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Simultaneous Kinetic-Potentiometric Determination of Levodopa and Carbidopa Using Multivariate Calibration Methods

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ABSTRACT

Partial least squares (PLS) and principle component regression (PCR) multivariate calibration methods were applied to the simultaneous determination of carbidopa and levodopa using kinetic data from novel potentiometry methods. These methods were based on the rate of chloride ion production in the reaction of carbidopa and levodopa with N-chlorosuccinimide (NCS) which was monitored by a chloride ion-selective electrode. The experimental data shows suitability of ion-selective electrodes (ISEs) to be used as detectors not only for the direct determination of chloride ion but also for simultaneous kinetic-potentiometric analysis using chemometric methods. These methods are based on the differences observed in the production rate of chloride ions. The results show that simultaneous determination of carbidopa and levodopa can be performed in their concentration ranges of 1.0 - 14.0 and 0.5 - 25.0 μg/mL, respectively. The total relative standard errors for applying PCR and PLS methods to 9 synthetic samples in the concentration range of 2.0 - 13.0 μg/mL for carbidopa and 1.0 - 18.0 μg/mL for levodopa were 4.81 and 4.29, respectively. The effects of certain additives used as excipients upon the reaction rate were determined to assess selectivity of the method. Both methods (PLS and PCR) were validated using a set of synthetic sample mixtures and then were successfully applied to the determination of carbidopa and levodopa in several commercially available mixture formulations. The recoveries were satisfactory and comparable to those obtained by applying the reference Pharmacopoeia method of high performance liquid chromatography.

Key words: simultaneous determination, kinetic-potentiometric, carbidopa, levodopa, PLS, PCR

INTRODUCTION

Levodopa [3-(3,4-dihydroxyphenyl)-*L*-alanine], an important neurotransmitter has been used for the treatment of neural disorders such as Parkinson's. Parkinson's disease is a progressive neurological disorder that occurs when the brain fails to produce enough dopamine. This drug is principally metabolized by an enzymatic reaction (dopa-decarboxilase) to dopamine compensating for the deficiency of dopamine in the brain. Carbidopa has been used as an inhibitor of the decarboxylase activity. These drugs as a combination (levodopa-carbidopa) are also used in ophthalmology for the treatment of tumors and spasms and controlling poor muscle control caused by CO and manganese intoxication[1-3]. By administering levodopa combined with carbidopa, the concentration of dopamine is controlled at an appropriate level effectively with generally reduced side effects[4]. 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. From this perspective, considerable work has been done on their detection and quantification. Various methods such as, spectrophotometry[5], spectrofluorimetry[6], gas chromatography[7], high performance liquid chromatography (HPLC)[8], radio immunoassay[9], chemiluminescence[10], potentiometry[11] and voltammetric determination[12] have been reported in the literature on the determination of these compounds in various biological samples and pharmaceutical preparations. Many of these methods cannot be applied for the simultaneous determination of these drugs and some are expensive and require complex pretreatment or toxic organic solvents. Damiani *et al.* designed and optimized

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a partial least squares (PLS) enhanced spectrophotometric method for the simultaneous determination of levodopa and carbidopa in pharmaceutical preparations, based on their oxidation with potassium periodate[12]. Madrakian *et al.* described two spectrophotometric methods for the simultaneous determination of binary mixtures of carbidopa and levodopa in pharmaceutical formulations, by mean centering of ratio spectra and H-point standard addition method (HPSAM)[13]. The methods are based on the difference in the absorption spectra for the products of the reaction of carbidopa and levodopa with 4-aminobenzoic acid in the presence of periodate ion. Safavi *et al.* also reported simultaneous kinetic spectrophotometric determination of these drugs by HPSAM[14]. The method was based on the difference in the rate of oxidation of these compounds with Cu(II)–neocuproine system and formation of Cu(I)-neocuproine complex. Recently, Chamsaz *et al.* reported a spectrophotometric method for the simultaneous determination of ternary mixtures of carbidopa, levodopa and methyldopa by multivariate calibration methods and artificial neural networks (ANN)[15]. The kinetic methodology was based on the difference of reaction rates between oxidizing agent of tris (1,10-phenanthroline)-iron (III) complex (ferriin, $[Fe(phen)3]^{3+}$) in the presence of citrate and spectrophotometrically. All the above mentioned chemometric methods[12-15] are based on the spectrophotometrically determination and to the best of our knowledge no one has reported any applications of the chemometric methods for the simultaneous determination of these drugs based on the electrochemical methods. Thus, the usage of potentiometry technique as a simple and inexpensive electroanalytical method is highly interesting and important.

Electroanalytical techniques are excellent and cheap procedures for the determination of trace chemical species. In recent years the usage of chemometrics methods including principal component regression (PCR), PLS, artificial neural network (ANN) and HPSAM in electroanalytical chemistry has received considerable attention for the extraction of more information from experimental data as in other areas of analytical chemistry[16-18]. In the field of potentiometry, several methods have been reported based on flow injection system and titration using PLS and ANN as modeling methods[19-22]. Recently, we reported the first application of PLS and PCR multivariate calibration methods and HPSAM to the simultaneous kinetic-potentiometric determination of binary mixtures of hydrazine and its derivatives[23,24]. The methods were based on the differences observed in the production rate of chloride ions in the reaction of hydrazine and its derivatives with N-chlorosuccinimide (NCS). The reaction rate of production of chloride ion was monitored by a chloride ion-selective electrode.

This work reports the first application of PLS and PCR multivariate calibration methods to the simultaneous kinetic-potentiometric determination of binary mixture of drugs using ion-selective electrodes (ISEs). These methods are based on the differences observed in the rate of production of chloride ion in the reactions of NCS with carbidopa and levodopa. The reaction rate of the production of chloride ion was monitored by a chloride ISE.

MATERIALS AND METHODS

I. *Apparatus and Software*

A solid-state chloride-selective electrode (Metrohm Model 6.0502.120) was used in conjunction with a double junction Ag/AgCl reference electrode (Metrohm Model 6.0726.100), whose outer compartment was filled with a 10% (w/v) KNO_3 solution. The Metrohm Model 780 potentiometer, attached to a Pentium (IV) computer, was used for recording the kinetic potentiometric data. All measurements were carried out in a thermostated (25 ± 0.2°C), double-walled reaction cell with continuous magnetic stirring. The electrode was stored in 1×10^{-3} M potassium chloride solution when not in use. For pH measurements, a Metrohm Model 780 pH meter with a combination glass electrode was used. The data were treated in an AMD 2000 XP (256 Mb RAM) microcomputer using MATLAB software. PLS and PCR analysis were performed using PLS and PCR toolboxes in the MATLAB 7.0 program.

A high-performance liquid chromatograph (Shimadzu, Japan) equipped with a Hypersi column (150 mm × 3.9 mm i.d., 5 µm Japan), an UV detector and a recording digital integrator (C-R6A, Shimadzu, Japan) were used. The UV detector was set at 280 nm, and the recorder was set at 1.0 cm/min.

II. *Reagents and Chemicals*

All chemicals were of analytical reagent grade and doubly distilled water was used throughout the experiment. Stock solutions (1000 µg/mL) of levodopa (Fluka) and carbidopa (Fluka) were prepared in 100-mL flasks by dissolving appropriate amounts in water and diluting to the mark. Both solutions were stored in a refrigerator at 4°C and protected from light. Under these conditions, the solutions were stable for 15 days. A 0.05 M N-chlorosuccinimide stock solution was prepared by dissolving 0.6667g of NCS (Serva) in water and diluting to 100 mL. The solution was prepared daily and kept at 4°C in an amber-coloured bottle in the dark. Acetate buffer solution (0.05 M, pH 5.0) was prepared using acetic acid and NaOH solutions and its pH was monitored with a pH meter and adjusted as needed.

III. *Procedure*

Twenty five milliliters of doubly distilled water, 2.0 mL of buffer solution and 1.0 mL of 5×10^{-2} M NCS solution were transferred into a thermostated (25ºC) reaction

cell. Ten milliliters of the standard or sample solution of levodopa, carbidopa or a mixture of them were injected into the cell quickly, and after the stabilization of the potential, all data were recorded. The potential changes versus time were recorded at the time intervals of 1s. Simultaneous determination of levodopa and carbidopa was performed by recording the potential changes for each solution from 10 to 300 s. After each run, the cell was emptied and washed twice with distilled water.

Using the standard analyte solutions, a calibration graph of ($10^{\Delta E/S}$-1) *versus* concentration (fixed-time method) was constructed[25] (Table 1), where ΔE is the potential change in a selected time interval Δt (usually 290 s) and S is the slope of the chloride electrode response, which was determined periodically by successive additions of micro-amounts of 100 μL of 1.0×10^{-2} - 3.0 M KCl standard solutions in 25.0 mL of water mixed with 2.0 mL of buffer solution. The simultaneous determination of carbidopa and levodopa can be performed in the concentration ranges of 1.0 - 14.0 and 0.5 - 25.0 μg/mL, respectively.

PLS and PCR algorithms and their general principles are similar. PLS and PCR have been used in this study to resolve simultaneously mixtures of levodopa and carbidopa by recording the potential for each solution from 10 to 300 s. In this research, a synthetic set of 33 solutions including different concentrations of levodopa and carbidopa was prepared. A collection of 24 solutions were selected as the calibration set (Table 2) and the other 9 solutions were used as the prediction set (Table 3). Their composition was randomly designed to obtain more information from the calibration procedure. Changes in the potential of the solutions were recorded between 10 and 300 s, which implies experimental points (at 1- second intervals) were selected for analysis. In this potential region, the maximum difference in kinetic behavior is shown. Kinetic-potentiometric data were collected in the Excel program and fed into the MATLAB program. Data were mean-centered before being input to the appropriate algorithms.

For the analysis of real samples, twenty tablets of each sample containing levodopa and carbidopa were accurately weighted and their solutions were prepared through dissolving a certain amount of grounded tablets in the weak acidic solutions and filtering the solutions. The prepared solutions containing aliquot amounts of levodopa and carbidopa were analyzed (n = 4).

For the HPLC experiments, the above stock solutions of levodopa and carbidopa were employed. The mobile phase and buffer were prepared based on the reference method of US Pharmacopeia[26].

RESULTS AND DISCUSSION

In order to accomplish simultaneous kinetic potentiometric determination of levodopa and carbidopa to be implemented through PLS and PCR, a series of experiments were conducted to establish the optimum conditions to achieve maximum sensitivities. In the proposed analytical method, using suitable reagent to react with levodopa and carbidopa under different rates as the first step is very important. Under this condition, the rate of halide ion production through the reaction of reagent with levodopa and carbidopa will differ; and consequently the potentiometric responses for levodopa and for carbidopa will be different. Various reactants such as 1-fluoro-2,4-dinitrobenzene (FDNB), 1-chloro-2,4-dinitrobenzene (CDNB), 1-bromo-2,4-dinitrobenzene (BDNB), N-bromosuccinimide (NBS), sodium dichloroisocyanurate (SDCC) and NCS were investigated as reagents for reaction with levodopa and carbidopa to produce halide ions. Preliminary studies showed that NCS is a suitable reagent for our purpose. By using this reagent, we realized that the linear range and differences of reaction rate for both species (levodopa and carbidopa) were better than those for any other reagents.

NCS, a chlorinating and oxidizing agent is more stable than alkali hypochlorite which contains chlorine in the same oxidation state but disproportionates easily to chlorate and chloride ions[27]. In oxidation reactions, NCS undergoes a two electron reduction to succinimide (NHS) and chloride ion. Alternatively, NCS may be regarded as a source for hypochlorous acid, which is generated by hydrolysis (Scheme 1):

Hypochlorous acid is probably responsible for the oxidizing properties of NCS. Levodopa and carbidopa are oxidized by NCS to ketonic and azoic derivatives, respectively. Moreover, NCS is reduced to NHS (Scheme 2)[24-31]:

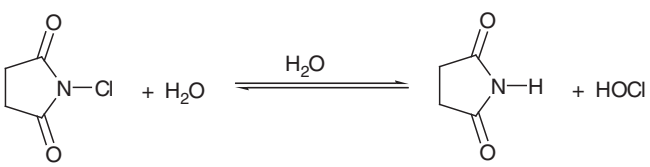
All experimental parameters affecting the reaction rate of NCS with levodopa and carbidopa (electrode characteristics, concentration of NCS, pH, etc) were optimized by "one at a time" studies.

I. *Study of Electrode Characteristics*

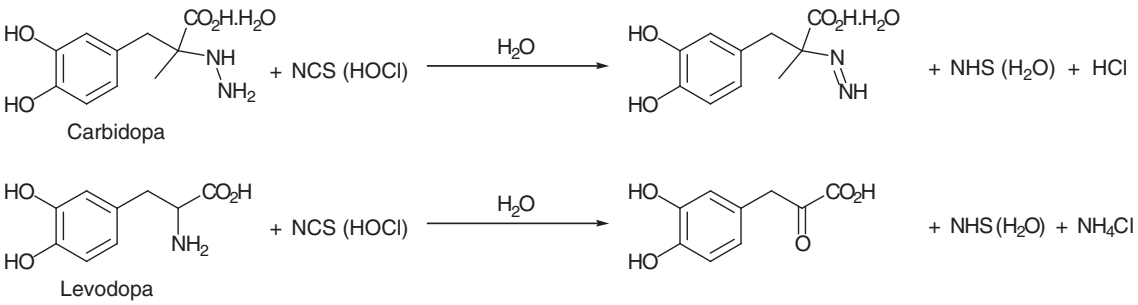
Characteristics of the chloride-selective electrode in the acetate buffer were studied. Typical calibration graphs for the chloride-selective electrode showed a linear response in the range of 3×10^{-6} - 1 M chloride. The slope was found to be 56.4 mV/decade and remained almost constant at 0.4 mV over 10 months of use in this system.

II. *Effect of NCS Concentration and pH*

The effect of NCS concentration on the reaction



Scheme 1



Scheme 2

rate of levodopa and carbidopa and mixture of them was investigated. The reaction rates of both species increase with the concentration of oxidant (NCS) in the range of 5 × 10^{-5} - 5 × 10^{-2} M. Further increase in oxidizing agent concentration was avoided due to the limited solubility of NCS in water. Therefore, a concentration of 5 × 10^{-2} M NCS was selected as the optimum concentration for further studies.

The effect of pH on the reaction rates of levodopa and carbidopa with NCS over the pH range of 2.0 to 9.0 was examined. Since, maximum differences in kinetic behavior levodopa and carbidopa were observed at pH 5.0; this pH was selected as the optimum pH for both analytes.

The electrode potential was also investigated at different pHs. The electrode potential is stable in pure NCS solutions. Therefore, NCS solutions behave like dilute chloride solutions. The concentration of chloride, equivalent to NCS (having the same potential), increases with pH, and provides a constant background response before injection of the sample solutions. Concentration of chloride was minimized at pH 5.0.

III. *Effect of Surfactants*

The effect of each three types of surfactants (anionic, cationic and nonionic) and their concentrations on the reaction rates of levodopa and carbidopa with NCS were investigated. The results showed that each type of surfactants of sodium dodecyle sulfate (SDS), cetyltrimethyl ammonium bromide (CTAB) and Triton X-100 (TX-100) reduces the reaction rate of both species (levodopa and carbidopa). Therefore, the reaction should be performed without surfactants.

IV. *Potential-times Behavior*

Figure 1 shows the potential-time behavior of reactions of NCS with levodopa and carbidopa and mixtures of them at the optimized conditions. Figure 2 shows typical reaction curves for the reaction of NCS with levodopa and carbidopa at different concentrations. As shown Figures 1 and 2, the reaction of carbidopa was faster than levodopa and was almost completed in 40 s after the initial reaction but reaction of levodopa is completed in 300 s. This difference in the reaction rates allowed us to

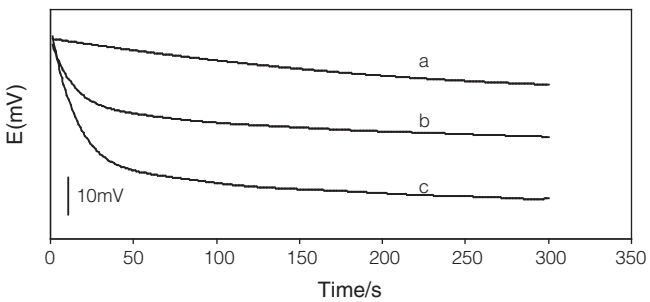


Figure 1. Potential-time curves for the reaction of NCS with 5 μg/mL of levodopa (a), 15 μg/mL of carbidopa (b) and mixture of them (c).

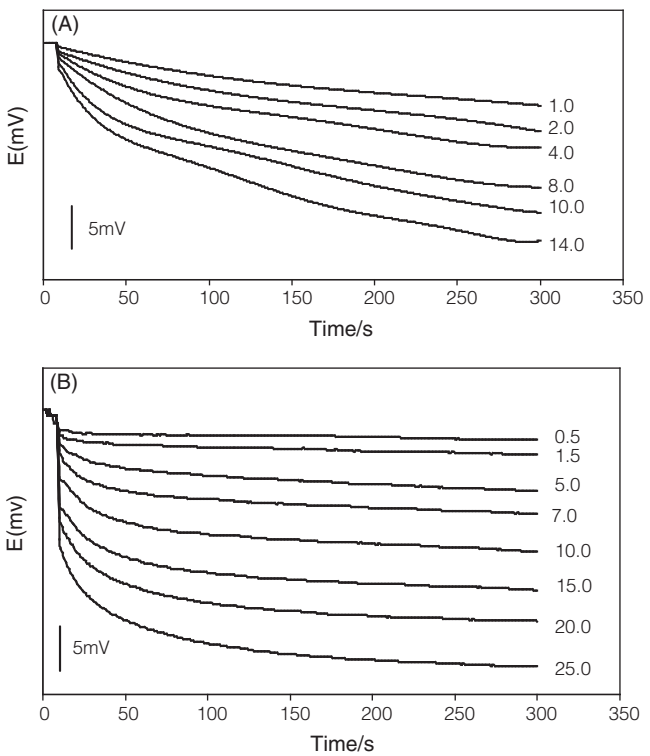


Figure 2. Typical potential-time curves for the reaction of NCS with levodopa (a) and carbidopa (b) at different concentrations (μg/mL).

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Table 1. Characteristics of calibration graphs for the determination of levodopa and carbidopa

Species	Linear range (μg/mL)	Slope (μg/mL)	Intercept	Correlation coefficient (n = 10)	Detection limit[a] (μg/mL)
Levodopa	1.0-14.0	0. 430	0.0706	0.9982	0.70
Carbidopa	0.5-25.0	0.752	-0.0306	0.9981	0.20

[a] concentration corresponding to a net analytical signal equal to three times the standard deviation of the blank.

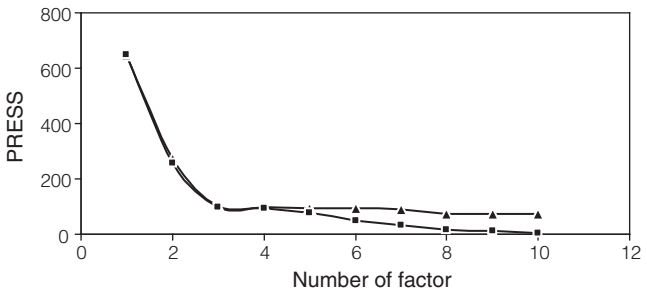

Figure 3. Plot of PRESS against the numbers of factors for mixture of levodopa and carbidopa for PCR (▲) and PLS (■).

design the PLS and PCR methods for simultaneous determination of levodopa and carbidopa.

Under the optimum conditions, the amount of $(10^{\Delta E/S}\text{-}1)$ of the produced at the reaction of NCS with levodopa and carbidopa is linear with their concentrations (Figure 3). Characteristics of calibration graphs for determination of levodopa and carbidopa are given in Table 1.

V. *Multivariate Calibration and Statistical Parameters*

Multivariate calibration consists of establishment of a relationship between matrices of chemical data. These methods are based on a first calibration step in which a mathematical model is built using a chemical data set (e.g., potential values) and a concentration matrix data set. The calibration is followed by a prediction set in which this model is used to estimate unknown concentrations of a mixture from kinetic profile. Multivariate calibration methods are being successfully applied to the multi-component kinetic determination to overcome some of the drawbacks of classical methods. Soft algorithms such as PCR, PLS and ANN, which avoid colinearity problems, have been used for simultaneous determination of the analytes having the same chemical properties that cannot be resolved with common methods. The theories and applications of chemometric methods such as PLS and PCR to the analysis of multi-component mixtures have been discussed by several workers[32-35]. These multivariate calibration methods pave the way to deal with the problem of ill conditioned matrices. In particular, PCR and PLS techniques are called "factor methods" because transform the high number of original variables in to a smaller number of orthogonal variables called "factors" or "principal components", which are linear. PLS regression and PCR are methods used for building regression models based on the latent variable decomposition and set a relationship between the two blocks, matrices X and Y, containing the independent, x, and dependent, y, variables, respectively. These matrices can be simultaneously decomposed into a sum of latent variables, as follows:

$$X = TP^T + E \quad (1)$$

$$Y = UQ^T + F \quad (2)$$

Table 2. Calibration set for constructing PLS and PCR method in determination of levodopa and carbidopa (μg/mL)

Sample number	Levodopa	Carbidopa
1	2.0	1.0
2	2.0	3.0
3	2.0	5.0
4	2.0	10.0
5	5.0	1.0
6	5.0	7.0
7	5.0	10.0
8	5.0	15.0
9	7.0	3.0
10	7.0	5.0
11	7.0	7.0
12	7.0	18.0
13	9.0	5.0
14	9.0	7.0
15	9.0	10.0
16	9.0	15.0
17	11.0	7.0
18	11.0	10.0
19	11.0	15.0
20	11.0	18.0
21	13.0	1.0
22	13.0	3.0
23	13.0	5.0
24	13.0	1.0

Table 3. Prediction set for constructing PLS and PCR method in determination of levodopa and carbidopa

Sample	Actual (μg/mL)		Predicted (μg/mL)				Recovery (%)			
			PCR		PLS		PLS		PCR	
	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa	Levodopa	Carbidopa
1	2.0	7.0	2.12	7.12	2.05	7.03	106.0	101.7	102.5	100.4
2	2.0	15.0	2.12	15.78	2.18	15.80	106.0	105.2	104.0	105.3
3	5.0	3.0	5.22	3.23	5.13	3.13	104.4	107.6	102.6	104.3
4	5.0	5.0	5.19	5.13	5.08	5.07	103.8	102.6	101.6	101.4
5	7.0	1.0	7.25	0.98	7.12	0.94	103.5	98.0	101.7	94.0
6	7.0	10.0	7.18	9.63	7.10	9.65	102.5	96.5	101.4	96.5
7	9.0	18.0	9.41	18.84	9.36	18.80	104.5	104.6	104.0	104.4
8	11.0	3.0	11.39	3.09	11.38	3.15	103.5	103.0	103.4	105.0
9	13.0	7.0	13.80	6.76	13.67	6.87	106.1	96.5	105.1	98.1
Mean Recovery (%)							104.4	101.7	102.9	101.0
RSEP (%)							4.94	4.22	4.71	4.33
$RSEP_t$ (%)							4.81		4.29	

in which *T* and *U* are the score matrices for *X* and *Y*, respectively; *P* and *Q* are the loading matrices for X and Y, respectively; E and F are the residual matrices. In this procedure, it is necessary to find the best number of latent variables for both methods (PLS and PCR), which normally is performed by using cross-validation based on the determination of minimum prediction error. The first step in the simultaneous determination of species through these methodologies involves constructing the calibration matrix for the binary mixture of levodopa and carbidopa. To construct the calibration set, we applied random design to five levels to extract a great deal of quantitative information using only a few experimental trials.

To select the number of factors (latent variables) in the PLS and PCR algorithm a cross-validation without one-sample methods was employed[32]. An important chemometric tool is called cross-validation. The basis of the method is that the predictive ability of a model formed on part of a dataset can be tested out by how well it predicts the remainder of the data. It is possible to determine a model using *i*-1 samples leaving out one sample (*i*).

The optimum number of factors (latent variables) to be included in the calibration model was determined by computing the prediction error sum of squares (PRESS) for cross-validated models using a high number of factors (half the number of total standard + 1), which is defined as follows:

$$PRESS = \sum_{i=1}^{m} \left(\hat{C}_i - C_i \right)^2 \quad (3)$$

where m is the total number of calibration samples, $\hat{C}i$ represents the estimated concentration, and Ci is the reference concentration for the *i*th sample left out of the calibration during cross validation. One reasonable choice for the optimum number of factor would be the number which yields the minimum PRESS. Since there are a finite number of samples in the training set, in many cases the minimum PRESS value causes over fitting for unknown samples that were not included in the model. In order to find the fewest number of factors, the F-statistic was used to carry out the significant determination[32]. Figure 3 shows a plot of PRESS against the factors involved in the mixture of components. The optimum number of factors for two components was 3 for both PLS and PCR.

For the evaluation of the predictive ability of a multivariate calibration model, the root mean square error of prediction (RMSEP) and the relative standard error of prediction (RSEP) can be used [32] :

$$RMSEP = \left(\sum_{i=1}^{N} \left(\hat{C}_i - C_i \right)^2 / n \right)^{1/2} \quad (4)$$

$$RSEP\,(\%) = \left(\sum_{i=1}^{N} \left(\hat{C}_i - C_i \right)^2 / \sum_{i=1}^{N} (C_i)^2 \right)^{1/2} \times 100 \quad (5)$$

where $\hat{C}_i$ represents the estimated concentration, C_i and *n* are the actual analyte concentration and the number of samples, respectively.

The squares of correlation coefficient (R^2), which is an indication of the quality fit of all the data to a straight line is presented by:

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$$R^2 = \sum_{i=1}^{N}\left(\hat{C}_i - C'\right)^2 / \sum_{j=1}^{N}\left(C_i - C'\right)^2 \quad (6)$$

Table 4 shows values of RSEP, RMSEP and R^2 for each component using PLS and PCR. It is shown that the obtained values for the statistical parameters are almost the same for both PLS and PCR methods.

VI. *Interference Studies*

In order to assess the possible analytical applications of the proposed methods, the effects of common excipients used in pharmaceutical preparations were studied. Potential changes of a solution containing levodopa (2.0 μg/mL) and carbidopa (2.0 μg/mL) were analyzed for four times. Afterwards the effect of interfering species at different concentrations on the potential of this solution was studied. A species was considered an interference when its presence produced a variation in the concentration of the levodopa and carbidopa mixture (in the period of 300 s) with more than 5% relative error. The following excipients did not interfere in the maximum tested concentrations (μg/mL) shown in parentheses: starch (1000), sugar (500), galactose (500), glucose (500), sucrose (500), riboflavin (100). Some other compounds with significant redox properties (e.g., ascorbic acid) may interfere in the proposed procedure. But our goal was the determination of levodopa and carbidopa in tablets. In these tablets, other redox compounds are absent.

VII. *Application*

The proposed methods were applied to determine simultaneously levodopa and carbidopa in several commercially available pharmaceutical formulations. Twenty tablets of each sample were accurately weighed and their solutions were prepared by dissolving them in water and filtering the solutions. The prepared solutions containing aliquot amounts of levodopa and carbidopa were analyzed (n = 4). The accuracy of proposed methods was determined by analyzing the recoveries of known amounts of analytes into samples and comparing test results from the proposed methods (PCR and PLS) with those obtained applying the reference method proposed in the based on HPLC. The reference method was conducted using UV monitoring at 280 nm and Hypersi column (150 mm × 3.9 mm i.d. 5 μm Japan). A mixture of 11.04 g of monobasic sodium phosphate, 1.3 mL of sodium 1-decanesulfonate solution (0.24 g of sodium 1-decanesulfonate dissolved in 1 liter of water) and 950 mL of water in a beaker was prepared. The flow rate, about 2 mL per minute, was adjusted until the retention times for levodopa and carbidopa were 4 and 11 minutes, respectively. For sample preparation, 50 mg of levodopa was transferred to a 100-mL volumetric flask. An accurately weighed quantity of carbidopa, which is in a ratio with levodopa that corresponds with the ratio of carbidopa to levodopa in the tablets, was added. 10 mL of 0.1M phosphoric acid was added warmed gently to dissolve the standard mixed and then diluted with water to the mark. Chromatograph five replicate injections of the standard preparation, and recorded the peak response as directed for procedure. The relative standard deviation was not more than 2.5% and the resolution factor between levodopa and carbidopa was not less than 7. For assay preparation, twenty tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 50 mg of levodopa, was transferred to a 10-mL volumetric flask. Then 10 mL of 0.1 M phosphoric acid was added and mixed and then diluted with water to the mark.

The quantitative results of this analysis are summarized in Table 5. Good agreements between results obtained and the nominal values labeled and reference method indicate that PCR and PLS can be applied successfully for simultaneous determination of levodopa and carbidopa in pharmaceutical samples. Although commercial samples always contain excipients, good recoveries confirmed that they do not seriously interfere under the present conditions. A comparison of PLS and PCR results with those provided by HPLC using standard *t*-test statistics revealed no significant differences between the chemometrics-assisted methodologies and the reference technique (Table 5).

CONCLUSIONS

This work as the first application of chemometrics methods to the simultaneous determination of binary mixture of levodopa and carbidopa shows the ability and excellent performance of ISEs as detectors not only for individually determination of produced or consumed ions, but also in simultaneous kinetic-potentiomet-

Table 4. Statistical parameters calculated for the prediction set using PLS and PCR methods

Component	RSEP (%)		RMSEP		R^2	
	PCR	PLS	PCR	PLS	PCR	PLS
Levodopa	4.33	4.22	0.4570	0.4192	0.9786	0.9988
Carbidopa	4.71	4.94	0.3997	0.3362	0.9916	0.9989

Table 5. Simultaneous kinetic-potentiometric determination of levodopa and carbidopa in pharmaceutical samples using PCR and PLS methods and comparison with HPLC[26].

Sample	Levodopa (mg)					Carbidopa (mg)				
	PCR	PLS	HPLC	t^d	t^d	PCR	PLS	HPLC	t^d	t^d
1[a]	105.7 (± 1.2)[c]	104.7 (± 1.1)[c]	104.3 (± 2.6)[c]	1.0	0.3	9.4 (± 0.6)[c]	9.8 (± 0.6)[c]	10.3 (± 2.4)[c]	0.8	0.4
2[b]	257.4 (± 1.2)[c]	256.8 (± 1.3)[c]	262.8 (± 1.8)[c]	1.9	1.5	24.4 (± 0.7)[c]	24.6 (± 1.0)[c]	25.5 (± 2.1)[c]	1.0	0.9

[a] (Levodopa,100 mg; Carbidopa,10 mg per tablet); Ramofarmin Co. IRAN

[b] (Levodopa, 250 mg; Carbidopa, 25 mg per tablet); Alborz Daruo Co. IRAN

[c] Standard deviation (*s*) is in parenthesis. The results are averages of four replicates and are given in mg per sample.

[d] Calculated values of the statistical *t* coefficient for the PCR and PLS methods comparison with HPLC. The critical value at 95% confidence level and 6 d.f. is t (ν = 6, 95%) = 2.45.

ric analysis. In addition, the abilities and advantages of chemometrics methods such as PLS and PCR in the analysis of multi-component mixtures and advantages of ion-selective electrodes i.e. high selectivity, sensitivity and freedom from optical interferences along with the selectivity, sensitivity, rapidity, flexibility and simplicity of kinetic methods, produce very attractive and versatile techniques. Reproducibility and accuracy tests were successful, and the recovery results were statistically comparable to those obtained by the reference method based on HPLC. The proposed methods are cheaper than chromatographic separation methods. Furthermore, we don't need to use complex pretreatment or toxic organic solvents in these methods. In other words, they belong to green chemistry.

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