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Electrochemical Investigation and Determination of Levodopa on Poly(Nile Blue-A)/Multiwalled Carbon Nanotube Modified Glassy Carbon Electrodes

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Abstract: The electrochemical behaviour of levodopa (LD) has been investigated at multiwalled carbon nanotube (MWCNT) and poly(Nile blue-A) (PNB) modified glassy carbon electrodes (GCE). MWCNT/PNB modified electrodes were prepared by NB potential cycling electropolymerisation in 0.1 M phosphate buffer solution (PB), pH 6.0, followed by MWCNT deposition, and PNB/MWCNT/GCE by coating in the inverse order. The oxidation behaviour of LD was investigated in 0.1 M PB at pH values between 5.0 and 8.0. Quantitative determination of LD by differential pulse and square wave voltammetry was carried out in 0.1 M PB at pH 6.0. Analytical parameters were compared with the literature.

Keywords: Levodopa • Nile blue-A • Polyphenoxazines • Carbon nanotubes • Modified glassy carbon electrodes

1 Introduction

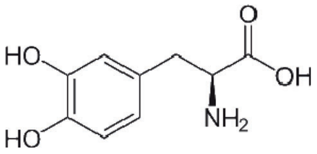
Levodopa (LD) is a unique drug for Parkinson's disease (PD) and currently the most important drug for its treatment. It is used for the treatment of degenerative disorder of the central nervous system that affects movement, muscle control, balance, and other functions [1–3]. It helps to decrease motor-related movements such as tremor, rigidity, slowness of movement, and postural instability. The structure of LD, a large neutral amino acid (3,4-dihydroxy-L-phenylalanine) is shown in Scheme 1.

The symptoms of Parkinson's disease are related to the loss of dopamine in the brain. The medication of the disease aims to increase the level of dopamine and therefore the progression of the disease and the motor-symptoms slow down. Levodopa is converted into dopamine and stored in dopaminergic neurons [4,5]. Only 5–10% of LD crosses the blood-brain barrier and the rest is taken up by skeletal muscle, liver, and kidney. Most of LD in the body is decarboxylated to dopamine before it reaches the brain and as a result, the side effects are reduced when high doses of LD are administered [6,7].

Phenothiazines, phenazines, and phenoxazines are dyes which can be used as redox mediators and can be electropolymerised in aqueous solution on the surface of solid electrodes to obtain electroactive polymers [8,9]. They can be used in sensors and biosensors for the investigation of the electrochemical characteristics of, for example, some carboxylic acids [10], NADH [11,12], and hemoglobin [13] due to their advantages such as simple one-step preparation, high stability, and reproducibility. Poly(Nile blue-A) (PNB) is obtained by the electropolymerisation of the phenoxazine dye Nile blue-A (NB), in aqueous media [14], and has been used as a mediator for the electrocatalytic oxidation of NADH and NADPH [15,16], for detecting nitrite [17] and in biofuel cells [18].

Carbon nanotubes (CNT), as single-wall carbon nanotubes (SWCNT) and multiwalled carbon nanotubes (MWCNT), exhibit high tensile strength, high electrical conductivity, chemical stability, high surface area, and insolubility in all solvents [19,20]. CNTs are used to obtain modified electrodes showing better electrochemical performance than conventional carbon electrodes and they have been shown to enhance the performance of sensors and biosensors e.g. [21–24], for the immobilisation of enzymes [25] and DNA [26]. In recent years, CNT and conducting polymers have been used together to obtain new modified electrodes e.g. [27].

The objective of this work was to carry out an electrochemical investigation and perform the electroanalytical determination of levodopa (LD) at glassy carbon electrodes (GCE) modified with MWCNT and poly(Nile blue-A), using cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV).



Scheme 1. Structure of levodopa.

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2 Experimental

2.1 Reagents

Nile blue-A (NB) and *N,N*-dimethylformamide (DMF) were purchased from Fluka (Switzerland). Levodopa was from Sigma (M_w: 197.2 g/mol, USA). They were used without further purification. Multiwalled carbon nanotubes were from NanoLab, U.S.A., with ~95 % purity, 30 ± 10 nm diameter, and 1–5 µm length.

Stock solutions of LD (1×10^{-3} M) were prepared in nanopure water and stored in the dark at +4 °C. Voltammetric experiments were done with working solutions of LD prepared by direct dilution of the stock solution with the selected supporting electrolyte.

Solutions of 0.1 M phosphate buffer (PB) were prepared at different pH values and used both for electropolymerisation of NB and as supporting electrolyte. They were prepared from sodium di-hydrogenphosphate and di-sodium hydrogenphosphate (Riedel-de-Haën, Germany), the pH then being adjusted with 5 M NaOH (Riedel-de Haën, Germany) solution.

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

2.2 Instrumentation

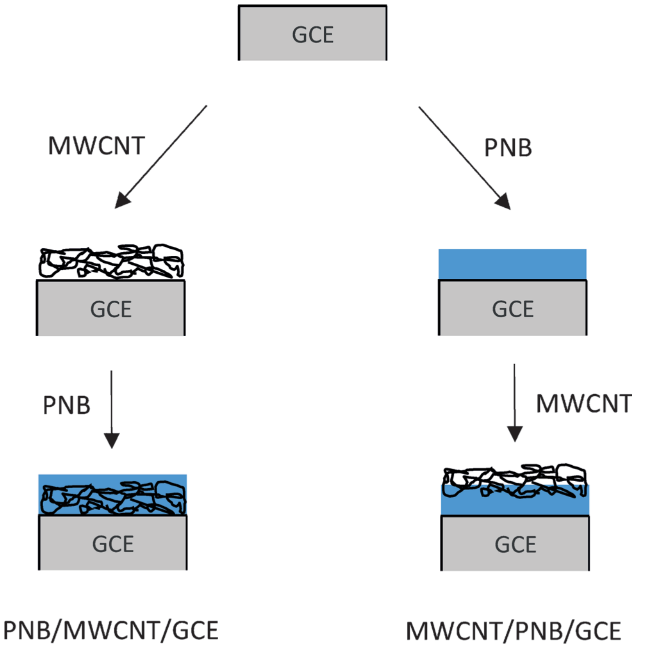
A three-electrode electrochemical cell was used for voltammetry measurements. It has a glassy carbon (GC) working electrode with a diameter of 5.5 mm, a platinum foil as counter electrode, and a saturated calomel electrode (SCE) as reference. The GCE surface was polished using diamond spray (Kemet International, UK) on a polishing cloth down to 1 µm particle size. It was then rinsed and ultrasonicated in Milli-Q ultrapure water.

The measurements were performed using a computer-controlled µ-Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm-Autolab, Utrecht, The Netherlands).

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

2.3 Preparation of PNB/MWCNT and MWCNT/PNB Modified Electrodes

Modification of GC electrodes was carried out by electropolymerisation of NB to form PNB films on the bare GCE or on GCE modified by functionalised MWCNT, as shown in Scheme 2. MWCNT/PNB modified electrodes, MWCNT/PNB/GCE, were prepared by first electropolymerising NB on the surface of the GC electrode and then coating by MWCNT/DMF dispersion. PNB/MWCNT modified electrodes, PNB/MWCNT/GCE, were prepared by first coating with the MWCNT/DMF dispersion, and then electropolymerising NB.



Scheme 2. Preparation of PNB and MWCNT modified electrodes.

Functionalised MWCNT were prepared as follows. A chosen amount of MWCNT was stirred in 3 M nitric acid solution for 24 h. The solid product was filtered, washed with nanopure water until the filtrate solution became neutral, and dried in an oven at 80 °C for 24 h. The functionalised MWCNTs were dispersed in DMF with loading 0.2 % (mg mL^{-1}) and sonicated for 4 h to obtain a homogeneous mixture. Then, 20 µL of the dispersion was dropped either directly on the GCE surface or on the PNB film, and then dried overnight at room temperature.

NB was electropolymerised by cycling in the potential range between −0.6 and +1.2 V vs. SCE at a scan rate of 50 mV s^{-1} in 0.1 M PB at pH 6.0 containing 0.5 mM of NB. The optimum number of cycles of NB electropolymerisation for MWCNT/PNB and PNB/MWCNT modified electrodes was 17 and 5, respectively [14]. After polymerisation, the modified electrodes were dried for 24 h at room temperature.

2.4 Validation of the Analytical Methods

Validation of the studied method was carried out with regard to ruggedness, precision, and accuracy. Five replicate samples were assayed on the same day and on different days over a week. Relative standard deviations (RSD %) were also calculated to check the ruggedness and precision of the method [28,29].

All working solutions were freshly prepared just before the experiments and protected from the light. Measurements were done at room temperature (25 ± 1 °C). The calibration equations were constructed using results from DPV and SWV experiments, by plotting the peak current against LD concentration.

3 Results and Discussion

3.1 Electrochemical Behaviour of LD at the Modified Electrodes

Modified electrodes PNB/MWCNT/GCE and MWCNT/PNB/GCE were prepared. The electropolymerisation of Nile blue-A has been studied in detail previously [14]. The optimised procedure was carried out in 0.1 M PB at pH 6.0 containing 0.5 mM NB monomer by potential cycling on the surface of the GCE (17 cycles) or MWCNT-coated GCE (5 cycles). Cyclic voltammograms during and after NB polymerisation give two redox couples: oxidation/reduction peaks for NB monomer and PNB polymer at around −0.3 V and 0.0 V, respectively. Peak currents for the electropolymerisation of NB onto MWCNT-coated electrodes are much higher than those on the surface of the GCE, since modification by MWCNT leads to a larger electroactive surface area [14,30]. Differences in morphology are also expected as can be deduced from Scheme 2.

The electrochemical behaviour of LD was investigated using cyclic voltammetry (CV) at both MWCNT/PNB and PNB/MWCNT modified electrodes in 0.1 M PB at different pH values between 5.0 and 8.0. More acidic and basic buffer solutions were not used, since PNB films are unstable in media more acidic than pH 5.0 [14] and LD decomposes instantly in media more basic than pH 8.0.

CV measurements were carried out in the potential range between 0.0 V and 0.5 V at a scan rate of 50 mV s^{-1} in 0.1 M PB at pH 5.0, 6.0, 7.0, and 8.0 containing 100 μM LD. Typical cyclic voltammograms are shown in Figure 1. As seen, LD has a single anodic peak at all pH values studied for both modified electrodes, at ~0.240 V vs. SCE in 0.1 M PB at pH 6.0. A small cathodic response was observed in 0.1 M PB at pH 5.0 and a very small response at pH 6.0, which were at ~0.240 V and ~0.197 V, respectively. These results show that LD undergoes an irreversible electrochemical oxidation process on PNB-modified electrodes. Repetitive cyclic voltammograms showed a decrease of LD peak height in the second and later cycles due to blocking of the surfaces of the modified electrodes by oxidised adsorbed species. Regeneration of the modified electrodes was able to be carried out easily by simple potential cycling in 0.1 M PB between 0.0 V and 0.60 V, the response being recovered after 2 cycles.

The effect of pH on the peak potential and peak current was investigated in 0.1 M PB at pH 5.0, 6.0, 7.0, and 8.0 using both CV and DPV. Since the results obtained with CV and DPV were similar, only CV results are shown in Figure 2. The anodic peak potential of LD moved to less positive potentials with increasing pH. A linear dependence of anodic peak potentials of LD versus pH was obtained with a negative slope of 61.9 mV/pH (correlation coefficient 0.998) for MWCNT/PNB and 59.5 mV/pH (correlation coefficient 0.999) for PNB/MWCNT modified electrodes. The values obtained are both close to the theoretical value of 59 mV/pH that corresponds to an equal number of protons and electrons being involved in the oxidation process [8,31]. The variation of peak current *vs.* pH shows a maximum value and best peak shape at pH 6.0 for MWCNT/PNB/GCE. At PNB/MWCNT/GCE, the peak current is higher at pH 7 and pH 8, but the stability of LD at these values of pH was found to be less good, signs of degradation (turbidity and colour change) appearing after only 15 min. Thus, 0.1 M PB at pH 6.0 was selected as supporting electrolyte for the quantitative determination of LD.

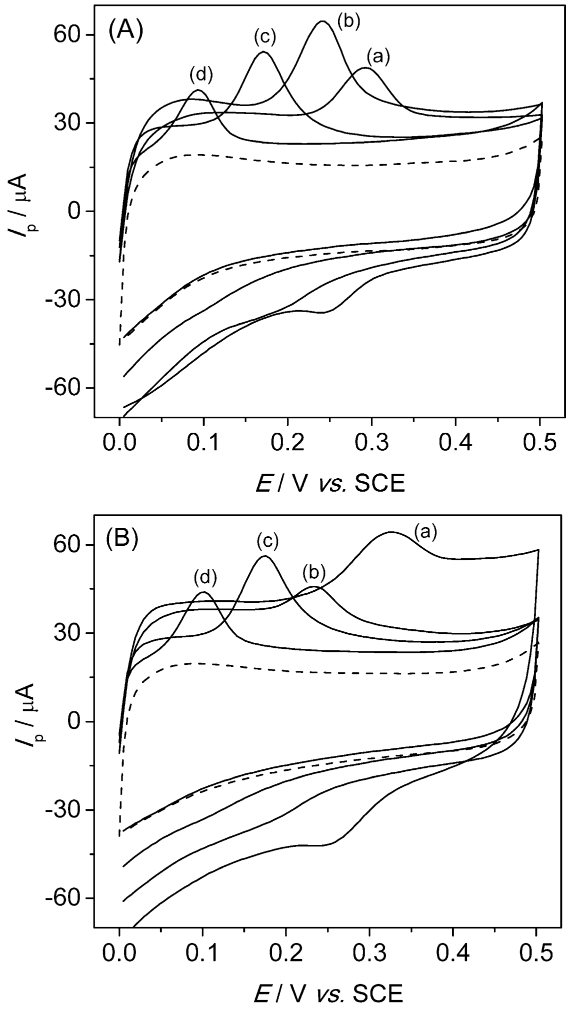


Fig. 1. Cyclic voltammograms of LD in 0.1 M PB at pH (a) 5.0, (b) 6.0, (c) 7.0, and (d) 8.0 with (A) MWCNT/PNB and (B) PNB/MWCNT modified GCE. Dashed lines: 0.1 M PB at pH 8.0; LD concentration: 100 μM; scan rate 50 mV s^{-1}.

CV scan rate studies were also performed in the range 5 to 250 mV s^{-1}. The anodic peak potentials shifted to more positive values by 73 mV for both MWCNT/PNB and PNB/MWCNT modified GCE when the scan rate was increased in this range. Plots of anodic peak current *vs.* scan rate (surface-confined electrode process) were linear for both types of modified electrode [32], with slopes of 0.31 and 0.43 μA mV^{-1} for MWCNT/PNB and PNB/MWCNT modified electrodes, respectively. This in-

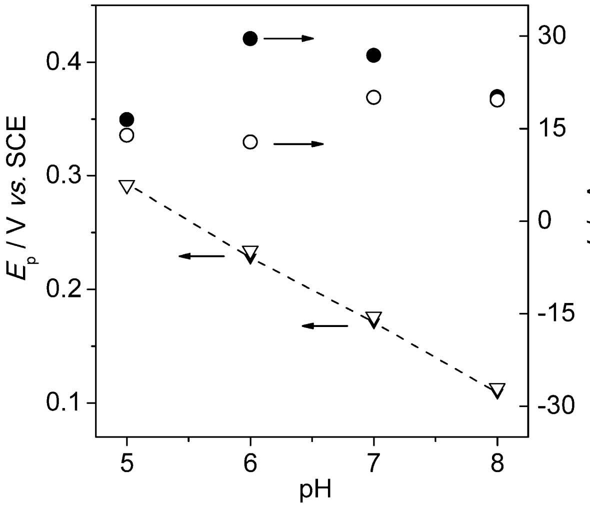


Fig. 2. Plots of peak potential, E_p, and peak current, I_p, vs. pH from cyclic voltammograms of 100 µM LD in 0.1 M PB. Triangles indicate E_p and circles I_p. Filled symbols refer to MWCNT/PNB/GCE and unfilled to PNB/MWCNT/GCE.

dicates adsorption or thin-layer effects due to the porous nature of the modifier layer on the surface of the glassy carbon electrode (see Scheme 2). The higher response in the latter case occurs when PNB is deposited on the MWCNT, suggesting an increased available surface area.

3.2 Electroanalytical Determination and Validation

Validation studies for the quantitative analysis of LD were carried out using both differential pulse voltammetry (DPV) and square wave voltammetry (SWV), chosen due to their selectivity, sensitivity with low detection limits, and speed of analysis [33,34].

Since the modified electrodes showed adsorption behaviour, adsorptive stripping techniques, AdSDPV and AdSSWV, and with variation of the parameters accumulation time, accumulation potential, and stirring rate were tested for the determination of LD. However, a linear response could not be obtained with any adsorptive stripping techniques. Thus, DPV and SWV were used for the quantitative analysis of LD, with similar results. The supporting electrolyte for the quantitative analysis of LD was selected as 0.1 M PB at pH 6.0 for both modified electrodes, since the best results such as peak shape, peak current sensitivity, reproducibility, and LD stability were obtained in this buffer. All solutions were freshly prepared before the measurements in order to ensure the stability of the analyte.

Differential pulse voltammograms for various concentrations of LD are shown in Figure 3. The peak-width at half height of the peaks gives values of ~50 mV. Taken together with the slope of the plot of E_p vs. pH in Figure 3 from which it is deduced that an equal number of electrons and protons are involved in the oxidation mechanism, it can be inferred that this number is two. The oxidation of LD thus involves the oxidation of the two –OH groups on the aromatic ring to give the corresponding dopaquinone, which is in agreement with the literature, e.g. [31].

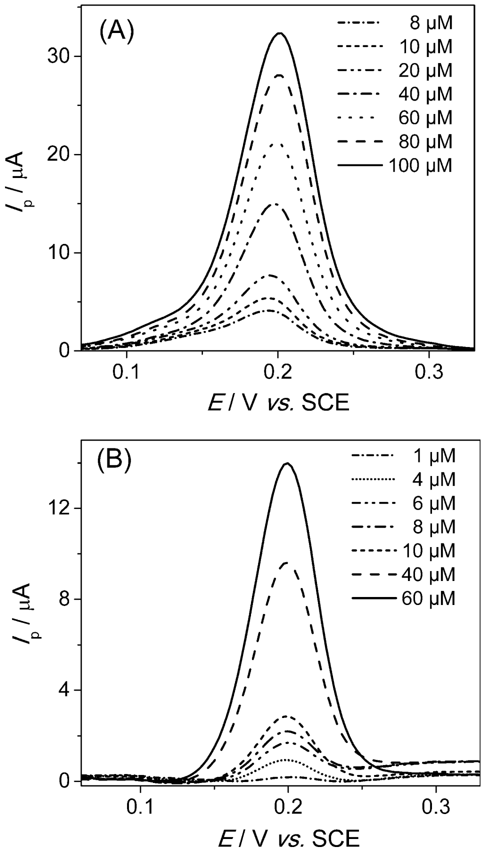


Fig. 3. Baseline-subtracted differential pulse voltammograms of LD at different concentrations in 0.1 M PB at pH 6.0 using (A) MWCNT/PNB and (B) PNB/MWCNT modified GCE.

Plots of peak current vs. the concentration of LD were linear in the range between 1×10^{-6} and 1×10^{-4} M for MWCNT/PNB modified electrodes and between 1×10^{-6} and 6×10^{-5} M for PNB/MWCNT modified electrodes. Higher LD concentrations led to a loss of linearity of the calibration plots due to saturation effects. Table 1 shows the analytical parameters obtained. The precision of the proposed methods was confirmed by the low standard error values of the slopes and intercepts and correlation coefficients greater than 0.998 for both MWCNT/PNB/GCE and PNB/MWCNT/GCE.

Standard validation procedures were applied to the proposed methods [28], the results of such analyses being shown in Table 1. Accuracy, precision, reproducibility, and the other parameters of the proposed methods for LD were evaluated by performing replicate analysis of the standard solutions in 0.1 M PB at pH 6.0 [29,33]. Limits of detection (*LOD*) and limits of quantification (*LOQ*) were calculated from the peak currents using $LOD=3s/m$ and $LOQ=10s/m$ equations, where s is the standard deviation of the peak currents (three runs) and m is the slope of the related calibration equation [35]. The precision of the proposed methods was evaluated

Table 1. Data of the calibration lines for the quantitative determination of LD in 0.1 M PB at pH 6.0 by DPV and SWV for MWCNT/PNB and PNB/MWCNT modified GCE.

	MWCNT/PNB/GCE		PNB/MWCNT/GCE	
	DPV	SWV	DPV	SWV
Peak potential (V vs. SCE)	0.197	0.212	0.195	0.204
Linearity range (μM)	1–100	1–100	1–60	2–60
Slope ($\mu A\,\mu M^{-1}$)	0.27 ± 0.003	0.40 ± 0.003	0.21 ± 0.002	0.16 ± 0.002
Intercept (μA)	0.66 ± 0.16	0.70 ± 0.17	0.41 ± 0.05	−0.39 ± 0.05
Correlation coefficient	0.998	0.999	0.999	0.999
LOD (μM)	0.37	0.69	0.16	0.51
LOQ (μM)	1.13	2.08	0.47	1.54
Repeatability of peak potential (*RSD*%)[a]	0.45	0.36	0.45	0.39
Repeatability of peak current (*RSD*%)[a]	0.62	0.78	0.84	0.81
Reproducibility of peak potential (*RSD*%)[a]	0.91	0.50	0.56	0.54
Reproducibility of peak current (*RSD*%)[a]	0.89	1.61	1.19	1.21

[a] LD concentration: 40 μM

Table 2. Electrochemical detection of LD at different modified electrodes.

Electrode	Method	pH	Reference electrode	Peak potential (V)	Linear range (μM)	Sensitivity ($\mu A\,\mu M^{-1}\,cm^{-2}$)	*LOD* (μM)	Ref.
Ru–red/NaY/CPE [a]	CV	4.8	SCE	0.580	120–10000	0.072	0.85	[36]
Au-TiO_2-NTs/Ti [b]	DPV	7.0	SCE	0.110	10–70	0.402	–	[37]
FCMCNPE [c]	DPV	7.0	$Ag/AgCl_{(sat.)}$	0.370	2–500	1.032	1.20	[38]
Q/fMWCNT-MGCE [d]	DPV	7.0	$Ag/AgCl_{(sat.)}$	0.156	0.9–85	4.409	0.38	[39]
CAMCPE [e]	DPV	10.0	SCE	0.500	3–20	3.848	0.95	[40]
GCE [f]	DPV	5.0	$Ag/AgCl_{(sat.)}$	0.304	10–80	4.775	2.52	[41]
$Co(DMG)_2ClPy$/MWCNT/BPPG [g]	SWV	6.4	$Ag/AgCl_{(sat.)}$	0.180	3–100	4.430	0.86	[42]
MWCNT/PNB/GCE	DPV	6.0	SCE	0.197	1–100	3.803	0.37	This work
PNB/MWCNT/GCE	DPV	6.0	SCE	0.195	1–60	2.958	0.16	This work

[a] Carbon paste electrode modified with trinuclear ruthenium ammine complex $[(NH_3)_5Ru^{III}–O–Ru^{IV}(NH_3)_4–O–Ru^{III}(NH_3)_5]^{6+}$ (Ru-red) incorporated in NaY zeolite. [b] Gold nanoparticles doped on titanium dioxide nanotubes. [c] Ferrocene modified carbon nanotube paste electrode. [d] Electrodeposition of quercetin on a functionalised multiwalled carbon nanotube immobilised on the surface of a glassy carbon electrode. [e] Chloranil modified carbon paste electrode. [f] Bare glassy carbon electrode. [g] Basal plane pyrolytic graphite (BPPG) electrode modified with chloro(pyridine)bis(dimethyl glyoximato)cobalt(III) ($Co(DMG)_2ClPy$) absorbed in a multiwalled carbon nanotube.

from five replicate experiments on the same day (repeatability) and over a week (reproducibility) in different solutions having the same concentration of LD, 4×10^{-5} M, using DPV and SWV at MWCNT/PNB and PNB/MWCNT modified electrodes. The precision and accuracy were determined as RSD %. These results demonstrate good precision, accuracy, and sensitivity.

Electroanalytical studies of LD can also be found in the literature, which use cyclic, differential pulse, and square wave voltammetry techniques: values of peak potential, linear range, sensitivity, and *LOD* are given in Table 2. The modified electrodes in this work exhibited a wider linear range, higher sensitivity, and lower detection limit than the electrodes reported in [36,37]. Although the modified electrode described in [38] showed a wider linear range, it had a lower sensitivity and higher detection limit, and the higher sensitivity values obtained with the modified electrodes in [39–42], are offset by narrow linear ranges and higher detection limits. Combining all the analytical parameters, MWCNT/PNB and PNB/MWCNT modified GCE exhibited better results than the electrodes in the literature in Table 2 and are easy to prepare with good stability.

4 Conclusions

Glassy carbon electrodes modified by MWCNT and by poly(Nile blue-A) to form MWCNT/PNB/GCE and PNB/MWCNT/GCE have been used to investigate the electrochemical behaviour of the important antiparkinsonian agent levodopa (LD). Cyclic voltammetry of LD showed irreversible electrochemical behaviour in 0.1 M PB in the pH ranges from 5.0 to 8.0. Analysis of peak currents and peak shapes led to the supporting electrolyte being selected as 0.1 M PB at pH 6.0 for further studies. Scan rate studies showed a complex anodic behaviour, indicating adsorption or thin-layer effects due to the porous nature of the modifier layer. Quantitative analysis of LD was carried out using DPV and SWV as rapid, selective, sensitive, and simple techniques, the linear calibration curves demonstrating good precision, accuracy, and sensitivity for LD.

These new stable and easy-to-prepare modified electrodes represent a useful alternative strategy for the electroanalysis of LD.

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