phosphate buffer (a) and DA (1.0×10^{-5} M) (b and c) with prior exposition to 0.050 M phosphate buffer (pH 7.4) for 300 s at open circuit with (a and b) and without (c) AA (1.0×10^{-3} M). EP as in Fig. 1. DPV operational parameters: pulse amplitude: 50 mV; sample width: 17 ms; pulse width: 50 ms; pulse period: 200 ms; quiet time: 2 s; scan rate: 20 mV s^{-1}.

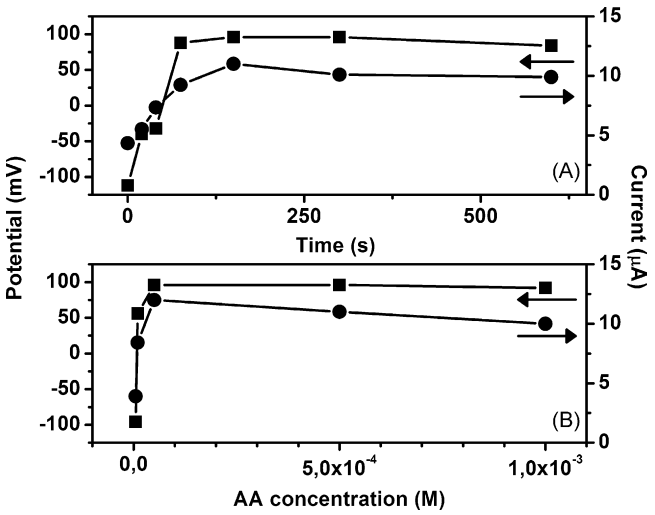

Fig. 3. Effect of AA on differential pulse voltammetry peak of DA (1.0×10^{-5} M) in 0.050 M phosphate buffer (pH 7.4). Effect of the interaction time (A): AA concentration: 1.0×10^{-3} M; effect of AA concentration (B): interaction time: 200 s. EP and DPV operational parameters as in Fig. 2.

an interaction time of 200 s. Fig. 3B shows that DA peak current increases with AA concentration up to 1.0×10^{-4} M, to remain constant thereafter. A similar trend was obtained for the peak potential which shifted to more positive values with AA concentration up to 1.0×10^{-4} M.

The results obtained suggest that no further improvement could be achieved in DA oxidation signal with larger AA concentration or longer interaction time. In view of these results and taking into account the concentration range of AA in biological samples, the selected conditions for AA interaction were 5.0×10^{-4} M AA in a 0.050 M phosphate buffer (pH 7.4) for 200 s. In this way, the DA voltammetric signal should be independent of the concentration of AA in the sample.

3.3.2. *Electrochemical characterization of the effect of AA interaction after EP*

As it was already mentioned in Section 3.1, during anodic EP several processes take place on SPE surface producing important changes, such as increase of surface active area, water oxidation to molecular oxygen (and other reactive species) [30,31] and formation of different carbon oxides [26]. When carbon nanotubes are present, EP could lead, in addition, to open their capped ends or side-walls, introducing in this way new edge plane like sites [31]. Usually, after these treatments the electrochemical performance of such electrodes is finally improved by introducing catalytic oxygenated groups and edge plane like sites within their graphene layers structure, that increase the electron transfer rate of many analytes [23,26].

Changes induced by an AA exposition on an anodized SPE-MWCNT surface could be related to its reducing properties towards carbon oxides and oxygenated species formed after EP. In order to evaluate such effect, CV of representative electrochemical benchmarks, namely hexaammineruthenium (III), hexacyanoferrate (III), iron (II), oxygen, AA and DA, were performed at untreated and pretreated SPE-MWCNT with and without AA interaction and the obtained results are summarized in Table 2.

The first tested probe was $Ru(NH_3)_6^{3+/2+}$ system, classified as an outer-sphere electrochemical process and typically unaffected by the chemical condition of the carbon electrode surface [26]. As expected, the observed electron transfer kinetics, expressed by the measured ΔE_p, was similar and relatively fast in all cases. The EP effect observed was just the increase of active surface, which was denoted by the peak current and capacitance (not shown) rise, and a slight increase in ΔE_p.

Concerning the electrochemical behavior of $Fe(CN)_6^{3-/4-}$ system, it has been reported that its electrochemistry is somehow surface dependant in several conditions [26,32]. The results obtained show that untreated SPEs display noticeable better reversibility than the treated ones, with larger ΔE_p after EP than after EP followed by AA interaction. This fact indicates that favorable electroactive sites for hexacyanoferrate were blocked after EP and part of them were recovered after AA treatment. Compton et al. have reported slower electron transfer kinetics for this electrochemical system when oxygenated species were generated in pyrolytic graphite and carbon nanotubes [32,33]. The suggested explanation was the formation of epoxy-like bridges between graphene layers in the edge plane of pyrolytic graphite which prevent the electron transfer in these sites. MWCNT employed in the SPE-MWCNT under study were functionalized with carboxylic groups by the manufacturer so these groups were present at untreated electrodes. Therefore, they did not affect the electron transfer rate as severely as EP did. It is very often assumed that EP of carbon electrodes only introduces new oxides (typically carboxylic, carbonyl and quinone groups) in their graphene planes structure but the generation of molecular oxygen and other reactive species at the edge plane like sites is usually ignored. Since most of these species were not removed from the electrode surface after EP, probably they could be adsorbed at edge plane sites and disturbing the electron transfer of redox systems such as hexacyanoferrate, especially sensitive to these sites. After AA treatment, these reactive oxygen species were reduced, liberating in this way the edge plane sites and subsequently increasing the observed hexacyanoferrate electron transfer. This rationale could also explain the slight increase on ΔE_p of hexaammineruthenium system after EP.

Next, attention was turned to $Fe^{2+/3+}$ system, whose electron transfer kinetics is well-known to be accelerated when carbonyl groups are present in the electrode surface [34]. Untreated SPE-MWCNT displayed large ΔE_p which indicated low density of carbonyl groups in MWCNT surface and poor catalytic activity of carboxylic substituents or edge plane like sites towards this system. As expected, EP largely improved the reversibility of the electrochemical system as a consequence of the introduction of new carbonyl groups. When SPE-MWCNTs were treated with AA after EP, measured ΔE_p was the same to the one obtained with SPE-MWCNT subjected only to EP. Thus, the generated carbonyl groups were not modified by AA and the same electron transfer kinetics was observed. These results reinforce the idea that AA interacts with other reactive substances or substituents distributed along MWCNT surface rather than with carboxylic, carbonyl or quinone ones.

The next electrochemical system studied was the molecular oxygen reduction which is favored when a high density of edge plane like sites is present at the electrode surface and it is also catalyzed by quinone groups [35,36]. The overpotential needed for oxygen reduction moderately decreases after EP while a larger decrease was observed after AA interaction. The peak currents were proportional to scan rate in all cases (not shown), indicating that this electrochemical process is adsorption-controlled. These results suggest again that after AA exposition, edge plane like sites are liberated, increasing the electron transfer rate and promoting the adsorption of oxygen. As a consequence of that, lower overpotentials and higher currents are obtained.

Similarly to oxygen reduction, AA oxidation is an example of inner-sphere electrochemical process [26] whose overpotential is typically decreased by the presence of edge plane like sites [37] as well as after EP [35,38]. As expected, EP of SPE-MWCNT produces a significant decrease of the overpotential and an important enhancement of the associated currents. The AA treatment only

Table 2
Summary of cyclic voltammetry data for different electrochemical benchmarks before and after the treatments[a].

Compound	Electrode treatment	Ep_a (mV)	Ep_c (mV)	ΔE_p (mV)	i_{pa} (μA)	i_{pc} (μA)	i_{pa}/i_{pc}
$Ru(NH_3)_6^{+3}$	Untreated	−194	−264	70	15.0	−19.9	0.75
	Pre-treated	−191	−271	80	13.4	−24.5	0.55
	Pre-treated + AA	−199	−276	77	12.2	−23.9	0.51
$K_3Fe(CN)_6$	Untreated	155	55	100	19.0	−19.2	0.99
	Pre-treated	188	35	153	15.1	−15.8	0.95
	Pre-treated + AA	184	60	124	17.5	−17.5	1.00
$FeSO_4{\cdot}7H_2O$	Untreated	591	68	523	13.6	−8.17	1.67
	Pre-treated	270	160	110	16.3	−21.5	0.76
	Pre-treated + AA	287	177	110	16.5	−22.7	0.73
AA	Untreated	358	–	–	21.8	–	–
	Pre-treated	205	–	–	19.0	–	–
	Pre-treated + AA	195	–	–	20.8	–	–
DA	Untreated	158	84	74	3.88	−1.72	2.26
	Pre-treated	147	100	47	9.36	−4.32	2.17
	Pre-treated + AA	150	110	40	10.4	−4.24	2.46
O_2	Untreated	–	−959	–	−43.6	–	–
	Pre-treated	–	−844	–	−53.1	–	–
	Pre-treated + AA	–	−495	–	−49.1	–	–

[a] Experimental conditions: 1.0×10^{-3} M $Ru(NH_3)_6^{+3}$ and 1.0×10^{-3} M $K_3Fe(CN)_6$ in KCl 0.1 M; 1.0×10^{-3} M $FeSO_4{\cdot}7H_2O$ in $HClO_4$ 0.1 M; 1.0×10^{-3} M AA and 1.0×10^{-4} M DA in 0.050 M phosphate buffer solution (pH 7.4). Scan rate: 50 mV s^{-1}. Results shown were obtained from the average at three measurements using different electrodes. Standard deviations of measured potentials were lower than 10 mV in all cases.

supposes a slight decrease in the observed peak potential indicating that the main improvement in the electron transfer kinetics was due to oxygenated groups generated after the EP. In all cases, this electrochemical process was diffusion-controlled as peak currents were proportional to the square root of scan rate. Since adsorption did not control this electrochemical process, the positive effect of AA treatment was lighter in this case.

Finally, the effect of EP and AA exposure on DA electrochemical oxidation was also studied. This process is known to be strongly affected by surface cleanliness, presence of oxygenated groups and presence of edge plane sites, since both electrocatalysis and adsorption play a key role in DA electrochemistry [39,40]. The results obtained were qualitatively equal to those described in the precedent section where EP promotes an enhancement in electron transfer kinetics as well as an electrocatalytic effect revealed by the increase of peak currents. The further improvement in ΔE_p and sensitivity after AA treatment should be ascribed to liberation of more edge plane sites for DA adsorption.

From the results exposed all along this section it could be concluded that the improvements achieved using the AA treatment after EP are related to the reduction and elimination of reactive species located preferentially at edge plane sites which decrease the electron transfer kinetics but also especially prevent the adsorption in these sites of electroactive targets as DA in our case. The result of such treatment is a noticeable improvement in the electron transfer process and an important favoring of adsorption-controlled processes.

3.3.3. *Adsorptive behaviour of Dopamine at pre-treated SPE-MWCNT*

Taking into account the capability of the pre-treated SPE-MWCNT for the selective adsorption of DA in the presence of AA, DA was determined by adsorptive stripping voltammetry (AdSV) in presence of a large excess of AA. The experiment was carried out as follows: a 5.0×10^{-4} M AA was placed onto the pre-treated electrode during 200 s, then it was rinsed with water and the given accumulation solution was placed on the electrode for 600 s at open circuit. Finally, after washing the electrode with water again, the DPV signal was recorded in a fresh phosphate buffer solution. Fig. 4 shows the voltammograms obtained employing different accumulation solutions: blank phosphate buffer (a), 5.0×10^{-4} M AA (b), 1.0×10^{-5} M DA (c) and a mixture of 5.0×10^{-4} M AA and 1.0×10^{-5} M DA (d). An oxidation peak around 120 mV was obtained only when DA was present in the accumulation solution, demonstrating that DA is selectively adsorbed on the electrode surface. These facts are consistent with the results obtained in the previous studies. The difference in the peak oxidation current for the neurotransmitter alone or in the presence of AA is 16% (16 μA *vs* 19 μA for DA and DA + AA, respectively). The same behavior was observed for an accumulation solution containing 10-fold lower DA concentration and the same AA excess.

The analytical features of DA detection using the proposed scheme were subsequently evaluated. Fig. 5A displays differential pulse voltammograms obtained from solutions containing DA in concentration between 5×10^{-8} and 1.0×10^{-6} M in the presence of 5.0×10^{-4} M AA and the resulting calibration plot (shown as inset). A linear dependence between DA concentration and stripping peak current was observed for the concentration range studied with a regression equation of i_p (μA) = (0.16 ± 0.04) + (3.01 ± 0.08) × 10^6 C (mol L^{-1}), $r = 0.993$, $n = 8$. The limit of detection was 1.5×10^{-8} M,

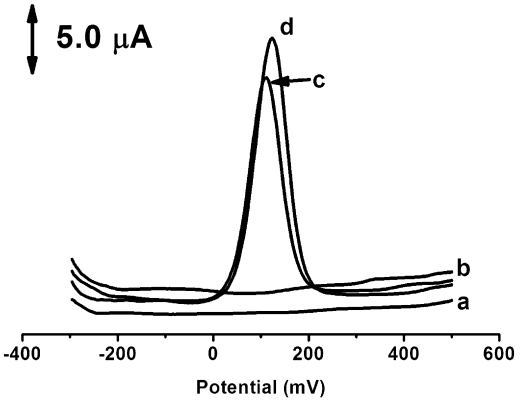

Fig. 4. AdSV obtained for different accumulation solutions: (a) blank 0.050 M phosphate buffer (pH 7.4); (b) 5.0×10^{-4} M AA; (c) 1.0×10^{-5} M DA and (d) mixture of 5.0×10^{-4} M AA and 1.0×10^{-5} M DA. Measuring procedure: (1) EP as in Fig. 2; (2) 50 μL drop of 5.0×10^{-4} M AA in 0.050 M phosphate buffer (pH 7.4) during 200 s; (3) 50 μL drop of accumulation solution during 600 s at open circuit; (4) DPV obtained in 0.050 M phosphate buffer (pH 7.4), operational parameters as in Fig. 2.

Table 3
Determination of dopamine in injections.

Sample no.	Content		Dopamine found ($\times 10^{-7}$ M)	Recovery (%)
	Dopamine ($\times 10^{-7}$ M)	Ascorbic acid ($\times 10^{-4}$ M)		
1	3.0	0	2.8	93
2	3.0	0	3.1	103
3	3.0	0	2.9	97
4	3.0	1.0	2.8	93
5	3.0	1.0	3.1	103
6	3.0	5.0	2.9	97
7	3.0	5.0	2.8	93

calculated using the $S/N = 3$ criterion, being S the sensitivity and N the standard deviation of 10 background signals. The reproducibility of the method was determined by performing 10 successive measurements of 1.0×10^{-6} M DA solutions in the presence of 5.0×10^{-4} M AA using different SPE-MWCNT. The relative standard deviation obtained was 5.6% ($n = 10$).

A closer approach to actual capabilities under physiological conditions was carried out using a synthetic serum as model matrix. Differential pulse voltammograms from samples with DA concentration between 5.0×10^{-8} and 1.0×10^{-6} M in the presence of 5.0×10^{-4} M AA in such saline serum (Na^+ 131, K^+ 5.4, Ca^{2+} 3.6, Cl^- 112, lactate 28 mEq L^{-1} with pH range 5.0–7.0) were obtained. As shown in Fig. 5B, the signal of DA was not affected by the presence of these ions and the sensitivity obtained working under these physiological conditions ($(3.2 \pm 0.2) \times 10^6$ (μA L mol^{-1})) was similar to that obtained measuring in phosphate buffer solution, indicating that the components of saline serum do not compromise the performance of the SPE and this methodology could be readily employed to DA analysis in serum samples. In addition the possible interference of coexisting 1.0×10^{-3} M glucose, 1.0×10^{-4} M uric acid or 5.0×10^{-5} M dopac was studied and variations on 1.0×10^{-6} M DA signal were similar to those observed for different SPE-MWCNT in the reproducibility study.

3.3.4. Analytical applications

The utility of the developed DA electrochemical detection scheme involving SPE-MWCNT was demonstrated in connection to the DA quantification in a pharmaceutical product (Dopamine chlorhydrate, 40 mg mL^{-1}) and in spiked samples of human urine.

Diluted samples of pharmaceutical product were determined using the proposed method in the presence or absence of different concentration of AA. Recovery results were obtained under these conditions by the standard addition method (three 2.0×10^{-7} M DA additions were made) and they are shown in Table 3. Recovery values close to 100% were obtained in all cases, demonstrating the ability of SPE-MWCNT for the selective determination of DA in real samples.

Fresh urine samples collected over a period of 24 h were treated as described in Section 2. Urine samples were 10-fold diluted with phosphate buffer (pH 7.4) and then spiked with increasing amounts of DA in the range of 1.0×10^{-7} to 2.0×10^{-6} M while keeping a constant AA concentration of 5.0×10^{-4} M. Fig. 6 shows the voltammograms obtained and the resulting calibration plot (shown as inset). Unspiked samples showed a weak DA response, insufficient for a suitable quantification. A linear dependence between DA concentration and stripping peak current was observed for spiked samples in the concentration range studied, with a regression equation of i_p (μA) = $(0.05 \pm 0.01) + (7.9 \pm 0.3) \times 10^5$ C (mol L^{-1}), $r = 0.9994$ $n = 4$. It could be noted that the sensitivity obtained in this matrix was lower than that achieved only in the presence of phosphate buffer, pointing that the proposed methodology is yet useful and applicable but the interference of urine components make mandatory the use of the standard

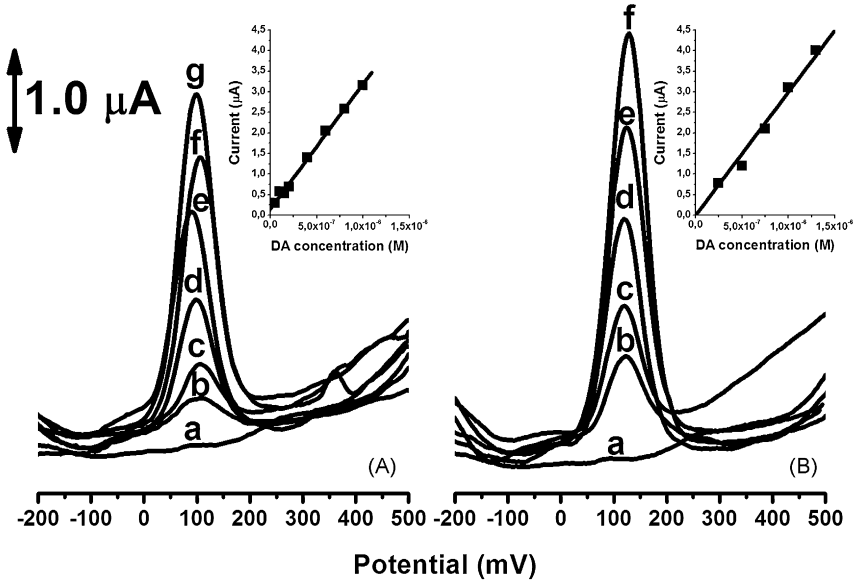

Fig. 5. Effect of DA concentration in the presence of AA 5.0×10^{-4} M upon the stripping voltammetric signal in 0.050 M phosphate buffer (pH 7.4) (A) and saline serum (B) at pre-treated SPE-MWCNT. Dopamine concentration: (A) (a) 0, (b) 5.0×10^{-8}, (c) 1.0×10^{-7}, (d) 4.0×10^{-7}, (e) 6.0×10^{-7}, (f) 8.0×10^{-7} and (g) 1.0×10^{-6} M; (B) (a) 0, (b) 2.5×10^{-7}, (c) 5.0×10^{-7}, (d) 7.5×10^{-7}, and (e) 1.0×10^{-6} and (f) 1.3×10^{-6} M. Measuring procedure as in Fig. 4.

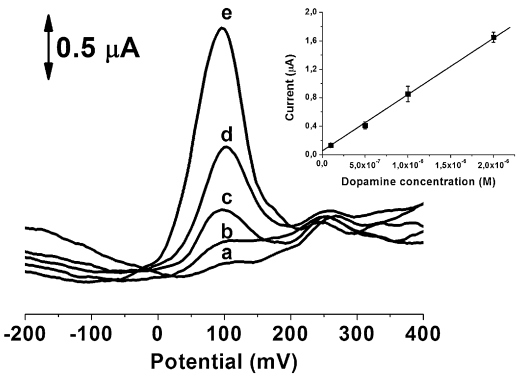

Fig. 6. Effect of DA concentration in the presence of AA 5.0×10^{-4} M upon the stripping voltammetric signal in urine sample at pre-treated SPE-MWCNT. Added DA concentration: (a) 0, (b) 1.0×10^{-7}, (c) 5.0×10^{-7}, (d) 1.0×10^{-6}, and (e) 2.0×10^{-6} M. Measuring procedure as in Fig. 4.

addition method for reliable DA quantification under these conditions.

4. Conclusion

The results presented in this work have demonstrated that the electrode pre-treatment and the presence of carbon nanotubes played a key role in the final electrochemical response of SPE-MWCNT. The proposed methodology have allowed the highly sensitive and selective DA quantification in the presence of large AA excess using low sample with little reagent consumption and simple sample treatment, showing potential application to DA determination in serum and urine. Therefore, the important advantages obtained when combining screen-printing devices with nanomaterials for one-shot electrochemical analysis, especially required for bionalytical applications, have been proved, opening the doors to the design of further bioanalytical devices.

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