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Simultaneous determination of vitamins C, B6 and PP in pharmaceutics using differential pulse voltammetry with a glassy carbon electrode and multivariate calibration tools

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Abstract

In this work, the artificial neural networks (ANN) and partial least squares (PLS) were applied to data obtained by differential pulse voltammetry for the determination of vitamins in synthetic and pharmaceutical samples. For calibration purposes, both synthetic and commercial samples were employed as standards. From the results it was possible to verify that ANN is the best method for modeling the data due to the fact that interactions among electro-active components result in non-linear response on the glassy carbon electrode. The results achieved for the determination of vitamins in pharmaceutical samples using ANN method provided a maximum value for relative error of 0.40% for VC, 8.3% for VPP and 9.1% for VB6. The proposed methodology is simple, rapid and can be easily used to control quality laboratories as an alternative analysis method.

Keywords: Vitamin C; Vitamin B6; Vitamin PP; Differential pulse voltammetry; Partial least squares; Artificial neural networks

1. Introduction

Vitamins are organic substances essential to human body function. They act mainly as coenzymes in numerous metabolic ways, with the systematic absence of vitamins in the diet the cause of some diseases [1]. Recently, enriched foods and pharmaceutics preparations have become an important means of acquiring vitamins for the organism. In fact of the large consumption of these products necessitates control methods to assure their quality. For most of the methods described in the literature involve prior chemical and physical separations. These methods include high-performance liquid chromatography with electrochemical and UV detection [2–4], titration and spectrophotometric techniques [5,6]. On the other hand, a few studies employing multivariate data analysis in conjunction with spectrophotometric [7,8] and electrochemical techniques [9] have also been reported.

In the present work, two calibration models were evaluated using data obtained by differential pulse voltammetry: artificial neural networks (ANN) and partial least squares (PLS).

Partial least squares [10,11] is the method normally used for multivariate calibration, where the multivariate signal, in this case current measured at different potentials of the voltammograms (variable *x*) and concentrations (variable *y*) of the samples are used to establish a linear regression model. First, the data are placed in matrix form: matrix **X** and **Y** which contain the independent, *x*, and dependent, *y*, variables respectively. These matrices are decomposed into a sum of latent variables and two sets of models are obtained:

$$\mathbf{X} = \mathbf{T}\mathbf{P}^{\mathrm{T}} + \mathbf{E} = \sum \mathbf{t}_{\mathrm{f}}\mathbf{p}_{\mathrm{f}}^{\mathrm{T}} + \mathbf{E} \quad (1)$$

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$$\mathbf{Y} = \mathbf{U}\mathbf{Q}^{\mathrm{T}} + \mathbf{F} = \sum \mathbf{t}_{\mathrm{f}}\mathbf{p}_{\mathrm{f}}^{\mathrm{T}} + \mathbf{F} \quad (2)$$

in which **T** and **U** are the score matrices; **P** and **Q** are the loading matrices and **E** and **F** are the residual matrices. The superscript T indicates a transposed matrix. The product of **T** and $\mathbf{P}^{\mathrm{T}}$ approximates to the independent variables (e.g. voltammograms data) and the product **U** and $\mathbf{Q}^{\mathrm{T}}$ to the dependent variables (e.g. concentrations). In the PLS method, significant information contained in the voltammograms is concentrated in a few latent variables that are optimized to produce the best correlation with the desired property to be determined (concentration). It is possible to obtain a scores matrix that is common to both the concentration (**Y**) and measurements (**X**). The concentration of new samples can be estimated from the new scores $\mathbf{T}^{*}$ and the model loading **Q**, which can be substituted in Eq. (2), leading to Eq. (3):

$$\mathbf{Y}_{\mathrm{new}} = \mathbf{T}^{*}\mathbf{Q}^{\mathrm{T}} \quad (3)$$

Using cross-validation which is based on the determination of the minimum prediction error performs the best number of latent variable necessary in this procedure.

Usually PLS method assumes a linear relationship between the measured sample parameter and the intensity of its signal. Small deviations from linearity are acceptable. In this case additional modeling factors are taken into account in the model. However, in the presence of substantial non-linearity, PLS tends to give large prediction errors and calls for more robust models. Intrinsically non-linear calibration methods such as artificial neural networks are applicable in the latter case. In many cases the principal disadvantage of neural network is the time necessary for trained it.

Artificial neural networks [12] is a multivariate calibration method used mainly for modeling non-linear data, although, some applications use the neural network for modeling linear data. This form of multivariate data analysis is becoming extremely important in many analytical applications [13].

The artificial neural network is a system composed of several simple units (artificial neurons), properly linked, producing a complex behavior. The neural network behavior is determined by their topologies. In this study feed-forward was used as topology and the neural network architecture composed of three layers: the first layer corresponds to data input (the input can be the current measured at different potentials or principal components), one hidden layer with an appropriate number of neurons and an output layer or responses of the neural network corresponds to concentration of vitamins in pharmaceutics.

To train a neural network, the data input multiplied by weights are integrated into an artificial neuron. The output of each layer is obtained by applying a linear, sigmoid or tangent hyperbolic transfer function to these data. Normally, the bias is added to transfer function for better adjustment. For multilayer networks the output of one layer becomes the input to the following layer and the outputs of the neurons in the last layer are considered the network outputs.

The network output or the estimated values obtained are compared to the expected value obtaining the mean square error (calibration error). The error is defined as the sum of the square resulting from the difference between the estimate value and the expected value. The next step is to correct the weights of all layers until the calibration error is minimized, which can be made through of a specified algorithm. The algorithm utilized to correct the weights and biases in this study was Marquardt–Levenberg algorithm. This can be represented by Eq. (4).

$$\Delta\mathbf{x}_{\mathrm{k}} = -[\mathbf{J}^{\mathrm{T}}(\mathbf{x}_{\mathrm{k}})\mathbf{J}(\mathbf{x}_{\mathrm{k}}) + \mu_{\mathrm{k}}\mathbf{I}]^{-1}\mathbf{J}^{\mathrm{T}}(\mathbf{x}_{\mathrm{k}})(\mathbf{v}_{\mathrm{k}}) \quad (4)$$

where **J** is the Jacobian matrix of the error for each weights, μ the non-negative scalar, **I** the identity matrix, ($\mathbf{x}_{\mathrm{k}}$) represents weights and ($\mathbf{v}_{\mathrm{k}}$) represents error. This learning method can be seen as an intermediate procedure between the Gauss–Newton method and the steeped descendent method. In this algorithm when μ assumes elevated values the descendent gradient method is obtained and when μ assumes small values the Gauss–Newton method predominates. The Marquardt–Levenberg method is faster in convergence and is more robust than the other algorithms.

After correcting the weights and biases and obtaining a satisfactory error, the artificial neural network is completely trained, and it is possible to evaluate the generalization properties of the neural network by adopting another group, a validation set, which has different data from those used in the calibration. The concentrations of vitamins in this new group can be predicted.

In this work, artificial neural networks and partial least squares in conjunction with differential pulse voltammetry (DPV) were used to substitute traditional methods for vitamins determinations. These methods are especially appealing for the determination of the active components in complex pharmaceutical samples whose other components may show analytical signals, which are severally overlapped with those from the analytes. In this study, although the determination in synthetic samples does not show overlap, this can be found in pharmaceutical sample which impossibility to use a univariate method for analysis.

The proposed methodology is fast, simple and does not generate hazardous chemical wastes, thus making it easily possible to use in quality control analysis.

2. Experimental

2.1. Reagent and commercial samples

All the chemicals were of analytical-reagent grade. Nicotinamide (VPP), pyridoxine hydrochloride (VB6) and ascorbic acid (VC) (all from Sigma, St Louis, MO, USA) were used without purification. Supporting electrolyte solution: Sodium dihydrogen phosphate salts (Merck) and phosphoric acid (Merck) were used to prepare buffer solutions (pH

6.0; 0.2 mol L^{-1}). Stock solutions were prepared daily by dissolving each sample in buffer solution, obtaining concentrations of 3.0 mg mL^{-1} for ascorbic acid (VC), 0.8 mg mL^{-1} for nicotinamide (VPP) and 0.09 mg mL^{-1} for pyridoxine hydrochloride (VB6).

The pharmaceutical preparations assayed had the following composition: Revitam® (Biolab-Sanus, Brazil), vitamin A–1250 U, VB1–0.4 mg, VB2–0.5 mg, VB6–0.6 mg, VB12–0.5 μg, VC–35.0 mg, vitamin D3–400 U, folic acid–35.0 μg, VPP–6.0 mg, D-Panthenol–3.0 mg, Na_2EDTA, polisorbate 80, ascorbate, magrogol, Etilmaltol, sacarin, sodium ciclamate, sucrose, orange flavor, water, and butylhydroxyanizole, as excipients per mL. Teragran® (Bristol-Meyers Squibb), vitamin A–2500 U, vitamin D3–200 U, VB1–1.5 mg, VB2–1.5 mg, VB6–0.5 mg, VPP–10.0 mg, D-Panthenol–2.5 mg, VB12–2.5 μg, VC–25.0 mg and Polisorbate, ferric ammonium citrate, sodium benzoate, glycerol, orange flavor, and liquid sugar, as excipients per 2.5 mL. No brand name formulation sample, vitamin A–1250 U, VB1–0.4 mg, VB2–0.5 mg, VB6–1.0 mg, VB12–0.5 μg, VC–25.0 mg, vitamin D3–400 U, folic acid–35.0 μg, VPP–12.0 mg, D-Panthenol–3.0 mg, per mL and the same excipients as Teragran®. This sample was furnished by a local manufacturer.

For analysis, stock solutions of each sample were prepared in phosphate buffer solution, obtaining the same concentration utilized for synthetic samples.

Deionised water obtained from a Millipore Milli-Q apparatus was used throughout.

2.2. Chromatographic analysis

The pharmaceutical preparations were first analyzed by HPLC technique, which was used as reference.

The HPLC system was composed of a Shimadzu (Tokyo, Japan) LC-10 AD pumps, a SPD-M10A UV–vis detector and an universal injector. The software to process the chromatographic data was Class-LC, 1.64 version. A NH_2 chromatographic column (Shimadzu-Shim-Pack) was utilized. The separation of the vitamins was carried out by an elution with a 1:1 v/v methanol–potassium dihydrogen phosphate (0.05 M) solution which pH was adjusted to 2.8 by adding proper amounts of a phosphoric acid 0.01 M solution. All measurements were carried out in a thermostated room (20 ± 1 °C) at a flow-rate of 1 mL min^{-1}. The UV detector at 254 nm revealed the peaks. Quantitative data were obtained using external standardization, and analytical curves were constructed by plotting peak areas versus concentration. The results using the HPLC technique are shown in Table 1.

2.3. Electrochemical measurements

Differential pulse voltammetric experiments were performed with a polarographic analyzer from (EG&G Princeton Applied Research (PAR) model 174, which was automated by the use of a twelve bits A/D&D/A Lab made interface connected to an IBM/PC compatible microcomputer where the voltammetric waveforms were computed and the acquired data were recorded. In this system for a differential pulse voltammogram a pair of data points (*E* versus d*i*) is obtained for each applied pulse that means the total number of data points is given by the potential interval divided by the potential increment that follows each potential pulse. The DPV curves for the real samples where registered from −1500 mV to 1300 mV covering all the available potential window for that solutions using 7 mV for the potential step, which results in 400 data points per voltammogram. From that data banks appropriated potential intervals were used to compose the matrix for the ANN and PLS calculations. A conventional three-electrode system was employed consisting of a glassy carbon electrode as working electrode, an $Ag/AgCl_{(s)}$ reference electrode and a Pt wire as counter-electrode. The electrode was polished with alumina and washed with water purified in a Milli-Q system and the sample solutions were degassed by a nitrogen flow for 15 min before measurements.

Table 1
HPLC results and label claim of VC, VPP and VB6 in Revitam®, no brand formulation (mg mL^{-1}) and Teragran® (2.5 mg mL^{-1})

Sample	Vitamin C	Vitamin PP	Vitamin B6
Revitam®[a]	35.20 ± 1.40	6.00 ± 0.30	0.60 ± 0.10
Revitam®[a]	35.40 ± 1.30	6.10 ± 0.30	0.70 ± 0.08
Revitam®	35.0	6.0	0.6
Teragran®[a]	25.10 ± 1.10	10.20 ± 0.80	0.50 ± 0.10
Teragran®[a]	25.20 ± 1.00	10.10 ± 0.45	0.50 ± 0.15
Teragran®	25.0	10.0	0.5
No brand formulation[a]	25.00 ± 1.30	12.10 ± 0.90	1.10 ± 0.10
No brand formulation[a]	25.20 ± 1.24	12.30 ± 0.90	1.20 ± 0.17
No brand formulation	25.0	12.0	1.0

[a] Average ± S.D. for three determinations.

Differential pulse voltammetry was carried out for the mixture of synthetic vitamins and for the pharmaceutical samples in phosphate buffer solutions (pH 6.0; 0.2 mol L^{-1}). The instrumental parameters of DPV were scan speed: 14 mV s^{-1}, pulse duration: 100 ms, pulse frequency: 0.5 s^{-1} and pulse amplitude: 50 mV.

2.4. Software

The data were handled using MATLAB software, 6.0 version (The Mathworks, Natik, USA). PLS toolbox, 2.0 version (Eigenvector Technologies, Manson, USA).

2.5. Calibration and validation set

For calibration purposes several models were proposed. In the first, synthetic samples of pyridoxine hydrochloride (VB6), nicotinamide (VPP) and ascorbic acid (VC) in a phosphate buffer (pH 6.0, 0.2 mol L^{-1}) were utilized as standards. The sample set was prepared in the concentration range of 0.07–0.8 mg mL^{-1} for nicotinamide, 0.05–3.0 mg mL^{-1} for

ascorbic acid and 0.008–0.08 mg mL^{-1} for pyridoxine hydrochloride, resulting in a total of 151 samples.

For validation of this model, three synthetic samples with different concentration of vitamin C, vitamin B6 and vitamin PP were utilized.

Another model was constructed using pharmaceutical sample as standard. A total of 166 solutions were prepared from dilutions of pharmaceutical samples in phosphate buffer (pH 6.0, 0.2 mol L^{-1}), with the same concentration range utilized for synthetic samples. For validation of this model, three commercial samples of the same type were used.

The model obtained using DPV and synthetic samples was able to predict the concentration of other synthetic samples but this calibration curve was not able to predict the concentrations of commercial samples, due to interference by the excipients.

On the order hand, the model constructed using commercial samples shows good results when applied to predict the concentrations of the same types of sample. Modeling only the excipients and other components not results in good predictions, in this case, due to the complexity of the samples utilized.

3. Results and discussion

3.1. VC, VPP and VB6 differential pulse voltammograms

The differential pulse voltammograms for synthetic and pharmaceutical samples are shown in Figs. 1 and 2, respectively. These figures show voltammograms of mixtures having different concentrations of the compounds pyridoxine (VB6), ascorbic acid (VC) and nicotinamide (VPP) for synthetic samples and for the three pharmaceutics samples using

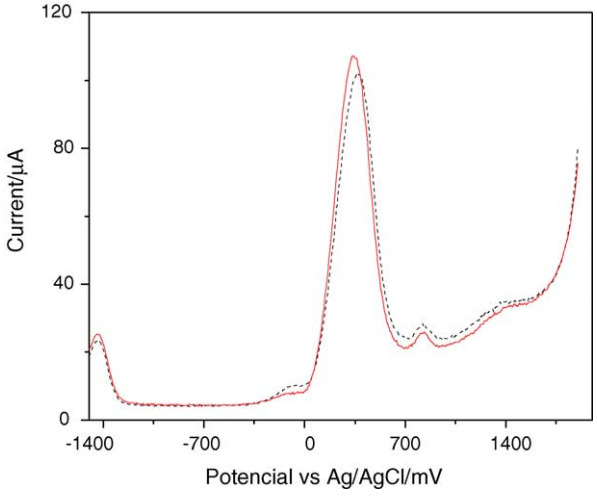

Fig. 1. Differential pulse voltammetric signal provided by a mixture of VC, VB6 and VPP in different concentrations: (----) 1.53 mg mL^{-1} VC, 0.51 mg mL^{-1} VPP and 0.05 mg mL^{-1} VB6; (. . .) 1.49 mg mL^{-1} VC, 0.49 mg mL^{-1} VPP and 0.04 mg mL^{-1} VB6 in 0.2 mol L^{-1} phosphate buffer of pH 6.0.

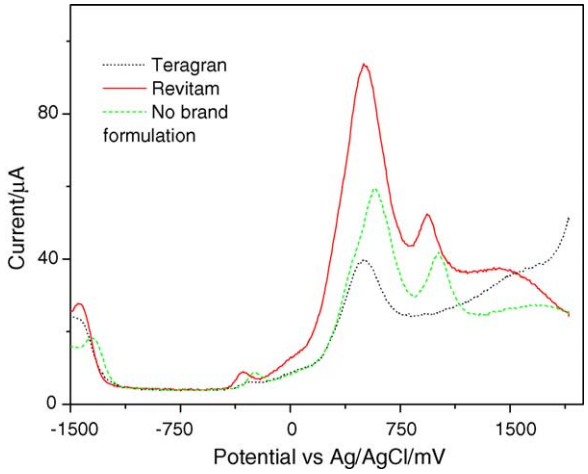

Fig. 2. Differential pulse voltammogram pf pharmaceutics samples: (----) Revitam® (----) no brand formulation and (. . .); Teragran®, in 0.2 mol L^{-1} phosphate buffer of pH 6.0.

the glassy carbon electrode in a phosphate buffer (pH 6.0, 0.2 mol L^{-1}). From these figures it is possible to observe that nicotinamide shows a reduction peak at a potential nearly −1400 mV and a small oxidation peak at −315 mV when the concentration of this compound is high. This can be better observed in Fig. 2 where the small anodic peak corresponds to oxidation of a nicotinamide reduction product. Ascorbic acid shows an oxidation peak at a potential near 400 mV and the last compound, pyridoxine, an oxidation peak at a potential near to 850 mV. From Fig. 2 it is possible to observe that the pharmaceutical preparation Teragran® presents an overlap of the ascorbic and pyridoxine peaks, not observed in the other compounds. This is probably due to the fact that in commercial samples the excipients can cause interference in this determination. For this reason, a calibration curve employing synthetic samples as standards is not useful for calibration purposes of commercial samples because potential matrix effect do not consider these facts. The potential matrix effect was verified in this study. However this problem can be overcome by using multivariate methods and samples of the same type in the calibration model, as observed in the obtained results.

3.2. Calibration and validation of PLS and neural network for synthetic samples

Multivariate calibration methods require a suitable number of samples present in calibration set. The ANN method require a high number of samples in order to trained it to the contrary this method will not provide a good prediction. Although the PLS not require an equal number of samples, the same group of samples were utilized for comparison purposes. A PLS model constructed with a smaller group of samples did not show different results.

The partial least squares model using synthetic samples was constructed with the data obtained from differential pulse voltammetry (variable *x*) and values of concentrations

Table 2
Results for the validation set modeled with PLS for VC, VPP and VB6 in synthetic samples (mg mL^{-1})

Sample	Real value			PLS			Error (%)		
	VC	VPP	VB6	VC	VPP	VB6	VC	VPP	VB6
1	1.00	0.20	0.030	0.98	0.25	0.038	−2.0	25.0	26.7
2	1.10	0.30	0.030	1.05	0.28	0.035	−4.8	−6.8	16.7
3	0.70	0.20	0.040	0.68	0.17	0.029	−2.9	−15.0	−27.5
RMSEP							3.4	17.3	24.1

(variable *y*). These data were disposed in matrix form: Matrix **X** and matrix **Y**, corresponding to data from variables *x* and *y*, respectively. The data pre-treatment used in this process was mean centering that is a pre-treatment for columns. Mean centered data is obtained by subtracting the average value of the column, for each point in this column. This pre-treatment consists of a translation of the coordinate axis to the coordinate center. The number of latent variables to be used in this model was chosen by full cross-validation where all calibration samples were validated one by one. From cross-validation five latent variables were necessary to construct the PLS model for these vitamins.

After these analyses, the PLS calibration model constructed was utilized to determine vitamins in other synthetic samples. The relative performance of the different models for each vitamin was evaluated in terms of relative error and root mean square of error prediction (RMSEP), represented by Eq. (6).

$$\mathrm{RMSEP} = \sqrt{\frac{\sum (y_{\mathrm{real}} - y_{\mathrm{prev}})^2}{n}} \quad (6)$$

where y_{real} are the real values for y, y_{prev} are the values found by the model constructed and n the number of samples used. The results obtained from PLS and the criteria of validation method based on relative error and RMSEP are shown in Table 2.

The other multivariate model, artificial neural networks, was also utilized for modeling data from synthetic samples. After pre-treatment using mean and the standard deviation, these data were reduced by applying principal component analysis. From this, it is possible to verify that five principal components were capable of explaining 99.99% of data variance. These five components were the data inputs in the first layer. A hidden layer with six neurons and an output layer with one neuron followed the first layer. This was the architecture used in the neural network .The model was specific for each vitamin.

To train the neural network, a tan-sigmoidal function was utilized in the hidden layer as a transfer function. For the output layer a linear function was utilized. The neural network was trained by the Marquardt–Levenberg algorithm, using a maximum number of iterations equal to three hundred and the error value employed as criterion for stopping was 1×10^{-2}. The results, the relative error and RMSEP values are presented in Table 3.

3.3. Calibration and validation of PLS and neural network for pharmaceutical samples

The PLS model using pharmaceutical samples was constructed with the data obtained from differential pulse voltammetry (variable *x*) and values of concentrations (variable *y*) based on HPLC analysis. These data were disposed in matrix **X** and **Y** form corresponding to variables *x* and *y*, respectively. The same data pre-treatment (mean centering) was used. From cross-validation six latent variables were necessary to construct the PLS model for the three vitamins using DPV. The PLS model constructed was utilized to determine vitamin concentrations in other commercial samples of the same type that was used in the calibration model.

The results obtained from PLS by DPV analysis, the RMSEP and relative error value are shown in Table 4.

The other multivariate model, artificial neural network was applied for modeling data from DPV using these samples. For this analysis, a pre-treatment using mean and the standard deviation was applied and the data were reduced by applying principal component analysis. Six principal components were capable of explaining 99.99% of data variance and they were employed as data inputs in the first layer which is followed by a hidden layer with six neurons and an output layer with

Table 3
Results for the validation set modeled with ANN for VC, VPP and VB6 in synthetic samples (mg mL^{-1})

Sample	Real value			ANN			Error (%)		
	VC	VPP	VB6	VC	VPP	VB6	VC	VPP	VB6
1	1.00	0.20	0.030	1.00	0.21	0.028	0	5.0	−6.7
2	1.10	0.30	0.030	1.09	0.30	0.029	−0.9	0	−3.3
3	0.70	0.20	0.040	0.70	0.19	0.039	0	−5.0	−2.5
RMSEP							0.5	4.1	4.8

Table 4
Results for the validation set modeled with PLS for VC, VPP and VB6 in pharmaceutical samples

Sample	HPLC results			PLS			Error (%)		
	VC	VPP	VB6	VC	VPP	VB6	VC	VPP	VB6
Revitam®	35.20	6.00	0.60	39.10	8.00	0.80	11.1	33.3	33.3
Teragran®	25.10	10.20	0.50	27.10	10.10	0.40	7.9	−1.0	−20.0
No brand	25.00	12.10	1.10	26.00	12.60	1.20	4.0	4.1	9.1
RMSEP							8.2	19.4	23.0

Revitam®, no brand sample (mg mL^{-1}) and Teragran® (2.5 mg mL^{-1}).

Table 5
Results for the validation set modeled with ANN for VC, VPP and VB6 in pharmaceutical samples

Sample	HPLC results			ANN			Error (%)		
	VC	VPP	VB6	VC	VPP	VB6	VC	VPP	VB6
Revitam®	35.20	6.00	0.60	35.30	6.50	0.60	0.30	8.3	0
Teragran®	25.10	10.20	0.50	25.20	10.10	0.50	0.40	−1.0	0
No brand	25.00	12.10	1.10	25.00	12.10	1.20	0	0	9.1
RMSEP							0.28	4.8	5.3

Revitam®, no brand sample (mg mL^{-1}) and Teragran® (2.5 mg mL^{-1}).

one neuron. This is the architecture used in neural network. The model was specific for each vitamin. To train the neural network, a tan-sigmoidal function was utilized in the hidden layer as a transfer function and for the output layer a linear function was utilized. The neural network was trained by the Marquardt–Levenberg algorithm. This neural network was trained with a maximum number of iterations equal to 300 and the error value employed as a stopping criterion was also 1×10^{-2}.

The results obtained for ascorbic acid, nicotinamide and pyridoxine analyze, are shown in Table 5.

4. Conclusions

This works demonstrates that differential pulse voltammetry in conjunction with chemometric techniques is a powerful analytical tool to determine vitamins in synthetic and also in pharmaceuticals samples. The method using neural network shows better results in comparison with PLS data possible due to the fact that interactions among electroactive components give a non-linear response on the glassy carbon electrode. The method is fast, simple and does not generate hazardous chemical wastes, thus making it easily possible for use in quality control laboratories.

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References

[1] Kirk-Othmer, Encyclopedia of Chemical Technology, Wiley, New York, 1984, v.24.
[2] A. Rizollo, S. Polesello, J. Chromatogr. 624 (1992) 103–152.
[3] P.C.H. Hollman, J.H. Slanger, P.J. Wagstafe, U. Faured, A.A.T. Southgate, P.M. Finglas, Analyst 118 (1993) 481–488.
[4] T.S. Agostini, H.T. Godoy, J. High Resol. Chromatogr. 20 (1997) 245–248.
[5] K.K. Verma, A. Jain, B. Sahasrabuddhey, K. Gupta, S. Mishira, J. AOCS 79 (1996) 1236–1243.
[6] A.V. Pereira, O. Fatibelo-Filho, Talanta 47 (1998) 11–18.
[7] H. Wu, K. Oguma, R.Q. Yu, Anal. Sci. 10 (1994) 875–880.
[8] J. Wang, R. Han, B. Su, C. Lin, N. Wamg, J. Hu, Anal. Sci. 14 (1998) 965–969.
[9] S.R. Hernández, G.G. Ribeiro, H.C. Goicoechea, Talanta 61 (2003) 743–753.
[10] H. Martens, T. Naes, Multivariate Calibration, Wiley, New York, 1989.
[11] B.M. Wise, N.B. Gallagher, PLS-Toolbox 2.1 for use with Matlab, EigenVector Research Inc., Manson, W.A., 1998.
[12] F. Despagne, D.L. Massart, Analyst 123 (1998) 157R.
[13] H. Demuth, M. Beale, Neural Network Toolbox for use with Matlab, EigenVector Research Inc., Manson,W.A., 1998.