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Electrochemistry

SIMPLE AND EFFICIENT EPINEPHRINE SENSOR BASED ON CARBON NANOTUBE MODIFIED CARBON FILM ELECTRODES

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A simple electrochemical sensor for epinephrine (EP) has been developed by modification of a carbon film electrode (CFE) with multiwalled carbon nanotubes (MWCNTs) in a chitosan matrix. Cyclic voltammetry (CV) was employed for the evaluation of the electrochemical oxidation of EP at modified electrodes MWCNT/CFE in different pH electrolytes. Under the optimum conditions (pH 7.0), the MWCNT/CFE electrode showed significant electrocatalytic oxidation of EP: a decrease of about 200 mV in the overpotential and an 11-fold increase in the peak current, compared with the unmodified CFE. Detection of EP was carried out by CV, fixed potential amperometry, and differential pulse voltammetry (DPV); the most sensitive response with the lowest detection limit of 0.9 μM obtained by DPV with preconcentration. At MWCNT/CFE, a separation of 175 mV between EP and ascorbic acid peaks was found. The sensor exhibited excellent stability over a period of 6 months and was successfully applied to the analysis of injectable adrenaline solutions.

Keywords: Adrenaline; Ascorbic acid; Carbon film electrodes; Carbon nanotubes; Chitosan; Epinephrine

INTRODUCTION

Epinephrine (EP), also known as adrenaline, a benzene derivative with two hydroxyl groups and an alkylamine chain, is one of the most important catecholamine neurotransmitters with an important role in health and disease: at high levels they are associated with stress and thyroid hormone deficiency, while low amounts are typical of idiopathic postural hypotension (Xiaorong et al. 1997). Therefore, a simple, fast, and accurate method for detection and quantification of epinephrine in physiological pH conditions is of great interest not only for direct analysis, but also for pharmacology research and life science.

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Different methods for epinephrine determination have been developed, including chromatography (Mishra et al. 2009), a photokinetic method (Martinez-Lozano et al. 1991), flow injection chemiluminescence (Deftereos, Calokerinos, and Efstathiou 1993), and capillary electrophoresis (Wei, Song, and Lin 2005). However, these methods are time consuming and require expensive instruments and well-controlled experimental conditions.

Because epinephrine is easily oxidized (Hawley et al. 1967), electrochemical methods are advantageous for quantitative determination due to rapidity, low cost, high sensitivity, and low detection limit. However, at bare electrodes, the oxidation products strongly adsorb on the electrode, blocking its surface. Another drawback is that epinephrine exists in its natural environment together with other chemical species, such as ascorbic or uric acid, which are oxidized at similar potential values (Luczak 2009b).

The electrochemical determination of epinephrine has been carried out mainly at carbon electrodes with different polymer modifications: poly(caffeic acid) (Ren, Luo, and Li 2006a, 2006b), poly(L-aspartic acid) (Yu et al. 2011), poly(indoleacetic acid) (Zhou et al. 2012), poly(L-methionine) (Ma and Sun 2007), poly(eriochrome black) (Yao et al. 2007), poly(3-methylthiophene) (H. S. Wang, Huang, and Liu 2004), and also with peroxidase catalysis (Goyal, Rana, and Chasta 2012). Gold electrodes have also been used, normally modified by self-assembled-monolayers (SAM) of different compounds such as cysteamine and metal-octacarboxyphthalocyanine (MOCPc) (Agboola and Ozoemena 2008), penicillamine (L. Wang et al. 2006), or triazole (Sun et al. 2006). Bulk-modified carbon paste electrodes have also been employed (Beitollahi et al. 2012; Mazloum-Ardakani et al. 2012) as has glassy carbon modified by MnO_2 with Nafion (Liu et al. 2012). Carbon nanotube (CNT) modified electrodes have been employed previously for epinephrine determination, but in most of these studies more complex architectures that include CNT with other components are involved: cyclodextrin (G. Wang et al. 2004); Nafion (Yogeswaran, Thiagarajan, and Chen 2007; Ou et al. 2009); hematoxylin (Zare and Nasirizadeh 2010); cobalt phthalocyanine (Agboola, Vilakazi, and Ozoemena 2009; Moraes et al. 2010); or dyes (Yi et al. 2008b), which make the procedure more time consuming, difficult, and expensive. There are only a few reports on the use of electrodes modified only by carbon nanotubes (Salimi, Banks, and Compton 2004; J. Wang et al. 2005; Valentini et al. 2007; Goyal and Bishnoi 2011) and in another the nanotubes are decorated by gold nanoparticles on a platinum substrate (Adekunle et al. 2011). There are very recent reports involving graphene modification (Li, Chen, and Ma 2012; Cui and Zhang 2012), one of these using graphene/gold nanocomposites (Cui and Zhang 2012).

In the present work, a simple, low-cost, and easy-to-prepare sensor is proposed for the measurement of epinephrine. The sensor was prepared by modification of carbon film electrodes with multiwalled carbon nanotubes (MWCNT) covalently immobilized in a chitosan matrix. In addition to the uncomplicated preparation method, the proposed sensor has the advantage of being small and its cylindrical shape makes it attractive for miniaturization for using as an implantable device, due to the fact that analyte can easily reach its surface. Furthermore, it is shown that the sensor is usable by cyclic voltammetry, amperometry or differential pulse voltammetry with good sensitivity and low detection limits, well below that necessary for clinical applications. The determination of epinephrine in physiological conditions

is discussed, as well as possible interferences, and the results are compared with those in the literature. In order to evaluate its applicability, the sensor is used for recovery measurements in injectable adrenaline solutions.

EXPERIMENTAL

Reagents and Solutions

Multiwalled carbon nanotubes (MWCNTs) were obtained 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% was obtained from Aldrich (Steinheim, Germany). Epinephrine (EP) and ascorbic acid (AA) were from Sigma (St. Louis, USA). The electrolytes used in the electrochemical studies were McIlvaine buffer with pH values from 3.9 to 8.0 prepared by combining 0.1 M citric acid and 0.2 M disodium hydrogen phosphate, both from Merck (Darmstadt, Germany) and phosphate buffer, NaPB (0.2 M $Na_2HPO_4 + 0.2$ M NaH_2PO_4, Riedel de Haën, Seelze, Germany) with pH between 5.0 and 8.0. Millipore Milli-Q nanopure water (resistivity > 18 MΩ cm) was used for the preparation of all solutions.

Injectable adrenaline chlorhydrate solutions were from Labesfal (Campo de Besteiros, Portugal) with labeled concentration 1 mg/mL.

Instrumentation and Methods

A Bioanalytical Systems CV-50 W electrochemical analyzer (BAS, West Lafayette, IN, USA), or a potentiostat/galvanostat (Autolab PGSTAT30) with GPES v4.9 software (Metrohm-Autolab, Netherlands), were used in all electrochemical measurements. A three-electrode system was employed throughout, which consisted of a (modified) carbon film electrode as working electrode, a platinum wire as counter electrode, and a saturated calomel electrode (SCE) as reference.

The working electrodes were made from carbon film resistors (2 Ω nominal resistance, 15 μm carbon film thickness). The resistors were fabricated from ceramic cylinders, 6 mm in length and 1.5 mm in diameter, by pyrolytic deposition of carbon from methane in a nitrogen atmosphere (Brett, Angnes, and Liess 2001). One of the two tight-fitting metal caps, linked to an external 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.

Scanning electron microscopy (SEM) images were obtained using a Jeol JSM-5310 scanning electron microscope.

Pretreatment and Immobilization of Carbon Nanotubes

Multi-walled carbon nanotubes (MWCNT) were purified and functionalized as described elsewhere (Gouveia-Caridade, Pauliukaite, and Brett 2008). Briefly, this was done by stirring the MWCNTs with nitric acid (5 M), then collecting on filter paper and neutralizing to pH 7.0 and finally drying in an oven.

The immobilization of carbon nanotubes in chitosan has been reported previously (Ghica et al. 2009). A 1% chitosan mixture was prepared in 1% acetic acid solution which was further used for the dispersion of 1% functionalized carbon

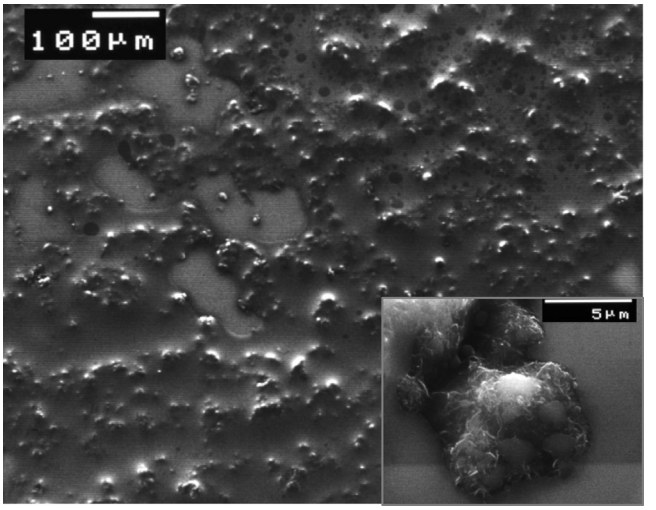


Figure 1. SEM images of CNT in chitosan on ITO-modified electrodes. Inset shows a detail at higher magnification.

nanotubes. After vigorous sonication, the nanotube dispersion was used to cover carbon film electrodes by twice pipetting a 10 µL aliquot on their surface, allowing it to dry after each modification. Figure 1 shows an SEM image of an indium tin oxide (ITO) electrode modified in this way with the MWCNT held in the chitosan matrix and forming small agglomerates; the well-known small bundle morphology of MWCNT is seen in Fig. 1 inset.

RESULTS AND DISCUSSION

Cyclic Voltammetry

Comparison of epinephrine behavior at modified and unmodified electrodes. The electro-oxidation of epinephrine was first examined by cyclic voltammetry at both bare (CF) and multiwall carbon nanotube modified carbon film (MWCNT/CF) electrodes in pH 7.0 phosphate buffer (Fig. 2). At the bare CFE a small oxidation peak appears at +0.4 V with a peak current of $\sim 5\,\mu A\,cm^{-2}$ (inset Fig. 2). At the MWCNT-modified electrode an irreversible oxidation occurs at around +0.2 V (peak A) and a redox couple (peaks B and C) with mid-point potential of −0.15 V is also observed. Peak A is due to oxidation of epinephrine to open chain epinephrinequinone (Luczak 2009).

At pH $\geq$ 3 the open chain epinephrinequinone follows a cyclization reaction; the cyclized form is further reduced generating leucoepinephrinechrome. Leucoepinephrinechrome can be electrochemically oxidized to form epinephrinechrome. The redox couple (peaks B and C) in Fig. 2 is related to the reversible couple epinephrinechrome/leucoepinephrinechrome. The proposed mechanism is shown in Scheme 1.

The MWCNT on CFE allow electro-oxidation of EP under excellent conditions: the peak potential is reduced by 200 mV and the current peak is enhanced

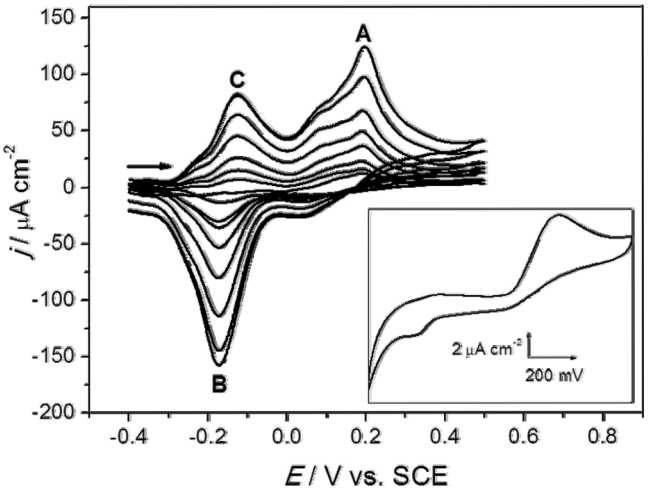
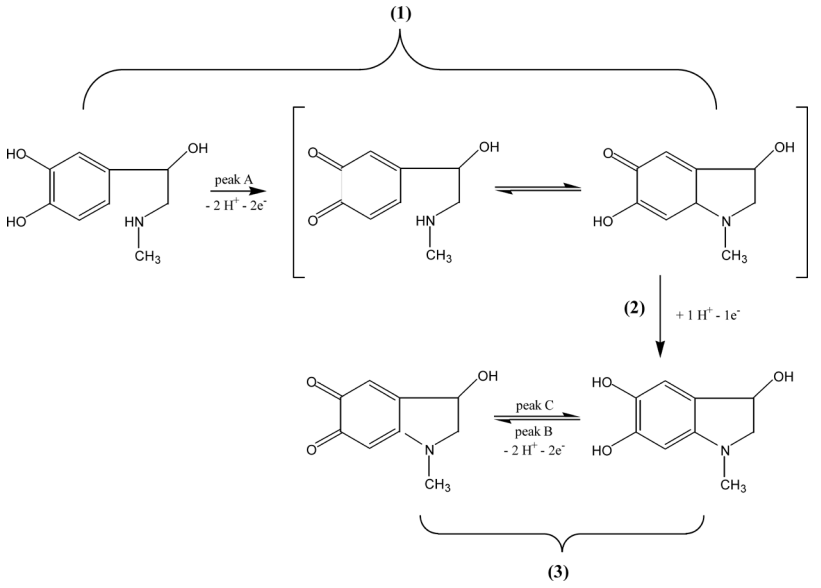


Figure 2. Cyclic voltammograms (baseline subtracted) of EP at MWCNT/CFE for concentrations from 10–200 μM in 0.1 M NaPB pH 7.0. The inset shows the CV of 50 μM EP at a bare CFE. Scan rate 10 mV s^{-1}.

by about 11 times, compared with bare electrodes. These improvements are better than those obtained with basal plane pyrolytic graphite (bppg) modified with CNT (Salimi, Banks, and Compton 2004) where the current response to epinephrine



Scheme 1. Proposed mechanism for the electrochemistry of epinephrine at pH > 3: (1) oxidation of epinephrine to epinephrinequinone; (2) reduction of epinephrinequinone to leucoepinephrinechrome; and (3) oxidation of leucoepinephrinechrome to epinephrinechrome.

Table 1. Comparison of the epinephrine response characteristics at different modified electrodes

Electrode	Linear range/ μM	Sensitivity/ $\mu A\,cm^{-2}$ μM^{-1}	LOD/ μM	Method	Reference
GCE-MWCNT-CoTSPc	3.0–15	1.860	0.450	Amp	Agboola and Ozoemena 2008
DH-CN/CPE	5.0–20	1.040	1.000	DPV	Mazloum-Ardakani et al. 2012
	20–600	0.175			
MnO_2/Nafion/GCE	0.5–100	2.500	0.100	CV	Liu et al. 2012
	100–700	1.420			
	0.03–10	5.780	0.005	DPV	
	10–100	2.660			
paraffin/MWCNT/CoPc	1.3–5.5	2.400	0.016	DPV	Moraes et al. 2010
CNT/GCE	1.0–50	18.500	0.100	CV	Wang et al. 2005
CNT/SSE	2.0–100	28.100	2.000	DPV	Valentini et al. 2007
GME/GCE	0.4–13	28.100	0.089	CV	Li et al. 2012
	13–109	4.450			
GR/Au/GCE	0.05–8.0	14.800	0.007	CV	Cui and Zhang 2012
PolyCafA/GCE	2.0–80	1.980	0.200	CV	Ren, Luo, and Li 2006b
FePc/CPE	1.0–30	1.440	0.500	DPV	Shahrokhian et al. 2009
Os-$(PVP)_{10}$/Nafion	2.0–113	0.316	*	Amp	Ni et al. 1999
RuOHCF/MWCNT/GCE	0.1–10	412.000	0.052	DPV	Raoof et al. 2011
MWCNT/CFE	up to 200	0.730	3.400	CV	present work
MWCNT/CFE	up to 100	2.400	0.900	DPV	present work
MWCNT/CFE	up to 100	0.420	2.000	Amp	present work

*Not specified.

increased only 4 times. More complex architectures, see Table 1, such as graphene/gold nanocomposites (Cui and Zhang 2012) did not exhibit better results than MWCNT/CFE: the epinephrine oxidation current peak was 6.5-fold higher than at the unmodified electrode and the peak potential was reduced by only 33 mV. The 5-amino-3′4′-dimethoxy-biphenyl-2-ol modified carbon nanotube paste electrode (5ADMBCNPE) also had less good results: the current increase was around 5.5 times and peak potential was reduced by 160 mV (Beitollahi et al. 2012). Thus, the modified electrode developed in this work demonstrates a significant electrocatalytic effect in facilitating the electrochemical oxidation of EP, besides increasing the electro-active surface area.

Study of pH and scan rate dependence of epinephrine. The effect of the pH on the oxidation peak of epinephrine, peak C, was studied in McIlvaine buffer, in the pH range 3.9–8.0 and in phosphate buffer between pH 5.0 and 8.0. Plots of peak current and peak potential vs. pH are shown in Fig. 3. In both electrolytes, the peak current associated with epinephrine oxidation increases with an increase in pH up to pH 7.0, and then decreases. The peak potential moves to more negative values with an increase in pH; linear regression gave slopes of 0.065 V (McIlvaine) and 0.062 V (phosphate) per pH unit (data not shown), indicating that the transfer of electrons is accompanied by an equal number of protons in the oxidation mechanism of epinephrine to epinephrinequinone, as already reported (Zhou et al. 2012; Beitollahi et al. 2012; Cui and Zhang 2012).

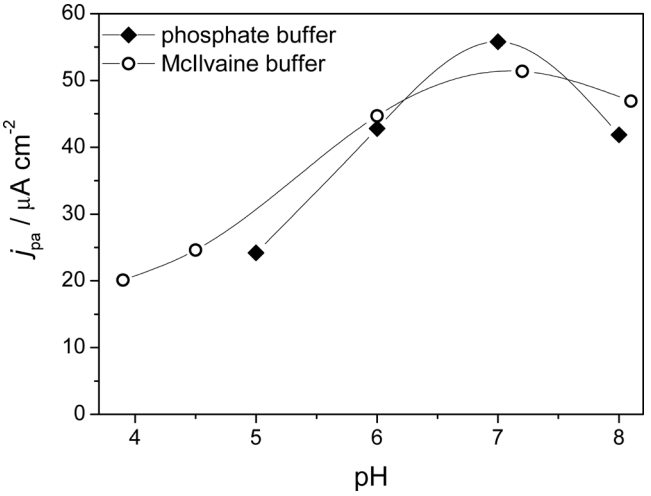


Figure 3. Effect of pH on the EP oxidation peak current, concentration 50 μM, from CV at scan rate 10 mV s^{-1}, in 0.1 M electrolytes with different pH values.

The influence of scan rate on the anodic and cathodic current peaks of EP (Fig. 4), gives a good linear relationship between I_p and $v^{1/2}$ for scan rates of 5–100 mV s^{-1} (for the redox couple) and of 5–50 mV s^{-1} (for the irreversible peak) confirming diffusion-controlled processes at the modified electrode, as observed previously (Salimi, Banks, and Compton 2004). For the redox couple, the slopes are almost identical, consistent with the same rate for oxidation and reduction reactions. The slope of the plot corresponding to the epinephrine irreversible peak was lower, as this process is

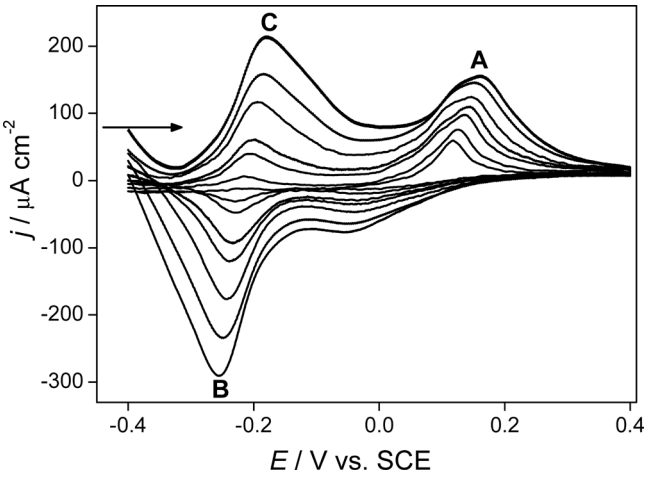


Figure 4. Cyclic voltammograms (baseline subtracted) at MWCNT/CFE for 50 μM EP, in 0.1 M NaPB pH 7.0, at different scan rates, from 5 to 100 mV s^{-1}.

slower. These results confirm that leucoepinephrinechrome is more easily oxidized than epinephrine.

Determination of epinephrine by CV. Cyclic voltammetry and all further measurements were performed in phosphate buffer pH 7.0, in which the highest response to EP was obtained (see previous section) and which also better simulates physiological conditions. The determination of epinephrine by CV was carried out at $50\,mV\,s^{-1}$ with linear response between 10 and 200 μM, sensitivity of 730 ± 22 nA $cm^{-2}\,\mu M^{-1}$, and detection limit of $3.4 \pm 0.1\,\mu M$. Cyclic voltammetry has not often been used to determine epinephrine, due to its relatively low sensitivity, but there are a few reports as noted on Table 1. At activated MWCNT/GCE (J. Wang et al. 2005), EP was determined by CV, using preconcentration during 120 s, which helps to improve sensitivity ($18.5\,\mu A\ cm^{-2}\,\mu M^{-1}$) the linear range was much shorter (50 μM) and detection limit lower (0.1 μM). A shorter linear range (2–80 μM) was also obtained with GCE modified with poly(caffeic acid), PolyCafA/GCE (Ren, Luo, and Li 2006b), but the detection limit was lower (0.2 μM). Graphene modified GCE (Li et al. 2012; Cui and Zhang 2012) exhibited higher sensitivities than reported herein (28.1 and $14.8\,\mu A\ cm^{-2}\,\mu M^{-1}$), but the linear ranges are much shorter (up to 13 and 8.0 μM, respectively).

Fixed Potential Amperometric Detection

The determination of epinephrine was also carried out by fixed potential amperometry. In order to obtain the best response, different applied potentials were tested between −0.22 and +0.20 V vs. SCE. Up to −0.20 V a reduction process is observed, and between −0.15 and +0.20 V oxidation occurs. The oxidation current increases with increase in applied potential and more positive than +0.125 V the increase in response is small. Thus, in order to reduce possible interferences from oxidation of other components in complex matrices, measurements were carried out at +0.125 V. Using these experimental conditions, a linear response was found up to 100 μM, with a sensitivity of $425 \pm 30\,nA\ cm^{-2}\,\mu M^{-1}$ ($n = 4$) and a detection limit of $2.0 \pm 0.05\,\mu M$, see Fig. 5. Although the detection limit obtained by this method is lower than by cyclic voltammetry, the sensitivity is much lower but is sufficiently good for many applications.

To our knowledge there exist few amperometric studies for the detection of epinephrine in the literature (Table 1). One reports a GCE modified with cobalt (II) tetrasulphophthalocyanine-multiwalled carbon nanotubes (GCE-MWCNT-CoTSPc). This sensor worked at +0.38 V (higher than here) and exhibited a linear response to EP between 3.0–15 μM (much shorter than here) that led to a higher sensitivity ($1.86\,\mu A\,cm^{-2}\,\mu M^{-1}$) and a lower detection limit (0.45 μM) (Agboola and Ozoemena 2008). A lower sensitivity ($0.316\,\mu A\,cm^{-2}\,\mu M^{-1}$) for a slightly longer linear range (2.0–113 μM) was achieved with a rotating disk electrode modified with an osmium complex and Nafion, Os-$(PVP)_{10}$/Naf (Ni et al. 1999). There is only one amperometric epinephrine sensor based exclusively on a CNT modified electrode (Salimi, Banks, and Compton 2004); although its sensitivity is higher than here and the detection limit of 0.02 μM lower, both due to using a rotating disk electrode,

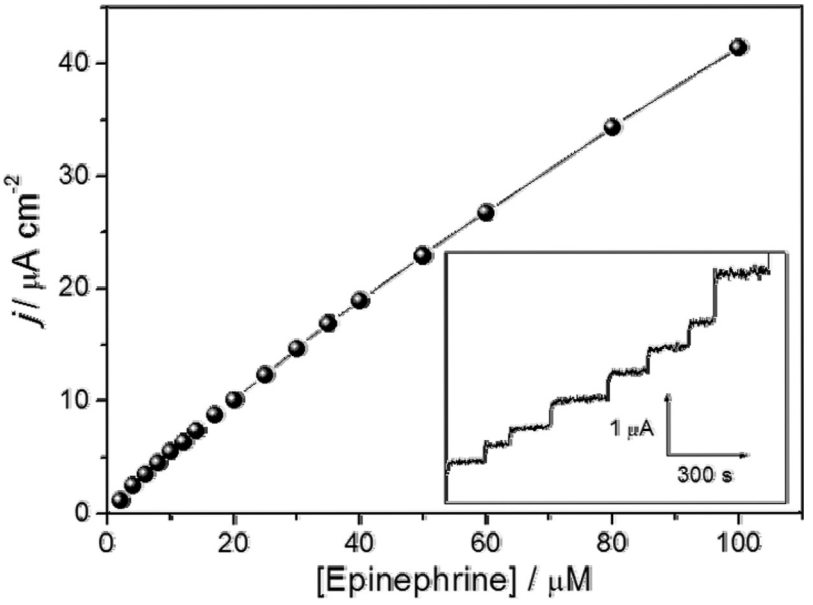


Figure 5. Calibration curve for EP at MWCNT/CFE in NaPB pH 7.0, using amperometry at +0.125 V vs. SCE. The inset shows the typical response for epinephrine.

the linear range was much shorter, up to 6.5 μM. Additionally, this sensor operates at a higher potential (+0.25 V) than the present one (+0.125 V).

The results obtained are encouraging for using the proposed sensor for the determination of EP by fixed potential amperometry. It is a simple means for epinephrine determination, exhibiting sufficiently low detection limit, fast response time (around 25 s for reaching 95% of the final response) and also good sensor stability, the response decreasing only 5% after one week, storing the electrodes dry at room temperature.

Differential Pulse Voltammetry

In order to achieve a more rapid and sensitive detection for epinephrine, differential pulse voltammetry (DPV) was investigated. DPVs were recorded between −0.50 V and +0.50 V at 20 mV s^{-1}, using a pulse amplitude of 50 mV (optimized conditions) with preconcentration at a fixed potential for a chosen time in order to increase the signal response. Preconcentration has been previously used to improve sensitivity: at poly(neutral red) carbon fiber micro electrodes (PNR/CFME) (Xiaorong et al. 1997) where EP was determined by anodic stripping voltammetry, at a triazole self-assembled monolayer modified gold electrode (TA SAM/Au) by square wave adsorptive stripping voltammetry (Sun et al. 2006) and at graphene modified GCEs by cyclic voltammetry (Li et al. 2012; Cui and Zhang 2012).

The optimization of accumulation potential and time was performed, keeping one of these parameters constant and varying the other. The influence of accumulation potential was studied between −0.40 and +0.30 V vs. SCE (the region where

the peaks appear) using 20 μM of EP in 0.1 M NaPB pH 7.0, with 100 s accumulation time. The peak current increases as the accumulation potential becomes more positive from −0.40 up to −0.10 V (at which the highest response was obtained), and above this decreases. Hence, −0.10 V was chosen as accumulation potential.

The accumulation time was varied between 100 and 500 s, applying an accumulation potential of −0.10 V, and it was found that increasing accumulation time in this range had little influence on the EP response, suggesting that nearly all surface sites where adsorption can occur are already occupied after 100s. In light of these results, 100 s was chosen as the accumulation time, less than both literature reports: 180 s for TA SAM/Au (Sun et al. 2006) or 2 min for graphene/gold nanocomposite modified GCEs (Cui and Zhang 2012).

The determination of epinephrine performed under these optimized conditions, see Fig. 6, exhibited a linear response up to 100 μM, a sensitivity of $2.4 \pm 0.2\,\mu A\,cm^{-2}\,\mu M^{-1}$ and a detection limit of $0.90 \pm 0.02\,\mu M$.

These analytical parameters are comparable or better than others in the literature using the DPV technique (Table 1). For example, the sensitivity is the same as with MWCNT modified by cobalt phthalocyanine (CoPc) in a paraffin composite electrode, paraffin/MWCNT/CoPc (Moraes et al. 2010), but higher than the $1.4\,\mu A\,cm^{-2}\,\mu M^{-1}$ found with a carbon paste electrode modified with iron (II) phthalocyanine, FePc/CPE (Shahrokhian, Ghalkhani, and Amini 2009). There are reports of lower detection limits of 16 nM (Moraes et al. 2010) or of 0.5 μM (Shahrokhian et al. 2009); however, these sensors exhibited smaller linear ranges (up to 5.5 and 30 μM) which could be a significant drawback. When using a stainless steel microelectrode modified with CNT, CNT/SSE (Valentini et al. 2007), the analytical parameters were a linear range of 2.0–100 μM (similar to that here), a higher sensitivity ($28.1\,\mu A\,cm^{-2}\mu M^{-1}$) but, conversely, a higher detection limit (2 μM). GCEs modified with MWCNT and ruthenium oxide hexacyanoferrate RuOHCF/MWCNT/GCE exhibited a very high sensitivity ($412\,\mu A\,cm^{-2}\,\mu M^{-1}$), but this might be due to the very short linear range

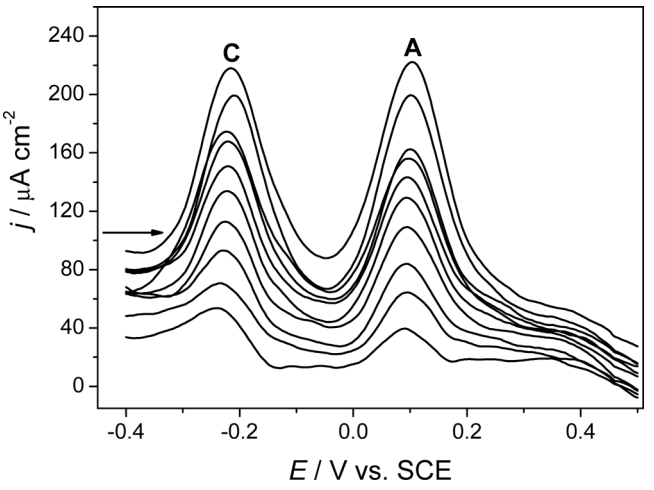


Figure 6. Differential pulse voltammograms (baseline subtracted) at MWCNT/CFE in 0.1 M NaPB pH 7.0 for EP concentrations from 10 to 100 μM. Scan rate $20\,mV\,s^{-1}$.

response to EP (0.1–10 μM) (Raoof, Ojani, and Baghayeri 2011). Epinephrine was determined with a lower sensitivity than here (1.04 $\mu A\,cm^{-2}\,\mu M^{-1}$) at carbon nanoparticle/hydroquinone derivative modified carbon paste electrodes; the linear range was smaller (5.0–20 μM) and the detection limit a little higher at 1.0 μM (Mazloum-Ardakani et al. 2012).

Interelectrode reproducibility was investigated, assembling four electrodes on the same day and recording the sensitivity towards epinephrine by DPV in phosphate buffer pH 7.0; the relative standard deviation (RSD) of voltammetric responses (sensitivity) was 8.3%. Intraelectrode repeatability was measured by recording the sensitivity from the response to epinephrine at the same electrode, using it 5 times on the same day and the RSD was 6.7%.

The long term stability of the modified electrodes was investigated by measuring their electrochemical responses once every two weeks. Between each measurement the electrodes were stored dry at room temperature (≅25°C). In these conditions, after 6 months, the sensitivity to epinephrine decreased to about 85% of the initial value. The stability is much better than the electrode reported with gold nanoparticle decorated CNTs (Adekunle et al. 2011) which was stable for only one month. Other sensors were stable for less time than here (when stored under the same conditions): 3 weeks (Beitollahi et al. 2012), 2 weeks (Zhou et al. 2012; Mazloum-Ardakani et al. 2012) and in the best in the literature the same drop in sensor response (as here) was reached after only one month (Liu et al. 2012).

The results obtained by DPV are thus very promising for using these modified electrodes as sensors for epinephrine, with high sensitivity, low detection limit, linear range wide enough for applications, and very high stability, when stored at room temperature.

Determination of Epinephrine in the Presence of Ascorbic Acid

Improving the detection selectivity and sensitivity for neurotransmitters in the presence of ascorbic acid (AA) has attracted much attention (Ou et al. 2009; Raj, Okajima, and Ohsaka 2003) due to the close oxidation potentials of these compounds and the reaction between AA and the oxidation products of the neurotransmitters.

In order to assess the selectivity of the sensor, epinephrine determination was carried out in the presence of AA. Cyclic and differential pulse voltammograms were recorded in two different ways: adding first ascorbic acid and then epinephrine and also by adding first epinephrine and then ascorbic acid. In both cases, Fig. 7, it was observed that distinct peaks appear for ascorbic acid and epinephrine, with a separation of around 175 mV, which is enough for avoiding undesired interferences. In the solution containing AA, different aliquots of EP were added and a calibration curve was recorded. It was observed by both CV and DPV that the sensitivity towards epinephrine is higher by 9% than in the absence of ascorbic acid. However, it can be expected that this increase will not influence the determination of epinephrine by the standard addition method.

Determination of Epinephrine in Adrenaline Injection Solution

The applicability of the MWCNT/CFE was evaluated by measurements performed in adrenaline (epinephrine) injection solutions with a labeled content of

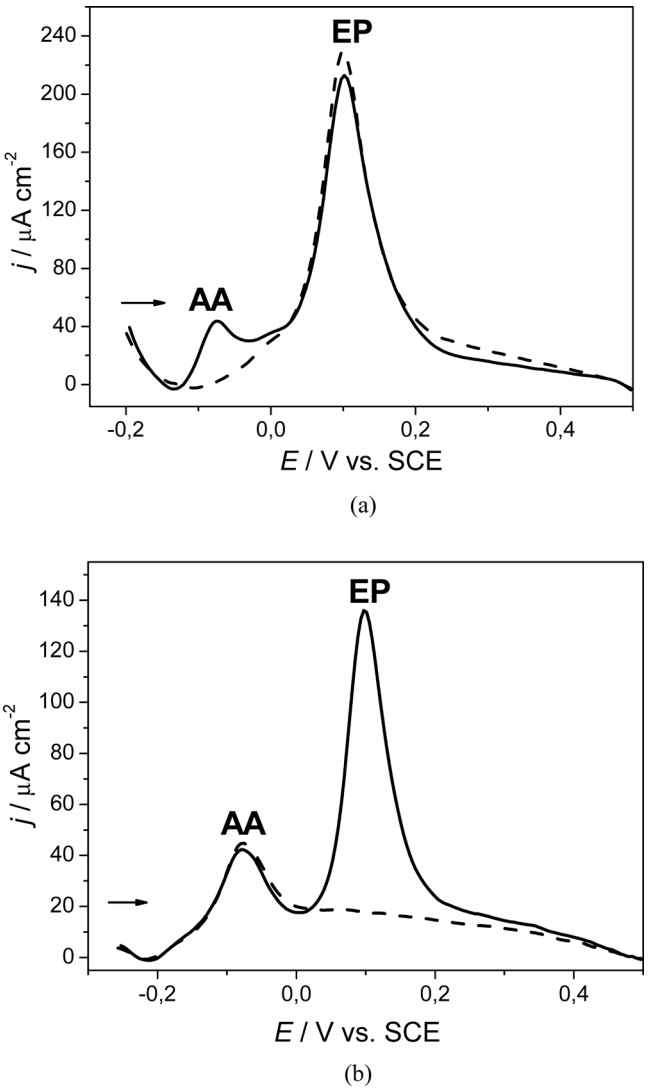


Figure 7. Differential pulse voltammograms (baseline subtracted) at MWCNT/CFE in 0.1 M NaPB pH 7.0, scan rate 20 mV s^{-1}, for: (a) 200 μM EP (dash line) and after addition of 700 μM AA (solid line); and (b) 200 μM AA (dash line) and after addition of 200 μM EP (solid line).

1 g L^{-1} on the ampoules. The standard addition method was used in DPV with 100 s preconcentration by first adding a known aliquot of epinephrine injection solution and then further adding known amounts of standard epinephrine solution. This determination procedure was repeated three times for two different concentrations (20 and 40 μmol L^{-1} epinephrine) and the standard deviations were 3.4% and 1.8%. The concentrations of epinephrine determined by the proposed method were 20.2 ± 0.70 and 41.6 ± 0.75 μmol L^{-1}, which led to recoveries of 101 and 104%,

indicating good agreement and the excellent applicability of the proposed sensor for the fast determination of epinephrine.

CONCLUSIONS

A simple and easy-to-prepare multiwall carbon nanotube/carbon film modified electrode sensor has been developed for epinephrine determination. Detection can be performed by any of the three electrochemical techniques: cyclic voltammetry, fixed potential amperometry, or differential pulse voltammetry. The highest sensitivity and the lowest detection limit were obtained with differential pulse voltammetry. The range of epinephrine concentrations which can be reliably estimated by the method proposed as well as detection limit is comparable or better than other reports in the literature with more complex architectures. Additionally, the sensors show good reproducibility and excellent stability for more than six months. One important advantage is the large peak separation between ascorbic acid and epinephrine using DPV, indicating high selectivity. Application to the analysis of epinephrine injection solutions was successfully carried out.

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