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Chemometric assisted for the simultaneous spectrophotometric determination of calcium and magnesium in natural water

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Abstract : A method for simultaneous determination of calcium and magnesium in natural waters using multivariate calibration methods are proposed. The method is based on the development of the reaction between the analytes and Bromopyrogallol red at pH 9.50. Principal Component Regression (PCR), Partial Least Squares (PLS) and Artificial Neural Network Calibration (ANN) methods were successfully applied to the multi - component analysis of the binary containing substances in natural waters. PCR, PLS and ANN methods are suitable for the quantitative resolution of the mixtures of these substances and this approach doesn't require any separation and extraction steps. Under the working conditions, the proposed method were successfully applied to simultaneous determination of calcium and magnesium in natural waters.

Keywords : Ca^{2+}; Mg^{2+}; PCR; PLS; ANN; Natural waters.

INTRODUCTION

Calcium and magnesium are two of the most commonly determined cations in drinking waters. From a physiological point of view, calcium and magnesium, along with sodium and potassium, are the most important ions affecting cardiology, owing to their role in nervous impulse conduction and cell contraction. From an industrial point of view, the main problem is related to the formation of deposits of their carbonates. The control of the concentrations of magnesium and calcium in waters is important, as they are the responsible for water hardness, and their presence at high concentrations lowers the quality of drinking waters. For this reason calcium and magnesium are among the major ions to be determined routinely.

In spectrophotometry the formation of an absorbent complex by reaction between the analyte and an external reagent is normally used to determine an analyte. The absorbance of the formed complex is calculated by subtracting from the measured absorbance value, the one corresponding to the "reagent blank", added in the initial concentration, if it absorbs at the measurement wavelength. The blank absorbance at the end of the reaction can be lower than that measured at the beginning, because of the reagent consumption. Therefore, the real absorbance of the formed product will be larger than the measured value. If we consider a calibration line, this difference will be greater the higher the concentration assayed, and it will affect the accuracy of the analytical results obtained.

The traditional method in quality control of calcium and magnesium in water and waste water is complexometry using EDTA as titrant[18]. Although this is an inexpensive method, it requires some skill. The main disadvantage of complexometric methods is that they are time consuming and subject to operational errors. Several instrumental approaches, including FAAS and ICPAES (Franson, 1992, have been proposed for determining these analytes individually in water. Adaptations of highly selective techniques to a continuous-flow scheme, *e.g.*, FAAS [4] and potentiometry using ion-selective electrodes [29, 16]make it easier to automate the analysis. Several reagents have been proposed to carry out the spectrophotometric determination of those cations, some of them are; 4-(2-pyridylazo)-resorcinol (PAR)[21, 19], arsenazo (I)[27], arsenazo (III)[7, 26, 14, 15, 2], emodin (Pal and Jana, 1993), 3, 3'-bis [N, N-bis (carboximethyl) amino methyl)][31], bromopyrogallol red (BPR)[1]etc.

PAR and arsenazo (III) are generally the most employed reagents for the simultaneous determination of both ions. PAR presents a too high molar absorptivity in relation to that of the Ca (II) and Mg (II) complexes in the working wavelength range, and it offers a narrow spectral window from a multivariate perspective[20].

In recent years, chemometric calibration techniques can be summarized as multiple linear regression (MLR) (classical least square, CLS and inverse least squares, ILS calibrations), principal component regression (PCR), partial least regression (PLS) and artificial neural network (ANN) techniques[22, 28, 5, 17, 9, 3]. Several researchers used these techniques for the simultaneous analysis binary and a ternary mixture[10, 11, 12,]. All the multivariate approaches are useful for the resolution of spectral band overlapping in quantitative determination. In the multivariate analysis, a calibration is build from spectral response values for a set of standard samples as known concentrations corresponding to the analytes of interest. The obtained calibration is used to predict the component concentrations from the sample spectrum. The multivariate calibration-prediction techniques use the full spectrum, full automation, multivariate data analysis and the reduction of noise and the advantages of the selection of the calibration model. In addition these multivariate calibrations do not need any separation procedure, they are very cheap, very easy to apply and very sensitive. For these reasons these multivariate techniques are very popular today.

In this paper, simultaneous spectrophotometric determination of calcium and magnesium, in mineral waters, with bromopyrogallol red (BPR) at pH 9.50 is chemometrically studied from the point of view of the model used for the calibration and from the point of view of the prediction. The usefulness of the method for the prediction of the concentration of calcium and magnesium in commercial waters is discussed.

EXPERIMENTAL

Instruments

Absorbance measurements were carried out by using a Perkin –Elmer Lambda 20 double beam UV-visible spectrophotometer interfaced to an IBM SX-486 microcomputer for the spectral acquisition provided with a UV-Win Lab software and subsequent manipulation of the experimental data. The absorbance measurements were carried out in two matching quartz 1.0 cm cells with a 1 mm path length.

Chemometric software

Application of PCR and PLS algorithms was supported by the software package "Minitab® 16". The software is dedicated to both multivariate analysis and experimental design and is equipped with several multivariate methods. It allows to optimize the calibration models and to develop validation procedures. The back-propagation neural network algorithm three layers were used in MATLAB (version 7.0, Math Work Inc.) using NN toolbox. All programs were run on a Pentium, personal computer, with windows XP home edition.

Reagents and standards

All solutions were prepared with analytical grade reagents. Stock solutions of Ca^{2+} and Mg^{2+} (1000 mg L^{-1}) were prepared by dissolving $Ca(NO_3)_2.4H_2O$ (Merck, Darmstadt - Germany) and $Mg(NO_3)_2.6H_2O$ (Merck, Darmstadt – Germany) respectively. A $5.0x10^{-3}$ mol L^{-1} bromopyrogallol red (BPR) (Merck, Darmstadt - Germany) was prepared by dissolving it in minimum amount of ethanol and diluting to the mark with doubly distillated water. A pH 9.50 buffer solution was prepared by mixing 95.0 mL of 0.05 mol L^{-1} $Na_2B_4O_7.10H_2O$ and 5.0 mL of 0.1 mol L^{-1} potassium dihydrogen phosphates[1].

General procedure

Suitable amounts of each metal, 1 mL of $5.0x10^{-2}$ mol L^{-1} BPR solution and buffer solutions were added to a 25 mL volumetric flask. The solution was diluted to the mark with water and allowed to stand for 5 min at room temperature. A portion of the solution was then transferred into a 1.0 cm quartz cell to record the absorption spectra in the wavelength range 400-700 nm.

Sampling and analytical procedure

Water samples were collected in Isparta city 5 different stations. Samples from wells were collected by using 1.0 L plastic bottles, avoiding the formation of headspace of air, and transported into the laboratory within 1 h. Once there, samples were filtered through Whatman No.42 filter paper and stored until their analysis, which was accomplished within 1 week. Samples were kept at their natural pH. The determinations of Ca^{2+} and Mg^{2+} by the proposed methodology and by UV-Visible spectrophotometry were performed on the same day in order to avoid any possible bias.

RESULTS AND DISCUSSION

Preliminary investigations

The chromogenic reagent bromopyrogallol red (BPR) can be use as a reagent for the determination of amounts Ca^{2+} and Mg^{2+}. The compositions of complexes were determined by molar ratio method[6]. The values of molar ratio indicated a 1:1 and 1:2 metal:ligand ratio for Ca-BPR and Mg-BPR. The absorption spectra of Ca^{2+} and Mg^{2+} complexes are shown in Figure 1. As Figure 1 shows, the spectra of the complexes, overlap with each other, and therefore each compound interfere in the spectrophotometric determination of the other. But this system

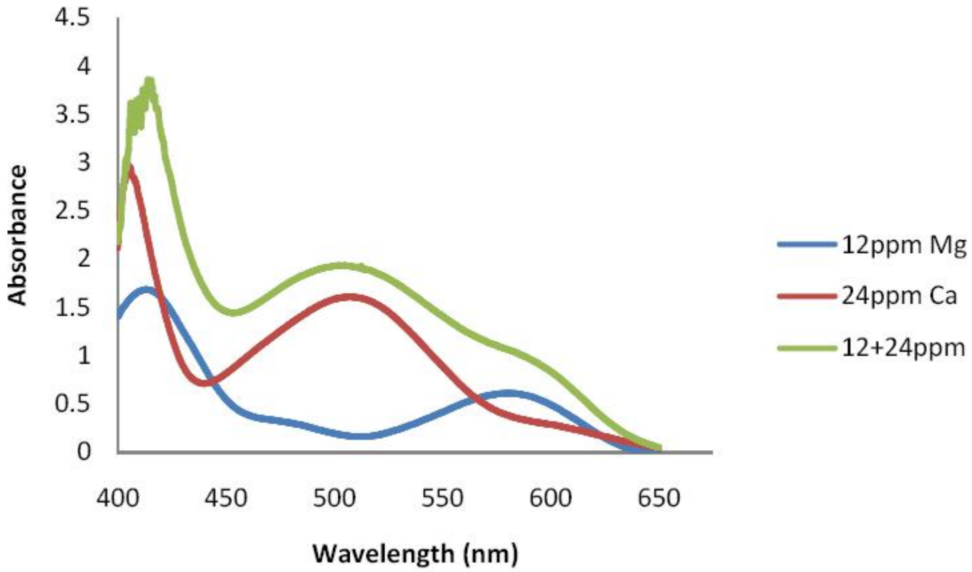

Figure 1 : Absorption spectra of 12 ppm Mg, 24 ppm Ca, and their mixture

can be suitable for simultaneous determination of Ca^{2+} and Mg^{2+} using PCR, PLS and ANN.

Optimization of the experimental conditions

For receiving in the best sensitivity, we need to obtain the best conditions. A study on the influence of the pH on the formation of Ca-BPR and Mg-BPR complexes were conducted in the pH 4-11. The study revealed that the maximum color formations of Ca-BPR and Mg-BPR complexes were obtained 9.0-9.9. Therefore pH 9.5 was used for further works. Borax-potassium dihydrogen phosphate and ammonia-ammonium chloride buffer solutions of pH 9.5 were tested and the Borax solution was found as the best. A 5.10^{-2} mol L^{-1} BPR was used as optimum concentration.

Spectrophotometric determination

Calibration sets

The absorption spectra were recorded between 400 and 700 nm with an interval of 1.0 nm between each two points and were contrasted with the corresponding blanks. The calibration matrix was prepared from 10 solutions containing mixtures of the two components in different ratios and optimized and calculated by using PCR, PLS and ANN calibration both to analyses the spectra obtained and to calculate the concentration of the analytes in the real samples.

Multivariate analysis

PLS is factor analysis method, based on a two-stage procedure; a calibration step, in which a mathematical model is built by using component concentrations and spectral data from a set of references, followed by a prediction step in which the model is used to calculate the concentrations unknown sample from its spectrum. These methods are also called “factor methods” because they transform the original variables into a smaller number of orthogonal variables called factors or principal components (PCs), which are linear combinations of the original variables. When multivariate calibration approaches are applied in spectrophotometric multi component analysis, a relationship between spectral and concentration data from reference samples, representing the variables of the system, is established. A new matrix constituted by the new variables PCs and scores is built. The calculation of this new matrix is planned by algorithm specific to the regression method adopted. The most used regression methods are PLS. The theory of such techniques has been fully described by several authors [32, 30, 13].

Artificial neural network is a multivariate calibration method used mainly for modeling non-linear data, although, some applications use the neural network for modeling linear data. It is important to state that this method is computationally more complex than linear methods, they have limitation of being prone to over fitting and they heavily depend on amount and quality of data available. In many cases the principal disadvantage of the neural network is time required for its training.

Wavelength selection

Multivariate calibration methods have generally been considered as full-spectrum in the processes of calibration and prediction. But, most often, selecting the undesired regions of the spectrum evaluated is provided more noise in the analytical results[8]. Rossi and Pardue showed that accuracy can be improved by careful wavelength selection[25]. So far, various criteria have been developed to allow for wavelength selection. In the present work, the UV-spectra of Ca^{2+} and Mg^{2+} solutions were recorded, in the wavelength range of 400 – 700 nm with 1 nm interval. Spectrum as shown the simultaneous determination of the related compounds in samples is not possible by using classical spectrophotometric approaches. In this study, without any separation step using PCR, PLS and ANN chemometric approaches Ca^{2+} and Mg^{2+} are focused two mixtures of quantitative resolution.

Multivariate methods

The first step in simultaneous determination of the binary mixture of natural waters by multivariate calibration methods involves constructing the calibration matrix for binary mixture of Ca^{2+} and Mg^{2+}. Nineteen binary mixtures were selected by random design as the calibration set. The composition of the

TABLE 1 : Composition of the calibration set for applying PCR, PLS and ANN methods

	Concentration (µg/mL)	
No.	**Ca^{2+}**	**Mg^{2+}**
1	4	8
2	4	16
3	4	24
4	4	32
5	4	36
6	12	8
7	12	16
8	12	24
9	12	32
10	12	40
11	20	8
12	20	16
13	20	24
14	20	32
15	28	8
16	28	16
17	28	24
18	36	8
19	36	16

samples was randomly designed in order to obtain non-correlated concentration profiles (TABLE 1). In order to minimize the correlation between concentration vectors, the correlation coefficient matrix is considered as a criterion. The calibration model in each chemometric method was validated with 14 synthetic mixtures set containing the water under study in different proportions selected randomly. The predictive abilities of PCR, PLS and ANN were examined for simultaneous determination Ca^{2+} and Mg^{2+} in sample mixtures. The common requirement for all mentioned methods is that unknown samples and standards be of the same nature.

Partial least squares calibration

In the UV-VIS spectra, the absorbance data (A) and concentration data (C) are mean centered to give data matrix A_o and vector C_o. The orthogonalized PLS algorithm has the following steps. The loading weight vector W has the following expression:

$$W = \frac{A_o^T C_o}{C_o^T C_o}$$

The scores and loadings are given by:

$$t_1 = A_o W \quad ; \quad P_1 = \frac{A_o^T t_1}{t_1^T t_1} \quad ; \quad q_1 = \frac{C_o^T t_1}{t_1^T t_1}$$

The matrix and vector of the residuals in A_o and C_o are:

$$A_1 = A_o - t_1 p_1^T$$

$$C_1 = C_o - t_1 q_1^T$$

From the general linear equation, the regression coefficients were calculated by;

$$\mathbf{b} = \mathbf{W} (\mathbf{P}^T\mathbf{W})^{-1}\mathbf{q}$$

$$a = C_{mean} - A_{mean}^T b$$

The builded calibration equation is used for the estimation of the compounds in the samples. *3.6.2. Artificial neural network calibration*

A feed-forward ANN model with three layers of nodes was constructed as in Figure 2. The artificial neuron is the building component of ANN designed to simulate the function of biological neuron. The arriving signals, called inputs, multiplied by the connection weighted (adjusted) are first summed (combined) and then passed through a transfer function to output that neuron. The activation function is the weighed sum of the neuron's inputs and the most commonly used transfer function is sigmoid function (Figure 2).

The logistic function was used as the activation function in a neural network. The training and testing data sets must be normalized into a range 0.1-0.9. The input and the output data sets were normalized by using following equation:

$$X_N = 0.1 + \frac{0.8(X - X\min)}{(X\max - X\min)} \qquad (1)$$

Where X_N is normalized value of a variable (the network input or the network output), *X* is a original value of a variable, and Xmax *and* Xmin are maximum and minimum original values of the variables, respectively. In order to produce sufficient data for training and testing of the model shown in Figure 2, 14 different standard solutions were prepared using different Ca^{2+} and Mg^{2+} concentrations and each standard solution was subjected to spectrophotometric determination. Randomly chosen 600 data pairs from these 3000 data pairs were used in the training of the neural network, and the rest of the data were

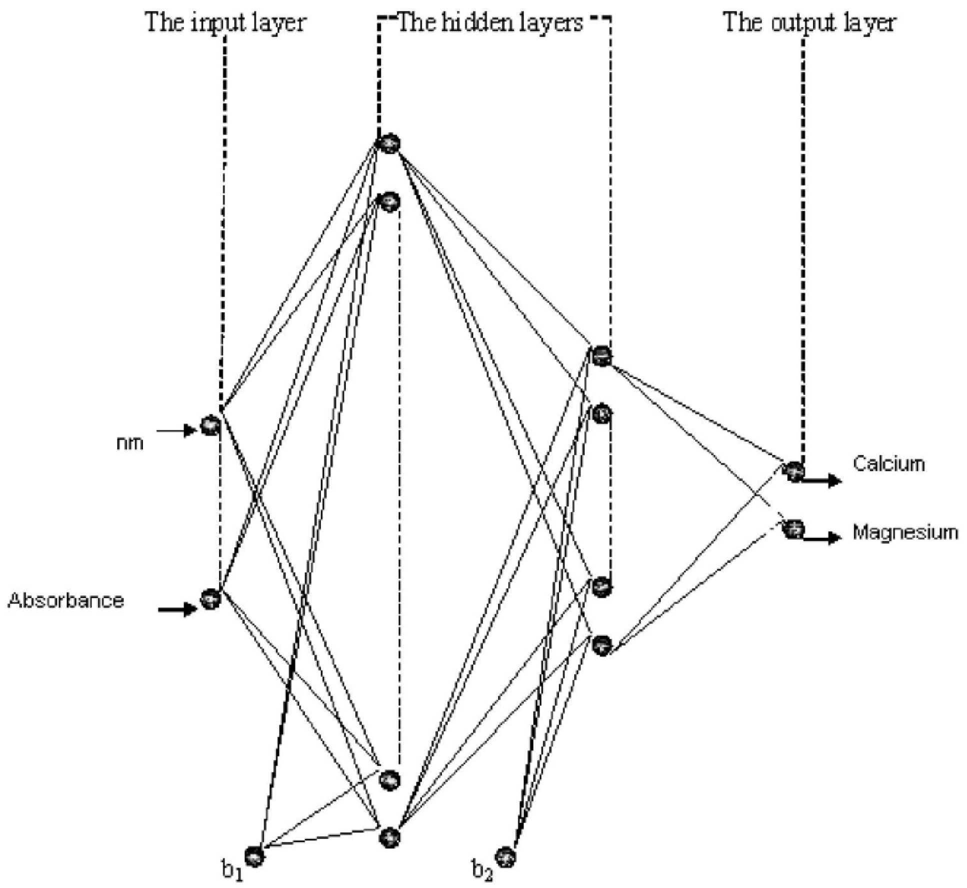

Figure 2 : Network architecture used in the spectrophotometric determination

used in the testing. The root mean square error values were calculated from following equation to prove quantitatively the accuracy of the testing results of neural network models:

$$RMS = \sqrt{0.5N^{-1}\sum_{i=1}^{N}(X_1^{'} - X_1)^2} \quad \textbf{(2)}$$

Where N is the number of testing data and $X_1^{'}$ is target value.

MATLAB 7.0 software was used to construct ANN models which have sigmoidal logistic function with back propagation of error algorithm. For this neural network modeling an input layer, one or two hidden layers and output layer were used.

To obtain the best network performance, the op-timal network architecture and parameters must be chosen. Studies of the network structure include the selection of the number of layers and number of nodes in each layer. The number of layers used for this neu-ral network modeling was four, i.e. an input layer, one or two hidden layers and an output layer. As can be seen from Figure 2, two neurons were used in the input layer, which were the absorbance and wavelength (nm). The absorbance and wavelength of the solution were considered as independent variables of the spectrophotometric method. Therefore, these variables were used as input variables in the network architecture. In TABLE 1, the concentration of standard solutions is represented (or output data of the network).

The various neural network models, which have the logistic function, were trained and tested. In this step, the number of the hidden layer units of the network was determined by performance evaluating of the network models defined in TABLE 2. According to RMS errors given in TABLE 2, the NN4 2-12-2 model, which performs best on a testing data set, were selected as neural network model to predict the Ca^{2+} and Mg^{2+} concentrations.

Chemometric parameters

The application competence of a calibration model can be explained in several ways. These re-

TABLE 2 : Comparison of the performances of the neural network models

	RMS error			
Model	**Ca^{2+}**		**Mg^{2+}**	
	Training	**Testing**	**Training**	**Testing**
NN1 2-1-2	0.052055	0.058584	0.062146	0.0612822
NN2 2-8-2	0.034128	0.040248	0.052246	0.0581231
NN3 2-11-2	0.0152854	0.125664	0.034897	0.0425870
NN4 2-12-2	0.0010003	0.021258	0.001516	0.0012485
NN5 2-15-2	0.0168124	0.082802	0.179586	0.0148762

sults can be examined numerically. One of the best ways to do this, by examining the predicted residual error sum of squares (PRESS). To calculate PRESS computed the errors between the expected and predicted values for all the samples, square them, and sum them together.

$$\text{PRESS} = \sum_{i=1}^{n}(C_i^{added} - C_i^{found})^2$$

Strikingly speaking, this is not a correct way to normalize the PRESS values when not all of the data sets contain the same number of samples. If want correctly compare PRESS values for data sets that contain differing numbers of samples, should convert to standard error of prediction (SEP), which is given by following formula.

$$\text{SEP} = \sqrt{\frac{\sum_{i=1}^{n}(C_i^{added} - C_i^{found})^2}{n-1}}$$

Where C_i^{added} the added concentration of water is, C_i^{found} is the found concentration of water and n is the total number of the synthetic mixtures. The SEP can provide a good measure of how well, on average, the calibration model performs. Often, however, the performance of the calibration model varies depending on the analyte level.

In the application of two chemometric techniques to the synthetic mixtures containing teas in variable compositions, the mean recoveries and relative standard deviations for PCR, PLS and ANN were found to be 99.49 and 0.30% ; 98.36 and 0.53%, 99.08 and 0.81% respectively for Ca^{2+}, 100.01 and 0.87%; 98.36 and 0.72%, 99.08 and 0.60% respectively for Mg^{2+} (TABLE 3). These experimental results indicate that these three methods are suitable for simultaneous determination of the above mentioned compounds in samples.

According to the added concentration and the concentration found in samples, the SEP and PRESS values of PCR, PLS and ANN techniques were calculated 0.0134 and 0.1157; 0.0485 and 0.2204; 0.0363 and 0.1905 respectively for Ca^{2+}, 0.0485, and 0.2204; 0.0253 and 0.1590; 0.0223 and 0.1494 respectively for Mg^{2+} (TABLE 4).

The linear regression analysis of the added concentration and the concentration found in the synthetic mixtures were realized for each analyte and for each calibration technique. In this regression analysis, the correlation coefficient (r), intercept, slope and relative standard deviation values were found satisfactory for the proposed chemometric techniques in TABLE4. In order to test the proposed calibrations, an independent set of the validation set in TABLE 4 was analyzed and used for the calculations of the standard error of prediction (SEP) in the validation step. The standard error of calibration (SEC) and the errors of prediction (SEP) for n=14 calibration – prediction samples were calculated PCR, PLS and ANN method and their values were summarized in TABLE 3. In same table, the results of linear regression analysis were applied to rela-

TABLE 3 : Composition of synthetic samples, their recoveries by PCR, PLS and ANN models

Mixtures added (μg/mL)		Recovery (%)					
		PCR		PLS		ANN	
Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
4	8	99.99	100.24	99.43	99.82	99.68	99.92
12	16	99.98	100.27	99.97	101.45	98.45	101.40
20	24	99.32	100.46	99.41	100.09	100.02	100.21
28	32	99.61	100.98	100.43	99.93	99.20	99.98
20	8	99.30	99.86	99.46	98.36	99.81	98.88
20	16	99.16	99.04	98.74	99.58	98.76	100.02
20	24	99.74	100.46	100.05	100.01	100.84	100.08
20	32	99.29	100.94	100.00	99.10	100.26	99.68
20	40	99.19	99.57	99.99	101.04	97.85	98.80
4	24	99.83	98.25	99.87	99.51	98.98	100.06
12	24	99.04	100.09	98.72	100.35	100.29	100.24
20	24	99.60	99.75	100.11	99.91	99.64	100.24
28	24	99.43	101.74	100.19	99.98	98.68	99.82
36	24	99.66	99.01	99.33	99.98	100.02	100.18
Mean		99.49	100.04	98.96	100.36	99.08	100.32
RSD[a]		0.30	0.87	0.53	0.72	0.81	0.60

a: Relative standard deviation

TABLE 4 : Statistical parameters in the calibration - prediction

Parameter	Method	Ca^{2+}	Mg^{2+}
PRESS	PCR	0.0134	0.0485
	PLS	0.0135	0.0253
	ANN	0.0363	0.0223
SEP	PCR	0.1157	0.2204
	PLS	0.1162	0.1590
	ANN	0.1905	0.1494
r	PCR	1.0000	0.9993
	PLS	0.9998	0.9997
	ANN	0.9996	0.9998
Intercept	PCR	0.0126	0.0535
	PLS	0.0207	0.1140
	ANN	0.0196	0.1890
Slope	PCR	0.9954	1.0032
	PLS	0.9998	1.0053
	ANN	0.9959	0.9909
RSD	PCR	0.30	0.87
	PLS	0.53	0.72
	ANN	0.81	0.60

tionships between actual and predicted concentrations in the calibration and validation sets. Their statistical results were presented in TABLE 4. As can be seen, all the statistic values indicated that all techniques are convenient for the determination of Ca^{2+}, Mg^{2+}in synthetic mixtures.

TABLE 5 : Comparison of results of CAF, TBR and TPH analyses obtained two different methods

Natural Waters	PCR	PLS	ANN
Ca^{2+}/mg g^{-1} Mean ±	0.3358± 0.01	0.3826±0.04	0.3417±0.04
Mg^{2+} mg g^{-1} Mean ±	0.2738± 0.03	0.2895± 0.05	0.2693±0.08

Applications

In order to assess the applicability of the proposed method to the analysis of real samples, they were applied to the determination of Ca^{2+} and Mg^{2+} in natural water sample. Six replicate measurements were made. The results are shown in TABLE 5. The good agreement between the results and the label claims indicates the successful applicability of the proposed procure for simultaneous determination of Ca^{2+} and Mg^{2+} in real sample. To check the validity of the proposed method, after the addition of the known amounts of Ca^{2+} and Mg^{2+} to the real samples, we found that the amount of these samples did not change. Moreover we compare the spectra obtaining from the mixture Ca^{2+} and Mg^{2+} in standard and real sample formulation solutions that showed similar patterns in their spectra. These findings indicate that excipients placed in real sample preparation did not interfere in the measurement of Ca^{2+} and Mg^{2+} in real sample formulation.

CONCLUSIONS

Three chemometric technique in spectrometric analysis, PCR, PLS and ANN were proposed for the simultaneous determination of Ca^{2+} and Mg^{2+} in their binary mixtures. These techniques were applied with great success to natural waters. The resolution of highly overlapping mixtures was achieved by the use of PCR, PLS and ANN techniques. A selection of working wavelength having high correlation values with concentration due to interference coming from matrix sample or additional analytes outside the working range. Comparison of the results of the ANN method with those of the revealed the lower prediction errors and higher correlation coefficients for ANN. Meanwhile, the data acquisition with ANN was easier than that with the PCR and PLS methods. The proposed chemometric techniques can be applied for the routine analysis of natural waters without any a priori chemical separation and without time consuming.

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