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A simple and high throughput methodology for simultaneous determination of levodopa and carbidopa

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Abstract

Parkinson's disease (PD) is a degenerative disorder of the central nervous system. The motor symptoms of PD disease result from the death of dopamine-generating cells in a region of the mid brain and the dopamine precursor levodopa (L-Dopa) is used for the treatment. Carbidopa (Car) is administered in association with L-Dopa in pharmaceutical formulation as an inhibitor on the decarboxylase activity. Thus, their simultaneous determination is of great importance because of their co-existence in pharmaceutical preparations. Present study deals with a simple method development for simultaneous voltammetric determination of L-Dopa and Car at a pencil graphite electrode (PGE) via monitoring the reduction peak of L-Dopa and the second oxidation peak of Car. The developed method exhibited a linear range for L-Dopa and Car between 0.29 – 3.06 and 0.22 – 3.3 μM and the limit of detection was calculated as 0.096 and 0.073 μM for L-Dopa and Car, respectively. The sensitivity of the method was found comparable to other methods depending on the sophisticated electrode modifications and the limits of detection were calculated as sub micromolar levels.

Keywords: Levodopa, carbidopa, pencil graphite electrode, assay method, differential pulse voltammetry

1. Introduction

Pharmaceuticals play an important part in human health; however, these compounds serve their purpose only if they are given in an appropriate amount and do not contain impurities. [1]. Every step of the process, starting with the development of a new molecule, up to clinical trials to reveal the optimum dose, requires analytical methods to monitor drug interaction and quality control purposes. Therefore, pharmaceutical analyzes require fast, precise, and selective methods with high efficiency to analyze trace amounts and screening approaches in mixtures [2].

Chromatographic methods have been widely used for quantitative and qualitative analysis of drug substances in tablets and biological fluids. Recently, liquid chromatography with tandem mass spectrometry (LC-MS/MS) is the method of choice in many laboratories [3]. As an economical alternative to the chromatographic techniques, voltammetric methodologies are emerging that offer high precision, accuracy, and precision for many analytical applications. In the past two decades, several review articles have been presented in the field of drug analysis using solid electrodes [5] and carbonaceous materials for electrode fabrication [4]. Gupta and co-workers have reviewed the voltammetric techniques for drug analysis to assess the performance of various electrodes and surface-active agents in determination [6]. In particular, pencil graphite electrodes are promising materials for drug analysis [7].

Levodopa (L-Dopa, L-3,4-dihydroxyphenylalanine) is the precursor of catecholamines produced via biosynthesis from L-tyrosine. L-Dopa is commonly known as the drug used for Parkinson's disease, but its activity suffers from its conversion to dopamine by a decarboxylation process [8,9]. Since a small amount of L-Dopa can be transported across the cerebral tissue to the central nervous system, another reagent is required for inhibiting the decarboxylase activity. Car has widely served for this purpose, and it is administered with L-Dopa for the treatment [10]. Consequently, their simultaneous analysis is important task in pharmaceutical quality control purposes.

Due to the electroactive nature of catecholamines, voltammetric methods are widely used for their determinations in pharmaceutical formulations [11].

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For complex sample matrices, on the other hand, electrochemical methods are prone to interferences and often require special precautions. In addition, electrochemical behavior of catecholamines may differ significantly due to minor changes in their structure allowing us to determine these compounds simultaneously. Accordingly, carbon paste electrode (CPE) modified by meso-tetrakis (3-methylphenyl) cobalt porphyrin (CP) and TiO_2 nanoparticles was utilized for this purpose [12]. In a more recent study the carbon paste electrode was functionalized with $NiFe_2O_4$ nanoparticle and 2-(4-ferrocenyl-[1,2,3]triazol-1-yl)-1-(naphthalen-2-yl) ethanone for simultaneous determination of Car and L-Dopa [13]. For glassy carbon electrode (GCE) this selectivity was maintained by covering the electrode with a Nafion film, which is selective for Car in the presence of L-Dopa [14].

Alternatively, the GCE has been modified with graphene nanosheets by chemically reducing graphene oxide by using hydrazine allowing the simultaneous analysis of L-Dopa and Car in micromolar levels [15]. Chemometric approach can also be a solution for the peak convolution issue and an analytical method based the partial least-squares algorithm enabled the simultaneous determination of L-Dopa, Car and benserazide in pharmaceutical formulations [16]. However, in real sample analysis, other precautions should be considered. Beitollah et al have developed a CPE containing of multiwall carbon nanotubes (MWCNT) and an ionic liquid (1-methyl-3-butylimidazolium bromide) which showed good electrocatalytic effect on Car by shifting the potential in negative direction in comparison to the bare CPE and increasing the peak potential as well [17]. It was also reported that the stability of the CPE was enhanced greatly due to the introduction of ionic liquid as a binder. The sensor was successfully applied for the determination of Car in human urine and serum and the interference of ascorbic acid was minimized by using ascorbic oxidase enzyme.

Although proper modification of the electrodes provides the desired selectivity and sensitivity by changing the peak potentials and enhancing the peak formation, modification procedures are often more complex, and it is rather difficult to maintain reproducible surfaces in fabrication. On the other hand, PGE provides an inexpensive alternative to other carbonaceous electrodes due to its low background current along with high electrochemical reactivity [18]. Since these electrodes can be readily used by eliminating time-consuming cleaning and polishing processes, PGEs are widely utilized in detection of a wide variety of electroactive species [19,20] and in developing genosensors [21].

In this study, a simple and sensitive voltammetric method was developed for the simultaneous determination of L-Dopa and Car in pharmaceutical formulations. To the best of our knowledge, this is the first study dealing with the simultaneous determination of L-Dopa and Car by using the PGEs without any modification and not requiring further chemo-metric modelling for achieving required selectivity. The anodic and cathodic peak were both used for quantification of Car and L-Dopa with aid of Differential pulse voltammetry (DPV) technique. Two different PGEs were utilized for the purpose and their response was evaluated in terms of sensitivity and selectivity.

2. Materials and methods

All chemicals were of analytical grade. L-Dopa (99.98%) and Car (99.83%) were purchased from Sigma Aldrich. Stock solutions of L-Dopa and Car were prepared in 0.01 M chloroacetic acid solution. Sinemet 25/250 mg tablets were purchased from a local drug store. The chloroacetic acid was purchased from Merck and 0.01 M chloroacetic acid solution was used for buffer preparation along with the 3.0 M NaOH solution.

The developed sensor was used for the quantification of L-Dopa and Car in Sinemet 25 / 250 mg tablets with the aid of standard addition method. Briefly, Sinemet 25 / 250 mg tablet was ground into fine particles by using agate mortar and a 1.000 g portion of tablets were weighted precisely. Ultrapure water: methanol mixture (1:1, v/v) is added into the beaker and placed into an ultrasonic bath for the extraction of active substances and filtered through the Teflon syringe filter. Then, the filtrate was added into the cell for voltammetric measurement. Subsequently, known amount of standard solution of L-Dopa and Car was added into the same cell three times. The obtained peak currents were plotted against to added concentration and sample concentrations were calculated extrapolated the obtained curve the concentration axis.

Electrochemical measurements were recorded with Metrohm PGST 204 Potentiostat equipped with conventional three electrodes system. In typical experiment, Ag / AgCl (sat'd. KCl), platinum wire was used as the reference and counter electrode, respectively. Faber 0.7 / 2B pencil graphite electrode denoted as PGE-A, and Tombow 0.3 / HB pencil lead denoted as PGE-B were used as the working electrodes. Chloroacetic acid buffer was used during electrochemical measurements. The potential was cycled between -0.5 and 1.0 V at a scan rate of 50 mV/s during the cycling voltammetry scan.

All measurements were tripled for obtaining a steady state current. Differential pulse voltammetric

measurements were carried out in a range of 0 – 1.0 V at a rate of 10 mV/s and pulse amplitude was set as 25 mV.

3. Results and Discussion

3.1. Voltammetric behavior of L-Dopa and carbidopa

Initial studies were devoted to reveal voltammetric behavior of L-Dopa and Car at both electrodes at pH 2.0 chloroacetic acid buffer solution. Cyclic voltammograms (CVs) of 2.54 × 10^{-4} M L-Dopa recorded at PGE-A and PGE-B at a scan rate of 50 mV/s reflect the differences in the chemical composition of both graphite materials (Fig. 1a). CVs recorded at former electrode have revealed a quasi-reversible peak formation at 0.57 V and a corresponding cathodic peak at 0.39 V while, rather small but closer peak formations were observed for the latter electrode having the peak potentials of 0.46 and 0.42 V for anodic and cathodic processes, respectively. The mineral content that gives the hardness of the PGE-B could be responsible for the reversible behaviors of both analytes while the polymeric content of PGE-A results in more analytes to attach onto the surface.

For 2.21 × 10^{-4} M Car on the other hand, two oxidation peaks with equal heights have been observed at 0.58 V and 0.95 V at PGE-A (Fig. 1b), with a very small cathodic counterpart in agreement with a previous study at a GCE [22]. For PGE-B, the subsequent oxidation peak potentials were 0.44 V and 0.82 V, where the first peak was higher than the second peak. Considering the higher sensitivity for L-Dopa, PGE-A was chosen for further studies.

Since the electrochemical behaviors of L-Dopa and Car are dependent on the pH of the solution, the influence of the medium pH on these peak characteristics was investigated. Fig. 2 has shown that the peak potentials of L-Dopa have shifted into more negative potentials as the pH increases, and irreversible electrode reactions have been observed in alkaline media. For Car, on the other hand, irreversible electrode process has been observed for all pHs and the peak potentials of the anodic peaks have given linear curves with the pH with the slopes of -42 and -64 mV pH^{-1}, respectively, suggesting that the reaction mechanism includes equal number of proton and electron evolved during the oxidation processes [23]. The peak currents were also altered with the solution pH as expected. Since the highest peak currents have been observed at pH 2.0 for both compounds, this medium pH was selected for further studies.

Analytical characteristics of the method for L-Dopa and Car were studied by recording DP voltammograms individually at pH 2.0 chloroacetic acid medium.

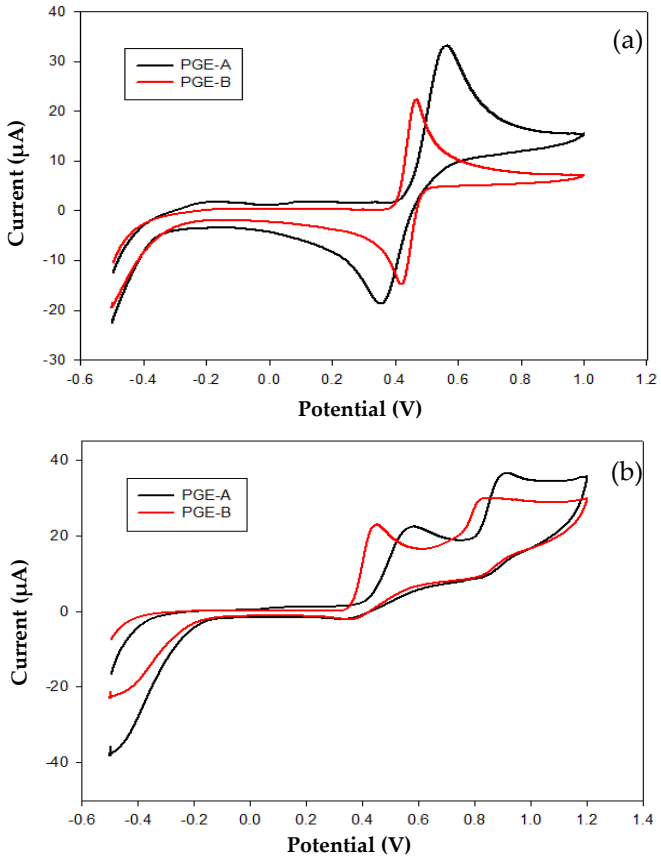

Figure 1. Cyclic voltammetric behavior of (a) 2.54 × 10^{-4} M L-Dopa and (b) 2.21 × 10^{-4} M Car in pH 2.0 chloroacetic acid buffer at two different PGEs at 50 mV/s scan rate

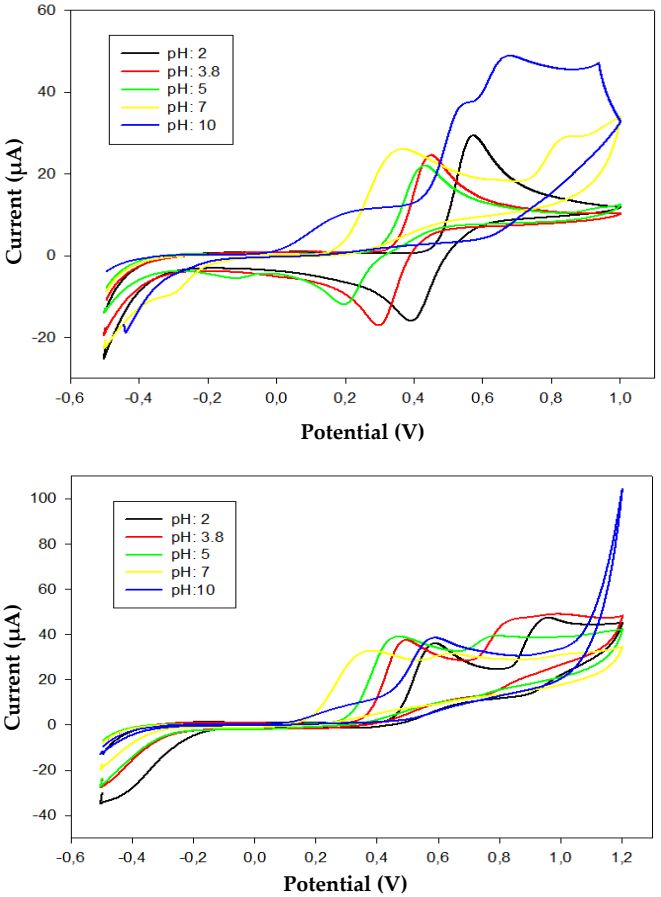

Figure 2. The influence of the medium pH on the cyclic voltammetric behaviour of a) 2.54 × 10^{-4} M L-Dopa and b) 2.21 × 10^{-4} M Car at PGE-A at a scan rate of 50 mV/s

Table 1. Analytical merits of the individual voltammetric analysis of the analytes

	Measured Peak	Dynamic range	Equation	LOD / LOQ	RSD (%)	Recovery
L-Dopa	**Anodic peak at 0.42 V**	$2.9 \times 10^{-7} – 2.8 \times 10^{-6}$ M	y = 0.00275x-0.0034 R^2 = 0.9947	LOD: 0.88×10^{-7} M LOQ: 2.90×10^{-7} M	8.26×10^{-7} M: 3.54% 1.94×10^{-6} M: 4.63%	8.26×10^{-7} M: 86.81% 1.94×10^{-6} M: 93.68%
	Cathodic peak at 0.45 V	$2.9 \times 10^{-7} – 3.06 \times 10^{-6}$ M	y = 0.0011x-0.0055 R^2 = 0.9915	LOD: 0.88×10^{-7} M LOQ: 2.90×10^{-7} M	8.26×10^{-7} M: 6.67% 1.94×10^{-6} M: 5.08% 2.81×10^{-6} M: 2.99%	8.26×10^{-7} M: 83.75% 1.94×10^{-6} M: 90.37% 2.81×10^{-6} M: 92.78%
Car	**Anodic peak at 0.46 V**	$6.54 \times 10^{-7} – 1.94 \times 10^{-6}$ M	y = 0.0138x-0.0012 R^2 = 0.9901	LOD: 1.98×10^{-7} M LOQ: 6.54×10^{-7} M	8.26×10^{-7} M: 16.8% 1.94×10^{-6} M: 7.49% 2.81×10^{-6} M: 2.44%	8.26×10^{-7} M: 76.0% 1.94×10^{-6} M: 90.4% 2.81×10^{-6} M: 96.19%
		$2.25 \times 10^{-6} – 3.29 \times 10^{-6}$ M	y = 0.0293x-0.0189 R^2 = 0.9936			
	Anodic peak at 0.82 V	$6.54 \times 10^{-7} – 1.60 \times 10^{-6}$ M	y = 0.0301x+0.0042 R^2 = 0.9758	LOD: 1.98×10^{-7} M LOQ: 6.54×10^{-7} M	8.26×10^{-7} M; 0.79% 1.94×10^{-6} M: 2.23% 2.81×10^{-6} M: 10.9%	8.26×10^{-7} M: 92.67% 1.94×10^{-6} M: 96.23% 2.81×10^{-6} M: 97.92%
		$1.94 \times 10^{-6} – 3.29 \times 10^{-6}$ M	y = 0.0462x-0.0311 R^2 = 0.9656			

For L-Dopa, both anodic and cathodic peak currents have been employed for constructing the calibration curve and a linear relation between anodic peak current at 0.42 V in the concentration range of $2.9 \times 10^{-7} – 2.8 \times 10^{-6}$ M (S-1). The repeatability of the electrode was tested by measuring the samples spiked with 8.26×10^{-7} and 1.94×10^{-6} M L-Dopa with taking account the anodic peak and relative standard deviation (RSD) was found less than 5% (Table 1).

Cathodic scan has also yielded a linear calibration graph for L-Dopa by monitoring the cathodic peak at 0.45 V in the concentration range of 2.9×10^{-7} - 3.1×10^{-6} M (S - 2). Limit of detection (LOD) and limit of quantification (LOQ) was calculated based on a signal-to-noise ratio (S / N = 3) and the limit of quantitation (LOQ) was determined to be 3.3 times the LOD. On the other hand, two linear segments were found for two oxidation peaks of Car in the concentration range studied in agreement with former studies [13]. The fact that the decrease in the slope of the second linear segment is likely due to kinetic limitation [18]. For the first anodic peak at 0.46 V, the calibration curve was linear in the range of $6.54 \times 10^{-7} – 1.94 \times 10^{-6}$ M while the other calibration graph was drawn in a rather narrow concentration range in micromolar level (S-3). The equations and other characteristics of the curve were listed in the Table 1. The accuracy of the individual analysis method was tested by analyzing quality control samples and the satisfactory recoveries were obtained for both analytes.

3.2. Simultaneous determination of L-Dopa and carbidopa

As can be deduced from the above studies, the anodic peak of L-Dopa at 0.42 V overlaps with the first anodic peak of Car at 0.46 V, which makes it difficult their simultaneous determination. Considering the irreversible behavior of Car in the potential range studied, L-Dopa can be determined free from the interference of Car in their binary mixture, by monitoring its reduction peak. A previous study utilized this peak for sensitive determination of L-Dopa without the interference of Car but, they could manage to determine the Car upon coating the electrode with a selective Nafion film. Here, by taking the advantage of sensitive determination of Car through using the both anodic peaks in micromolar levels, the second peak at 0.82 V which is laid down on the potential region was utilized for quantization free from the interference of L-Dopa.

Calibration studies have been repeated with binary mixtures for controlling the selectivity of the method. The cathodic peak of L-Dopa in micromolar level was recorded in pH 2.0 chloroacetic acid buffer solution by scanning the potential in DPV mode between 0.8 and 0 V at a rate of 10 mV/s and Car was added in equal concentrations into the cell to see any interference. No significant change was observed in the cathodic peak of L-Dopa. Here, the concentration ratio of the analytes may be important on the other peak formation. Considering that the method is intended to be applied to commercial formulations which contain 25 mg Carbidopa and 250 mg Dopa in dosages, the ratio of 1:10 was adopted.

The calibration curve was constructed for L-Dopa in a concentration range of $1.0 \times 10^{-5} – 2.0 \times 10^{-4}$ M (R^2: 0.9894). Reproducibility was studied for 5.0×10^{-5} M L-Dopa concentration, and the mean signal was calculated as 0.1183 ± 0.01607 µA. The RSD value was calculated as 13.58% for the studied concentration. The mean background current was calculated as 0.0055 µA and the LOD and LOQ values were calculated as 3.58×10^{-6} M and 1.19×10^{-5} M, respectively.

Fig. 3 shows the DP voltammograms of Car in pH 2.0 chloroacetic acid buffer solution upon addition of standard solution containing tenfold concentration of L-Dopa. The first anodic peak at 0.55 V is the overlapped peak obtained for both L-Dopa and Carbidopa, and the second anodic peak given inset belongs to Car alone.

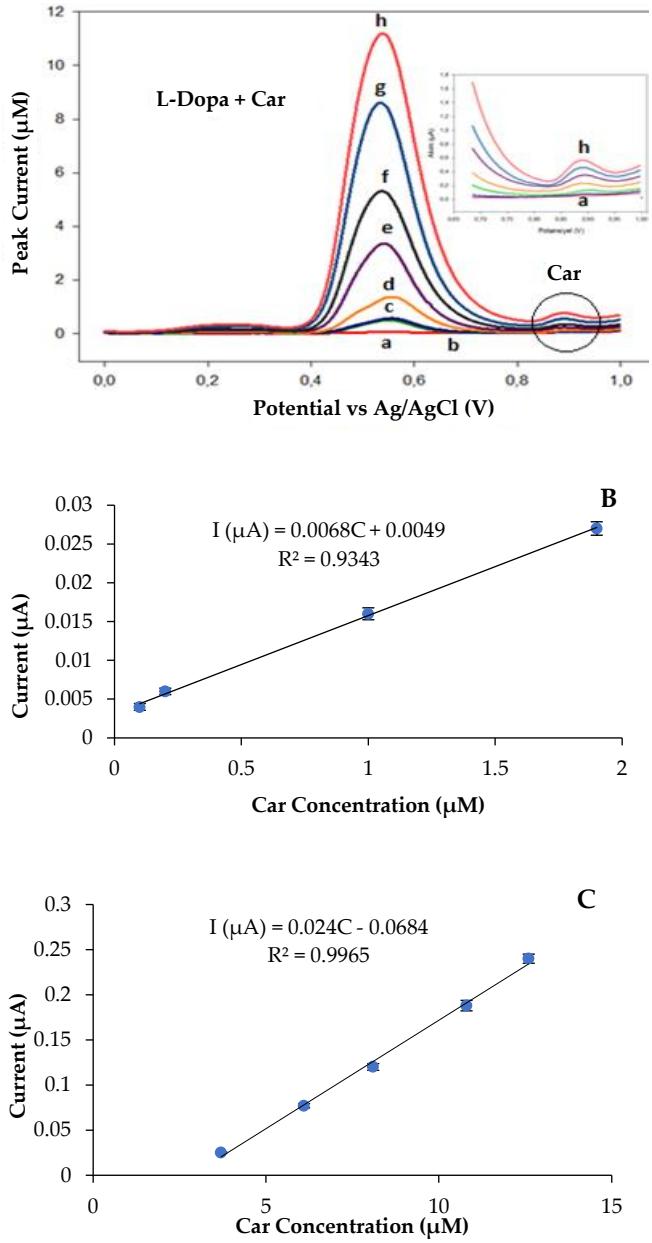

Figure 3. A) DP voltammograms recorded at 10 mV/s scan rate in pH 2.0 chloroacetic acid buffer upon addition of standard solution containing tenfold concentration of L-Dopa to be a) 2.0×10^{-7} M, b) 9.88×10^{-7} M, c) 1.94×10^{-6} M, d) 3.73×10^{-6} M, e) 6.13×10^{-6} M, f) 8.05×10^{-6} M, g) 1.08×10^{-5} M, h) 1.26×10^{-5} M Car in the cell and B) the lower range and C) higher range calibration curves drawn for the second anodic peak of Car.

Again, two linear calibration curves have been observed for the Car in the concentration ranges of $2.0 \times 10^{-7} - 1.94 \times 10^{-6}$ M (R^2: 0.9343) and $3.73 \times 10^{-6} - 1.26 \times 10^{-5}$ M (R^2: 0.9971). Reproducibility was studied for low, medium and high concentration levels of the calibration graph and for 3.73×10^{-6} M, the RSD was estimated as 6.97% while it was calculated as 6.43% for 8.05×10^{-6} M. Higher concentrations for Car (1.26×10^{-5} M) has resulted an RSD of % 4.29 which is in the acceptable limits. The LOD and LOQ values were calculated as 1.24×10^{-7} M and 4.13×10^{-7} M, respectively. The accuracy of the method was tested by recovery measurements. Recovery was calculated for 84% for 3.73×10^{-6} M, 97% for 8.05×10^{-6} M and 98% for 1.26×10^{-5} M.

Table 2. Analytical merits of the standard addition method for the commercial tablet analysis

Parameter	L-Dopa	Car
Dynamic range	$1.01 \times 10^{-6} - 1.81 \times 10^{-4}$ M	$2.9 \times 10^{-7} - 1.3 \times 10^{-5}$ M
Equation	y = 0.4452x + 0.0169	y = 0.0314x - 0.0209
R^2	0.9956	0.9849
LOD	0.3×10^{-7} M	1.0×10^{-7} M
LOQ	1.01×10^{-6}	2.9×10^{-7} M
Labeled value	4.80×10^{-6} M	4.20×10^{-7} M
Measured value	5.19×10^{-6} M	3.92×10^{-7} M
%Recovery	108.12%	93.33%
Confidence Interval (α=0.05, n=3)	89.2 – 111.8 %	86.8 – 113,2 %
Precision (RSD%)	(1.01×10^{-6} M)	(2.9×10^{-7} M)
Intraday	6.43	7.21
Interday	7.71	8.95

3.3. Method application

Overall results have indicated that a disposable bare pencil lead electrode can be used for simultaneous determination of Car and L-Dopa sensitively and selectively. The performance of developed method was applied for commercial tablets. The tablet was homogenized and prepared as described above. Then, the sample solution was transferred to the electrochemical cell for subsequent determination. The DP anodic scan was initiated from 0.8 to 0.0 V and the cathodic peak signals of L-Dopa was recorded upon standard addition onto the sample solution. In order to avoid the possible matrix effect of Sinemet tablet excipients, standard addition method was used for sample application. Table 2 shows the obtained results and the recovery values for the Sinemet tabled studied.

The comparison of the developed sensor with the literature was given in Table 3. The proposed electrode exhibits great sensitivity towards to L-Dopa and Car. The LOD and LOQ values are comparable with those studies which use electrode modifiers. Since, the other studies have modifiers, the sensor developed is found more economic, less hazardous for environment and ready to use without time consuming fabrication.

4. Conclusion

Present study describes a practical solution for the overlapped peaks of Car and L-Dopa which are important to be determined simultaneously in commercial tablets. A disposable pencil graphite electrode was utilized as the electrode via monitoring the reduction peak of L-Dopa and the second oxidation peak of Car without any significant interference. Even though the LOD calculated for individual analytes are sub micromolar levels, in their binary mixtures the electrode performance was found comparable with the modified electrodes as given in Table 3.

Table 3. Comparison of the performances of the electrochemical methods developed for simultaneous analysis of Car and / or L-Dopa

Analyte	Matrix	Detection Method	Electrode	Linear Range	LOD	Reference
L-Dopa **Car**	Water, Urine, Blood serum	DPV	Coumarin derivative / TiO_2 / IL / CPE	0.10 – 900 µM 20.0 – 900 µM	41 nM 0.38 µM	[9]
L-Dopa **Car**	Water, Urine Blood serum Pharmaceutical tablets	DPV	Meso-tetrakis (3-methyl phenyl) (CP) / TiO_2NPs / CPE	0.1 – 100 µM —	69 ± 2 nM —	[12]
L-Dopa **Car** **Benserazide**	Pharmaceutical tablets	DPV / Multivariate calibration	GCE	110–1300 µM 31– 470 µM 31–620 µM	5.12 µM 2.16 µM 2.77 µM	[16]
L-Dopa **Car** **Tryptophan**	Pharmaceutical tablets	CV	EBNBH/CNT CPE	0.2 – 700 µM — —	0.094 µM 7.2 µM 12.3 µM	[22]
Car **Droxidopa**	Urine, Blood serum	SWV	CNT / 5-amino-2′-ethyl-biphenyl-2-ol / CPE	0.2 – 700 µM 0.12 – 225 µM	— 50 nM	[24]
L-Dopa **Uric acid** **Folic acid**	Pharmaceutical tablets, Urine, Blood serum	SWV	DHB / AuNPs / RGO / GCE	0.05–1200 µM — —	0.018 µM — —	[25]
Car **Uric acid** **Folic acid**	Urine	SWV	MWCNT / CPE	0.09– 400 µM — —	0.071 µM — —	[26]
L-Dopa **Car**	pharmaceutical formulations	DPV	lead dioxide immobilized in a polyester resin	260 – 1200 µM 32 – 150 µM	25 µM 3.7 µM	[27]
L-Dopa **Car**	Pharmaceutical tablets	DPV	PGE	1.01–18.1 x10^{-5} M 0.98–8.05 µM	2.72 µM 0.44 µM	Present Study

CPE: Carbon paste electrode, EBNHB: 2, 2′-[1,2-ethanediylbis (nitriloethylidyne)]-bis-hydroquinone, CP: cobalt porphyrin, TiO_2 NPs: Titanium dioxide nanoparticles, IL: ionic liquid, DHB: 2-(3,4-dihydroxyphenyl benzothiazole), AuNPs: Gold nanoparticles, RGO: Reduced graphene oxide, MWCNT: multiwalled carbon nanotube, PGE: Pencil graphite electrode, SWV: Square wave voltammetry, CV: Cyclic voltammetry

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