Spectrophotometric Determination of Trace Amounts of Beryllium in Natural Water Using Mean Centering of Ratio Spectra Method and Partial Least Squares Regression

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Two simple chemometrics methods for spectrophotometric determination of trace amounts of beryllium by mean centering of ratio spectra (MCRS) and partial least squares (PLS) regression are described. Thorin at pH = 10.5 was used as a chelating agent. The proposed methods evaluate and eliminate the blank bias error present in such procedure. Under the optimized conditions, analytical characteristics of the mean centering of ratio spectra method were obtained. The calibration curve was linear over the range of 10-600 ng mL^{-1} of Be^{2+}. The concentration detection limit of 2.56 ng mL^{-1} was achieved. The relative standard deviation for six replicate measurements of 200 ng mL^{-1} beryllium was 4.0 %. Relative error of prediction for applying the partial least squares method to 5 synthetic samples in the range of 10-600 ng mL^{-1} was 3.2 %. Analysis of spiked tap water, using both proposed methods, gave results which are in good agreement with their beryllium contents.

Key Words: Beryllium, Thorin, Spectrophtometry, Mean centering of ratio spectra, Partial least squares regression.

INTRODUCTION

It is well known that beryllium is a micronutrient and plays a beneficial role. But at the moment, it can enter into human's body and result in cancer, so it plays a harmful (toxic and carcinogenic) biological roles. It is also involved in environmental pollution and is an occupational hazard[1].

The carcinogenic risks to humans of beryllium and beryllium compounds have been considered on a number of occasions by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO)[2]. Different national guidelines suggested that beryllium concentrations should not exceed a limit of 0.1 µg L^{-1} for tap water and 0.2 µg L^{-1} for surface water[3]. The average beryllium concentration in natural waters is in the ng L^{-1} range[4]. Therefore it is vital to determine the corresponding content of beryllium at trace and ultra trace levels.

Over the recent years numerous spectroscopic analytical techniques have been employed for detection of Be^{2+} in water samples. These include spectrophotometric

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methods[5,6], spectrofluorimetric methods[7], flame atomic absorption spectroscopy (FAAS)[8], electrothermal atomic absorption spectroscopy (ET-AAS)[9-11], inductively coupled plasma atomic emission spectroscopy (ICP-AES)[12] and inductively coupled plasma coupled with a mass spectrometry (ICP-MS)[13].

Spectrophotometric methods are very simple, fast and inexpensive but have low selectivity and sensitivity. Spectroscopic interferences can be a problem when working with real samples and so many published methods require the incorporation of some sort of separation/extraction methodology prior to analysis.

Additionally, in spectrophotometry, the reagent blank response usually is subtracted from the standard response, when the product and one of the reagents absorb at the same wavelength and hence, the blank absorbance at the end of reaction can be lower than that measured in the beginning, as a result of 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 increase by increasing the concentration of analyte and therefore affect the accuracy of the analytical response. Furthermore, excess reagents cannot be used in spectrophotometric determination, because if the blank absorbs near the absorbance maximum of the product formed, the presence of excess amounts of reagents can affect the molar absorptivities and therefore the calibration line is distorted.

Multivariate calibration can be used for determination of the analyte concentration free from bias error. These techniques present the advantages over other methods as they are faster, sample treatment is usually reduced and the removal of interference is not strictly required in many cases[14].

Partial least squares (PLS) regression is a powerful multivariate statistical tool that has been successfully applied to the quantitative analysis of spectroscopic data[15,16]. Partial least squares calibration can be used as model with some interference, whenever the calibration solutions and samples have similar compositions and interferences are included in variable concentrations in the calibration set (such as interferences due to spectral overlapping of unreacted ligand in complex formation). This overcomes the interference without any previous separation and makes the method more robust PLS is capable of being a full-spectrum method and therefore enjoys the signal averaging advantages of other full-spectrum methods such as principal component regression (PCR) and classical least squares (CLS). Partial least squares also has characteristics and advantages of the inverse least squares (ILS) method, which is limited in the number of spectral frequencies that can be included in the analysis[16].

By using derivative spectrophotometry, selectivity could be improved and it is a powerful tool for overcoming interferences due to spectral overlapping. Unfortunately, the advantages of derivative spectra are at least partially offset by degradation in signal-to-noise ratio that accompanies with derivatives.

More recently Afkhami *et al.*[17-21] have shown some new approaches for simultaneous analysis of binary and ternary mixtures which they called mean centering of ratio spectra (MCRS).

This method uses some mathematical equations to give correct responses, which can be used for determination of the analyte concentration free from bias error. This method eliminates derivative steps and therefore signal-to-noise ratio is enhanced.

In the present work, partial least squares regression and mean centering of ratio spectra method were employed, for the trace level determination of Be^{2+} using thorin *i.e.*, (1-(2-arsonophenylazo)-2-hydroxyl-3,6-naphthalenedisulfonic acid). The results related to the determination of beryllium using suggested methods, showed that these methods can be used where the analyte determination requires the use of an absorbent reagent. The suggested methods were applied for the analysis of synthetic and several natural water samples.

EXPERIMENTAL

All solutions were prepared using analytical-reagent grade substances and triply distilled water.

A 10.000 g L^{-1} standard stock solution of Be^{2+} (Merck) was used for preparation of working solutions. Thorin solution (0.1 % w/v) was prepared by dissolving 0.1 g of the powder in distilled water and diluting with water to 100 mL.

1.2 mL of stock thorin solution, as the chromogenic reagent and appropriate volumes of Be^{2+} were added to a 25 mL beaker, diluted to near 24 mL and its pH was adjusted to 10.5 with 0.01 mol L^{-1} NaOH. This solution was transferred to a 25 mL volumetric flask and made up to the mark with water. The absorbance spectrum of the solution was recorded and stored in the range of 400-600 nm. A calibration data matrix for Be^{2+} was constructed by recording and storing the absorbance spectra for the solutions containing different concentrations of Be^{2+} submitted to the same procedure. The blank solution was submitted to the same procedure and its spectrum was recorded and stored.

The UV-visible absorbance spectra were recorded on an Agilent 8453 spectro-photometer which is equipped with a 1 cm path length fused silica cell. The spectro-photometer was interfaced to a personal computer, furnished with the G1115A software. A Metrohm 620 pH meter furnished with a combined glass-saturated calomel electrode was used for pH measurements. Data processing (PLS1calibration and other programs in the computing process) were performed using a laboratory written program in MATLAB 7.1 and Microsoft Excel 2007 for windows.

Theoretical background

Mean centering of ratio spectra method: Consider a mixture of two compounds "x" and "y". If there is no interaction among the compounds and Beer's law is obeyed for each compound, it can be written[22]:

$$A = a_x c_x + a_y c_y \tag{1}$$

where A is the vector of the absorbance of the mixture, a_x and a_y the molar absorptivity vectors of "x" and "y" and c_x and c_y are the concentrations of "x" and "y", respectively.

If eqn. 1 is divided by a_y corresponding to the spectrum of a standard solution of "y" in binary mixture, the ratio spectrum is obtained in the form of eqn. 2 (for possibility of dividing operation, the zero values of a_y should not be used in the divisor):

$$B = \frac{A}{a_x} = \frac{a_x c_x}{a_y} + c_y \qquad (2)$$

If the eqn. 2 is mean centered (MC), since the mean centering of a constant (c_y) is zero, eqn. 3 would be obtained:

$$MC(B) = MC\left[\frac{a_x c_x}{a_y}\right] \qquad (3)$$

The minimum or maximum of these vectors with respect to wavelength is used for the construction of the calibration graph for analyte.

Partial least square regression: The basic principle of PLS is as follows: principal component analysis[23] of the absorbance matrix $A_{n \times p}$ for n mixtures at p wavelengths and the concentration matrix $C_{n \times m}$ for m components of n mixtures gives the equations:

$$A_{n \times p} = T_{n \times a} \cdot P_{a \times p} + E_{n \times p} \qquad (4)$$
$$C_{n \times m} = U_{n \times a} \cdot Q_{a \times m} + E_{n \times m} \qquad (5)$$

where T and U are characteristic matrices, P and Q are loading matrices, E and F are error matrices, n, m, p and a are the sample number, component number, number of wavelength points and abstracted factor number, respectively. The deviation of A usually occurs for the same reason as those of C. Variations in C are related to those of A. Their factor lengths are different, but their factor directions are the same. Thus, one may obtain:

$$U_{n \times a} = T_{n \times a} \cdot B_{a \times a} \qquad (6)$$

where B is a diagonal matrix indicating the internal relation between the latent dimension matrices T and U, P, Q and B are determined according to the calibration models. For n' unknown samples:

$$A_{n' \times p} = T_{n' \times a} \cdot P_{a \times p} \qquad (7)$$

Then the characteristic variable matrix U of C is evaluated:

$$U_{n' \times a} = T_{n' \times a} \cdot B_{a \times a} \qquad (8)$$

Therefore, the evaluated concentration matrix C is[24]:

$$A_{n' \times m} = T_{n' \times a} \cdot B_{a \times a} \cdot Q_{a \times m} \qquad (9)$$

RESULTS AND DISCUSSION

Effect of operational parameters: To take full advantage of the procedure, assay conditions must be investigated for optimization. Various experimental parameters such as pH, ionic strength and reagent concentrations, were studied in order

to obtain optimized system. These parameters were optimized by setting all parameters to be constant and optimizing one at each time. This optimization procedure may not lead to the actual optimum, although it certainly leads to an improvement of the analytical method.

Selection of working wavelength: Thorin formed colored complex with Be^{2+}. As Fig. 1a-b shows the absorbance spectra of thorin and complex formed with Be^{2+} in the range of 220-320 and 400-600 nm are strongly overlapped. This prevents a determination of Be^{2+} by direct UV-vis absorbance measurements. On the other hand the sensitivity is low because of a colored reagent. Derivative spectrophotometric method is a powerful tool for overcoming interferences due to spectral overlapping, however in this method signal-to-noise ratio is degraded but it is appropriate for optimization procedure. The first and second derivative spectra of thorin and Be-thorin complex were overlapped, too. But at 260 nm for third derivative the errors due to the blank absorption were completely removed (Fig. 1c). It should be noted that in
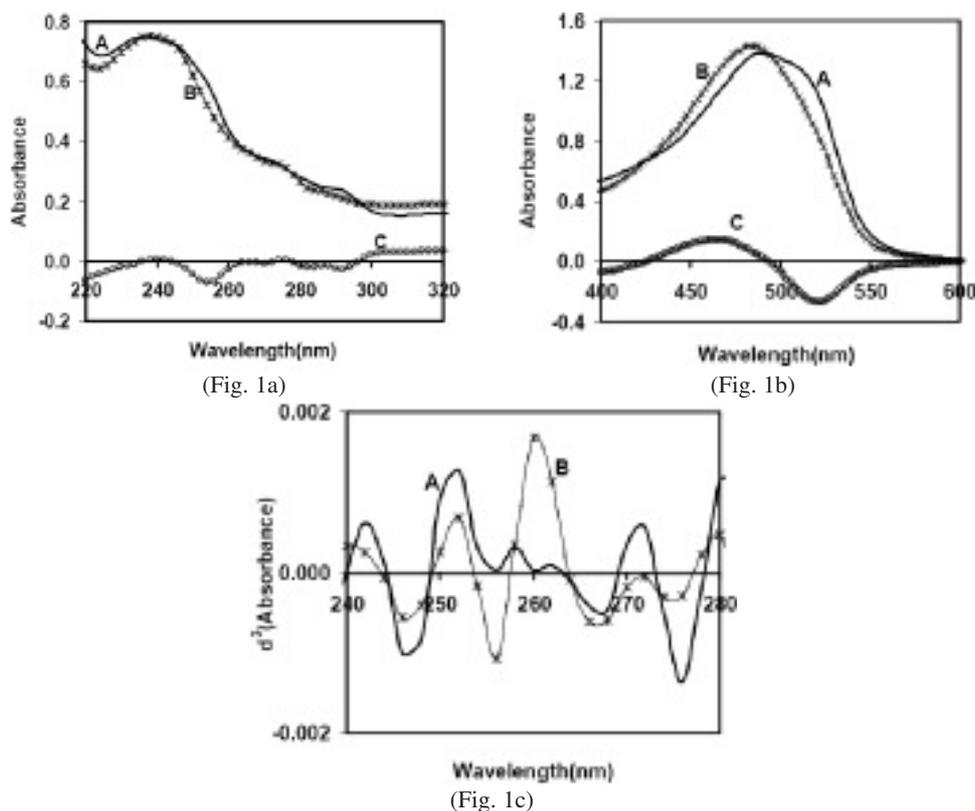

(Fig. 1a)   (Fig. 1b)

(Fig. 1c)

Fig. 1.   Absorbance spectra of thorin and Be-thorin in the range of (a) 220-320 nm, (b) 400-600 nm and (c) third derivative in the range of 240-280 nm, at pH 10.5. Curve A is reagent blank (thorin 4.8×10^{-3} %) (reference, water), curve B is mixture of 0.2 $\mu g\ mL^{-1}$ of Be^{2+} and 4.8×10^{-3} % of thorin (reference, water), curve C is Be-thorin; their concentrations are as in curves B (reference, reagent blank)

various concentration of thorin the absorbance value was constant and near to zero. Using of different type of surfactants such as Triton-X-100, sodium dodecyl sulphate (SDS) and cetyltrimethyl ammonium bromide (CTAB), in an amount greater than the CMC (critical micelle concentration), has no significant effect on spectral overlapping.

Effect of pH: The pH of the medium had a great effect on the existing form of reagent and also affected the absorbance of Be-thorin complex. The absorbance of thorin and its beryllium complex is pH dependent in the pH range 4-12. The optimum pH of the complex formation was determined by calculating the difference in the absorbance of the ligand and the complex at 260 nm as a function of pH as spectra recorded against water. A constant and maximum relative change in the absorbance of the complex observed in the pH range of 9.5-12.0, but respect to the ligand the pH 10.5 was better and taken as optimum pH during all subsequent studies.

Effect of the concentration of the ligand: The influence of thorin concentration on the absorbance of the complex was investigated. The influence of the amount of thorin on the absorbance of the solutions containing 0.2 μg mL^{-1} of beryllium was studied under the conditions established above. The absorbance of complex increased with increasing the amount of thorin up to 3.2×10^{-3} % and remained constant between 3.2 and 4.8×10^{-3} % of ligand and the absorbance of the ligand showed no significant variations. Thus, 4.8×10^{-3} % was selected to ensure a sufficient excess of the reagent throughout the experimental work.

Effect of other experimental condition: The Be-thorin complex is stable up to 48 h, at room temperature. The effect of ionic strength was investigated using 0.01-1.0 mol L^{-1} KNO$_3$ solution. This parameter had no effect on the complexation process.

Mean centering of ratio spectra method: The absorption spectra for the samples with different concentrations (5-1000 ng mL^{-1}) of beryllium were recorded in the wavelength range of 400-600 nm (Fig. 2a) and divided by the normalized spectrum of thorin, according to eqn. 2 and the ratio spectra were obtained in the wavelength range of 425-495 nm (Fig. 2b). Mean centering of the ratio spectra were obtained (Fig. 2c) according to eqn. 3. The concentration of beryllium was determined by measuring the amplitude at 458 nm corresponding to a maximum wavelength shown in Fig. 2c.

Analytical characteristics of mean centering of ratio spectra method: Table-1 summarizes the analytical characteristics of the optimized method, including regression equation, linear range, limit of detection, reproducibility factors. The calibration curve was linear over the range of 10-600 ng mL^{-1} of Be^{2+}. The limit of detection, defined as LOD = 3 S$_B$/m[25] (where LOD, S$_B$ and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), for five replicate measurements of the blank, was 2.56 ng mL^{-1}. The relative standard deviation (RSD) for six replicate measurements of 0.2 μg mL^{-1} beryllium was 4.05 %.
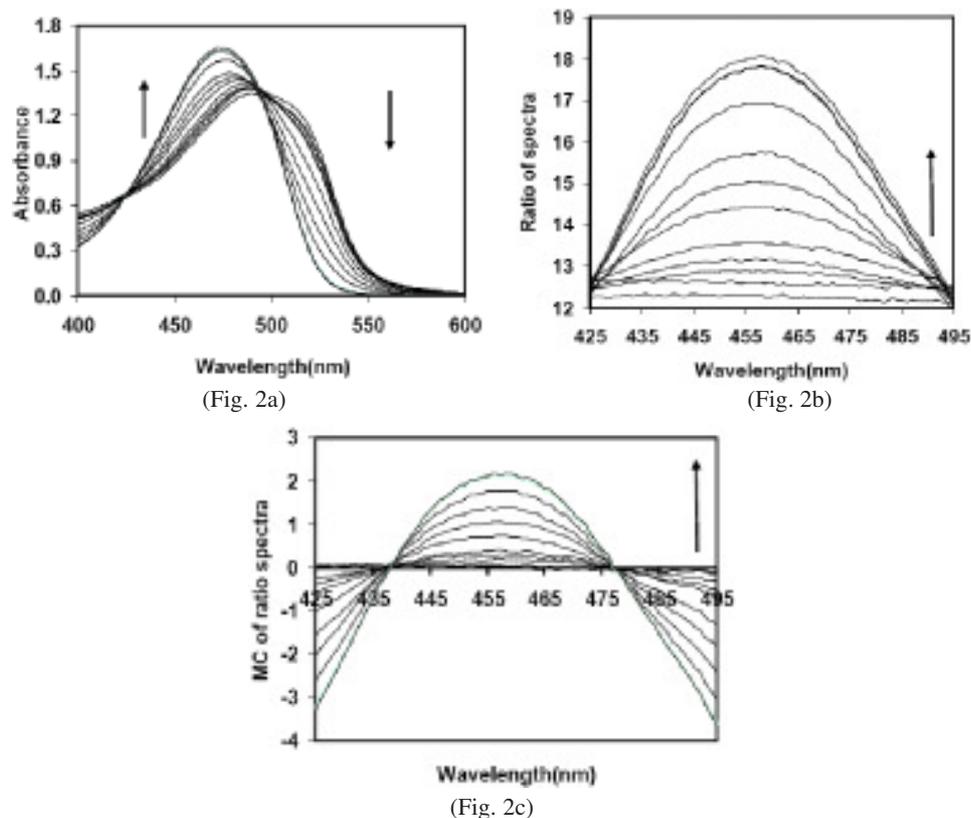
(Fig. 2a)

(Fig. 2b)

(Fig. 2c)

Fig. 2.   (a) The absorption spectra of Be-thorin (thorin 4.8×10^{-3} %) for different concentrations of Be^{2+} (5-1000 ng mL^{-1}), (b) The ratio spectra that were obtained by dividing spectra of Be-thorin by normalized spectrum of the thorin and (c) Mean centering of the ratio spectra

TABLE-1
ANALYTICAL CHARACTERISTICS OF THE MEAN
CENTERING OF RATIO SPECTRA METHOD

Analyte	Calibration equation[a]	(R^2)[b]	Linear range (ng mL^{-1})	RSD (%)[c]	LOD (ng mL^{-1})[d]
Be^{2+}	Y = 3.5194C + 0.0066	0.9989 (n = 10)	10-600	4.1	2.56

a: C is the concentration of Be^{2+} in µg mL^{-1}. b: Squared correlation coefficient. c: Relative standard deviation. d: Limit of detection.

Partial least squares regression: To perform the determination, the PLS1 method was evaluated for the resolution of beryllium. For this purpose a calibration set of 10 solutions of beryllium were prepared randomly that covers concentrations between 10 and 600 ng mL^{-1}. The calibration model was validated with 5 synthetic samples containing the considered beryllium in different concentrations. The obtained results are given in Table-2.

TABLE-2
CONCENTRATION OF SYNTHETIC SAMPLES OF Be^{2+}, THEIR PREDICTIONS BY
PLS1 REGRESSION AND STATISTICAL PARAMETERS FOR THE SYSTEM

Solution No.	Synthetic ($\mu g\ mL^{-1}$)	Prediction ($\mu g\ mL^{-1}$)	Recovery (%)
1	0.050	0.049	98.0000
2	0.500	0.502	100.4000
3	0.350	0.354	101.1000
4	0.070	0.068	97.4000
5	0.250	0.267	106.8000
Mean recovery	–	–	100.7000
RMSD[a]	–	–	0.0079
REP (%)[b]	–	–	3.2478

a: Calculated by eqn. 5. b: Calculated by eqn. 6.

Selection of optimal number of factors: The selection of the number of factors used in the calibration with PLS is very important for achieving the best prediction. As a first approach, the number of factors were estimated by cross-validation method, leaving out one sample at a time and plotting the prediction residual sum of squares (PRESS) *versus* the number of factors for each individual component. The prediction residual sum of squares for each number of factors was calculated by comparing the predicted concentration of compounds in each sample with known concentration of compounds in standard solutions (eqn. 10).

$$PRESS = \Sigma_{i=1}^{N}(\hat{C}_i - C_i)^2 \qquad (10)$$

where N is the number of samples, C_i and $\hat{C}_i$ the concentration of the analyte in the ith sample and the estimated concentration.

Fig. 3 shows a plot of PRESS *versus* the number of factors. For finding the smallest model (fewest number of factors), the F-statistic was used to carry out the significance determination[16]. The optimal number of factors for Be^{2+} was obtained as two and PRESS in the optimum number of factors was calculated 0.0006.
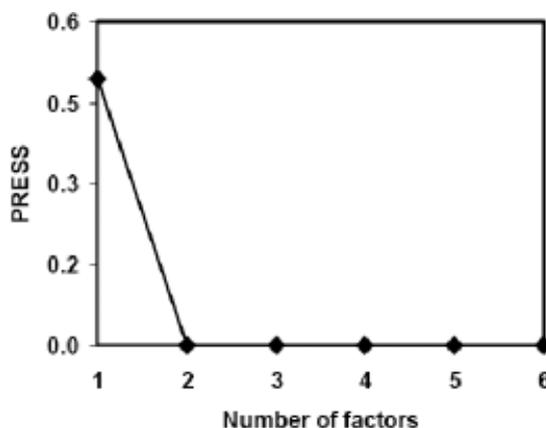

Fig. 3. Plot of PRESS against the number of factors for Be^{2+}

Statistical parameters: For the optimized model three parameters were selected to evaluate the prediction ability of the model for determination of Be^{2+} in the prediction set. The root mean square difference (RMSD), which is an indication of the average error in the analysis, for each component:

$$RMSD = \left[\frac{1}{N}\Sigma_i^N(\hat{C}_i - C_i)^2\right]^{0.5} \tag{11}$$

The RMSD values are an estimate of the absolute error of prediction for each component. Another parameter was relative error of prediction (REP) that shows the predictive ability of each component and is calculated as:

$$RPE\,(\%) = \frac{100}{C}\left[\frac{1}{N}\Sigma_i^N(\hat{C}_i - C_i)^2\right]^{0.5} \tag{12}$$

where $\hat{C}$ is the mean of true concentrations in the prediction set and N is the total number of samples used in the prediction sets. The value of RMSD, REP calculated for beryllium concentration in the prediction set are summarized in Table-2.

Effect of foreign ions: The effects of interferences were studied for 0.2 μg mL^{-1} of Be^{2+} in the presence of 100 fold of foreign ions. The precipitate, if formed, was removed by filtration. Cations of Cu^{2+}, Zn^{2+}, Mg^{2+}, Cr^{2+}, Co^{2+}, Fe^{3+}, Mn^{2+}, Al^{3+}, Bi^{3+} and Ni^{2+} were interfered in Be^{2+} determination. However, the cations of Se^{6+}, Ca^{2+}, Na^+, K^+, Ba^{2+} did not interfere. Most anions were found to have no interference.

(An ion was considered as interference when its presence produced a variation in the absorbance, due to mean centering of ratio spectra, of the sample greater than 5 %).

Attempts were made to overcome the interferences of the above mentioned metal ions using masking agents. Be^{2+} is selectivity unreactive towards EDTA[26]. So, EDTA can be used as a masking agent for various metal ions. For this purpose EDTA at 1000 fold level was added to each solution and as a result most of the interferences were removed. The interference due to Fe^{3+} ions was eliminated by using citrate as a masking agent.

Application: In order to evaluate the analytical applicability of the both proposed methods (PLS1 and mean centering of ratio spectra), they were applied to the determination of beryllium in several tap water samples collected from Mashhad, Iran. The results are given in Table-3. The good agreement between these results and known values indicates the successful applicability of the proposed methods for determination of beryllium in water samples.

The results shown in Table-3 also indicate that there are no significant difference between the outputs of the PLS1 and mean centering of ratio spectra method.

Conclusion

Two relatively simple, selective, rapid and inexpensive spectrophotometric procedures have been described for the determination of beryllium free from absorbent reagent blank. Mean centering of ratio spectra has been used to obtain pure analyte

response. Hence, the calibration lines shows no influence of the free reagent, because at each sample spectra its absorbance is cancelled using mean centering of ratio spectra, even when its contribution to the absorbance value differ from one solution to other. Also, using partial least-squares multivariate calibration was an effective and accurate way. The proposed methods have been applied to the determination of beryllium in natural water samples with good results.

TABLE-3
RESULTS OF DETERMINATION OF Be^{2+} IN WATER SAMPLES BY PLS1
AND THE MEAN CENTERING OF RATIO SPECTRA METHODS

Tap water	Added	Be^{2+} concentration ($\mu g\ mL^{-1}$)			
		Found		Recovery (%)	
		PLS1	MCRS[b]	PLS1	MCRS
Sample 1	0.000	–	–	–	–
Sample 2	0.500	0.512 ± 0.03[a]	0.501 ± 0.04	102.4	100.2
Sample 3	0.150	0.143 ± 0.03	0.145 ± 0.03	95.3	96.7
Sample 4	0.080	0.083 ± 0.02	0.085 ± 0.03	103.8	106.2

a: Mean ± standard deviation (three replicates). b: Mean centering of ratio spectra.

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