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Flow Injection Determination of levodopa in tablets using a solid-phase reactor containing lead(IV) dioxide immobilized

Luiz H. Marcolino-Júnior [a], Marcos F. S. Teixeira [a], Airton Vicente Pereira [b], Orlando Fatibello-Filho [a,*]

[a] *Grupo de Química Analítica, Departamento de Química, Centro de Ciencias Exatas e de Tecnologia, Universidade Federal de São Carlos, Caixa Postal 676, CEP 13.560-970, São Carlos, SP, Brazil*
[b] *Laboratório de Química Farmacêutica, Departamento de Ciências Farmacêuticas, Universidade Estadual de Ponta Grossa CEP 84010-970, Ponta Grossa, PR, Brazil*

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Abstract

A flow injection spectrophotometric procedure was developed for determining levodopa in tablets. The determination of this drug was carried out by reacting it with lead(IV) dioxide immobilized in polyester resin packed in a solid-phase reactor and the dopachrome yielded was monitored at 520 nm. The analytical curve for levodopa was linear in the concentration range from 1.0×10^{-4} to 1.0×10^{-3} mol l^{-1} with a detection limit of 8.0×10^{-5} mol l^{-1}. The relative standard deviation (R.S.D.) was 0.2% for a solution containing 4.0×10^{-4} mol l^{-1} levodopa ($n = 10$), and 90 determinations per hour were obtained. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Levodopa; Flow injection spectrophotometry; Solid-phase reactor; Lead dioxide

1. Introduction

The levodopa ((−)3-(3,4-dihydroxyphenyl)-L-alanine)), a naturally occurring amino acid, is the immediate precursor of the neurotransmitter dopamine. Unlike dopamine, levodopa easily enters the central nervous system and is used in the treatment of Parkinson's disease. It is usually given together with a peripheral *decarboxilase* inhibitor such as benserazide or carbidopa to permit a considerably higher proportion of levodopa to enter the brain [1].

Levodopa is a white, odorless, microcrystalline powder which is rapidly oxidized by atmospheric oxygen [2]. This drug can be chemically oxidized by different reagents such as metaperiodate [3], and potassium permanganate [4]. The oxidation reaction involves several steps that occur during the transient formation of dopachrome, which presents a strong absorption at 480 nm.

Several techniques have been reported in the literature for the determination of levodopa in

* Corresponding author. Tel.: +55-16-2608206; fax: +55-16-2608350.
E-mail address: bello@dq.ufscar.br (O. Fatibello-Filho).

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pharmaceutical formulations such as amperometry [5], ion-selective electrode [6], spectrofluorimetry [7], spectrophotometry [8–12], HPLC [13–22], and gas chromatography [23]. Flow injection procedures for the determination of levodopa have also been reported [24–27].

The enzyme *polyphenol oxidase* catalyses the oxidation of levodopa to dopaquinone. Further, dopaquinone undergoes a rapid spontaneous auto-oxidation to leucodopachrome, which is in turn oxidized to dopachrome that absorbs light at 480 nm. This reaction has been exploited in a flow injection system for determining levodopa and carbidopa in pharmaceutical formulations [28].

Solid-phase reactors coupled in flow systems is an interesting strategy because it is possible to use reagents that are not available in soluble form, such as zinc amalgams or cadmium copperized used as reductans and ion-exchange resins utilized for the separation/pre-concentration of metals. On the other hand, it is be more convenient to use reagents in the insoluble forms in order to avoid the time consuming steps of preparing reagent solutions. Other advantageous features, such as, simplification of manifolds, saving reagents, and increased of sensitivity have been discussed [29].

This paper reports on the application of a flow injection procedure for the spectrophotometric determination of levodopa in tablets using a solid-phase reactor containing PbO_2(s) immobilized in polyester resin. The method is based on the oxidation of levodopa with Pb(IV) to produce dopachrome which is monitored at 520 nm. The Scheme 1 shows the oxidation of the levodopa.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and all solutions were prepared with water from a Millipore (Bedford, MA, USA) Milli-Q system (model UV Plus Ultra-Low Organics Water).

The acetate buffer solution (pH 4.8) was prepared by mixing appropriate volumes of 0.4 mol l^{-1} sodium acetate and 0.4 mol l^{-1} acetic acid (Merck).

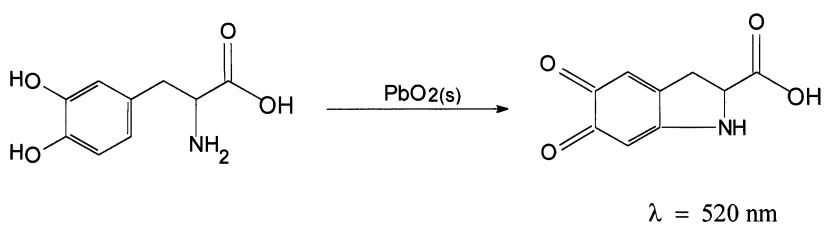
A 1.0×10^{-2} mol l^{-1} levodopa stock solution was prepared by dissolving 194 mg of levodopa (Roche, Brazil) in 100 ml of 0.2 mol l^{-1} acetate buffer solution previously de-oxygenated with nitrogen.

The immobilization of PbO_2(s) (Merck) was made using a commercial polyester resin solution (Resapol T-208, Resana, SP, Brazil) and methyl ethyl ketone (Ibere, Ramires and Cia, Taboão da Serra, SP, Brazil) as catalyst.

2.2. Apparatus

A model 8452A Hewlett-Packard (Boise, ID, USA) UV-visible spectrophotometer was used for the levodopa determination by enzymatic method.

A model 7618–50 12-channel Ismatec (Zurich, Switzerland) peristaltic pump supplied with Tygon pump tubing was used for the propulsion of the solutions. The manifold was constructed with polyethylene tubing (0.8 mm i.d.). All the reference or sample solutions were injected manu-



Scheme 1.

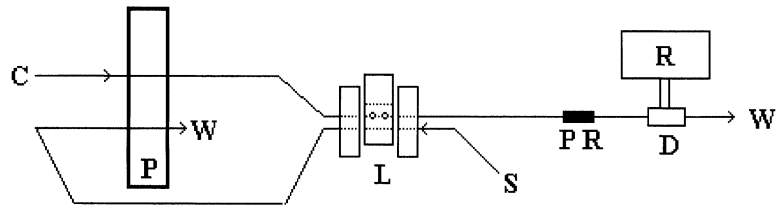


Fig. 1. Schematic diagram of the flow injection system for spectrophotometric determination of levodopa at 25°C. The rectangular piece (P) represents a peristaltic pump; *C*, 0.2 mol l^{-1} acetate buffer (pH 4.8) carrier solution flowing at 2.2 ml min^{-1}; L, is a scheme of the sliding-bar manual commutator; S, sample or reference solutions; PR, packed reactor (7.5 cm × 2.0 mm i.d.) containing PbO_2(s); D, spectrophotometer at 520 nm; R, recorder; W, waste

ally into the carrier stream using a laboratory-constructed three-piece injector-commutator [30] made of Perspex®, with two fixed side bars and a sliding central bar, that is moved for sampling and injection. FI spectrophotometric measurements were carried out using Model 435 Femto (São Paulo, Brazil) spectrophotometer equipped with a glass flow-cell (optical path, 1.0 cm) connected to a Model 1202–0000 Cole Parmer (Chicago, IL, USA) two-channel strip-chart recorder.

2.3. Preparation of pharmaceutical samples

To prepare commercial samples, ten tablets were ground to a fine powder and an accurately weighed mass corresponding to about 200 mg of levodopa was transferred to a 100 ml calibrated flask, dissolved and made to volume with 0.2 mol l^{-1} acetate buffer (pH 4.8). This solution was filtered through a filter paper and the filtrate was further diluted with 0.2 mol l^{-1} acetate buffer to obtain concentrations in the range from 1.0×10^{-4} to 1.0×10^{-3} mol l^{-1} of levodopa.

Two Brazilian pharmaceutical formulations containing levodopa such as Cronomet (Merck Sharp and Dohme, Rio de Janeiro, RJ) and Sinemet (Prodome, Guarulhos, SP) were analyzed using the proposed method.

2.4. Immobilization method

The immobilization of PbO_2(s) was similar to that reported earlier [31]; a mass of 10 g of polyester resin solution was transferred to a polyethylene flask, then 20 g of PbO_2(s) was added and after manual homogenization, 0.5 ml of the catalyst (methyl ethyl ketone) was added and stirred until an increase of viscosity was observed. After 3 h, a rigid solid was obtained, which was broken with a hammer and a grinder coffee was used to obtain small particles. The particle size was selected by passing the particles in known mesh sieves.

The solid phase reactor was prepared by packing a polyethylene tube (7.0 cm × 2.0 mm i.d.) with one end plugged with glass-wool to prevent the packing material escaping from the reactor with 300 mg of PbO_2(s) immobilized in polyester beads (particle size 100–350 μm) with aid of a syringe. The solid phase reactor was inserted in the flow manifold between the injector and the detector.

2.5. Flow injection procedure

A schematic diagram of the flow manifold is shown in Fig. 1. A solution of 0.2 mol l^{-1} acetate buffer (pH 4.8) flowing at 2.2 ml min^{-1} was used as carrier solution. The analytical length from the solid phase reactor to spectrophotometer was the minimum required (50 cm). When the solution of levodopa is injected, the drug is oxidized by Pb(IV) to produce dopachrome which is monitored at 520 nm.

3. Results and discussion

3.1. Preliminary studies

The oxidation of levodopa with a solid phase reactor containing PbO_2(s) in acetate buffer medium was studied. The visible spectrum of the dopachrome was obtained after injection of

levodopa solution (solution collected at flow cell point and the spectrum measured off-line). Dopachrome showed a maximum absorbance at 480 nm. However, as carbidopachrome has a slight absorbance in 480 nm, in order to minimize the interference by carbidopa the wavelength to measure dopachrome was displaced to 520 nm.

3.2. Solid phase reactor parameters

The parameters influencing the reactor performance, such as, weight ratio PbO_2:polyester resin, particle size and length, and inner diameter of the reactor were studied. All solid phase reactors tested were previously conditioned by passing the carrier solution 10 min before starting the first injection in order to minimize compaction of the particles in the reactor. At least ten injections were necessary to obtain reproducible absorbance signals after the system was started.

The proportion of PbO_2(s) immobilized in polyester beads has a important effect on reactivity of the oxidant column. Three different weight ratios of immobilized PbO_2 in polyester resin were used in the preparation of the solid-phase reactors; 1/2, 1/1 and 2/1 (m/m). An increase of the sensitivity was observed with an increase of the PbO_2:polyester ratio up to (2:1) with the polyester solution used.

The effect of particle size was studied in three size ranges (< 100, 100–350 and 350–500 μm). The signal decreased with increase in particle size, in the range studied. As particles < 100 μm permit only modest carrier flow rates, a particle size of 100–350 mm was chosen for further experiments. These findings were similar to the results obtained in our earlier works [31–34].

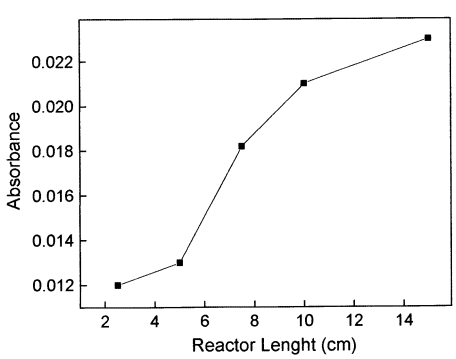


Fig. 2. Effect of solid-phase reactor length on the absorbance for a 2.0 10^{-4} mol l^{-1} levodopa at 25°C.

For the oxidation of levodopa to dopachrome by PbO_2(s) immobilized in polyester resin, the reaction time depends on both, the reactor length and carrier solution flow rate. The PbO_2 solid phase reactor was studied by testing two parameters alternately, namely, length and diameter of the reactor. The influence of reactor length on the absorbance was studied in the 2.5–15 cm range at a carrier flow rate of 2.2 ml min^{-1} (Fig. 2). It was found that the highest absorbance signal was obtained when a 15 cm column length was used. Nevertheless, a 7.5 cm column length was used, once excellent precision and baseline stability were obtained with this column length.

The effect of internal diameter on peak height was evaluated in columns with the same length (7.5 cm) and three different internal diameters (1.0, 2.0, and 3.0 mm). The 2.0-mm i.d. polyethylene tube led to highest absorbance signals and was chosen for further experiments.

The lifetime of the oxidant column depends on the volume of levodopa which passes through it. Therefore, the lifetime was investigated as a function of volume and/or concentration of levodopa injected in the system. It was found that each solid phase reactor prepared by the described procedure gave reproducible results after injection of at least 200 times for insertions of 250 μl of 1.0×10^{-3} mol l^{-1} levodopa solution. After this experiment, the solid phase reactor retained 80% of its efficiency in terms of being able to oxidize levodopa to levodopachrome. For levodopa concentration of 2.0×10^{-4} mol l^{-1} the solid-phase reactor presented at least 380–400 reproducible results.

3.3. Flow injection parameters

The effect of flow injection variables for the flow system performance such as, carrier and reagent flow rates, and sample volume was studied. The values chosen as optimum were those that resulted in the best compromise between absorbance magnitude, reproducibility and sample throughput.

The influence of the sample volume on the peak

Table 1
Results of the addition-recovery experiments

Sample	Levodopa[a] (10^{-3} mol l^{-1})		Recovery (%)
	Added	Found	
Cronomet	0.20	0.21	105.0
	0.40	0.41	102.5
	0.60	0.62	103.3
Sinemet	0.20	0.20	100.0
	0.40	0.40	100.0
	0.60	0.60	100.0

[a] $n = 5$.

height was studied; the range tested was from 62.5 to 750 μl by changing the length of sample loop in the injector-commutator. The absorbance magnitude resulting from 2.0×10^{-4} mol l^{-1} levodopa solution passed through the column and immobilized PbO_2 was measured. The sensitivity was found to increase with sample volume to 750 μl. A 250-μl volume was chosen as a compromise between sensitivity and the sample throughput.

The carrier flow rate was optimized by using an univariant approach. When the carrier flow rate was increased from 1.1 to 4.3 ml min^{-1}, it was found that the sensitivity increased to 2.2 ml min^{-1}, above which it showed a slight decrease. Then, a carrier flow rate of 2.2 ml min^{-1} was selected as optimum.

3.4. Interferences and recovery studies

The selectivity of the flow-injection procedure was investigated using solutions containing 4.0×10^{-4} mol l^{-1} levodopa added to foreign compounds that are commonly found in tablets containing levodopa. No interference in the flow procedure was observed upto a 10-fold excess for carboxymethylcellulose, lactose, saccharin, starch, magnesium stearate, and carbidopa. It is important to emphasize that this last analyte/concomitant concentration ratio studied is much higher than those normally found in the commercial pharmaceutical products. As mentioned before, the presence of carbidopa caused an increase in the peak height on the levodopa determination. But this interference was removed by displacing the wavelength to 520 nm where absorption of carbidophacrome is negligible.

To study the recovery of the levodopa from pharmaceutical formulations, two commercial samples of pharmaceutical formulations were used. The recovery of levodopa was examined by adding levodopa reference solution at three levels (2.0×10^{-4}, 4.0×10^{-4} and 6.0×10^{-4} mol l^{-1}) to the samples and results obtained (Table 1) were compared with the added concentrations. The average recoveries varied from 100.0 to 105.0%, evidencing the absence of matrix effect on the flow injection procedure.

3.5. Analytical curve and applications

Levodopa in commercial pharmaceutical formulations was determined by the proposed and enzymatic methods. The results were compared with those obtained from the enzymatic method [28] and are presented in Table 2. The results obtained by the proposed flow procedure are in good agreement with those obtained by the enzymatic method, since the value of F obtained (1.00) for 95% confidence level is lower than the critical value of 5.05 ($F_{0.05/5,5}$) [35], showing that the flow procedure can be satisfactorily applied for the determination of levodopa in pharmaceutical products.

Table 2
Results obtained for levodopa in pharmaceutical formulations by enzymatic [28] and proposed flow injection methods

Samples	Label value (mg/g)	Levodopa[a] (mg ± S.D.)		Relative error (%)	
		Enzymatic method	FI method	Re_1	Re_2
Cronomet	710	750.0 ± 0.7	768.1 ± 0.7	+8.2	+2.4
Sinemet	704	707.0 ± 0.3	713.8 ± 0.3	+1.4	+1.0

[a] $n = 5$; Re_1, flow injection method vs. label value; Re_2, flow injection method vs. enzymatic method; FI, flow injection.

A set of reference or sample solutions of levodopa was injected into the manifold depicted in Fig. 1. Typical transient signals corresponding to a linear analytical curve for levodopa and injections of two samples of tablets containing levodopa were obtained. The analytical curve was linear in the 1.0×10^{-4}–1.0×10^{-3} mol l^{-1} concentration range ($A = -0.00143 + 26.14\,C$; $r = 0.9999$, where A is the absorbance and C the concentration of levodopa in mol l^{-1}). The precision of the proposed method was tested by ten repeated runs of a solution containing 4.0×10^{-4} mol l^{-1} of levodopa. The R.S.D. was 0.2% ($n = 10$) and the analytical frequency was 90 determinations per hour. Under optimized experimental conditions, a detection limit of 8.0×10^{-5} mol l^{-1} levodopa can be obtained.

4. Conclusions

The proposed flow injection with an online packed reactor containing PbO_2(s) immobilized in polyester resin with spectrophotometric detection is precise, accurate and sensitive to permit the determination of levodopa in pharmaceutical formulations. The reactor was stable enough to permit at least 380–400 reproducible results.

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