Simultaneous Derivative Spectrophotometric Determination of Levodopa and Carbidopa in Pharmaceutical Preparations

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The present work reports on the investigations based on the interaction of sodium hydroxide with catechol derivatives. The first derivative spectra allowed the simultaneous determination of levodopa and carbidopa. The calibration graph was constructed from the first derivative signals by measuring ^{1}D at 350 nm for standard samples containing between 3 and 30 mg L^{-1} of levodopa and at 305 nm for standard samples containing between 3 and 80 mg L^{-1} of carbidopa. This method offers the advantages of simplicity, rapidity, selectivity and higher sensitivity than most of the existing spectophotometric methods.

Key Words : Derivative spectrophotometry, Simultaneous determination, Levodopa, Carbidopa

Introduction

Levodopa [(−)3-(3,4-dihydroxylphenyl)-L-alanine] is an important neurotransmitter, which has been used for the treatment of neural disorders such as Parkinsons disease. The cause of this disease is the significant depletion of dopamine.[1] After its oral administration, levodopa is absorbed through the bowel and converted into dopamine by decarboxylase. Hence, levodopa can relax the symptoms of Parkinsons disease and also decrease muscular rigidity, oculogyric crises, and tremor. However, elevated levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias. Carbidopa [(3,4-dihydroxybenzyl)-2-hydrazinopropionic acid] has been used as an inhibitor of the decarboxylase activity. By administering levodopa combined with carbidopa, the concentration of dopamine is controlled at appropriate level effectively and with generally reduced side effects.[2] In order to achieve better curative effect and lower toxicity, it is very important to control the content of levodopa and carbidopa in pharmaceutical tablets. In view of their importance, considerable work has been done on their detection and quantification. Various methods such as, spectrofluorimetry,[3,4] gas chromatography,[5,6] high performance liquid chromatography (HPLC),[7-9] radio immunoassay,[10] chemiluminescence[11-13] and voltammetric determination[14,15] have been reported in the literature for the determination of these compounds in various biological samples and pharmaceutical preparations. Many spectrophotometric methods have been proposed. Some of them require long heating[16] or involve nonaqueous media.[17] Some other spectrophotometric methods have very narrow limits of detection.[18]

Several methods have also been reported for simultaneous determination of levodopa and derivates of cathecholamines. Michotte and coworkers[19] determined levodopa, carbidopa, 3-O-methyldopa and dopamine simultaneously in plasma by HPLC with electrochemical detection. Sagar and Smyth[20] reported simultaneous determination of levodopa, carbidopa, and their metabolites by electrochemical detectors combined with HPLC (LC-EC). The peak currents obtained for the different analytes were directly proportional to the analytes over the concentration range 0.02-4.0 μg mL^{-1}. Coello *et al.*[21] studied simultaneous kinetic- spectrophotometric determination of levodopa and benserazide by bi- and three-way partial least squares calibration that permit the quantification of both analytes with a precision on the order of 0.7% for levodopa and 1.5% for benserazide. Talebpour *et al.*[22] used ^{1}H NMR spectroscopy analysis for simultaneous determination of levodopa, carbidopa, and methyldopa in human serum and pharmaceutical formulations. The detection limit was estimated as 4.2, 1.7 and 1.6 μg mL^{-1} for levodopa, carbidopa and methyldopa, respectively. Uslu and Ozkan[23] determined the binary mixtures of levodopa and benserazide by derivative spectrophotometry. The linear ranges were 10 to 70 μg/mL for levodopa and 5 to 50 μg/mL for benseazide. Many of these methods are either not sensitive enough or require complicated and expensive instruments, or are subjected to interferences from other ions, suffer from small calibration range, toxicity of the reagents used, or more or less are time consuming.

The present work reports on the investigations based on the interaction of sodium hydroxide with catechol derivatives. The first derivative spectra allowed the simultaneous determination of levodopa and carbidopa. This method offers the advantages of simplicity, rapidity and specificity without the need of extraction or heating besides having higher selectivity range than most of the existing spectophotometric methods.

Experimental Section

Apparatus. A Perkin-Elmer-Lamba45 (1.23) Model UV-Visible spectrophotometer with 1.0-cm matched cells was used for the electronic spectral measurements.

Reagents. Levodopa, LDP, and carbidopa, CDP, were

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prepared from Ramin laboratory (Tehran), and sodium hydroxide was prepared from Merck. All other chemicals used were of analytical reagent grade. Deionised water was used to prepare all solutions and in all experiments.

Solutions. Freshly prepared aqueous solutions of the pure drugs were used as the standard solution for analytical purposes. A 1 mol/L of aqueous sodium hydroxide was used.

General procedure. Aliquots of standard solutions of LDP or CDP were transferred into a 10-mL calibrated flask, 1 ml of 1 mol L^{-1} sodium hydroxide was added to the catecholamines solution, and the mixture was allowed to stand for 7 min at room temperature. The contents were then diluted to the mark and mixed well. The derivative absorbance at ^{1}D-max was measured against water blank.

Results and Discussion

Preliminary studies. Figure 1 shows the absorption spectra of levodopa and carbidopa in distilled water, it was observed that the spectra of these two compounds completely overlap and each compound interferes in the spectrophotometric determination of other one, but a shift in maximum absorbance wavelength of levodopa is attained after adding sodium hydroxide.

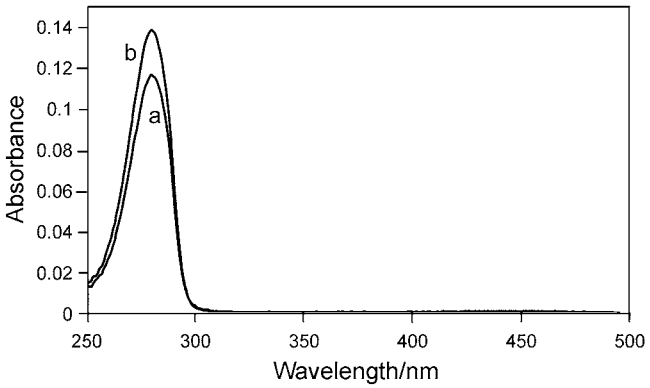


Figure 1. The UV-Vis spectra of (a) 10 mg/L of carbidopa and (b) 10 mg/L of levodopa in distilled water.

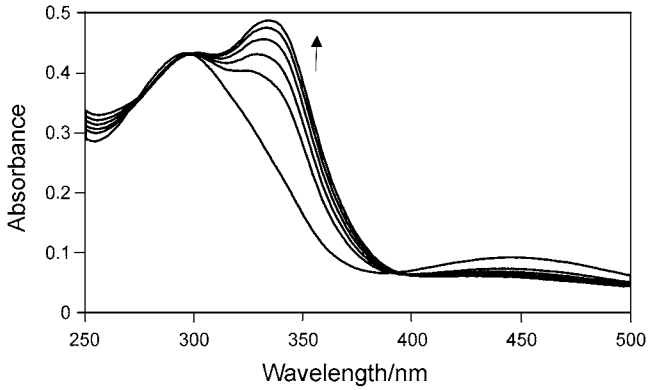


Figure 2. The UV-Vis spectra of 10 mg L^{-1} levodopa in the presence of 1 mol L^{-1} NaOH at different times between 1-7 minutes. From bottom to up 1, 3, 4, 5, 6 and 7 min.

Figure 2 shows the interaction between levodopa and sodium hydroxide produces a yellow solution, which presents two absorption bands at 300 and 440 nm. However, the band at 440 nm decreased gradually and after 3 min a new absorption band appeared at 330 nm. This behaviour can be explained by the oxidation of levodopa to the open-chain quinone (II), which showed absorption at 440 nm and after that evolves to leucodopachrome (III)[24] (Scheme 1).

Figure 3 shows the interaction between carbidopa and

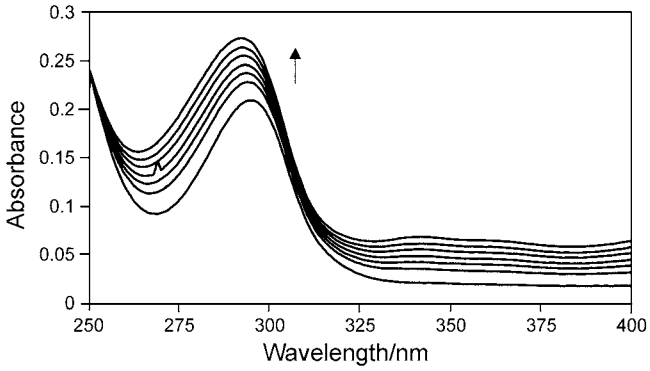
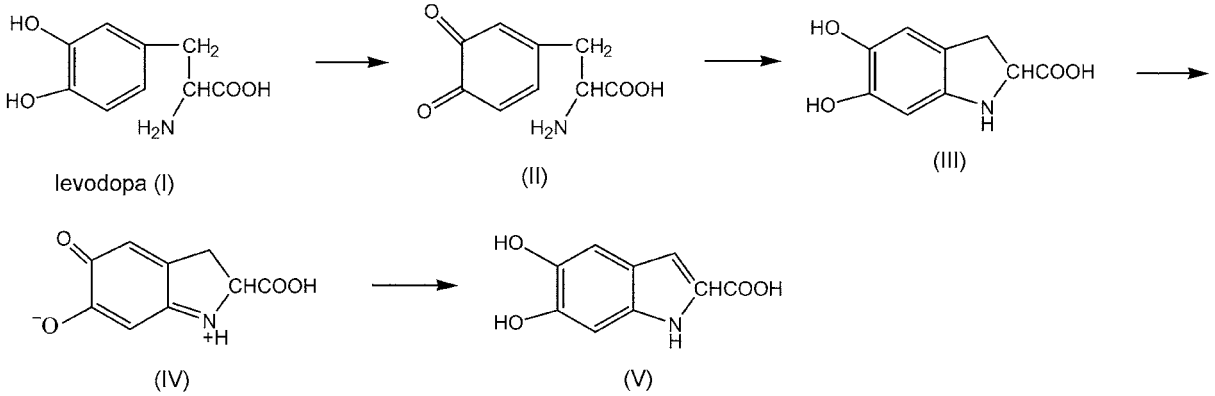
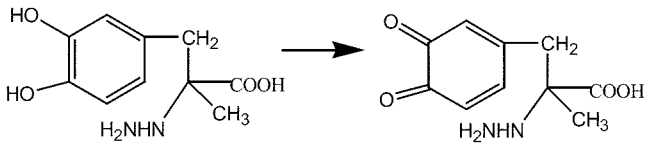


Figure 3. UV-Vis spectra of carbidopa in the presence of 1 mol L^{-1} NaOH at different times between 1-7 minutes. From bottom to up1, 3, 4, 5, 6 and 7 min.



Scheme 1. Oxidation of levodopa in basic media.



Scheme 2. Oxidation of carbidopa in basic media.

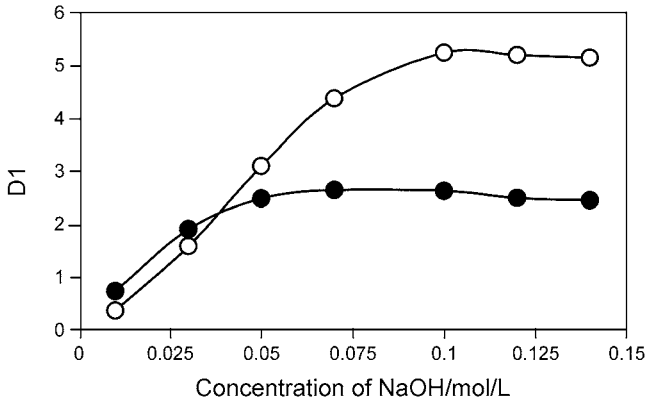


Figure 4. Effect of sodium hydroxide concentration on the oxidation of 10 mg/L of levodopa (●), 10 mg/L of carbidopa (○) after 7 min.

sodium hydroxide produces a yellow solution, which presents one absorption band at 290 nm (Scheme 2).

Optimum hydroxide concentration. The effect of sodium hydroxide concentration on the absorbance of 10 mg L^{-1} of levodopa and carbidopa solutions was studied in the range of 0.00-0.15 mol L^{-1}. The results are shown in Figure 4. As Figure 4 shows, 1 mol L^{-1} of sodium hydroxide is necessary to achieve maximum color intensity.

Optimum instrumental conditions. The main instrumental parameters that affect the shape of the derivative spectra are wavelength scanning speed and the wavelength increment over which the derivative is obtained ($\Delta\lambda$). These parameters need to be optimized to give a well-resolved large peak *i.e.* to give good selectivity and large sensitivity in the determination.

Generally, the noise level decreases with an increase in $\Delta\lambda$, thus decreasing the fluctuations in the derivative spectrum. However, if the value of $\Delta\lambda$ is too large, the spectral resolution is very poor. Therefore, the optimum value of $\Delta\lambda$ determined by taking into account the noise level, the resolution of the spectrum and the sample concentration. Some values of $\Delta\lambda$ were tested and 2.0 were selected as the optimum.

The scanning speed fast, medium and slow was studied and a medium scanning speed was selected.

First derivative spectrophotometry. Figure 5 shows the absorption spectra of 10 mg L^{-1} levodopa, carbidopa and their mixture in the presence of 1 mol L^{-1} sodium hydroxide after 7 min. As Figure 5 shows, the large overlap of the spectral bands of the drugs between 230.0-330.0 nm prevents the simultaneous determination of these two compounds in the mixture from their zero- order spectra. But the first derivative spectra allowed the simultaneous

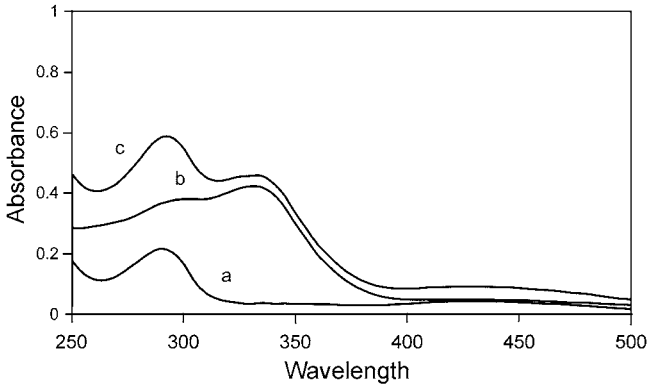


Figure 5. Absorption spectra of: (a) 10 mg L^{-1} carbidopa, (b) 10 mg L^{-1} levodopa, and (c) their mixture in 1 mol L^{-1} of sodium hydroxide after 7 min.

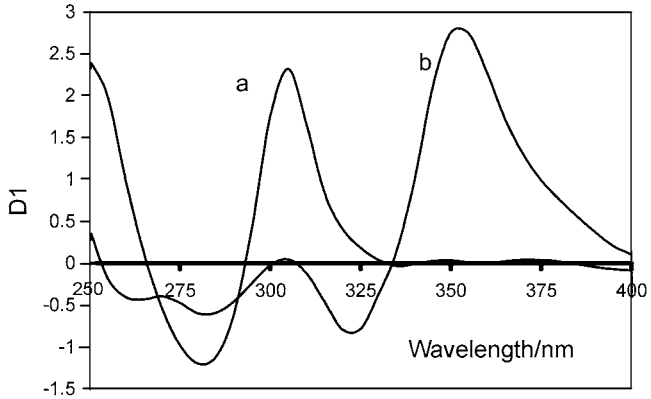


Figure 6. First derivative spectra of (a), 10 mg L^{-1} carbidopa, and (b), levodopa in the presence of 1 mol L^{-1} NaOH after 7 min.

determination of levodopa and carbidopa. Figure 6 shows the first derivative spectra of levodopa and carbidopa. The ^{1}D spectrum of carbidopa shows a well-defined maximum at 305 nm while levodopa has a zero ^{1}D value at this wavelength. Levodopa has a maximum ^{1}D value at 350 nm at which carbidopa exhibits no contribution. Therefore, at

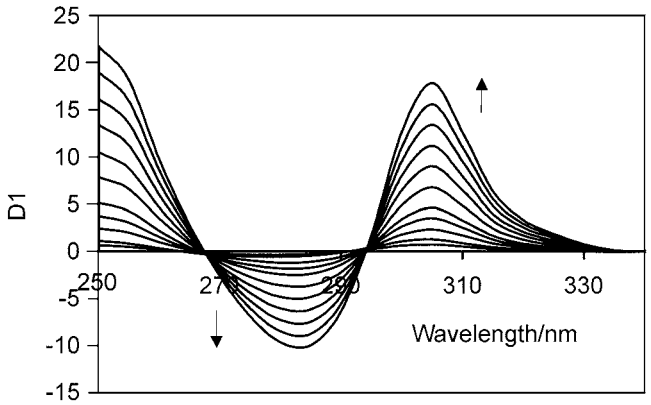


Figure 7. First derivative spectra of different concentrations of carbidopa in 1 mol/L sodium hydroxide (3 mg L^{-1}-80 mg L^{-1}). From bottom to up 3, 5, 10, 15, 20, 30, 40, 50, 60, 70, and 80 mg L^{-1} after 7 min.

Table 1. Regression equation for the determination of levodopa and carbidopa in the mixtures by first-derivative spectrophotometry

Compound	Measurment	Linearity range (mg L^{-1})	Regression analysis (n = 4)				
			Detection limit (mg L^{-1})	Intercept	Slope	r [a]	RSD%
Levodopa	$^1D_{350}$	3.00-30.00	0.08	–0.12	0.2765	0.9936	0.14
Carbidopa	$^1D_{305}$	3.00-80.00	0.10	0.1123	0.22155	0.9998	0.04

[a]Correlation coefficient

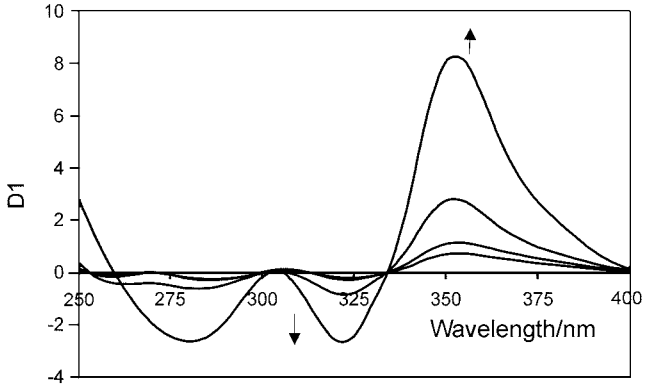


Figure 8. First derivative spectra of different concentration of levodopa in 1 mol/L of sodium hydroxide (3 mg L^{-1}-30 mg L^{-1}) From bottom to up 3, 5, 10, and 30 mg L^{-1} after 7 min.

these selected wavelengths the two drugs can be quantified in the presence of each other without interference from each other.

Calibration graphs and statistical analysis.

Linearity: The calibration graphs were constructed under the optimum conditions from the first derivative signals by measuring the ^1D signal at 350 nm for levodopa, (Fig. 7) and at 305 nm for carbidopa, (Fig. 8). Table 1 shows the statistical analysis of the experimental data. The regression equation calculated from the calibration graph, along with standard deviations of the slope and the intercept of the ordinate. The high value of the correlation coefficient indicates the good linearity of the calibration graph.

Accuracy and precision. The precision was ascertained by carrying out four replicate determinations of synthetic mixtures of levodopa and carbidopa. The relative standard deviation for four replicate determinations of a mixture containing 10 μg mL^{-1} of levodopa and 10 μg mL^{-1} of carbidopa were obtained 0.14% and 0.04%, respectively, indicating reasonable repeatability of the proposed method. These results are given in Table 1. The accuracy was tested by the determination of mixture containing different concentrations of levodopa and carbidopa. The relative error of 0.14% and 0.04% for levodopa and carbidopa, respectively, indicated that the accuracy was satisfactory.

Interferences. Some common excipients such as lactose, starch, glucose, sucrose, and fructose, which are usually present in preparation of tablets and capsules, show no influence on the determination of levodopa and carbidopa by proposed method.

Application. The proposed method has been successfully applied to the determination of levodopa and carbidopa in dosage form. The results are given in Table 2. As Table 2 shows there is no significant difference between the results obtained by the proposed method with the reported values.

Table 2. Determination of levodopa and carbidopa in Levodopa-C tablets by proposed method

Levodopa-C tablet	Found (mg L^{-1})	Known values (mg L^{-1})
Levodopa	29.20	30.00
Carbidopa	2.85	3.00

Conclusions

The proposed method is simple (as there is no need for solvent extraction), rapid (as it only requires measurements of ^1D values at a single wavelength) and direct (as it estimates each drug independently of the other). This paper demonstrates the potential of first derivative spectrophotometry method as an analytical technique and its usefulness to accurate, rapid, simple and simultaneous quantitation of levodopa and carbidopa in pharmaceutical preparation.

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