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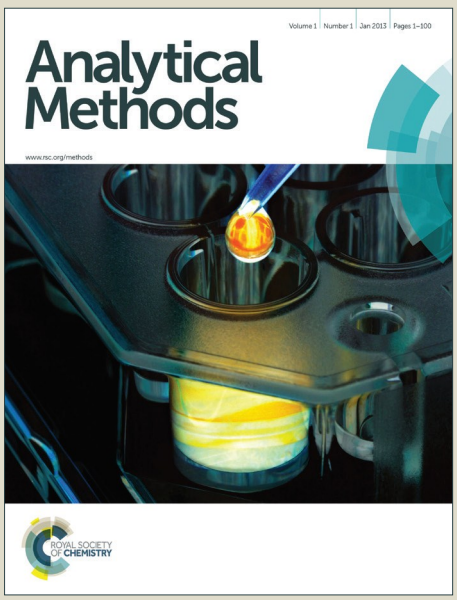

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Electrochemical sensing of levodopa or carbidopa using a glassy carbon electrode modified with carbon nanotubes within a poly(allylamine hydrochloride) film

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Abstract

In this study, the performance of a glassy carbon electrode (GCE) modified with functionalized multi-walled carbon nanotubes (MWCNT) immobilized within a poly(allylamine hydrochloride) (PAH) film for the electrochemical determination of the catecholamines levodopa (LD) and carbidopa (CD) was evaluated. The electrochemical behaviour of the MWCNT-PAH/GCE sensor was investigated by cyclic voltammetry. Under the optimum experimental conditions, the LD and CD analytes were determined by differential pulse voltammetry (DPV) using its oxidation redox processes at −24 mV and +490 mV (versus Ag/AgCl (3.0 mol L^{-1} KCl)), respectively. The obtained analytical curves were linear from 2.0 to 27 μmol L^{-1} for LD and from 2.0 to 23 μmol L^{-1} for CD, with limits of detection of 0.84 μmol L^{-1} for LD and 0.65 μmol L^{-1} for CD, respectively. The proposed DPV method was successfully employed in the LD and CD determination in commercial pharmaceutical formulation samples, and the collected results were consistent with those obtained by the respective standard methods at a confidence level of 95%. In addition, the potentiality of the DPV method was tested for LD and CD quantification in human serum samples.

Keywords: Levodopa; Carbidopa; Carbon nanotubes; Pharmaceutical analysis; Human serum sample; Voltammetric determination

1. Introduction

Carbon nanotubes (CNT) is one of the most explored carbon based nanostructures in the scenario of nanotechnology. Defined as graphene sheets rolled up in a cylindrical shape, the CNT presents a set of unique chemical and physical features, as excellent electrical and thermal conductivity, high stiffness and strength, versatility for different types of chemical functionalization and for immobilization of chemical and biological species, biocompatibility, among others[1-3]. Based on the CNT features, these nanostructures have been applied in a varied range of technological innovation areas, as well reviewed by Baughman et al.[4] and more recently by De Volder et al.[5]. A special application field of CNT is the establishment of new architectures of electrochemical sensors and biosensors[6,7].

The use of CNT for design of electrochemical sensors can be performed using these as the electrode material itself, as in the case of carbon nanotube paste electrodes[8-11], carbon nanotubes deposited on a metal substrate by chemical vapour deposition[12-14], or as an electrode modifier, e.g., by casting of a CNT dispersion on the surface of a bare electrode. In this last strategy, glassy carbon electrodes (GCE) have been used with success as substrate for deposition of CNT films. In fact, the modification of GCE surfaces with CNT provide a considerable improvement of the electrochemical response of this bare carbonaceous electrode, with high increments of analytical signal (peak current and/or charge) and reduction of the working potentials[15-18]. Both these effects are desired for electroanalytical determination, once the increase of analytical signal can generate analytical methods with excellent analytical parameters, and the second effect can provide a better selectivity[19].

In order to prepare the CNT dispersion designated for the construction of modified electrodes, two different practices are commonly employed: (i) direct

dispersion of CNT in organic solvents[20, 21] and (ii) dispersion using surfactants, polymers or biomolecules[22-24]. From these, the second strategy is the more indicated for prepare modified electrodes by two reasons. Firstly, the produced dispersion is more stable and contains a higher number of individualized CNT and, secondly, the final modified electrode presents a better stability of response, once the CNT are incorporated within a well-adhered polymeric matrice on the bare electrode. In this work, the poly(allylamine hydrochloride) (PAH) polyelectrolyte was applied as dispersing agent. PAH is composed by monomer units forming a macromolecular structure typical of polymers and, this polyelectrolyte is widely used in the preparation of thin films for sensor purposes[25-28]. Recently, we demonstrated the high stability of dispersions prepared using PAH and functionalized CNT[28]. Using zeta potential (ζ) measurements, higher ζ values were recorded in a wide range of pHs for a PAH-CNT dispersion in contrast with a CNT dispersion prepared only in water, indicating the greater stability of the proposed dispersion and its potentiality for preparation of modified electrodes [28].

Levodopa (LD) and carbidopa (CD) are pharmaceutics from the catecholamine class, and their respective chemical structures are showed in the Fig. 1. LD is a precursor of dopamine, and it is associated to the Parkinson's disease treatment[29]. This drug is able to cross the blood-brain barrier, and after this transposition, there is the conversion of LD to dopamine by an enzymatic reaction performed by L-dopa decarboxylase[30], thus leading to restitution of dopamine in the brain. To make the LD treatment more efficient is common to administer this active principle together with CD[31, 32]. CD is a catecholamine of inhibitory action of the L-dopa decarboxylase enzymatic activity and, hence, the combined use of LD and CD promotes the controlled production of dopamine in the brain[30-32]. Despite of the good results for the Parkinson's

disease treatment with LD, a set of side effects has been reported from the LD consumption, as motor and non-motor fluctuations, dyskinesias, neuropsychiatric complications and sleep disturbances[33-35]. Therefore, the establishment of accurate analytical methods for the control of LD and CD in pharmaceutical formulations is of great relevance.

In this work, a novel high sensitivity electrochemical sensor is designed for the LD and CD determination in varied matrice samples. The proposed architecture sensor was assembled using multi-walled carbon nanotube (MWCNT) and a PAH film by a very simple strategy, and the proposed sensor was evaluated for the quantification of LD and CD in pharmaceutical and biological samples for the first time. The developed method is characterized by excellent precision, accurate and simplicity.

Insert Figure 1.

2. Experimental

2.1. Reagents, solutions and samples

LD and CD standards, multi-walled carbon nanotube (MWCNT) (95% purity, 20-30 nm in diameter, 1-2 nm in thick and 0.5-2 μm in length), and PAH were purchased from Sigma-Aldrich. The other employed reagents in the assays were of analytical grade and were purchased from Sigma-Aldrich. The supporting electrolyte solutions were prepared at a concentration of 0.1 mol L^{-1} and the stock solutions of each analyte prepared in the respective optimum supporting electrolyte solution.

Commercial pharmaceutical samples of LD (100 mg LD/tablet) and CD (12 mg CD/tablet) were purchased in a local drugstore and subjected to a simple sample

preparation step. For this, ten tables of each sample were pulverized to powder in a mortar and pestle. These pulverized tablets were weighed and the tablet average mass was calculated. From the tablet average mass the respective stock solutions of each sample were prepared in a 10 mL volumetric flask using supporting electrolyte. The non-dissolved excipients were removed by filtration. Next, the final stock solutions were directly employed in the voltammetric analysis.

Artificial human serum sample was prepared as previously reported by Parham and Zargar[36]. This sample was spiked with two LD and CD concentration levels and submitted to the electrochemical analysis in triplicate. The human serum sample was analysed immediately after its preparation.

2.2. Apparatus

An ultrasonic bath UltraCleaner 1400 A from Unique Ultrasonic Systems (Indaiatuba, Brazil) was used for GCE cleaning procedure and preparation of MWCNT dispersions. The voltammetric assays were conducted in a portable potentiostat PalmSens from PalmSens Instruments (Utrecht, The Netherlands) controlled by the PalmSens PC software and using a three electrode electrochemical cell of 15 mL. The modified GCE (Ø = 5.0 mm) was the working electrode, a Pt foil employed as auxiliary electrode and an Ag/AgCl (3.0 mol L^{-1} KCl) electrode as reference electrode.

2.3. Preparation of modified glassy carbon electrodes

The working electrode applied as electrochemical sensor was based on a GCE modified with MWCNT within a PAH film, which was named as MWCNT-PAH/GCE. The different steps followed for preparation of the MWCNT-PAH/GCE are summarized in the Fig. 2. In a first step, the MWCNT were subjected to a strong acid treatment with

the mixture of the concentrated acids H_2SO_4/HNO_3 3:1 v/v, as reported in our recent published works[19, 37, 38]. The pre-treatment was performed to remove possible metal impurities, open the MWCNT tips and add oxygenated functional groups onto the CNT surface[39]. Then, a stable black dispersion was prepared by sonication for 0.5 hour of a mixture containing 1.0 mg of MWCNT and 1.0 mL of a 0.1 mol L^{-1} PAH solution. An aliquot of 15 μL of this dispersion was dropped on the GCE surface (the GCE surface was previously polished with alumina on a polishing cloth and rinsed with ultra-pure water and isopropyl alcohol, respectively, under sonication). The solvent was evaporated at controlled temperature of 25 ± 0.5 ºC, and, thus, a very well-adhered PAH film containing the MWCNT was formed on the electrode surface.

Insert Figure 2.

3. Results and discussions

3.1. Morphological analysis of the MWCNT-PAH film

SEM images recorded for PAH/GCE and MWCNT-PAH/GCE were similar those reported on a previously published work[28]. In the first case, is possible to verify the formation of a polymeric cover homogeneously distributed on the GCE surface. As expected for the MWCNT-PAH/GCE case, the SEM image collected in a higher resolution clearly shows the presence of MWCNT within the PAH polymeric matrice.

3.2 Electrochemical behaviour of the modified glassy carbon electrodes

The effect of the GCE modification with PAH containing the MWCNT on the electrochemical behaviour was explored by cyclic voltammetric studies with the

electrochemical probe $Fe(CN)_6^{3-/4-}$. In Fig. 3 are presented the cyclic voltammograms recorded for a 1.0 mmol L^{-1} $K_3Fe(CN)_6$ solution in 0.1 mol L^{-1} KCl using the GCE, PAH/GCE and MWCNT-PAH/GCE. The incorporation of the PAH film onto GCE surface not changed the previous GCE voltammetric profile. In fact, a negative shift of the anodic and cathodic potentials was verified for the PAH/GCE, however, the ΔE_p value remained constant, indicating a similar electron transfer kinetics. The ΔE_p obtained for the MWCNT-PAH/GCE also was similar those registered for the GCE. However, when the MWCNT were incorporated within a PAH film, an increase of the faradaic current associated to redox couple of $Fe(CN)_6^{3-/4-}$ was observed. This effect can be associated with the higher electroactive surface area of the MWCNT-PAH/GCE. In order to prove this approach, cyclic voltammetric measurements were performed at different scan rates to estimate the respective electroactive areas. Typical cyclic voltammograms registered for the GCE, PAH/GCE and MWCNT-PAH/GCE in the scan rate range from 10 to 100 mV s^{-1} are showed in the Fig. SD-1 (Supplementary Information). Linear relationships between the anodic peak currents (I_a) and the square root of the scan rates ($v^{1/2}$) were obtained in all the cases (see the insets of the Fig. SD-1), as expected for an electrodic process controlled only by the diffusional mass transport[40, 41]. From the slopes obtained for the I_a *vs.* $v^{1/2}$ curves, the electroactive surface areas were calculated for each electrode using the Randles-Sevcik equation[40, 41]. The obtained values were, respectively: 0.184 cm^2 (GCE), 0.187 cm^2 (PAH/GCE), and 0.221 cm^2 (MWCNT-PAH/GCE) and, hence, proving the previous statement about the increase of peak current after the GCE modification with MWCNT.

Insert Figure 3.

3.3. Voltammetric behaviour of levodopa and carbidopa

The electrochemical responses of the LD and CD compounds were obtained by cyclic voltammetry using the GCE and MWCNT-PAH/GCE. In the Figs. 4 (a) and (b) are shown the cyclic voltammograms obtained for 20 μmol L^{-1} LD or 10 μmol L^{-1} CD solution prepared in 0.1 mol L^{-1} phosphate buffer solution (pH = 7.0). To LD molecule, from the result presented in the Fig. 4 (a) is clear the occurrence of anodic and cathodic processes when both electrodes were employed, which are equivalents to the quasi-reversible redox process between LD and dopaquinone[42-44]. Comparing the LD response on the GCE and MWCNT-PAH/GCE, the first observation was the lower value of ΔE_p when the modified electrode with MWCNT was employed, demonstrating an electrocatalytic activity of this nanomaterial on the LD redox processes. The ΔE_p changed from 840 mV on the GCE to 330 mV on the MWCNT-PAH/GCE, i.e., a significant reduction of 510 mV. Secondly, an extraordinary increase of peak current (I_p) was verified with the use of the modified GCE. The analytical signal increased approximately 100-fold on the MWCNT-PAH/GCE.

Cyclic voltammetric studies also were performed for the CD molecule. As can be seen from the Fig. 4 (b), two anodic peaks (peaks (i) and (ii) at +75 mV and +490 mV) and one cathodic peak (peak (iii) at −442 mV) were registered using MWCNT-PAH/GCE. In accordance to work reported by Vieira et al.[45], similar voltammetric response was noted for the CD molecule, being the peak (i) due the oxidation of two hydroxyl groups, the peak (ii) correspondent to the imine group oxidation and the peak (iii) is related to the dopaquinone reduction. Moreover, the MWCNT-PAH/GCE provided an improvement of voltammetric response to CD, with decrease of the working potentials and increase of the peak currents. The molecules of LD and CD were monitored by the oxidation processes at −24 mV and +490 mV.

Insert Figure 4.

3.4. Optimization of experimental conditions

In a first moment, it was evaluated the influence of the supporting electrolyte composition and the experimental parameters from the DPV technique on the LD and CD peak currents. Supporting electrolyte solutions containing LD or CD at 13 μmol L^{-1} were employed in the optimization assays. The following supporting electrolyte solutions were tested: sulfuric acid, acetic acid, potassium chloride, potassium nitrate, sodium chloride and sodium nitrate, all at a concentration of 0.1 mol L^{-1}. The best voltammetric response, regard to the peak definition, smaller potential work and higher current, for LD and CD oxidation was obtained in 0.1 mol L^{-1} sodium chloride (NaCl) solution. Therefore, this supporting electrolyte was selected for the further experiments. Next, the experimental parameters of the DPV technique were subjected to optimization. The selected values for LD and CD determination were: scan rate, *v* (mV s^{-1}): 60 (LD) and 20 (CD); amplitude, *a* (mV): 12 (LD) and 70 (CD); and modulation time, *t* (s): 0.1 (LD) and 0.01 (CD).

3.5. Analytical curves and applications

Under the optimized experimental conditions, the analytical curves for LD and CD were constructed. DP voltammograms registered in presence of 0.1 mol L^{-1} NaCl containing different LD and CD concentrations are showed in the Figs. 5 (a) and (b). In the insets of Figs. 5 (a) and (b) are presented the correspondent analytical curves. The obtained analytical curves were linear from 2.0 to 27 μmol L^{-1} for LD and from 2.0 to 23 μmol L^{-1} for CD, respectively, following the linear regression equations:

LD: I_p (μA) = −0.14 + 1.5 × 10^5 [LD] (mol L^{-1}); r = 0.999

CD: I_p (μA) = −2.8 + 1.3 × 10^6 [CD] (mol L^{-1}); r = 0.998

The limits of detection were also calculated to be 0.84 μmol L^{-1} for LD and 0.65 μmol L^{-1} for CD. The values of limit of detection were estimated using the relation (3 × σ) / *m*, where σ is the standard deviation of ten blank measurements and *m* is the slope of the analytical curve.

In Table 1 are organized the reported works in literature dedicated to LD and CD voltammetric determination, where the analytical parameters obtained using the MWCNT-PAH/GCE are compared with those from the literature. Regarding to LD voltammetric determination, most of the works also made use of DPV and SWV, with exception of the work reported by Teixeira et al.[46], which the cyclic voltammetry (CV) was applied to both electrochemical investigations and determination. The analytical features of the proposed DPV method were better than for some procedures using different modified carbon paste electrodes[45, 46] and similar those works involving the use of modified glassy carbon and carbon paste electrodes[43, 44, 47-50]. Similar conclusions were obtained in the CD case.

Insert Figure 5.

Insert Table 1.

The possible interference of some excipient compounds found in pharmaceutical formulation samples, such as mannitol, polyvinylpyrrolidone and starch, was evaluated

on the voltammetric determination of LD and CD. For this, DPV measurements were performed in presence of 0.1 mol L^{-1} NaCl solutions containing LD or CD at 10 μmol L^{-1} in the absence and presence of each potential interferent. The possible interferents were added in excess, using a ratio analyte:interferent of 1:10. At this ratio, relative standard deviations (RSD) lower of 5.0% were determined for all excipients, demonstrating the selectivity of the proposed voltammetric method relatively to excipients commonly found in commercial pharmaceutical formulations of LD and CD. In addition, the response of the sensor was investigated in presence neurotransmitters (dopamine and epinephrine), ascorbic acid (AA) and uric acid (UA), which are substances that can be found in biological sample matrices. AA and UA did not interfere the MWCNT-PAH/GCE response toward LD and CD at a ratio analyte:interferent of 10:1, with the following RSDs: AA – LD (RSD = 4.1%) and CD (RSD = 2.0%); UA – LD (RSD = –0.74%) and CD (RSD = 0.17%). Dopamine and epinephrine showed interference in LD and CD determination when they are in same concentration of the analytes. Therefore, some previous sample treatment step of the biological sample is necessary to eliminate dopamine and epinephrine interference when these substances are present in a considerable concentration level.

The addition and recovery studies were performed to verify the possible matrice effects from the pharmaceutical formulation samples. Table 2 contains the results of recovery obtained for three levels of analyte concentration added in four pharmaceutical samples of LD and CD. As can be seem, excellent results of recovery percentage were achieved in both cases, ranging from 92 to 105% for LD and from 96 to 102% for CD, respectively. These results proved the selectivity of the proposed DPV method, without occurrence of sample matrice effects.

Insert Table 2.

DPV measurements were performed with the MWCNT-PAH/GCE sensor in order to verify the intra-day repeatability of the proposed electrode. Thus, using only a MWCNT-PAH/GCE, voltammetric measurements were performed for 0.1 mol L^{-1} NaCl solutions containing LD or CD at 20 μmol L^{-1}. The RSD of 0.01% was obtained for both analytes, demonstrating high precision of the proposed sensor. Moreover, the inter-day repeatability of MWCNT-PAH/GCE was performed using three different modified electrodes during three different days. In this study, RSDs of 1.1 and 1.0% were obtained for LD and CD determination.

The voltammetric method developed was applied in the determination of LD and CD in various commercial pharmaceutical samples. These samples also were analysed by the respective reference methods, potentiometric titration in the LD case[51] and UV-Vis spectrometry in the CD case[52]. The obtained results by both the methods are organized in the Table 3. By comparing the results, low RSD values were verified ($-2.42\% < RSD < +2.33\%$), demonstrating the similarity of the results and, excellent recovery percentages in regarding the labelled values were also verified. Moreover, the equivalence of the results was demonstrated by applying the paired *t*-test, at a confidence level of 95%. The $t_{experimental}$ values (LD: 0.346; CD: 0.617) were lower than the $t_{critical}$ value (3.182) and, therefore, it can be concluded that there is no statistically significant difference between the results obtained using the two analytical methods. These results demonstrate the excellent accuracy of the voltammetric procedure and its effectiveness in the quantification of LD and CD in commercial pharmaceutical formulation samples.

Insert Table 3.

Finally, spiked artificial human serum samples were analyzed by the proposed voltammetric method. In Table 4 are presented the results of recovering LD and CD concentration in human serum samples spiked with two different LD and CD concentrations (5.0 and 10.0 μmol L^{-1}). As can be seen, excellent recovery percentages in the 89.4–104% range were obtained, which are indicative of the potentiality of the novel voltammetric procedure for the quantification of LD and CD in human body fluid samples.

Insert Table 4.

4. Conclusion

The applicability of a GCE modified with functionalized MWCNT within a PAH film as electrochemical sensor for the determination of levidopa and carbidopa in pharmaceutical formulation samples was explored in this work. The CNT increased the electroactive surface area of the GCE and the use of the PAH provided mechanical stability for the modified electrode. Using DPV technique under optimized conditions, the obtained analytical curves for LD and CD were linear in a wide concentration range, resulting in limits of detection of 0.84 μmol L^{-1} for LD and 0.65 μmol L^{-1} for CD, respectively. When applied for LD and CD quantification in commercial pharmaceutical samples, the proposed DPV procedure provided results in agreement with those obtained by the respective standard methods at a confidence level of 95%. Moreover,

the proposed DPV procedure was successfully applied for LD and CD determination in human serum samples.

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Figure Captions

Fig. 1. Chemical structure of the **(a)** levodopa and **(b)** carbidopa molecules.

Fig. 2. Schematic representation of the steps of acid MWCNT functionalization and modification of the GCE surface with functionalized MWCNT within PAH polymer.

Fig. 3. Cyclic voltammograms registered for 1.0 mmol L^{-1} $K_3[Fe(CN)_6]$ in 0.1 mol L^{-1} KCl using GCE, PAH/GCE and MWCNT-PAH/GCE.

Fig. 4. Cyclic voltammograms obtained using a GCE (—) and MWCNT-PAH/GCE (– . –) for **(a)** 20 μmol L^{-1} LD and **(b)** 10 μmol L^{-1} CD in 0.1 mol L^{-1} phosphate buffer solution (pH = 7.0); v = 50 mV s^{-1}.

Fig. 5. (a) Differential pulse voltammograms collected in presence of 0.1 mol L^{-1} NaCl solution containing different LD concentration levels: (1) 0; (2) 2.0; (3) 3.9; (4) 5.9; (5) 7.9; (6) 9.9; (8) 13; (9) 15; (10) 17; (11) 19; (12) 21; (13) 23; (14) 25 and (15) 27 μmol L^{-1}. v = 12 mV s^{-1}, a = 60 mV and t = 0.1 s. Inset: Analytical curve (I_a *vs.* [LD]) **(b)** Differential pulse voltammograms collected in presence of 0.1 mol L^{-1} NaCl solution containing different CD concentration levels: (1) 0; (2) 2.0; (3) 3.9; (4) 5.9; (5) 7.9; (6) 9.9; (7) 11; (8) 13; (9) 15; (10) 17; (11) 19; (12) 21 and (13) 23 μmol L^{-1}. v = 20 mV s^{-1}, a = 70 mV and t = 0.01 s. Inset: Analytical curve (I_a *vs.* [CD]).

Table 1. Comparison between the analytical parameters obtained for the proposed voltammetric procedure and other electroanalytical procedures reported in the literature for LD and CD.

Analyte	Electrode	Voltammetric technique	Linear range (µmol L^{-1})	Limit of detection (µmol L^{-1})	Reference
LD	SWCN-GCE	DPV	0.5 to 20	0.3	[43]
	PbO_2-CPE	DPV	260 to 1200	25	[45]
	AuNP-CNT/PGE	DPV	0.1 to 150	0.05	[44]
	Ru-red/NaY-CPE	CV	120 to 10000	85	[46]
	5AEBCNPE	SWV	0.25 to 200	0.09	[47]
	3,4'-AAZCPE	SWV	0.1 to 70	0.035	[48]
	poly(4-MoPD)/MWNT/GCE	DPV	0.416 to 400	0.101	[49]
	MWCNT-PAH/GCE	DPV	2.0 to 27.0	0.84	This work
CD	PbO_2-CPE	DPV	32 to 150	3.6	[45]
	MCNPE	SWV	0.09 to 400	0.071	[50]
	MWCNT-PAH/GCE	DPV	2.0 to 23.0	0.65	This work

SWCN-GCE – Glassy carbon electrode modified with single-walled carbon nanotubes; PbO_2-CPE – Carbon paste electrode modified with lead dioxide immobilized in a polyester resin; AuNP-CNT/PGE – Pyrolytic graphite electrode modified with gold nanoparticles and functionalized carbon nanotubes; Ru-red/NaY-CPE – Carbon paste electrode modified with trinuclear ruthenium ammine complex (Ru-red) incorporated in NaY zeolite; 5AEBCNPE - Carbon paste electrode modified with carbon nanotubes and 5-amino-2'-ethyl-biphenyl-2-ol; 3,4'-AAZCPE – Carbon paste electrode modified with ZnO nanorods and 5-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid; poly(4-MoPD)/MWNT/GCE - Glassy carbon electrode (GCE) modified with poly 4-methyl-*ortho*-phenylenediamine (4-MoPD) and multi-wall carbon nanotubes (MWNTs). MCNPE - Modified carbon nanotube paste electrode.

Table 2. Results obtained for the addition/recovery assays of LD and CD in pharmaceutical formulation samples (n = 3).

Analyte	Sample	Concentration (μmol L^{-1})		Recovery (%)*
		Added	Determined	
LD	A	7.9	8.0 ± 0.1	101
		9.9	9.7 ± 0.2	98
		12.0	12.0 ± 0.2	100
	B	7.9	8.3 ± 0.2	105
		9.9	10.0 ± 0.6	101
		12.0	11.0 ± 0.1	92
	C	7.9	7.7 ± 0.1	98
		9.9	9.8 ± 0.1	99
		12.0	12.0 ± 0.2	100
	D	7.9	7.6 ± 0.1	96
		9.9	9.8 ± 0.1	99
		12.0	11.7 ± 0.6	98
CD	A	11.8	11.63 ± 0.02	99
		15.7	15.03 ± 0.03	96
		17.6	17.97 ± 0.01	102
	B	11.8	11.59 ± 0.05	98
		15.7	15.00 ± 0.07	96
		17.6	18.01 ± 0.04	102
	C	11.8	11.65 ± 0.04	99
		15.7	15.02 ± 0.03	96
		17.6	17.97 ± 0.02	102
	D	11.8	11.64 ± 0.05	99
		15.7	15.93 ± 0.04	96
		17.6	17.98 ± 0.03	102

Table 3. Results for the determination of LD and CD in commercial pharmaceutical samples using the proposed voltammetric method and the respective reference methods (n = 3).

Analyte	Sample	Concentration (mg/tablet)			RSD* (%)	Recovery** (%)
		Labelled value	Reference method	Voltammetric method		
LD	A	100	99.7 ± 0.8	99 ± 1	−0.7	99
	B	100	98.7 ± 0.9	101 ± 1	+2.33	101
	C	100	101 ± 1	100 ± 2	−0.99	100
	D	100	102 ± 2	100 ± 2	−1.96	100
CD	A	12	12.2 ± 0.3	12.1 ± 0.2	−0.82	101
	B	12	12.4 ± 0.3	12.1 ± 0.2	−2.42	101
	C	12	12.1 ± 0.2	12.2 ± 0.1	+0.83	102
	D	12	11.9 ± 0.1	11.9 ± 0.1	0	99

*RSD = [(Voltammetric value – Reference value) / Reference value] × 100%;

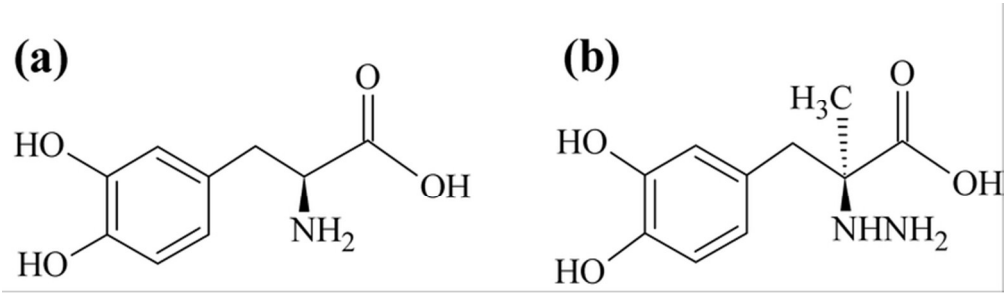
**Recovery = [(Voltammetric value) / (Labelled value)] × 100%.

Table 4. LD and CD determination in artificial human serum samples*.

Analyte	Added (μmol L^{-1})	Found (μmol L^{-1})	Recovery** (%)
LD	5.0	4.6 ± 0.6	92
	10.0	9.28 ± 0.03	92.8
CD	5.0	5.2 ± 0.2	104
	10.0	8.94 ± 0.06	89.4

* n = 3

** Recovery = $([Analyte]_{Found}/[Analyte]_{Added}) \times 100\%$



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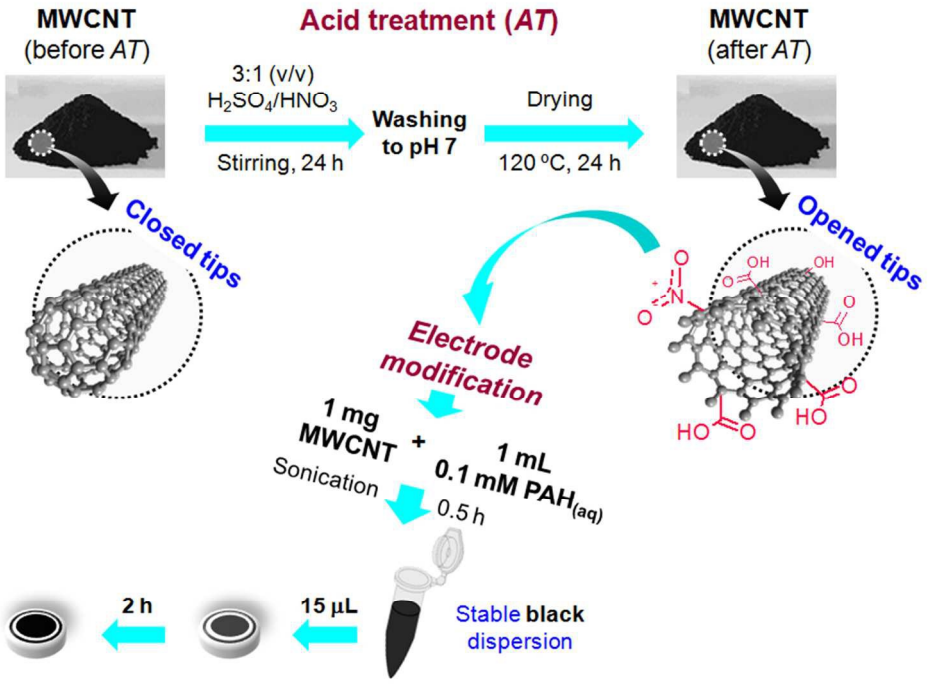

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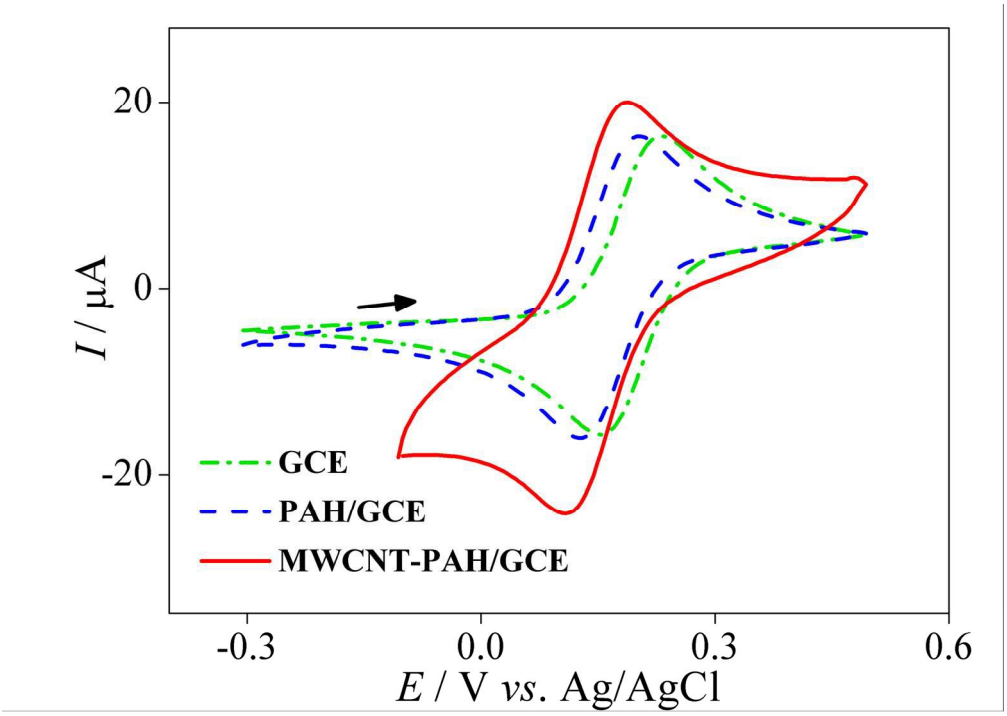

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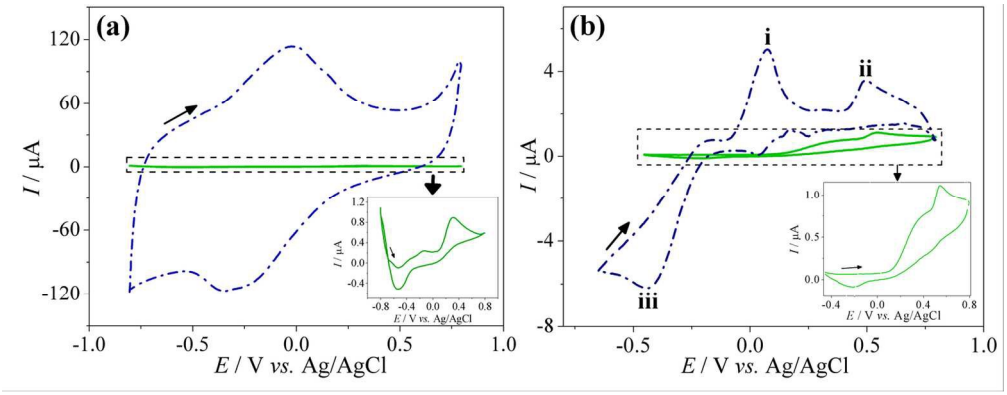

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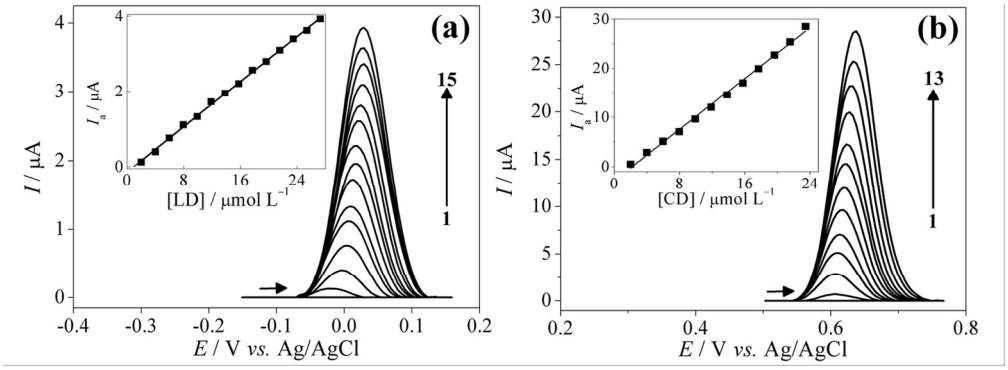

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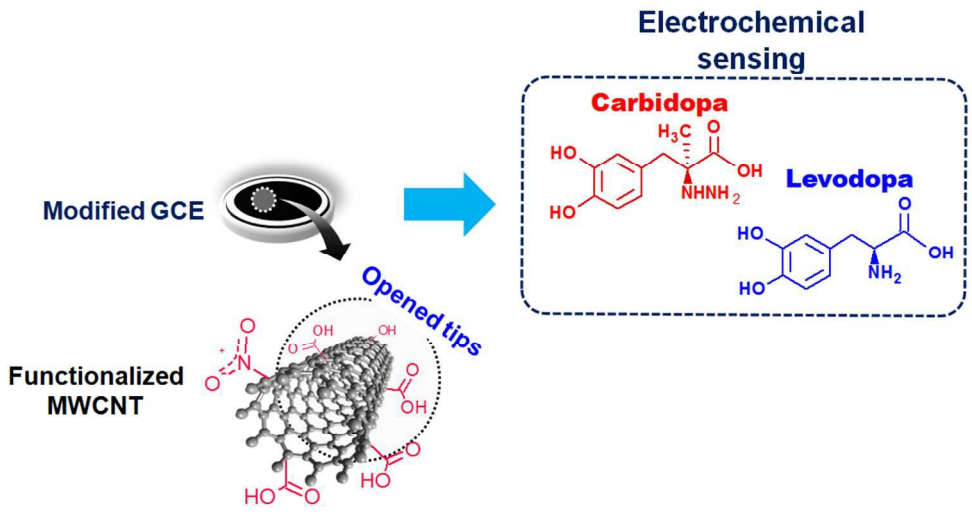

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