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Electrodeposition of Pt–Ru nanoparticles on multi-walled carbon nanotubes: Application in sensitive voltammetric determination of methyldopa

Saeed Shahrokhian^{a,b,*}, Shokoufeh Rastgar^a

^a Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran
^b Institute for Nanoscience and Nanotechnology, Sharif University of Technology, Tehran, Iran

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ABSTRACT

A modified glassy carbon electrode, prepared by potentiostatic electrodeposition of platinum–ruthenium nanoparticles (Pt–RuNPs) onto a multi-walled carbon nanotube (MWCNT) layer, offers dramatic improvements in the stability and sensitivity of voltammetric responses toward methyldopa (m-dopa) compared to glassy carbon electrodes individually coated with MWCNT or Pt–RuNPs. The surface morphology and nature of the hybrid film (Pt–RuNPs/MWCNT) deposited on glassy carbon electrodes was characterized by scanning electron microscopy (SEM), cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques. A remarkable enhancement in the microscopic area of the electrode together with the catalytic role of the composite modifier resulted in a considerable increase in the peak current (110 times) and a negative shift (–200 mV) in the oxidation peak potential of m-dopa. The mechanism of the electrocatalytic process on the surface of the modified electrode was analyzed via cyclic voltammetry was applied and shown to provide a very sensitive analytical method for the determination of sub-micromolar amounts of m-dopa, for which a linear dynamic range of 0.05–40 μ M and a detection limit of 10 nM was obtained. The modified electrode was successfully used for accurate determination of trace amounts of m-dopa in pharmaceutical and clinical preparations.

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1. Introduction

Recent activities in analytical chemistry have focused on the development of nano-scaled particles for sensor applications. Due to their small size (normally in the range of 1-100 nm), there are significant advantages to using nanoparticle-modified electrodes compared to traditional micro-electrodes. These include enhanced diffusion based on convergent rather than linear diffusion at the smaller nanoparticles (NPs), high active surface areas, improved selectivity, improved catalytic activity, higher signal-tonoise ratios and unique electrochemical properties. The use of NPs also provides control over the local microenvironment [1]. The substrates for supporting metal nanoparticles have a considerable influence on their morphology (size and distribution) and response characteristics. Carbon nanotubes have intrinsic properties that include high surface areas, unique physicochemical properties, and high electrical conductivities, and their inherent size and hollow geometry make them extremely attractive as supports for heterogeneous catalysis [2,3]. Furthermore, uniform dispersion of

* Corresponding author at: Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran. Tel.: +98 21 66005718; fax: +98 21 66012983. *E-mail address:* shahrokhian@sharif.edu (S. Shahrokhian). metallic NPs on MWCNT surfaces show promise as nanocatalysts for use in chemically modified electrodes [4–11].

Several methods for depositing metal NPs on carbon nanotubes have been proposed [12–16]. Among them, electrochemical techniques are particularly attractive for the electro-deposition of metallic nanostructures. Various experimental parameters including deposition potential, time and solution composition can be manipulated to control the nucleation and growth rate of metal NPs. Finally, electrochemical deposition is a clean, rapid and facile synthesis that facilitates control over the size and distribution of NPs [17,18].

Methyldopa (m-dopa) (l- α -methyl-3,4-dihydrophenylalanine) is an antihypertensive agent that is used in the treatment of high blood pressure (HBP) or hypertension (HTN), especially when it is complicated with renal disease. Its hypertensive proprieties are primarily due to its action on the central nervous system. M-dopa inhibits the enzyme DOPA decarboxylase, which converts L-DOPA into dopamine and is a precursor for norepinephrine (noradrenaline) and subsequently epinephrine (adrenaline). It is converted to α -methyl norepinephrine in adrenergic nerve terminals, and its antihypertensive action appears to be due to its stimulation of central adrenal-receptors, which reduces sympathetic tone and produces a fall in blood pressure. The therapeutic concentration of m-dopa in human plasma is usually in the range



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of 0.1–0.5 mg/L, and its average terminal elimination half-life is 2 h [19,20]. Clearly, detection and quantification of m-dopa is an important feature in pharmaceutical and clinical procedures [21–23]. Several analytical procedures have been reported for the analysis of m-dopa in bulk form, pharmaceutical form or biological fluids. These include titrimetry, chromatography [24,25], kinetic methods [23,26], spectrophotometry [22,27] and HNMR [28]. However, these methods have disadvantages, including high costs, long analysis times, the requirement of complex and tedious sample pretreatments and, in some cases, a low sensitivity and selectivity that makes them unsuitable for a routine analysis. On the other hand, electrochemical methods have attracted great interest because of their simplicity, rapidness and high sensitivity in detecting m-dopa and various other analytes without requiring tedious pre-treatments [29–34].

In the present work, we synthesized a composite of MWC-NTs modified with Pt–Ru nanoparticles by simple electrochemical deposition onto MWCNT films pre-casted on the surfaces of glassy carbon electrodes (GCE). Scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to characterize the electrode surface. The experimental results indicate that the Pt–RuNPs–MWCNT hybrid exhibits an increased electroactive surface area and enhanced electron-transfer at the electrode surface. The resulting electrochemical sensor is convenient, sensitive and applicable to the determination of m-dopa concentrations in pharmaceutical and clinical assays.

2. Experimental

2.1. Materials

Multi-walled carbon nanotubes synthesized by catalytic chemical vapor deposition (CVD, purity > 95%) and with an outer diameter (o.d.) of 10-20 nm, an inner diameter (i.d.) of 5-10 nm and a tube length of 0.5-200 nm were obtained from Nanostructured & Amorphous Materials (Houston, TX, USA). RuCl₃ was purchased from Across Chemical Company, France. Methyldopa (m-dopa, Supplement 1) was purchased from Aldrich, USA. Stock solutions of m-dopa were freshly prepared as required in 0.1 M of the prepared buffer solutions. All other chemicals were of analytical reagent grade and were obtained from Merck. All aqueous solutions were prepared with doubly distilled de-ionized water. A concentration of 0.1 M acetate was used for preparation of solutions of pHs 4 and 5, and 0.1 M phosphate was used for solutions of pHs 3, 6 and 7. Tablets of m-dopa (250 mg per tablet) were purchased from local pharmacies. Fresh frozen human blood serum was obtained from the Iranian Blood Transfusion Organization. Two percent (v/v) pure methanol was added to the serum sample. After vortexing each of the samples for 2 min, the precipitated proteins were separated by centrifugation for 10 min at 10000 rpm. Next, the sample was diluted 10-fold, spiked with different amounts of standard m-dopa without extraction or further treatments and used for the recovery tests in the spiked samples. Each sample was run in triplicate, and the relative standard deviation (RSD) for each sample was calculated.

2.2. Apparatus

Electrodeposition of Pt–RuNPs on MWCNTs and voltammetric experiments were performed using a Metrohm potentiostat/galvanostat model 797VA. A conventional three-electrode system was used with a glassy carbon working electrode (GCE, unmodified or modified, diameter=2 mm), a saturated Ag/AgCl reference electrode and a Pt wire counter electrode. The morphologies of MWCNTs and Pt-RuNPs electrodeposited on MWCNTs were obtained using a scanning electron microscope (SEM), and the composition(s) of Pt-RuNPs-MWCNT/GC electrodes were investigated by energy dispersive X-ray spectroscopy (EDS) (RONTEC, QUANTAX). Scanning electron microscopy (SEM) images were obtained with a VEGA\\TESCAN SEM that operated at an accelerating voltage of 15 kV. The tip of a glassy carbon electrode was used for SEM measurements. Before doing any SEM characterization, it was necessary to completely dry the surface of the modified electrode. The tips of the modified electrodes were held in sample holders/stubs by adhesives, and a thin gold coating (20-50 nm) was sputtered on the surface of the modified glassy carbon electrode to make it more conductive before SEM analysis. All the requirements mentioned for SEM were also necessary for EDS experiments. A digital pH/mV/Ion meter (CyberScan model 2500) was used for the preparation of the buffer solutions. Electrochemical impedance spectroscopy (EIS) measurements were performed with a Potentiostat/Galvanostat EG&G model 273A (Princeton Applied Research, USA) equipped with a Frequency Response Detector model 1025 (Power Suite software), which was used with a frequency between 100 MHz and 10 kHz and a 5 mV rms sinusoidal modulation (effective value of a varying AC amplitude) in 0.1 M KCl solution containing 1 mM of both K₄Fe(CN)₆ and K_3 Fe(CN)₆ (1:1 mixture) at the $E_{1/2}$ of the [Fe(CN)6]^{3-/4-} (0.13 V vs. Ag/AgCl). Voltammetric experiments were carried out in buffered solutions of m-dopa that were deoxygenated by purging with pure nitrogen (99.999% from Roham Gas Company). Nitrogen gas was also flowed over the surface of the test solutions during the experiments.

2.3. Preparation of the Pt-RuNPs-MWCNT/GCEs

Multi-walled carbon nanotubes were treated with a 1:3 (v/v)mixture of HNO₃ (65%) and H_2SO_4 (98%) at ca. 100 °C for 45 min to obtain more edge sites and a better dispersion of nanotubes by the creation of carboxylate groups. Before the modification step, each GCE was polished with alumina slurry $(0.1 \,\mu m)$ on a polishing cloth, rinsed thoroughly with water, sonicated in water for 5 min and air dried. A 2.0 mg portion of functionalized MWCNTs was dispersed in 2.0 mL of doubly distilled water and ultrasonicated for 15 min until a homogenous suspension of MWCNTs was obtained. For preparation of the MWCNT/GCE electrode, 3 µL of the prepared black suspension was applied directly onto a well-polished GCE using a micropipette and left to dry at room temperature. In the next step, the MWCNT/GCE electrode was immersed in 0.5 M H₂SO₄ aqueous solution containing 1.3 mM ruthenium (III) chloride and 1.3 mM hexachloroplatinic acid. The electrochemical deposition of the Pt–RuNPs was conducted for 15 s at -0.3 V (vs. Ag/AgCl). The resulting electrode, denoted as Pt-RuNPs-MWCNT/GCE, was removed and rinsed with water. Before voltammetric measurements, the modified electrode was cycled five times between 0 and 1 V (scan rate 100 mV s^{-1}) in a phosphate buffer solution of pH 3.0 to obtain a reproducible response. This modified electrode was also cycled 10 times in a buffer solution of pH 3.0 between measurements to remove any adsorbed m-dopa on the surface of the electrode and renew the electrode surface. The modification of the electrode surface was refreshed daily.

3. Results and discussion

3.1. Surface characterization of the Pt-RuNPs-MWCNT/GCE

The surface morphology of the Pt–RuNPs–MWCNT/GCE was characterized by SEM. As shown in Fig. 1A, a MWCNT Cover/Layer without aggregation was observed on the electrode surface,



Fig. 1. SEM images with different resolutions of (A) MWCNT and (B) Pt-RuNPs-MWCNT modified glassy carbon electrodes.

indicating that the MWCNTs were homogeneously dispersed on the surface of the GCE. The diameters of the MWCNTs were approximately 20–50 nm. As can be seen in Fig. 1B, the Pt–RuNPs deposited on the MWCNTs were spherical, with an average diameter of approximately 55 nm (40–80 nm), and well distributed.

The EDS results shown in Fig. 2 show that Pt, Ru, C and Au were the major elements on the electrode surface. The Au was obtained from the gold coating of the Pt–RuNPs–MWCNT/GC surface during SEM analysis. The EDS results clearly show that Pt and Ru are co-electrodeposited on the surface of MWCNT films. Furthermore, they indicate that Ru and Pt were not equally deposited on the MWCNT-GC electrode in terms of mass percent even under the same precursor salts concentrations. Therefore, loadings of Ru nanoparticles were less than those of Pt deposits at the deposition potential. This may be related to the fact that the reduction potentials for Ru precursor salts [7,35]. Thus, one would expect the reduction of the Pt metal to be more effective during the simultaneous deposition of Pt and Ru.

3.2. Electrochemical characterization of the *Pt–RuNPs–MWCNT/GCE*

The electrochemical response characteristics of the modified electrode with regard to m-dopa were investigated via cyclic and differential pulse voltammetry (CV and DPV). Fig. 3A depicts CVs for the electrochemical oxidation of 10 µM m-dopa at the surface of different electrodes including bare GCE and GCE coated with MWCNT, Pt-RuNPs and Pt-RuNPs-MWCNT, respectively, in a 0.1 M phosphate buffer solution of pH 3.0 using a scan rate of 100 mV s⁻¹. The m-dopa CVs display a broad oxidation peak at 0.65 V on the bare GCE. However, a reversible behavior was obtained for m-dopa within the potential window from 0.25 to 0.8 V on the surface of the modified GCEs. Compared to the bare GCE, these voltammograms exhibit an oxidation peak current that is enhanced by a factor of 40, 30 and 100 at the MWCNT/GCE, Pt-RuNPs/GCE and Pt-RuNPs-MWCNT/GCE, respectively. On the other hand, a 190 mV negative shift in the peak potential, together with enhanced sharpness of the anodic wave, indicate that the high surface area and electrocatalytic effect of the composite film caused a remarkable enhancement of the kinetics of the electrode process. This modified electrode also exhibited greater electrochemical activity toward the oxidation of m-dopa compared to its MWCNT and Pt-RuNPs counterparts, revealing a synergistic response rather than merely a combination of the individual nanostructures. The DPV peak currents of m-dopa on the surface of the MWCNT/GCE, Pt-RuNPs/GCE and Pt-RuNPs-MWCNT/GCE show remarkable enhancements of approximately 50, 26 and 110 times, respectively, as large as those on the bare GCE (Fig. 3B).



Element	Series	unn. C (wt%)	norm. C (wt%)	Atom. C (wt%)
Carbon Chlorine Ruthenium Platinum Gold	K series K series L series M series M series	78.61 0.37 1.00 4.16 15.86	78.61 0.37 1.00 4.16 15.86	98.17 0.16 0.15 0.32 1.21
	Total:	100.0 %		

Fig. 2. The EDS pattern of Pt-RuNPs-MWCNT/GCE.

We also recorded DPVs of 4 µM m-dopa oxidation on the surface of Pt-MWCNT/GCEs and Ru-MWCNT/GCEs. As shown in Fig. 3C, modification of the surface of the MWCNT/GCE with bimetallic Pt-Ru nanoparticles, in comparison to independent contributions from Ru or Pt nanoparticles, improved the voltammetric response toward m-dopa, increased the current (~2 times) and decreased the anodic potential of the oxidation peak (~30 mV). According to previous reports [7,36-39], deposition of bimetallic Pt-Ru nanoparticles at the surface of the electrode has shown improvement with respect to the separate deposition of Pt or Ru. Existence of adsorbed hydrogen on the Pt surface has been implicated in blocking the metal ions' access to the electrode surface, thereby inhibiting metal deposition. The disappearance of hydrogen adsorption/desorption peaks in the presence of Ru and the high current in the double-layer region ascribed to the adsorption of oxygen-containing species on Ru atoms led to more available sites for metal deposition and, consequently, improvement in the voltammetric response toward m-dopa. Electrochemical impedance spectroscopy (EIS) was employed to investigate the impedance changes and the interfacial characterization of the electrode surface during the modification process. The electrontransfer resistance (R_{ct}) at the electrode surface is equal to the

semicircle diameter of the Nyquist plot and can be used to describe the kinetics polarization of the electrode process. Fig. 4 shows the Nyquist plots of K_3Fe (CN)₆/ K_4Fe (CN)₆ at the bare GCE, the MWCNT/GCE and the Pt–RuNPs–CNT/GCE. A semicircle with a large diameter is observed at the surface of the bare GCE. The diameter of this semicircle is reduced at the Pt–RuNPs–CNT/GCE compared to the CNT/GCE and bare GCE, and the linear region is extended to more frequencies, suggesting that the surface of the Pt–RuNPs–CNT modified electrode exhibits lower electron-transfer resistance and greatly increases the electron transfer rate.

3.3. Optimization of the film thickness

3.3.1. Choice of the amount of MWCNTs

The effect of the amount of MWCNTs on the electrochemical response toward m-dopa was investigated via cyclic voltammetry. The results show a marked increase in the oxidation peak current of 4 μ M m-dopa obtained using the MWCNT/GCE when the amount of MWCNTs in the suspension was increased from 0 to 3 μ L. In our investigations, the optimal m-dopa sensitivity was obtained under conditions employing 3 μ L of the MWCNT suspension (Fig. 5). Greater suspension volumes resulted in inappropriate



Fig. 3. (A) CVs and (B) DPVs of m-dopa (10 μ M) on the surface of a bare GCE (inset), a MWCNT/GCE (dashed line), a Pt-RuNPs/GCE (dotted line) and a Pt-RuNPs-MWCNT/GCE (solid line) and (C) DPVs of MD (4 μ M) on the surface of a RuNP-MWCNT/GCE (dotted), a PtNs-MWCNT/GCE (dashed line) and a Ru-Pt-MWCNT/GCE (solid line) in 0.1 M PBS (pH 3.0), scan rate = 100 mV s⁻¹ and pulse amplitude = 50 mV.



Fig. 4. Nyquist diagram (-Z'' vs. Z') for the EIS measurements in 1 mM K_3 Fe(CN)₆/K₄Fe(CN)₆ and 0.1 M KCl at $E_{1/2} = 0.13$ V for a bare GCE (**■**), a MWCNT/GCE (**▲**) and a Pt-RuNPs-MWCNT/GCE (**●**).

mechanical properties and decreased the adherence of the MWCNT film onto the GCE surface. Such effects led to a remarkable decrease in the sensitivity and reproducibility of the modified electrode response.

3.3.2. Choice of deposition time for the MWCNT/GCE

The effect of the number of deposited Pt–RuNPs on the peak current was investigated using DPV, the corresponding results of which are shown in Fig. 6. The oxidation peak current of $4 \mu M$ m-dopa obtained with Pt–RuNPs–MWCNT/GCEs increased with increasing deposition time from 5 to 15 s. However, when the deposition time was longer than 15 s, the peak current gradually decreased. As previously reported, increasing the deposition time increases the average particle size of Pt–Ru nanoparticles, while



Fig. 5. Effect of the drop size of the MWCNT suspension cast on the GCE surface on the cyclic voltammograms of $4 \,\mu$ M m-dopa (down to up: 2, 3, 4 and 5 μ L).

the particles' density remains approximately constant [18,40]. Each NP has an independent diffusion layer and experiences convergent diffusion rather than linear diffusion, resulting in high signal-tonoise and high sensitivity. However, when the sizes of the NPs are increased, their independent diffusion layers interact, reducing the overall mass transfer rate of the electroactive material to the NP layer. Therefore, the current is less than that predicted for isolated NPs. If there is a great enough increase in the NPs size(s), the overlapping diffusion fields of neighboring particles results in linear rather than convergent diffusion, leading to a decrease in the peak current of m-dopa at the surface of the modified electrode [1,41–43]. For these reasons, the maximum sensitivity for m-dopa is obtained with a deposition time of 15 s.

3.4. Effect of potential scan rate

The electrochemical mechanism can usually be elucidated using CVs at different potential sweep rates. Fig. 7A shows CVs of 0.1 mM m-dopa at a modified electrode in a buffered solution of pH 3.0 at different scan rates from 10 to 400 mV s^{-1} . A slope of 0.957 for the linear variation of the logarithm of the peak current with respect to the logarithm of the sweep rate (Supplement 2A) describes a predominantly adsorptive mechanism for the reaction of m-dopa on the surface of the Pt–RuNPs–MWCNT film [44].

The investigations herein show that increasing the potential scan rate results in negative and positive shifts of the oxidation and reduction peak potentials, respectively, which are linearly dependent on the log υ (Supplement 2B). According to the Laviron theory [45], a plot of E_p vs. log υ yields two straight lines with slopes of $-2.3RT/\alpha nF$ and $2.3RT/(1-\alpha)nF$ for the reduction and oxidation peaks, respectively. Based on these linear relationships, the charge transfer coefficient (α) and the electron transfer number in the rate-determining step (n) can be calculated as 0.50 and 2 using the following equation:

$$\log \frac{k_{\rm a}}{k_{\rm c}} = \log \frac{\alpha}{1-\alpha},$$

where k_a and k_c are the slopes of the straight lines for $E_{p,a}$ and $E_{p,c}$ vs. log v, respectively.

3.5. Influence of pH

To achieve the optimum pH and to evaluate the ratio of electrons and protons involved in the anodic oxidation of m-dopa on the surface of the modified electrode, the electrochemical behavior of m-dopa was investigated in buffered solutions of varying pH (pH=3–8). The anodic peak potential shifted negatively with increasing pH, suggesting the participation of H⁺ in the oxidation process (Fig. 7B). The slope of the linear variation of $E_{p,a}$ vs. pH (-67.40 mV/pH) clearly indicates that equal numbers of electrons and protons are involved in the electro-oxidation of m-dopa (Supplement 3A) [46]. Integrating the results obtained in pH and scan rate studies, the electrochemical oxidation of m-dopa at the Pt–RuNPs–CNT/GCE should be a two-electron and two-proton process. From the results of cyclic voltammetric investigations, which are shown in Fig. 7B, one may conclude that the optimum pH for further measurements is 3.0 (Supplement 3B).

3.6. Effect of the time and potential of adsorption

The modified electrode was inserted in a buffered solution of m-dopa (pH=3.0) for a specified time after which multi-cyclic voltammograms were obtained. In the first scan, anodic and cathodic peaks corresponding to m-dopa were observed, and these gradually disappeared with subsequent cycles. On the other hand, increasing the adsorption time caused the m-dopa peak currents



Fig. 6. (A) Effect of deposition time on the DP voltammograms of 4 μ M m-dopa on the surface of Pt-RuNPs-MWCNT/GCEs (a-f: 0, 5, 10, 15, 20 and 30 s), (B) The plot of $I_{p,a}$ vs. deposition time.

to increase. These observations suggest the formation of adsorbed species on the electrode surface, indicating that m-dopa is chemically adsorbed on the electrode surface (through electrostatic and π - π interactions) and then diffused through the porous CNT layer. Such behavior has been previously reported by Compton et al. as a model of the inter-layer diffusion regime through the porous layer of the CNT on the electrode surface [47,48]. Therefore, time and potential are considered two paramount parameters for the adsorption step that can affect the sensitivity of determinations by changing the amount of adsorbed m-dopa at the modified electrode surface. Increasing the adsorption time increased the peak current (Fig. 8). The results indicate that, before reaching the equilibrium adsorption, longer accumulation times (up to 200 s) enhance the amount of adsorbed analyst. At longer times, saturation of the electrode surface is achieved.

The effect of the potential was also investigated from 0.4 to -0.5 V. The results show that, compared to open circuit conditions,

the oxidation peak current remained almost constant when the adsorption potential was applied. Therefore, adsorption of m-dopa was carried out under open circuit conditions.

3.7. Differential-pulse voltammetric determinations, reproducibility evaluations

A highly sensitive electrochemical method with a low detection limit, DPV was used to determine trace amounts of m-dopa under optimized experimental conditions at the surface of the Pt-RuNPs-MWCNT/GCE (Fig. 9A). The results show that the oxidation peak currents were proportional to the concentrations of m-dopa over two intervals in the range of $0.05-4.0 \,\mu$ M and $4.0-40 \,\mu$ M in 0.1 M phosphate buffer solution at pH 3.0 (Fig. 9B). These two linear ranges have different slopes (sensitivities) due to the different amounts of m-dopa adsorbed on the electrode surface in these two regions. It can be proposed that the adsorption



Fig. 7. (A) Effect of the potential sweep rate on the CV response to 0.1 mM m-dopa (10–400 mV s⁻¹) in 0.1 M PBS. (B) CVs of 10 μ M m-dopa at a Pt-RuNPs-MWCNT/GCE in various pHs (from 3 to 8) of buffer solutions, scan rate = 100 mV s⁻¹.



Fig. 8. (A) Effect of accumulation time (sec) on the DPV of 10 μ M m-dopa on the surface of the Pt–RuNPs–MWCNT/GCE. The supporting electrolyte was a 0.1 M phosphate buffer solution of pH 3, and the pulse amplitude was 50 mV. (B) Plot of $I_{p,a}$ vs. accumulation time.

of m-dopa directly onto the MWCNTs, as monolayer coverage at low concentrations ($0.05-4 \,\mu$ M) and as a multilayer at higher concentrations ($4-40 \,\mu$ M), caused the calibration curve to break. At concentrations higher than $40 \,\mu$ M, saturation of the modified electrode surface is obtained, and the m-dopa anodic peak current considerably decreases [49,50].

The linear regression equations related to two regions are inserted in the curves in Fig. 9. In these measurements, a detection limit of 10 nM (S/N = 3) is obtained, indicating that the system is sensitive toward trace amounts of m-dopa. Table 1 compares response characteristics of the introduced modified electrode with those of previously reported in the literature for the determination of m-dopa, showing that, due to its low detection limit and very wide linear dynamic range, the present method is preferred to the others for the successful determination of m-dopa. To study the reproducibility of the electrode preparation procedure, five electrodes modified by the same fabrication procedure

were prepared and used for the determination of $1 \mu M$ and $10 \mu M$ solutions of m-dopa. The RSD for the electrodes' peak currents (average of three determinations on each electrode) was calculated to be 3.05% and 3.00%. To determine the precision of the method, electrochemical experiments were repeated 5 times with the same Pt-RuNPs-MWCNT/GCE in solutions containing 1µM and 10 µM of m-dopa. The RSDs were calculated to be 2.33% and 2.86%, respectively. In this work, any adsorbed m-dopa was counteracted by applying ten CVs in supporting electrolyte (PBS) before each measurement, thereby obviating fouling problems. The modified electrode surface renewed in this way exhibited excellently reproducible responses. Thus, the results herein indicate that the modified electrode has high reproducibility and excellent repeatability in both the preparation procedure and the voltammetric determinations. In addition, after storing the Pt-RuNPs-MWCNT/GCE in air for two weeks, the electrode maintained 95.3% of its initial peak-current response for an m-dopa



Fig. 9. (A) DPVs for various m-dopa concentrations in the range of 0.05–40 μ M in 0.1 M PB solution (pH 3), and (B) corresponding linear calibration curve (peak current vs. m-dopa concentration). Pulse amplitude = 50 mV.

132 Table 1

Comparison of	of different methods	with the present v	work for the deter	mination of m-dopa.
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Method	Linear range (M)	Detection limit (M)	Electrode	Reference
DP voltammetry ^a DP voltammetry ¹ H NMR ^c FIS ^d Kinetic method	$\begin{array}{l} 1.00\times 10^{-7} \text{ to } 2.00\times 10^{-5} \\ 5.00\times 10^{-8} \text{ to } 4.00\times 10^{-5} \\ 1.39\times 10^{-4} \text{ to } 4.20\times 10^{-4} \\ 4.65\times 10^{-6} \text{ to } 2.32\times 10^{-4} \\ 1.85\times 10^{-4} \text{ to } 16.70\times 10^{-4} \end{array}$	$\begin{array}{l} 4.80 \times 10^{-8} \\ 1.00 \times 10^{-8} \\ 7.40 \times 10^{-6} \\ 1.80 \times 10^{-6} \\ - \end{array}$	Ppyox/NFR/Au ^b Pt-RuNPs-MWCNT/GC - - -	[22] This work [28] [27] [23]

^a Differential pulse voltammetry.

^b Ppyox/NFR/Au: polypyrrole film was prepared by incorporation of nuclear fast red (NFR) as the doping anion during the electropolymerization of pyrrole onto a gold (Au) electrode.

^c ¹H nuclear magnetic resonance spectroscopy.

^d Flow injection spectrophotometry.

concentration of $1\,\mu$ M, demonstrating the long-term stability of this thin-film modifier on the surface of the GCE during determinations in aqueous solutions.

3.8. Analytical applications

To evaluate the applicability of the proposed method in determinations in pharmaceutical preparations, the Pt-RuNPs-MWCNT/GCE was used to determine the m-dopa content of "methyldopa" tablets as real pharmaceutical samples using the DPV method. Three tablets containing labeled amounts of 250 mg m-dopa were accurately weighed, ground to a fine powder, diluted in PBS to a nominal concentration of 0.1 µM and then spiked with a standard solution of m-dopa in the range of 4.0-40 µM. The voltammetric responses and corresponding calibration plots of peak currents vs. concentrations are shown in Fig. 10A and B. Using the standard addition method, the m-dopa content was determined to be 249.92 mg with a RSD of 3.2% (n=3) per tablet, which is very close to the labeled amount of 250 mg. It is concluded that the tablet matrix does not result in any interferences in the electrochemical analysis of m-dopa. Moreover, for accuracy studies, the recoveries of the spiked tablet solutions at 8 concentration levels were evaluated (Table 2). The results are in the range of 92.50–105.50%, which are acceptable levels according to the US FDA Guidance for industry (bioanalytical method validation) [51]. The modified electrode was also used to analyze human blood serum samples using the standard addition method. Because there is binding between m-dopa and albumin, it was necessary

 Table 2

 Results of the recovery analysis of m-dopa spiked tablet samples.

No.	Spiked (μM)	Expected (μM)	Found ^a (μM)	Recovery (%)
1	0	0.1	0.0984	-
2	0.06	0.16	0.1480	92.50
3	0.1	0.2	0.1870	93.50
4	0.5	0.6	0.6330	105.5
5	0.8	0.9	0.9260	102.88
6	2	2.1	2.0130	95.85
7	4	4.1	3.80	92.68
8	10	10.1	10.12	100.19
9	20	20.1	18.92	94.13

^a Average of three replicate measurements (rounded).

Table 3

Results of the recovery analysis of m-dopa spiked plasma samples.

No.	Spiked (μM)	Found ^a (µM)	Recovery (%)
1	0	-	-
2	0.2	0.19 ± 0.14	96.66
3	0.6	0.62 ± 0.05	102.90
4	1	1.09 ± 0.09	109.00
5	2	$\textbf{2.36} \pm \textbf{0.22}$	118.00

^a Mean \pm standard deviation (*n*=3) (rounded).

to disrupt their interaction before the analysis. To do this, 2% (v/v) pure methanol was added to the serum sample, which was then thoroughly mixed. The resulting sample was diluted 10-fold and spiked with different amounts of standard m-dopa in the range of 0.2–2 μ M (DPs are shown in Supplement 4). In these tests, no



Fig. 10. (A) DPVs for the standard addition of different amounts of m-dopa (4, 5, 6, 8, 10, 20 and 40 μ M) to a real sample solution of m-dopa diluted with 0.1 M PB solution (pH 3), and (B) the plot of peak current vs. added concentration of m-dopa. Pulse amplitude = 50 mV.

m-dopa was detected in healthy blood serum. The accuracy was calculated by obtaining the recovery of known amounts of m-dopa spiked in the serum solutions at 4 concentrations. The results show an average recovery of 106.64% for m-dopa added to the serum samples (Table 3).

4. Conclusions

We have successfully demonstrated that Pt-RuNPs with diameters averaging 55 nm can be electrochemically deposited into the MWCNT matrix by applying a constant potential at room temperature for a short electrodeposition time. The prepared Pt-Ru-MWCNT nanocomposite was used as a modifier film on the surface of a GC electrode that showed an effective electrocatalytic role in the oxidation of m-dopa. This modified electrode exhibited greater electrochemical activity toward the oxidation of m-dopa compared to its Pt-RuNPs and MWCNT counterparts. The observed improvements are attributed to synergistic enhancement rather merely the combination of the two individual layers. High sensitivity and low detection limits, together with the very easy preparation and easy regeneration of the electrode surface as well as the reproducibility and repeatability of detection signals, make the system in question useful as an effective voltammetric sensor for the accurate and precise determination (with a sub-micromolar detection limit) of m-dopa in clinical and pharmaceutical preparations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.electacta.2011.09.023.

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