Talanta 82 (2010) 962–968

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Simultaneous voltammetric determination of levodopa, carbidopa and benserazide in pharmaceuticals using multivariate calibration

C. Zapata-Urzúa[a], M. Pérez-Ortiz[a], M. Bravo[b,1], A.C. Olivieri[c], A. Álvarez-Lueje[a,*,1]

[a] Bioelectrochemistry Laboratory, Chemical and Pharmaceutical Sciences Faculty, University of Chile, Santiago, Chile
[b] Instituto de Química, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Avenida Brasil 2950, Valparaiso, Chile
[c] Departamento de Química Analítica, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario e Instituto de Química Rosario (CONICET), Suipacha 531, Rosario 2000, Argentina

ARTICLE INFO

Article history:
Received 7 April 2010
Received in revised form 30 May 2010
Accepted 31 May 2010
Available online 8 June 2010

Keywords:
Levodopa
Carbidopa
Benserazide
Voltammetry
Multivariate calibration

ABSTRACT

An analytical methodology based on differential pulse voltammetry (DPV) on a glassy carbon electrode and the partial least-squares (PLS-1) algorithm for the simultaneous determination of levodopa, carbidopa and benserazide in pharmaceutical formulations was developed and validated. Some sources of bi-linearity deviation for electrochemical data are discussed and analyzed. The multivariate model was developed as a ternary calibration model and it was built and validated with an independent set of drug mixtures in presence of excipients, according with manufacturer specifications. The proposed method was applied to both the assay and the uniformity content of two commercial formulations containing mixtures of levodopa–carbidopa (10:1) and levodopa–benserazide (4:1). The results were satisfactory and statistically comparable to those obtained by applying the reference Pharmacopoeia method based on high performance liquid chromatography. In conclusion, the methodology proposed based on DPV data processed with the PLS-1 algorithm was able to quantify simultaneously levodopa, carbidopa and benserazide in its pharmaceuticals formulations using a ternary calibration model for these drugs in presence of excipients. Furthermore, the model appears to be successful even in the presence of slight potential shifts in the processed data, which have been taken into account by the flexible chemometric PLS-1 approach.

1. Introduction

The Parkinson's disease is related to a significant depletion of the dopamine neurotransmitter in the brain. Levodopa, a precursor of this neurotransmitter, is the principal drug used in the treatment of patients with Parkinson's disease. This catecholamine drug, in contrast to dopamine, is able to cross the blood–brain barrier and is metabolized into the central nervous system by dopadecarboxylase enzyme to dopamine. However, the enzymatic metabolization of levodopa does also occur in the peripheral system, producing different side effects associated to the increase of systemic dopamine. For this reason, levodopa is administered in pharmaceuticals in association with a peripheral dopadecarboxylase inhibitor, such as carbidopa or benserazide. The administration of these pharmaceuticals improves the efficiency of the treatment, because it makes possible a better control of dopamine levels, allowing to decrease the dose and the side effects [1]. The chemical structures of these compounds are shown in Fig. 1.

Different analytical methods have been employed for the determination of levodopa, carbidopa and benserazide in raw material, pharmaceutical formulations and biological fluids, mainly by high performance liquid chromatography [2–6], spectrophotometry [7–11], and capillary electrophoresis [12–15]. As with other catecholics and pyrogallics derivates, these drugs contain electroactive groups and can be electrochemically oxidized on carbon, platinum or gold electrodes. This has enabled the electrochemical characterization and determination of levodopa [16–21], carbidopa [22] and benserazide [23]. However, few electrochemical methodologies have been developed for simultaneous determination of these drugs, probably due to their similar structural patterns and electrochemical responses showing dramatic overlapping when using conventional electrodes. Recently, voltammetric methods have been reported for the simultaneous determination of these drugs using modified electrodes [24,25], but the treatment involved is time consuming and the associated cost is high. It is thus important to develop new methodologies for the simultaneous determinations of these drugs. An attractive possibility is the use of chemometric and multivariate calibration methods.

* Corresponding author at: P.O. Box 233, Santiago 1, Chile. Fax: +56 7378920.
E-mail address: aalvarez@ciq.uchile.cl (A. Álvarez-Lueje).
[1] These authors contributed equally to the manuscript.

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.05.071

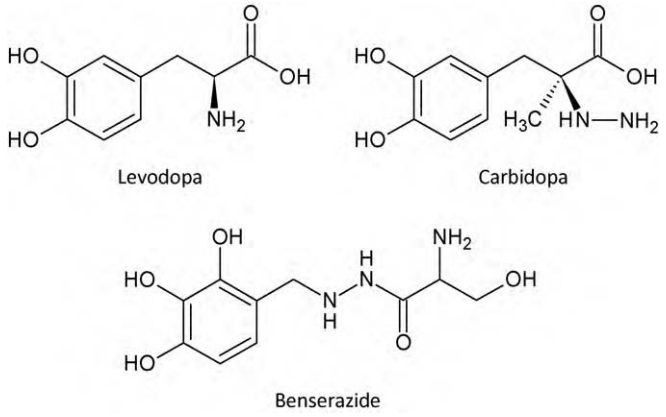

Fig. 1. Chemical structures of the drugs used in the treatment for Parkinson's disease.

In the recent years, multivariate calibration methods applied to absorptive spectral and electrochemical data are being increasingly used for the analysis of complex mixtures [26,27]. Several tools have been reported in the literature for processing these data [28], although the most popular are principal component regression (PCR) [29] and partial least-squares regression (PLS) [30]. All these techniques have the advantage of using the full spectral information and not only a characteristic peak value. Moreover, they allow a rapid determination of mixture components, often with no prior separation, and the calibration can be performed ignoring the concentrations of all components except the analyte of interest in complex samples. Recently, some spectrophotometric methods, assisted by multivariate calibration, have been described for the simultaneous determination of levodopa and carbidopa or levodopa and benserazide in pharmaceutical formulations [10,11]. Nevertheless, to the best of our knowledge, no reports exist in the literature about chemometric models applied to electrochemical data to resolve mixtures of these drugs.

In this work, we present the development of an electroanalytical methodology based on differential pulse voltammetry (DPV) on a glassy carbon electrode and the PLS-1 algorithm for the simultaneous determination of levodopa, carbidopa and benserazide.

2. Experimental

2.1. Reagents and standard solutions

Chile Laboratories (Santiago, Chile) supplied levodopa (99.3%) and carbidopa monohydrate (98.9%). Benserazide hydrochloride (98.5%) was supplied by Tecnofarma Laboratories (Santiago, Chile). Commercial tablets of Grifoparkin® (declared amount per tablet: 250 mg levodopa and 25 mg carbidopa, Chile Laboratories, Santiago, Chile) and Prolopa® (declared amount per tablet: 200 mg levodopa and 50 mg benserazide, Roche Laboratories, Santiago, Chile) were obtained commercially. All other reagents were of analytical grade unless indicated otherwise. Sodium hydrogen phosphate, phosphoric acid and acetonitrile HPLC grade were obtained from Merck. All solutions were prepared with ultrapure water (ρ = 18 MΩ) from Millipore-Milli-Q system.

Stock standard solutions of the drugs were prepared daily at a concentration of 1×10^{-2} mol L^{-1} in 0.1 mol L^{-1} perchloric acid solution and stored in amber glass material. Working solutions were prepared by diluting each stock standard solution before the measurements, using 0.1 mol L^{-1} Britton–Robinson buffer, 0.1 mol L^{-1} perchloric acid solution or 0.1 mol L^{-1} hydrochloric acid.

2.2. Apparatus

Differential pulse voltammetry measurements were performed with a BAS CV-50W electroanalyzer equipped with a 10-mL BAS cell. The components utilized in the three-electrode cell system were a glassy carbon (GC) (Ø = 3 mm, CHI) as working electrode, a platinum wire as auxiliary electrode and an Ag/AgCl (sat) as reference electrode.

High performance liquid chromatography (HPLC) measurements were carried out on a Waters assembly equipped with a model 600 Controller pump and a model 996 Photodiode Array Detector. The acquisition and treatment of data were made with the Millenium version 2.1 software. A Phenomenex C-8 column of 4.6 mm × 150 mm was used, and a C18 Bondapak (30 mm × 4.6 mm) was employed as column guard. The injector was a 20-μL Rheodyne valve. The column was kept at constant temperature using a Waters column heater cartridge model 600.

2.3. Calibration set for the PLS-1 model

For training the PLS-1 model, a calibration set of fourteen ternary mixtures was prepared using a central composite design with five concentration levels of each analyte: levodopa in the range 1.1×10^{-4} to 1.3×10^{-3} mol L^{-1}, carbidopa in the range 3.1×10^{-5} to 4.7×10^{-4} mol L^{-1} and benserazide 3.1×10^{-5} mol L^{-1} to 6.2×10^{-4} mol L^{-1}. A duplicated "center point" (level "0") solution was included in the calibration set, obtaining sixteen solutions for this set. The component ratios were selected considering the linear calibration ranges (previously established from univariate experiments for each drug) and the usual levodopa: carbidopa or levodopa: benserazide ratios present in commercial pharmaceutical formulations (4:1 to 10:1 for levodopa:carbidopa and 4:1 for levodopa:benserazide). All calibration samples were prepared by mixing appropriate volumes of levodopa, carbidopa and benserazide stock standard solutions and containing excipients according with manufacturer specifications. The excipients used for drug mixtures were cornstarch, microcrystalline cellulose, mannitol, polividone, ethylcellulose, titanium dioxide, talc, magnesium stearate and FD&C blue No. 2. Finally, the solutions were measured by triplicate and in random order.

2.4. Test samples for the PLS-1 model

An independent set of nine ternary mixtures was prepared by mixing appropriate volumes of each drug in the same concentration range used for calibration. The solutions were prepared containing the same excipients considered for calibration set. Each sample was measured by triplicate and in random order.

2.5. Electrochemical procedure

All DPV experiments were carried out at room temperature at the following operating conditions for the three drugs: sensitivity, 100 μA V^{-1}; potential range, −200 to 1400 mV at 4 mV intervals; sweep rate, 20 mV s^{-1}. Before each measurement, the working electrode surface was mechanically polished with 0.3 and 0.05 μm alumina slurries [31]. Working and sample solutions were analyzed in 0.1 mol L^{-1} perchloric acid.

2.6. Analysis of pharmaceutical forms

Assay and uniformity content of commercial samples were evaluated by both the DPV proposed method and HPLC official methods [32,33].

2.6.1. DPV method

2.6.1.1. Assay. A quantity of powder taken from 20 ground tablets, containing the equivalent of 50 mg levodopa (for levodopa:carbidopa mixtures) or 100 mg levodopa (for levodopa:benserazide mixtures) was dissolved with 10 mL of 0.1 mol L^{-1} phosphoric acid and completed to 100 mL with Milli-Q water. Appropriate dilutions of this solution in 0.1 mol L^{-1} perchloric acid were used for the measurements.

2.6.1.2. Uniformity content test. Ten commercial tablets were independently weighed, suspended and diluted as previously described for the *Assay*. Appropriate volumes of this solution were taken and diluted for measurements.

Each sample solution was measured by triplicate and the amount of drug was calculated using the adequate chemometric model.

2.7. Theory and software

2.7.1. PLS Calibration and prediction

PLS is a well-known first-order multivariate calibration methodology. It has been widely applied for different kind of instrumental data (i.e. spectroscopic, electrochemical or chromatographic) with satisfactory results [28,34]. This method involves a two-step procedure: (1) calibration, where the relation between instrumental signal (i.e. voltammograms or UV–vis spectra) and reference component concentrations is established from a set of standard samples or a reference method, and (2) prediction, in which the calibration results are employed to estimate the component concentrations in unknown samples from its instrumental profile [28,30].

In the PLS-1 version, all model parameters are optimized for the determination of one analyte at a time. During the model-training step, the calibration data are decomposed by an iterative algorithm, which correlates the data with the calibration concentrations using a so-called 'inverse' model [29,34]. This provides a set of loadings ($\mathbf{P}$, size $J \times A$, where J is the number of sensor and A the number of latent PLS variables), weight-loadings ($\mathbf{W}$, size $J \times A$) and regression coefficients to be applied to a new sample ($\mathbf{v}$, size $A \times 1$). Given the profile of an unknown sample $\mathbf{x}_u$ (size $J \times 1$), the latter is projected onto the space of the loadings and weight-loadings to provide the test sample scores ($\mathbf{t}_u$):

$$\mathbf{t}_u = (\mathbf{P}^T\mathbf{W})^{-1}\mathbf{P}^T\mathbf{x}_u \quad (1)$$

The sample scores are then multiplied by the regression coefficients to estimate the analyte concentration y:

$$y = \mathbf{v}^T\mathbf{t}_u \quad (2)$$

Before calibration, it is usual to assess the optimum number of latent variables in order to avoid overfitting, by applying the well-known cross-validation method described by Haaland and Thomas [34].

The PLS-1 algorithm was applied using the Toolbox MVC1 [35] written for MATLAB [36], because these routines allow one to evaluate the figures of merit based on Net Analyte Signal (NAS) theory [37]. Furthermore, MVC1 provides a convenient sensor selection procedure, where a moving-window strategy is implemented, and a comprehensive search of the optimum cross-validation variance is performed as a function of first sensor and window width.

Besides the problem arising from the presence of severely overlapping analyte profiles, in the presently studied case two additional complications may occur: (1) interactions among analytes and the background excipients, which may cause signal changes in comparison with pure analyte profiles, and (2) sample-to-sample potential shifts in the analyte profiles, which are common in voltammetric studies.

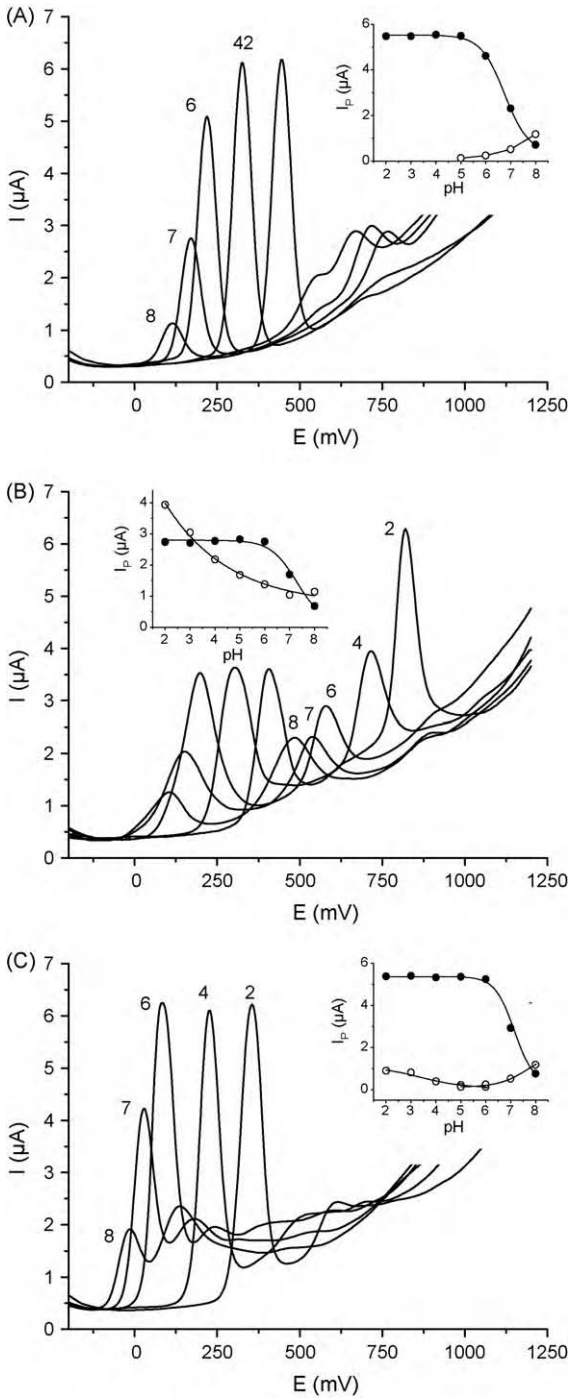

Fig. 2. Differential pulse voltammograms in 0.1 mol L^{-1} Britton Robinson buffer of levodopa (A), carbidopa (B) and benserazide (C) at 1×10^{-4} mol L^{-1} at different pHs. Inset: Dependence of peak current with pH (● main signal, ○ second signal).

For tackling the first of these problems, it was necessary to include the pharmaceutical excipients in the calibration set of samples, in order to allow PLS-1 to model the analyte-background interactions before prediction on new samples.

Concerning the second of the above commented problems, some preprocessing alternatives were independently applied on the electrochemical responses (entire voltammogram) before PLS-1 model building and validation.

(a) Background correction. This approach was applied using an in-house MATLAB based on linear interpolation between user-

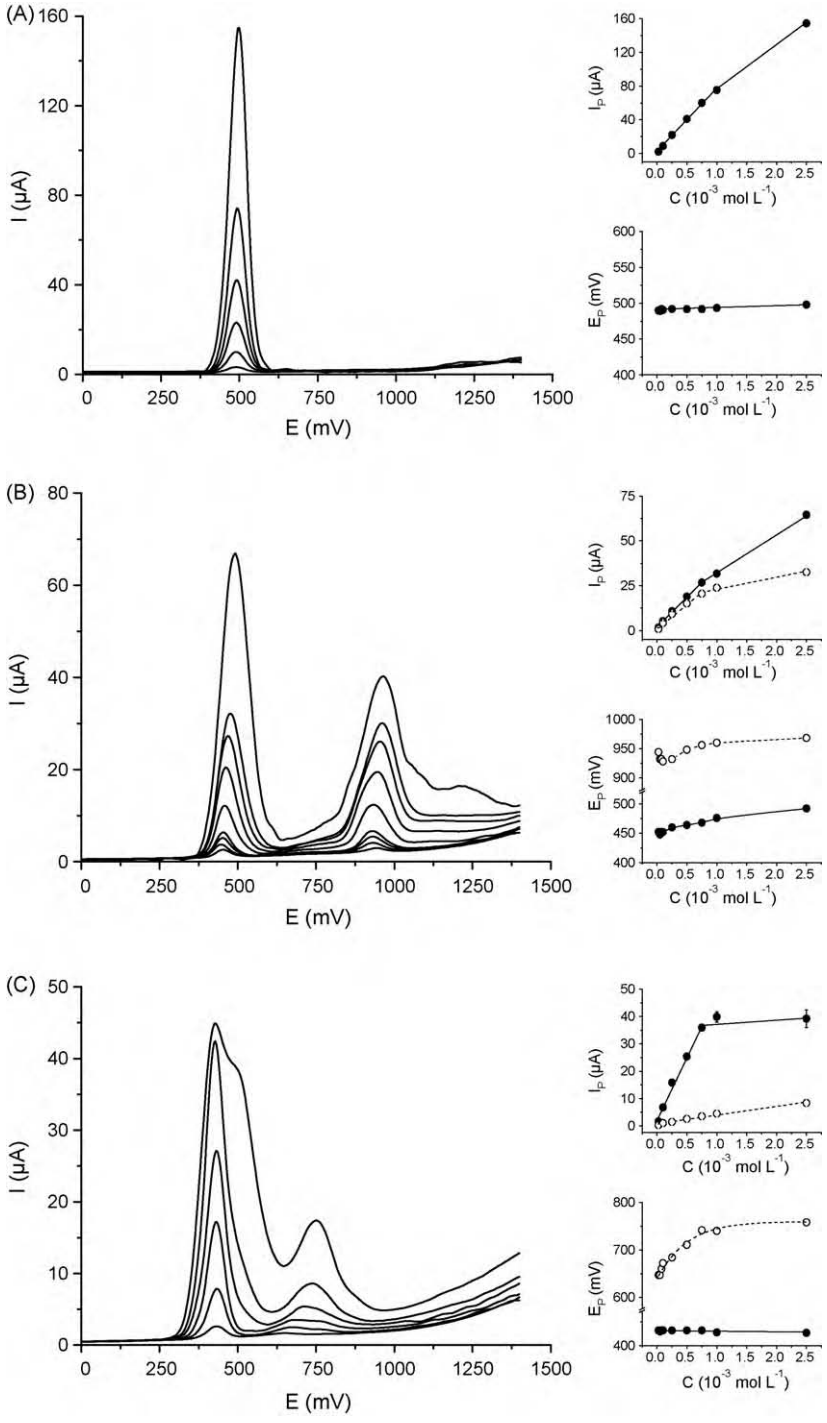

Fig. 3. Representative differential pulse voltammograms of levodopa (A), carbidopa (B) and benserazide (C) at different concentrations (2.5×10^{-5} to 2.5×10^{-3} mol L^{-1}) in 0.1 mol L^{-1} perchloric acid solution. Inset: Dependence of peak potential and peak current with concentration (● main signal, ○ second signal).

selected regions where the only present feature is the baseline. Two zones were considered for background correction: before maximum signals (E_p = 520 mV) and after the secondary signals (E_p = 968 mV). The quality of background correction was evaluated visually.

(b) Alignment of electrochemical signals. A correlation optimized warping (COW) routine written in MATLAB was used [38]. First, the section length (N) and slack (t) were optimized based on the Simplicity concept, using a simplex-like optimization routine. Different authors have described a detailed description of the algorithm and its optimization [39,40]. The mean voltammogram was selected as target "signal". After alignment, the worst Pearson's coefficient improved from 0.9178 to 0.9478 with respect to the target signal, which was considered satisfactory.

Finally, the PLS model was built using mean centering within an "optimal" potential region, the latter leading to a minimal cross-validation variance. Both options are available in the MVC1 Toolbox. A more detailed description of the algorithm can be found in reference [35].

3. Results and discussion

3.1. Electrochemical studies

As has been previously reported, the three drugs produce electrochemical responses which are due to the oxidation of their catecholic moieties in different supporting electrolytes, such as acetate buffer [17,18,20,21], sulfuric acid solution [16], perchloric acid solution [22,24,25], McIlvaine buffer [16] and phosphate buffer [13,14,19,23].

In this work, we have studied the oxidation of these drugs in different aqueous media: hydrochloric acid, perchloric acid and Britton–Robinson buffer (pH 2–8) by DPV and cyclic voltammetry (CV) on a glassy carbon electrode.

As expected, the three drugs exhibit a similar behavior in the three different evaluated media, with a main oxidation peak and two or three minor signals, being irreversible (data not shown) and pH-dependent in all cases. Fig. 2 shows the evolution of differential pulse voltammograms with pH in a Britton–Robinson buffer. Peak current for the main signal was both high and stable at acid pHs up to pH 6 and then decreased in all cases, but in the case of carbidopa the second signal increased at acid pHs (see inset in Fig. 2). Taking into account that for analytical purposes both maximal and stable currents are necessary, hydrochloric acid and perchloric acid were evaluated as solvents. Under these conditions, the signals of the three compounds were maximal and similar in both solvents assayed, but the perchloric acid solution showed some advantages, with a lower solvent discharge and a better voltammetric profile (data not shown). For this reason, and considering that in all media studied the drugs showed electrochemical signals in the same potential region, perchloric acid solution was chosen for the next experiments.

The effect of concentration on both current and peak potentials was also evaluated. Typical DPV at different concentrations and the evolution of peak potentials and currents for each drug are shown in Fig. 3. All drugs showed linear dependences between peak current and concentration at different concentrations intervals. Surprisingly, benserazide showed a loss of dependence between peak current and concentration to the highest concentrations tested (up to 7.5×10^{-4} mol L^{-1}), exhibiting both widening and unfold of the main signal. This result could be attributed to adsorption on the glassy carbon electrode observed for similar compounds (i.e., phenolic, catecholics and gallate derivatives) and was detected mainly for benserazide [41,42]. Furthermore, the peak potential values (E_p) of carbidopa and benserazide are strongly influenced by their concentrations in the range of 2.5×10^{-5} to 2.5×10^{-3} mol L^{-1}. The relationship between E_p and carbidopa concentration (C) can be described by the following linear regression: $E_p = 17279 \times C + 452$, allowing to confirm this hypothesis.

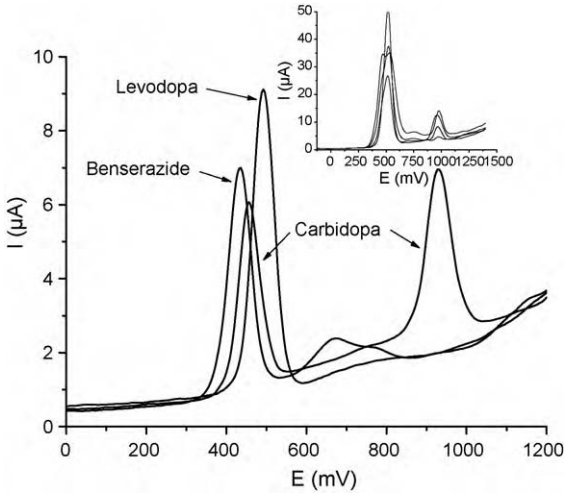

Fig. 4. Differential pulse voltammograms of each drug solution (1×10^{-4} mol L^{-1}) in 0.1 mol L^{-1} perchloric acid. Inset: Representative differential pulse voltammograms of calibration set mixtures.

Finally, in all conditions evaluated, a strong signal overlapping was observed for the simultaneous analysis of levodopa, carbidopa and benserazide (see Fig. 4). Thus, the quantification of any of these drugs will be biased if univariate calibration is used as analytical method.

3.2. Simultaneous determination of levodopa, carbidopa and benserazide by PLS-1

When the drugs mixtures were analyzed in presence of all excipients, the electrochemical profile revealed additional changes

Table 1
Error estimation for different calibration approaches for the simultaneous determination of levodopa, carbidopa and benserazide.

Calibration approach	Analyte	Optimal region (mV)	PLS-components	RMSE, C^a (mol L^{-1})	RMSE, P^a (mol L^{-1})	REP, P^b (%)
Mean voltammograms	Levodopa	484–1200	6	6.19×10^{-6}	1.51×10^{-5}	2.2
	Carbidopa	564–1160	6	1.21×10^{-6}	1.72×10^{-6}	1.7
	Benserazide	444–520	4	6.48×10^{-6}	6.02×10^{-6}	3.4
Mean-aligned voltammograms	Levodopa	(−116) to 920	4	1.74×10^{-5}	1.72×10^{-5}	2.5
	Carbidopa	464–1180	6	1.36×10^{-6}	1.87×10^{-6}	1.8
	Benserazide	(−16) to 520	6	4.41×10^{-6}	6.18×10^{-6}	3.4
Triplicates calibration voltammograms	Levodopa	484–1000	6	7.49×10^{-6}	1.80×10^{-5}	2.6
	Carbidopa	524–1280	5	2.39×10^{-6}	2.07×10^{-6}	2.0
	Benserazide	324–500	6	5.39×10^{-6}	4.81×10^{-6}	2.8
Mean-background corrected voltammograms	Levodopa	484–1000	5	1.07×10^{-5}	1.71×10^{-5}	2.4
	Carbidopa	644–1000	6	1.85×10^{-6}	2.60×10^{-6}	2.6
	Benserazide	444–500	4	5.92×10^{-6}	6.68×10^{-6}	3.8

[a] $\mathrm{RMSE} = \left[\frac{1}{n}\sum_{1}^{n}(C_{nom} - C_{pred})^2\right]^{1/2}$.

[b] $\mathrm{REP} = \frac{100}{C}\left[\frac{1}{n}\sum_{1}^{n}(C_{nom} - C_{pred})^2\right]^{1/2}$ with $n = 16$ and 48 for mean and triplicate voltammograms for calibration, respectively, and $n = 27$ for validation. C_{nom} and C_{pred} correspond to nominal and predicted concentrations, respectively. C, calibration; P, prediction.

to those observed in previous experiments. The main changes observed were minor alterations in the base line and displacement of peak potential, probably due to modification in viscosity of the solution and consequently the diffusion coefficient of analytes. This effect produce alterations in the chemometrics responses and for this reason, the calibration set was prepared by including the excipients in all samples, in order to provide PLS-1 enough information concerning the signals of the analytes when they are embedded into the real background. The addition of these excipients followed the relative proportions between analytes and excipients that are found in the real pharmaceutical formulations, as provided by the commercial manufacturers.

For chemometric model building, several strategies have been proposed to align shifted signals such as chromatograms, electropherograms or NIR spectra. One of the most popular ones is COW [39,40]. However, this situation has been scarcely described for electrochemical signals. Very recently, a study about shift correction for electrochemical data has been published [43]. According to the literature, the shift in electrochemical responses can be originated from adsorptive phenomena on the electrode surface, pH variations in the cell or fluctuations in the composition of cell solution, among others [44].

A basic assumption for application of multivariate calibration model is the data bi-linearity, which may be compromised by the above commented potential shifts. However, the use of flexible latent variables in PLS-1 may allow to take into account slight deviations of the bi-linearity, when sufficient information is provided in the calibration phase of the algorithm. Different approaches were therefore considered in order to circumvent the problem posed by the presence of potential shifts. One of them involved correction of the shifted signals by calibration with: (a) mean voltammograms, (b) COW aligned-mean voltammograms and (c) mean-background corrected voltammograms, where "mean" corresponds to the average of triplicate voltammograms, which were evaluated for each calibration solution. Fig. 5 presents the mean calibration voltammograms obtained for each of the latter three approaches. As can be seen, the baseline is satisfactorily corrected (see Fig. 5B). After alignment, the relative standard deviation based on E_p (~520 mV) improved from 8.2% to 2.6% (see Fig. 5C). The alternative consisted in providing more information to the model, in the form of triplicate calibration voltammograms.

In order to evaluate the performance of the above-mentioned approaches, each model was validated for prediction of the validation set, evaluating absolute and relative root-mean square errors (RMSE and REP, respectively). The results obtained for each of the above approaches are presented in Table 1. Notice that the optimum number of PLS-1 latent variables in all cases is larger than the theoretically expected value of three (which may arise from the presence of three overlapping signals), probably because of the need of modeling additional phenomena such as the above commented potential shifts in the studied signals.

According to the obtained RMSE, for levodopa and carbidopa the best models were obtained when the mean voltammogram calibration approach was used. In contrast, the best models for benserazide were obtained when triplicate and mean voltammogram approaches were used. The figures of merit obtained for these models are presented in Table 2. As can be seen, the application of the mean voltammogram for trainings the PLS-1 model shows an adequate predictive ability for the simultaneous quantification of levodopa and carbidopa contained in pharmaceuticals. For benserazide, this approach allows to obtain better figures of merit in comparison to the triplicate voltammogram approach. For this reason and considering the low level expected for this drug in the pharmaceuticals, the mean voltammogram was selected for analysis of new samples.

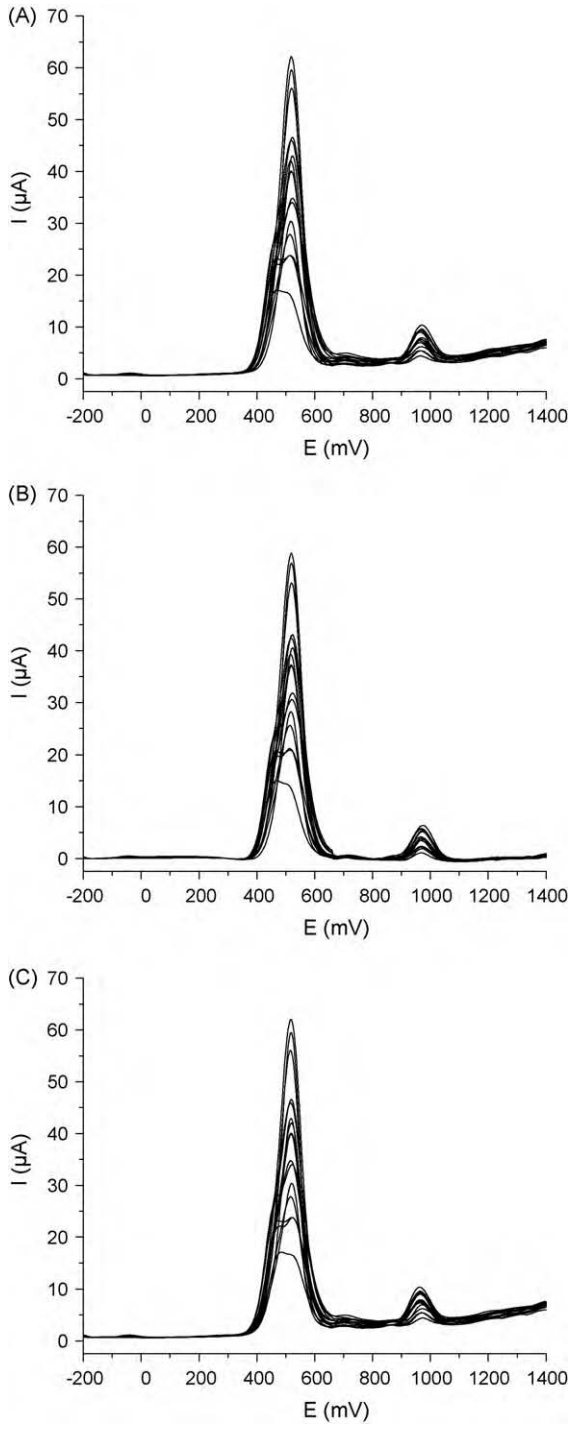

Fig. 5. Differential pulse voltammograms corresponding to the calibration set. (A) Raw data, and after preprocessing with (B) background correction and (C) alignment with correlation optimized warping.

3.3. Analysis of pharmaceuticals: assay and uniformity content (U.C.)

As can be seen in Table 3, the results obtained from the assay for the chemometric model are statistically comparable with the official method ($p < 0.1$). On the other hand, for uniformity content, the proposed method allows to obtain comparable results to the reference method based on HPLC. Furthermore, all percentages found are in accordance with the Pharmacopeia requirements for the assay (90.0–110.0% of the labeled amount). Also, the content

Table 2
Figures of merit for the simultaneous determination of levodopa, carbidopa and benserazide by PLS-1 algorithm.

	Approach[a]	Sensitivity ($L\,mol^{-1}$)	Analytical sensitivity ($L\,mol^{-1}$)	Limit of detection ($mol\,L^{-1}$)	Limit of quantification ($mol\,L^{-1}$)
Levodopa	MV	4.105×10^4	5.865×10^5	5.12×10^{-6}	1.53×10^{-5}
Carbidopa	MV	9.718×10^4	1.388×10^6	2.16×10^{-6}	6.48×10^{-6}
Benserazide	MV	7.574×10^4	1.082×10^6	2.77×10^{-6}	8.32×10^{-6}
	TV	1.870×10^4	2.672×10^5	1.12×10^{-5}	3.37×10^{-5}

[a] Calibration approach (MV: mean voltammogram; TV: triplicated voltamogram).

Table 3
Analysis of pharmaceuticals: assay and uniformity content (U.C.)[a].

Pharmaceutical	Drug	DPV		HPLC	
		Assay	U.C.	Assay	U.C.
Grifoparkin®	Levodopa	101.5 ± 1.0	100.8 ± 1.2 (98.1–102.0)	101.7 ± 0.5	99.2 ± 1.7 (95.2–102.4)
	Carbidopa	102.8 ± 2.5	103.8 ± 2.9 (99.2–106.0)	100.2 ± 1.2	98.1 ± 1.6 (97.0–101.67)
Prolopa®	Levodopa	103.2 ± 1.1	102.1 ± 1.0 (100.8–103.3)	101.4 ± 0.6	100.7 ± 1.0 (99.8–102.9)
	Benserazide	96.1 ± 0.7	100.7 ± 2.5 (99.5–102.1)	97.6 ± 0.3	98.6 ± 2.4 (96.0–102.1)

Grifoparkin® (levodopa 250 mg, carbidopa 25 mg). Prolopa® (levodopa 200 mg, benserazide 50 mg).
[a] U.C. are expressed as percentage founded ± SD and the ranges for 10 tablets assayed.

for all assayed tablets fulfill the Pharmacopoeia requirement, i.e., for uniformity content of tablets the content must be in the range 85.0–115.0% of the label claim and none of the individual values must be out of the range 75.0–125.0% of the label claim.

4. Concluding remarks

The three studied drugs (levodopa, carbidopa and benserazide), analyzed by DPV on glassy carbon, exhibited a similar anodic behavior, with a main oxidation peak and two or three minor signals, all of irreversible character and pH-dependent. Due to structural similarities, a strong voltammetric overlapping was produced for the simultaneous analysis of these compounds. The overlapping was successfully resolved using the PLS-1 algorithm, which did also take into account slight potential shifts in the voltammograms affecting the strict data bi-linearity.

The methodology proposed based on DPV data processed with the PLS-1 algorithm allowed the simultaneous determinations of levodopa, carbidopa and benserazide in any of its pharmaceutical formulations using a ternary calibration model for these drugs in the presence of excipients. The excipients produce little changes in the voltammograms profiles, for this reason the calibration set was prepared by including the excipients in all samples, in order to provide PLS-1 enough information concerning the signals of the analytes. Finally, the application of the developed method to both the assay and the uniformity content allowed to obtain satisfactory results which were in accordance with the HPLC reference method. This study allows one to propose the present method as a promissory, cheap and accessible alternative for routine control of common pharmaceutical formulations.

References

[1] S.C. Sweetman (Ed.), Martindale: The Complete Drug Reference, Pharmaceutical Press, London, 2007 (Electronic version).
[2] K.A. Sagar, M.R. Smyth, J. Pharm. Biomed. Anal. 22 (2000) 613.
[3] M. Karimi, J.L. Carl, S. Loftin, J.S. Perlmutter, J. Chromatogr. B 836 (2006) 120.
[4] G. Cannazza, A. Di Stefano, B. Mosciatti, D. Braghiroli, M. Baraldi, F. Pinnen, P. Sozio, C. Benatti, C. Parenti, J. Pharm. Biomed. Anal. 36 (2005) 1079.
[5] C. Muzzi, E. Bertocci, L. Terzuoli, B. Porcelli, I. Ciari, R. Pagani, R. Guerranti, Biomed. Pharmacother. 62 (2008) 253.
[6] A. Tolokán, I. Klebovich, K. Balogh-Nemes, G. Horvai, J. Chromatogr. B 698 (1997) 201.
[7] T. Madrakian, A. Afkhami, M. Borazjani, M. Bahram, B. Korean Chem. Soc. 25 (2004) 1764.
[8] B. Uslu, S.A. Özkan, Anal. Lett. 35 (2002) 303.
[9] E. Dinc, S. Kaya, T. Doganay, D. Baleanu, J. Pharm. Biomed. Anal. 44 (2007) 991.
[10] J. Coello, S. Maspoch, N. Villegas, Talanta 53 (2000) 627.
[11] P. Damiani, A. Moschetti, A. Rovetto, F. Benaven, A. Olivieri, Anal. Chim. Acta 543 (2005) 192.
[12] S. Fanali, V. Pucci, C. Sabbioni, M.A. Raggi, Electrophoresis 21 (2000) 2432.
[13] L. Zhang, G. Chen, Q. Hua, Y. Fang, Anal. Chim. Acta 431 (2001) 287.
[14] J. Wang, Y. Zhou, J. Liang, P.G. He, Y.Z. Fang, Chromatographia 61 (2005) 265.
[15] W.-W. He, X.-W. Zhou, J.-Q. Lu, J. Chromatogr. A 1131 (2006) 289.
[16] X. Liu, Z. Zhang, G. Cheng, S. Dong, Electroanalysis 15 (2003) 103.
[17] M. Teixeira, M. Bergamini, C. Marques, N. Bocchi, Talanta 63 (2004) 1083.
[18] A. Sivanesan, S.A. John, Biosens. Bioelectron. 23 (2007) 708.
[19] S. Viswanathan, W.C. Liao, C.C. Huang, W.L. Hsu, A.H. Ja-an, Talanta 74 (2007) 229.
[20] Y. Tu, Q. Xu, Q.J. Zou, Z.H. Yin, Y.Y. Sun, Y.D. Zhao, Anal. Sci. 23 (2007) 1321.
[21] M.F. Bergamini, A.L. Santos, N.R. Stradiotto, M.V.B. Zanoni, J. Pharm. Biomed. Anal. 39 (2005) 54.
[22] P. Tömpe, A.N. Halbauer, L. Ladanyi, Anal. Chim. Acta 273 (1993) 391.
[23] S.M. Wabaidur, S.M. Alam, M.A. Khan, Appl. Chem. 12 (2008) 85.
[24] M.S. Quintino, M. Yamashita, L. Angnes, Electroanalysis 18 (2006) 655.
[25] H. Corrêa de Melo, A.P. Seleghim, W.L. Polito, O. Fatibello-Filho, I.C. Vieira, J. Braz. Chem. Soc. 18 (2007) 797.
[26] J.A. Arancibia, G. Martínez-Delfa, C.E. Boschetti, G.M. Escandar, A.C. Olivieri, Anal. Chim. Acta 553 (2005) 141.
[27] J.A. Arancibia, A. Rullo, A.C. Olivieri, S. Di Nezio, M. Pistonesi, A. Lista, B.S. Fernández, Band. Anal. Chim. Acta 512 (2004) 157.
[28] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, 1989.
[29] H. Martens, M. Martens, Multivariate Analysis of Quality—An Introduction, Wiley, Chichester, 2000.
[30] S. Wold, M. Sjostrom, L. Eriksson, Chemom. Intell. Lab. Syst. 58 (2001) 109.
[31] I.F. Hu, D.H. Karweik, T. Kuwana, J. Electroanal. Chem. 188 (1985) 59.
[32] USP 30-NF 25, The United States Pharmacopeia Thirtieth Revision, The National Formulary, 25th ed., CD-ROM version, 2007.
[33] British Pharmacopoeia, Co-beneldopa Capsules, Stationery Office, London, UK, CD-ROM version, 2003.
[34] D.M. Haaland, E.V. Thomas, Anal. Chem. 60 (1988) 1193.
[35] A.C. Olivieri, H.C. Goicoechea, F.A. Iñón, Chemom. Intell. Lab. Syst. 73 (2004) 189.
[36] MATLAB Version 7.0, The MathWorks, Nattick, Massachusetts, USA, 2004.
[37] A. Lorber, Anal. Chem. 58 (1986) 1167.
[38] http://www.models.kvl.dk/source/ (May 28, 2010).
[39] N.-P.V. Nielsen, J.M. Carstensen, J. Smedsgaard, J. Chromatogr. A 805 (1998) 17.
[40] G. Tomasi, F. Van den Berg, C. Andersson, J. Chemometrics 18 (2004) 231.
[41] S. Gunckel, P. Santander, G. Cordano, J. Ferreira, S. Muñoz, L.J. Núñez-Vergara, J.A. Squella, Chem. Biol. Interact. 114 (1998) 45.
[42] I. Novak, M. Šeruga, Š. Komorsky-Lovrić, Electroanalysis 21 (2009) 1019.
[43] A. Alberich, J.M. Diaz-Cruz, C. Ariño, M. Esteban, Analyst 133 (2008) 112–125.
[44] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, 2nd ed., John Wiley & Sons, Inc., New York, 2001.