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Research Article

Electroanalytical Characterisation of Dopa Decarboxylase Inhibitors Carbidopa and Benserazide on Multiwalled Carbon Nanotube and Poly(Nile blue A) Modified Glassy Carbon Electrodes

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Modified glassy carbon electrodes have been made by deposition of functionalised multiwalled carbon nanotubes (MWCNTs) followed by formation of poly(Nile blue) (PNB) films by electropolymerisation, using potential cycling in 0.1 M phosphate buffer solution (PBS) at pH 6.0. The electrochemical oxidation of carbidopa (CD) and benserazide (BS) on these MWCNTs/PNB-modified electrodes was investigated using cyclic and differential pulse voltammetry in 0.1 M PBS at different values of pH between 5.0 and 8.0; both CD and BS gave one diffusion-controlled irreversible oxidation peak in cyclic voltammetry. Analytical characterisation of CD and BS was carried out in 0.1 M PBS, pH 5.0. Peak currents in differential pulse voltammetry were linear over the concentration range of 1×10^{-5} to 1×10^{-4} M for CD and 4×10^{-6} to 4×10^{-5} M for BS. The repeatability, precision, and accuracy of the method were also investigated. Higher sensitivities and lower detection limits, of 1.17 μ M for CD and 0.50 μ M for BS, were obtained with this new modified electrode compared with previous studies reported in the literature.

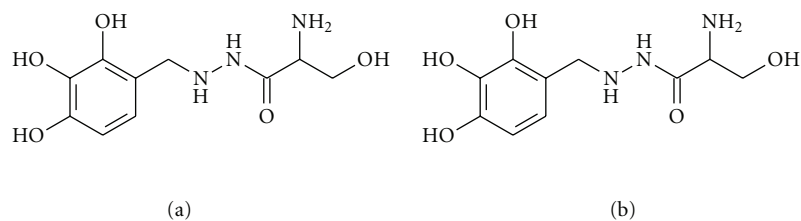
1. Introduction

Dopa decarboxylase inhibitors are used as part of the treatment for Parkinson's disease. Parkinson's disease (PD) is a degenerative disorder of the central nervous system that affects movement, muscle control, balance, and other functions. The most obvious symptoms are motor related such as tremor, rigidity, slowness of movement, and postural instability [1, 2]. There is currently no cure for Parkinson's disease. However, medications are used to increase levels of dopamine in the brain of patients with PD and in this way slow down the progression of the disease, since motor symptoms are produced by a lack of dopamine. The main drugs used for Parkinson's disease are levodopa, dopamine agonists, and MAO-B inhibitors [3].

Treatment using levodopa involves it being transformed into dopamine and increasing its level in the central nervous

system. However, only 5–10% of levodopa crosses the blood-brain barrier, and the rest is decarboxylated to dopamine in the metabolism before it reaches the brain, causing various side effects. Carbidopa, (2S)-3-(3,4-dihydroxyphenyl)-2-hydrazino-2-methylpropanoic acid, and benserazide, 2-amino-3-hydroxy-N'-(2,3,4-trihydroxybenzyl) propanehydrazide, (Scheme 1) are decarboxylase inhibitors of levodopa which are unable to cross the blood-brain barrier themselves and used in combination with levodopa for the treatment of PD. They prevent the carboxylation of levodopa before it reaches the brain and reduce the side effects when high doses of levodopa are used [4, 5].

Polymers of dyes such as phenothiazines, phenazines, and phenoxazines have recently been found to be attractive as redox mediators on the surface of solid electrodes [6, 7]. These dyes can be electropolymerised from aqueous solutions to obtain electrochemically active polymers. The



SCHEME 1: Structures of (a) carbidopa and (b) benserazide.

resulting polymers have many advantages such as simple one-step preparation, high stability, and reproducibility. Thus, they can be used as sensors and biosensors for the investigation of the electrochemical characteristics of some carboxylic acids [8] and NADH [9, 10].

Nile blue A (NB), one of the phenoxazine dyes, is a well-known water-soluble electroactive molecule. Electrochemical polymerisation of NB gives a semiconducting poly(Nile blue) (PNB) film. PNB has been used as a mediator for the electrocatalytic oxidation of the nicotinamide coenzymes NADH and NADPH [11, 12] and also used in biofuel cells [13].

Single- (SWCNT) and multiwalled (MWCNT) carbon nanotubes have a great interest due to their properties such as high electrical conductivity, chemical stability, high surface area, and insolubility in all solvents [14, 15]. The unique and one-dimensional structures of carbon nanotubes lead them to be used in sensors and biosensors [16–19]. CNTs have also been used with conducting polymers as redox mediators to obtain new modified electrodes having good electrical and mechanical properties [20, 21].

The goal of this work is to carry out investigation and analytical measurement of carbidopa (CD) and benserazide (BS), which are anti-Parkinsonian agents, with modified glassy carbon (GC) electrodes by using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Modification was achieved first with functionalised multiwalled carbon nanotubes on the surface of GC electrodes. Nile blue A was then electropolymerised onto MWCNT-modified electrodes by cyclic voltammetry. The analytical measurement of CD and BS was carried out at MWCNT/PNB-modified electrodes in the selected supporting electrolyte. The results obtained for CD and BS were compared.

2. Experimental

2.1. Reagents. Nile blue A and N,N-dimethylformamide (DMF) were purchased from Fluka (Switzerland). Carbidopa (CD, $\geq 98\%$, M_w : 226.23 g/mol) and benserazide (BS, $\geq 98\%$, M_w : 293.70 g/mol) were from Sigma (USA), and nitric acid was from Riedel-de Haën (Germany). They were used without further purification.

Multiwalled carbon nanotubes were from NanoLab, USA, with $\sim 95\%$ purity, 30 ± 10 nm diameter and 1–5 μm length. For carboxylate functionalisation, 120 mg of MWCNT was stirred in 10 mL of a 3 M nitric acid for 24 h at room temperature. The solid product was filtered, then

washed with nanopure water until the filtrate became close to neutral ($\text{pH} \approx 7$), and dried at 80°C for 24 h.

Phosphate buffer solutions (PBSs), ionic strength 0.1 M, with different pH values were used both for electropolymerisation of NB and as supporting electrolyte for CD and BS. They were prepared from sodium di-hydrogenphosphate and di-sodium hydrogenphosphate (Riedel-de-Haën, Germany), and the pH was then adjusted with 5 M NaOH (Riedel-de Haën, Germany) solution.

Stock solutions of CD and BS (1×10^{-3} M) were prepared in nanopure water and stored in the dark and at $+4^\circ\text{C}$. Working solutions of CD and BS for the voltammetric experiments were prepared by direct dilution of the stock solution with the selected supporting electrolyte just before use.

Millipore Milli-Q nanopure water (resistivity ≥ 18 M Ω cm) and analytical reagents were used for preparation of all solutions.

2.2. Instrumentation. A three-electrode electrochemical cell was used for voltammetric experiments. It contained 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. Measurements were performed using a computer-controlled μ -Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Metrohm-Autolab, the Netherlands).

The pH measurements were carried out with a CRISON 2001 micro-pH meter at room temperature.

2.3. Modification of GC Electrodes with MWCNT and PNB. Modification of GC electrodes was achieved with functionalised MWCNT and PNB films. Before modification, the GC electrode was polished with diamond spray (Kemet International Ltd., UK) down to 1 μm particle size, then sonicated in Milli-Q nanopure water and rinsed with nanopure water.

The functionalised MWCNTs were dispersed in DMF with loading 0.2% (mg/ μL) and sonicated for 4 h to obtain a homogeneous mixture. Then, 20 μL of the 0.2% MWCNT/DMF dispersion was dropped directly on the surface of the GC electrode using a micropipette and dried overnight at room temperature.

NB was electropolymerised onto MWCNT coatings (denoted as MWCNT/PNB) by cycling in the potential region from -0.6 to $+1.2$ V versus SCE at a scan rate of 50 mV s^{-1} in 0.1 M PBS at pH 6.0 containing 0.5 mM of NB. The optimum number of cycles for electropolymerisation of NB

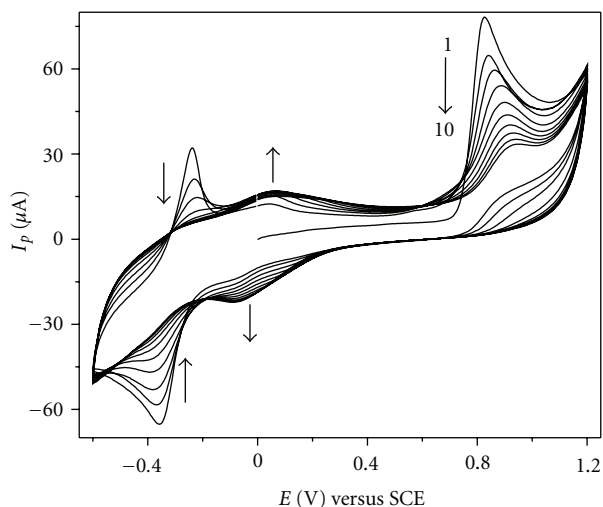


FIGURE 1: Cyclic voltammograms during potential cycling electro-polymerisation of 0.5 mM NB in 0.1 M PBS at pH 6.0. Scan rate 50 mV s^{-1} .

was found to be 5. After polymerisation, MWCNT/PNB-modified electrodes were dried for 24 h at room temperature.

2.4. Analytical Procedures. For analytical assays, all solutions were freshly prepared before the experiments and protected from the light. Measurements were carried out using differential pulse voltammetry (DPV) at room temperature ($25 \pm 1^\circ\text{C}$). The calibration equations for the DPV technique were constructed by plotting the peak current against CD or BS concentration.

Validation of the studied method was carried out with regard to ruggedness, precision, and accuracy by assaying five replicate samples [22, 23].

3. Results and Discussion

3.1. Electrochemical Polymerisation of Nile Blue. Electropolymerisation of NB was carried out in 0.1 M PBS at pH 6.0 containing 0.5 mM of NB by potential cycling on the MWCNT-modified electrodes. These polymerisation conditions were chosen according to a detailed study between pH 5–8, which showed that these conditions led to the polymer with the best electrochemical behaviour, with the most well-defined voltammetric peaks and the most stable PNB film [24]. Five cycles were used for the polymerisation of NB, since thicker PNB films, obtained with more than 5 cycles, onto MWCNTs coatings, led to unstable surface modifier films [24].

Cyclic voltammograms during and after NB polymerisation gave two redox couples: oxidation/reduction peaks for NB monomer and PNB polymer at around -0.4 V and 0.0 V , respectively. The irreversible oxidation peak at $\sim 0.9 \text{ V}$ is due to formation of the monomer radical (Figure 1). Peak currents for the electropolymerisation of NB onto MWCNT coatings were much higher than those of on the surface of the GC electrode, since modification by MWCNT leads to a larger electroactive surface area [15, 24, 25].

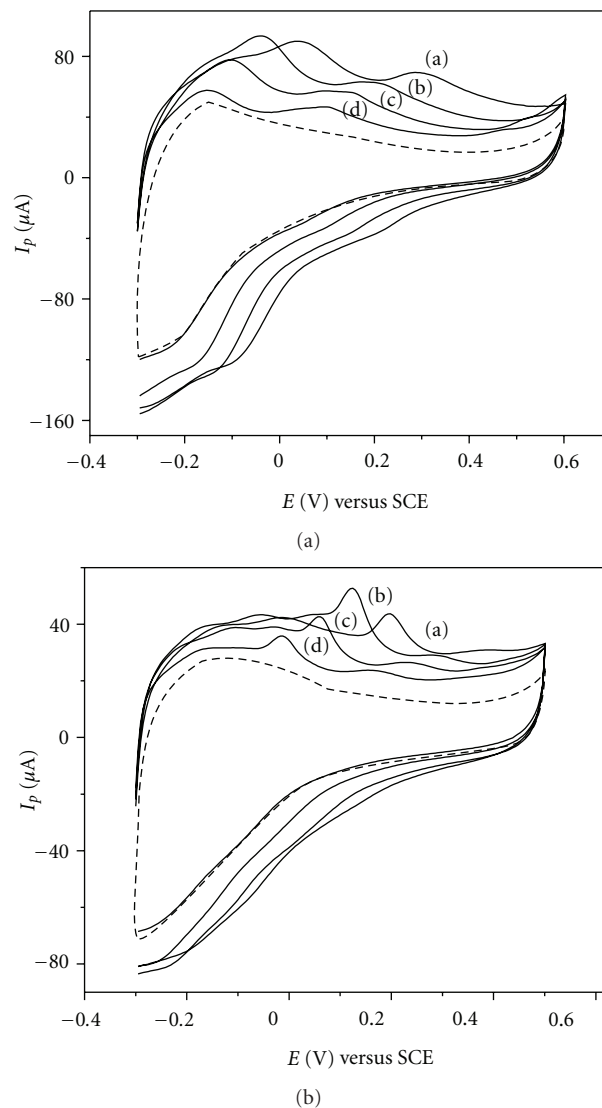


FIGURE 2: Cyclic voltammograms of (A) CD and (B) BS in 0.1 M PBS at pH (a) 5.0, (b) 6.0, (c) 7.0, and (d) 8.0 with MWCNTs/PNB-modified electrode. Dashed lines: 0.1 M PBS at pH 8.0. CD and BS concentrations: $100 \mu\text{M}$. Scan rate 50 mV s^{-1} .

3.2. Electrochemical Behaviour of CD and BS with Modified Electrodes. The electrochemical oxidation behaviour of CD and BS was investigated using cyclic voltammetry (CV) with MWCNTs/PNB-modified electrodes in 0.1 M PBS at different pH values between 5.0 and 8.0. More acidic buffer solutions were not used, since PNB films were unstable in media more acidic than pH 5.0 and media of pH > 8.0 cause decomposition of CD and BS.

CV measurements were made in the region between -0.3 V and 0.5 V , scanning in the positive direction at a scan rate of 50 mV s^{-1} in 0.1 M PBS at pH 5.0, 6.0, 7.0, and 8.0 containing $100 \mu\text{M}$ CD or BS; typical cyclic voltammograms are shown in Figure 2. Both CD and BS gave a single broad oxidation peak for all pH values tested. The oxidation peaks of CD and BS were at 286 and 198 mV versus SCE in 0.1 M

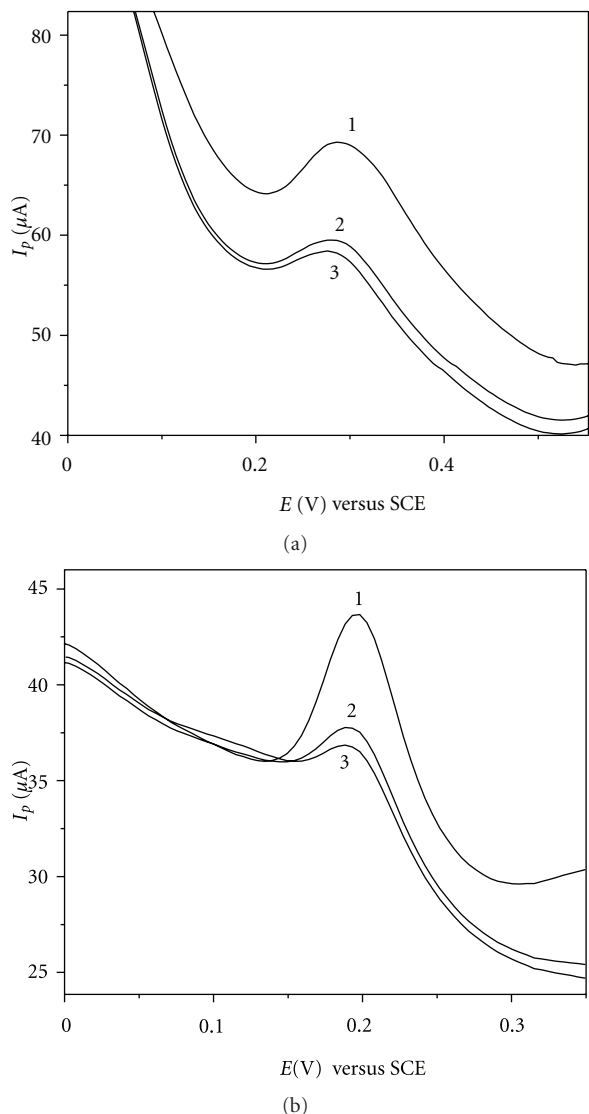


FIGURE 3: (1) First cycle, (2) second cycle, and (3) third cycle of the anodic responses of 1×10^{-4} M (a) CD and (b) BS from repetitive voltammograms in 0.1 M PBS at pH 5.0 with MWCNTs/PNB-modified electrode. Scan rate 50 mV s^{-1} .

PBS at pH 5.0. A very small cathodic response on sweep inversion was observed at around 210 mV for CD but only in pH 5.0 buffer solution. No reduction corresponding to the oxidation of BS was observed on the negative scan. These results demonstrate the irreversible nature of the CD and BS oxidation processes. The peaks of CD and BS decreased in the second and later cycles, due to blocking adsorption on the surface of the modified electrodes (Figure 3).

The effect of pH on the peak potential and peak current was investigated at pH 5.0, 6.0, 7.0, and 8.0 using both CV and DPV. Since the variations with pH seen by CV were similar to those with DPV, only the results obtained with DPV are shown in Figure 4. Both techniques showed that the peak potential of the anodic process moved to less positive potentials with increasing pH for both CD and BS.

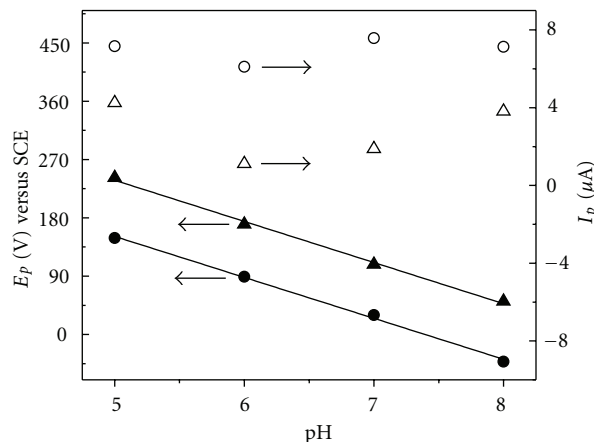


FIGURE 4: Plots of peak potential, E_p , and peak current, I_p , versus pH from DP voltammograms of CD and BS (concentrations $100 \mu\text{M}$) in 0.1 M PBS at MWCNT/PNB-modified electrodes. Triangles indicate CD and circles BS; filled symbols refer to E_p and unfilled ones to I_p .

Anodic peak potentials gave a linear dependence on pH with a negative slope of 63.5 mV/pH (correlation coefficient 0.996) and 63.2 mV/pH (correlation coefficient 0.997) for CD and BS, respectively, Figure 4. These slope values are close to the theoretical value of 59 mV/pH , obtained if the numbers of protons and electrons involved in the oxidation process are equal [6, 26]. The relationship between pH and peak current of CD and BS was also investigated. The maximum peak current and the better peak shape were obtained in 0.1 M PBS at pH 5.0 for CD and pH 7.0 for BS. However, BS was not stable at pH 7.0 for sufficient time to complete all of the studies. Thus, phosphate buffer at pH 5.0 was used as supporting electrolyte for the analytical characterisation of both CD and BS.

Scan rate studies were carried out in the range between 5 and 200 mV s^{-1} by CV to further assess the electrochemical oxidation of CD and BS. The oxidation peak potentials shifted to more positive potentials by about 132 mV for CD and 127 mV for BS when the scan rate was increased. Linear plots were not observed over this range neither from the plots of anodic peak current versus scan rate nor versus the square rate of scan rate, suggesting a more complex behavior. For this reason, plots of logarithm of peak current, $\log(I_p)$ versus logarithm of scan rate, $\log(\nu)$, for CD and BS were constructed, where a slope of 0.5 means a diffusion-controlled process and of 1.0 signifies an adsorption-controlled or surface-confined electrode process [27]. These plots showed the same tendencies for both CD and BS. At lower scan rates, $\leq 50 \text{ mV s}^{-1}$, the slope of the plot of $\log(I_p)$ versus $\log(\nu)$ was close to 0.5, indicative of diffusion control, and then began to increase, indicating that adsorption or thin-layer effects were beginning to have an influence on the observed behaviour. This can be explained taking into account the porous nature of the modifier layer into which the analyte will diffuse: the response is due to oxidation of species diffusing from bulk solution and to

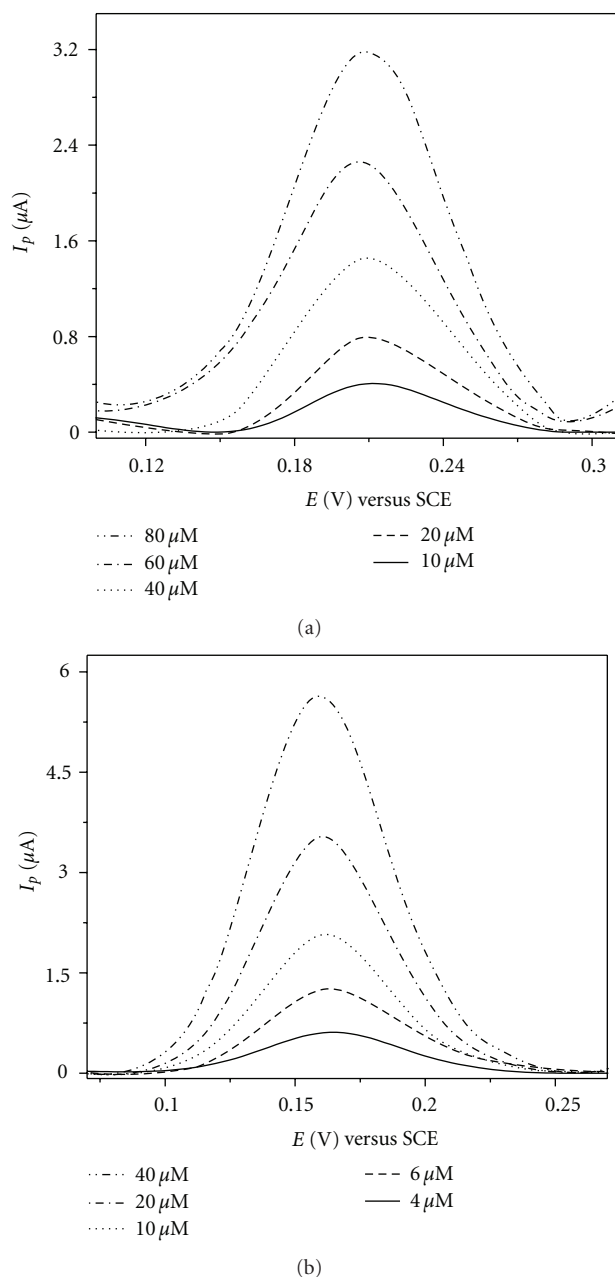


FIGURE 5: Baseline-subtracted differential pulse voltammograms of (a) CD and (b) BS at different concentrations in 0.1 M PBS at pH 5.0, using a MWCNT/PNB-modified electrode.

these species within the porous structure (similar to surface-confined species). As the scan rate increases, the timescale for diffusion decreases and the contribution of the latter becomes proportionally more important.

Tafel plots ($\log I$ versus E) were drawn for the scan rate of 5 mV s^{-1} beginning at a steady-state potential in 0.1 M PBS at pH 5.0 for both CD and BS [27]. The intercepts of the $\log I - f(E)$ plots gave the exchange current density (I_0) values, which were $9.73 \times 10^{-13} \mu\text{A cm}^{-2}$ and $4.48 \times 10^{-13} \mu\text{A cm}^{-2}$ for CD and BS, respectively. These values

TABLE 1: Data from the calibration plots for the analytical characterisation of CD and BS in 0.1 M PBS, pH 5.0 by DPV at the MWCNTs/PNB-modified electrode.

	Carbidopa	Benserazide
Peak potential (mV versus SCE)	210	160
Linearity range (μM)	10–100	4–40
Slope ($\mu\text{A } \mu\text{M}^{-1}$)	0.43 ± 0.001	0.15 ± 0.001
Intercept (μA)	0.49 ± 0.07	-0.32 ± 0.02
Correlation coefficient	0.997	0.999
LOD (μM)	1.17	0.50
LOQ (μM)	3.56	1.53
Repeatability of peak potential (RSD%)	0.50	0.56
Repeatability of peak current (RSD%)	0.96	0.44

confirmed the irreversibility of the oxidation reaction for both CD and BS.

3.3. Validation of the Analytical Methods. Differential pulse voltammetry (DPV) is an effective, selective, and sensitive technique which is suitable for the determination of organic drug compounds at low detection limits [28–30]. In this work, DPV was used for the quantitative evaluation of CD and BS, based on the linear correlation between oxidation peak current and concentration in 0.1 M PBS at pH 5.0 for both CD and BS, since the best response (regarding peak shape, peak current sensitivity, and reproducibility) and the best stability of CD and BS were obtained in this buffer. All solutions used for the analytical experiments were freshly prepared to ensure the stability of the analyte in the solutions.

Calibration plots under the selected conditions and using standard solutions of CD and BS are shown in Figure 5. The linear range was 1×10^{-5} to 1×10^{-4} M for CD and 4×10^{-6} to 4×10^{-5} M for BS; higher concentrations of CD and BS led to the loss of linearity and saturation effects that can be attributed to the effect of adsorption of species on the surface of MWCNT/PNB-modified electrode. The characteristics of the calibration lines and their related validation parameters are summarized in Table 1. Low values of standard error of the slopes and intercepts and correlation coefficients greater than 0.996 for both CD and BS confirmed the precision of the proposed method at MWCNT/PNB-modified electrodes.

The method was validated according to standard procedures [22, 31]. Accuracy, precision, and ruggedness were assessed by performing replicate analysis of the standard solutions in the supporting electrolyte. Limits of detection (LOD), limits of quantification (LOQ), repeatability (within day), precision, and selectivity were evaluated for both CD and BS [23, 29]. The LOD and LOQ were calculated from the peak currents using $\text{LOD} = 3 \text{ s/m}$ and $\text{LOQ} = 10 \text{ s/m}$, where s is the standard deviation of the peak currents (three runs) and m is the slope of the related calibration equation [32]. Also, these results indicated the reliability of the proposed voltammetric technique for the trace assay of CD and BS.

The precision of the method was calculated from five replicate experiments in different solutions having the same

concentration of CD or BS within the same day (repeatability) using DPV at the MWCNT/PNB-modified electrode. The concentrations of CD and BS for these studies were 8×10^{-5} M and 6×10^{-5} M, respectively. The precision and accuracy were determined as R.S.D.% (Table 1). These results demonstrate a good precision, accuracy, and sensitivity.

Electroanalytical studies of carbidopa and benserazide can also be found in the literature, which use differential pulse voltammetry. In [33], linear ranges between $31 \mu\text{M}$ and $470 \mu\text{M}$ with current sensitivity of $0.028 \mu\text{A} \mu\text{M}^{-1}$ and an LOD value of $2.16 \mu\text{M}$ were determined for CD and linear range $31 \mu\text{M}$ to $620 \mu\text{M}$ with current sensitivity $0.054 \mu\text{A} \mu\text{M}^{-1}$ and LOD of $2.77 \mu\text{M}$ for BS at GC electrodes. In another study [34], quantitative analysis of CD was carried out by DPV in the linear range from $5 \mu\text{M}$ to $600 \mu\text{M}$ with $3.6 \mu\text{M}$ of LOD and a current sensitivity of $0.041 \mu\text{A} \mu\text{M}^{-1}$ using ferrocene-modified carbon nanotube paste electrodes. In this work using MWCNTs/PNB-modified electrodes, as shown in Table 1, the sensitivity is significantly higher and the LOD lower than in the articles mentioned above.

4. Conclusions

Glassy carbon electrodes have been modified by coating with MWCNT and then poly(Nile blue), by drop coating with $20 \mu\text{L}$ of 0.2% MWCNT/DMF and then polymerising NB electrochemically in 0.1 M PBS at pH 6.0.

The MWCNT/PNB-modified electrode was used to investigate the electrochemical behaviour of the anti-Parkinsonian agents carbidopa and benserazide. Cyclic voltammetric measurements showed an irreversible behaviour in 0.1 M PBS at all pH values for both CD and BS. From the relationship of pH and peak currents and shapes, 0.1 M PBS at pH 5.0 was selected as supporting electrolyte for further studies. Scan rate studies showed that the process of MWCNT/PNB-modified electrodes was diffusion controlled with some evidence of thin-layer behaviour at high scan rate.

Analytical characterisation of CD and BS was achieved using DPV as a rapid, selective, sensitive, cheap, and simple technique. Linear calibration curves were obtained for both CD and BS. It was shown that the DPV method used gave good precision, accuracy, and sensitivity for CD and BS.

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