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Additional Information

Multivariate calibration applied to simultaneous chemiluminescence determination of cobalt and chromium

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Abstract The simultaneous determination of chromium and cobalt in water samples has been studied. Chemiluminescence registers based on the luminol–hydrogen peroxide reaction have obtained by a batch procedure. PLS algorithms have employed to model the time-response (formation and destruction of emitter). The influence of the presence of two metals and the non-linearity relationship between response and concentration have been evaluated in the signal. Different experimental designs and the selection of variables have been tested. The calibration set has been selected based on two criteria: unicomponent and/or bicomponent standard solutions and the slope calculated from linear univariate calibration. The response has been modelled providing high percentages of explained variance, robust models and low prediction errors. The proposed methodology has been validated using test standard solutions and a standard reference material of fresh water. Accurate results have proved the advantages of this method for the simultaneous determination of chromium and cobalt in water samples.

Keywords Chemiluminescence · Chromium and cobalt determination · Water · Luminol-hydrogen peroxide reaction · Simultaneous analysis

Introduction

Cobalt and chromium are widespread in water samples, existing in various chemical, physical and morphological forms. Generally, underground and surface waters contain very low levels of these metals but they are often introduced into hydric cycle from industrial waste. Many of their forms are toxic and their release must be carefully monitored. Accordingly, the maximum concentration for Co and Cr in drinking water is fixed by EU directives. The determination of trace elements in water samples requires analytical techniques with sufficiently high sensitivity and selectivity.

Several analytical methods have been proposed for cobalt and/or chromium determination as capillary electrophoresis [1], NAA [2], XRF [3, 4], AAS [5, 6], ICP-AES [7] or ICP-MS [3, 8]. Chemiluminescence methods provide low detection limits, rapid response and low cost. Although, the main disadvantage is the lack of selectivity for most chemiluminescence reactions. In this sense, several strategies have been proposed for selective or simultaneous determination, especially for luminol chemiluminescence reaction. Pre-treatment or separation procedures are usually required for the selective determination in flow injection. The use of masking agents [9], membrane phase separator [10] or discrete sample clean-up with ion exchange resin [11] have been reported. The ion chromatography with chemiluminescence detection has been proposed for simultaneous determination of some metals [12, 13]. However, the limitation is the incompatibility of the mobile phase with the reaction conditions required for post-column reaction [14].

A charge coupled device detector has been used for the acquisition of full chemiluminescence spectral profile. The simultaneous determination is achieved using wavelength discrimination or mathematical resolution [15, 16]. The use of two different reactions have described for Co and Fe determination in the same manifold [17]. Time-resolved emissions have also been reported for Co and Cu determinations [18]. Partial least squares (PLS) method has been applied to the simultaneous determination of mixture of compounds in water samples. Organic compound mixtures [19, 20] and metal ion mixtures [21, 22] have been resolved by the multicomponent calibration approach and/or interferent elimination technique.

We have studied the univariate and multivariate chemiluminescence determination of chromium in water samples, mainly by flow injection analysis [23, 24, 25]. In this paper, PLS models have been used for the simultaneous determination of Cr(III) and Co(II) in water samples. To our knowledge, it is the first application of simultaneous determination in chemiluminescence analysis by PLS regression. Chemiluminescence registers have obtained by a batch and nonautomatic procedure. Different models were built with time-response curves of calibration set selected by different criteria. Using standard solutions has assessed the prediction ability. The method proposed has been validated by the determination of concentrations of Cr(III) and Co(II) in a standard reference material of fresh water.

Experimental section

Apparatus and reagents

A Jasco FP-750 (Tokyo, Japan) fluorescence spectrophotometer was used for the measurements. The light emission was monitored at 425 nm. The chemiluminescence was measured with a 1-cm path length quartz cell and the chromium (III) solutions were injected using a Hamilton digital syringe (Nevada, USA). The following reagents were used: chromium (III) nitrate (Panreac, Spain), hydrogen peroxide (Panreac), luminol (Fluka, Switzerland), sodium carbonate (Merck, Germany), sodium hydroxide (Probus, Spain), chlorhydric acid (trace pur, Merck) and nitric acid (trace pur, Merck). The solutions were prepared in water (nanopure, Sybron, Barnstead, Spain). The concentration of chromium (III) or cobalt (II) in stock standard solutions was 100 mg/L.

Procedure

The reagent solution was prepared in the quartz cell. The reactive concentrations were 4×10^{-4} mol/L of luminol, 3.3×10^{-2} mol/L of hydrogen peroxide, 3.3×10^{-3} mol/L of EDTA and 0.1 mol/L of $\text{HCO}_3^- - \text{CO}_2$ buffer solution to adjust the pH to 10.8. The solutions were injected using a syringe throughout the septum and the solution in the cuvette was mixed with a stirrer. In all cases, the injection volume was 200 μL . The emission signal after the injection step was registered. Calibration sets Different calibration sets were analysed in function of concentration interval. So, the concentration interval was divided into linear response region and non-linear response region. Linear response The calibration set was composed of standard solutions of Cr(III) or Co(II). The concentrations were ranged 0–20 mg/L and 0–80 mg/L, respectively. Standard mixtures with different concentration of Cr(III) and Co(II) were also measured with a concentrations of 0–20 mg/L and 0–80 mg/L, respectively. Non-linear response for Cr(III) The calibration set consisted of 25 standards with different concentrations of Cr(III) and Co(II) following a two factorial design (52). A code with two numbers was assigned to each standard. The first one refers to Cr (III) concentration (0 – 80 mg/L) and the second to the Co(II) concentration (0 – 80 mg/L). Their concentrations are shown in Table 1.

Table 1 Code and concentration of standard calibration set (5² design)

Cr(III)\Co(II) ($\mu\text{g/L}$)	0	20	40	60	80
0	00	01	02	03	04
20	10	11	12	13	14
40	20	21	22	23	24
60	30	31	32	33	34
80	40	41	42	43	44

Analysis of standard material reference

The calibration set consisted of standard solutions in nitric acid. Calibrations of Cr(III) and Co(II) (ranging from 0 to 80 $\mu\text{g/L}$) were prepared in 0.07 mol/L HNO₃, before measurement step 0.2272 mol/L carbonate was added. Mixtures of Cr(III) and Co(II) were also prepared under the same conditions (15–10, 27–14.2, 15–25 and 45–25 $\mu\text{g/L}$, respectively). The certificate reference material SRM 1640 (NIST, USA) is composed of natural fresh water collected from Clark Creek, CO, which had been filtered and stabilised with nitric acid (Table 2). The SRM was diluted with standard carbonate solution. The time registers of calibration, mixture and standard reference material solutions were measured.

Table 2 Composition of standard reference material 1640: fresh water

Certified mass fractions				Reference mass fractions	
Element	($\mu\text{g/Kg}$)	Element	($\mu\text{g/Kg}$)	Element	($\mu\text{g/Kg}$)
Aluminum	52.0 \pm 1.5	Cobalt	20.28 \pm 0.31	Copper	34.3 \pm 1.6
Antimony	13.79 \pm 0.42	Iron	34.3 \pm 1.6	Lithium	27.89 \pm 0.14
Arsenic	26.67 \pm 0.41	Lead	27.89 \pm 0.14	Nickel	27.4 \pm 0.8
Barium	148.0 \pm 2.2	Manganese	121.5 \pm 1.1	Potassium	994 \pm 27
Beryllium	34.94 \pm 0.41	Molybdenum	46.75 \pm 0.26	Rubidium	2.00 \pm 0.02
Boron	301.1 \pm 6.1	Selenium	21.96 \pm 0.51	Zinc	53.2 \pm 1.1
Cadmium	22.79 \pm 0.96	Silver	7.62 \pm 0.25	Calcium	7.045 \pm 0.089
Chromium	38.6 \pm 1.6	Strontium	124.2 \pm 0.7	Magnesium	5.819 \pm 0.056
		Vanadium	12.99 \pm 0.37	Silicon	4.73 \pm 0.12
				Sodium	29.35 \pm 0.31
				Thallium*	<0.1

Calculations

The data were aligned according to the maximum emission signal. For all calculations, Unscrambler (CAMO, Norway) and Matlab for Windows (Math

Works, Natick, MA) were used. Some multivariate methods were carried out with modified routines of the PLS-Toolbox (Eigenvector Research, Manson, WA).

Results and discussion

Study of the chemiluminescence signal

In the presence of a catalyst, luminol is oxidised by peroxide in basic solution to form 3-aminophthalate in an excited state, which exhibits an intense luminescence ($\lambda=425$ nm). In a stopped-flow system, the chemiluminescence emission describes a typical response curve (intensity vs time). According to Gonzalez-Robledo et al. [26]. and Merenyi et al. [27], the response curve corresponds to a first-order consecutive sequence: formation and destruction of light emitter. The reaction rate is increased by different metal ions. According to Mottola and Lan [28], these rate-increasing effects have been better designated as promotions than catalytic effects. In most cases, the rate-affecting species is consumed during the reaction. The response curve depends on such different experimental factors as pH or mixing rate [23] and it is also a function of ion metal present in the solution. pH and other experimental parameters are kept constant so that their influences are avoided. In the experimental conditions, assayed Cr and Co contributed mainly to chemiluminescence signal, other species usually present in water samples did not interfere [23, 29]. The full analysis of the SMR 1640 (shown later), which is in fresh water (Table 2), corroborated this statement. In Fig. 1, normalised intensity- time profiles for Cr and Co determination are shown. The formation profile is similar for both metals but the decay of chemiluminescence signal is different. The Co (II) shows a decreasing of the signal more significant than for Cr (III). Robust profiles were obtained in both cases independently of the concentration of the metal in the solution.

Evaluation of calibration models

As it was explained in the previous section, the most significant differences between both metals are obtained in the chemiluminescence decay profile. Then, a variable selection could improve the system modelling. Two possible options are the study of entire register (61 variables) or the study of final region (46 variables). Moreover, PLS regression has two approaches based on the different algorithms PLS-1 (unicomponent models) and PLS-2 (uni and multicomponent models). The schedule of calibration study is shown in Fig. 2.

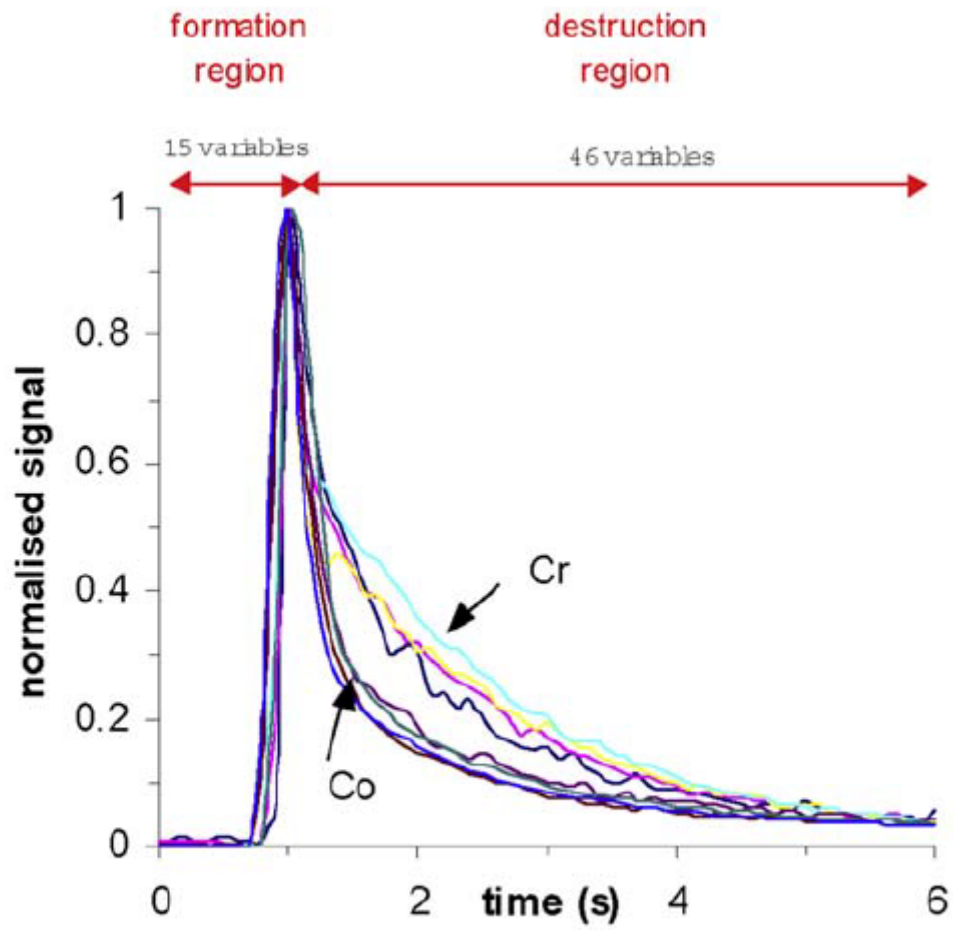


Fig.1 Normalised chemiluminescence intensity-time profiles

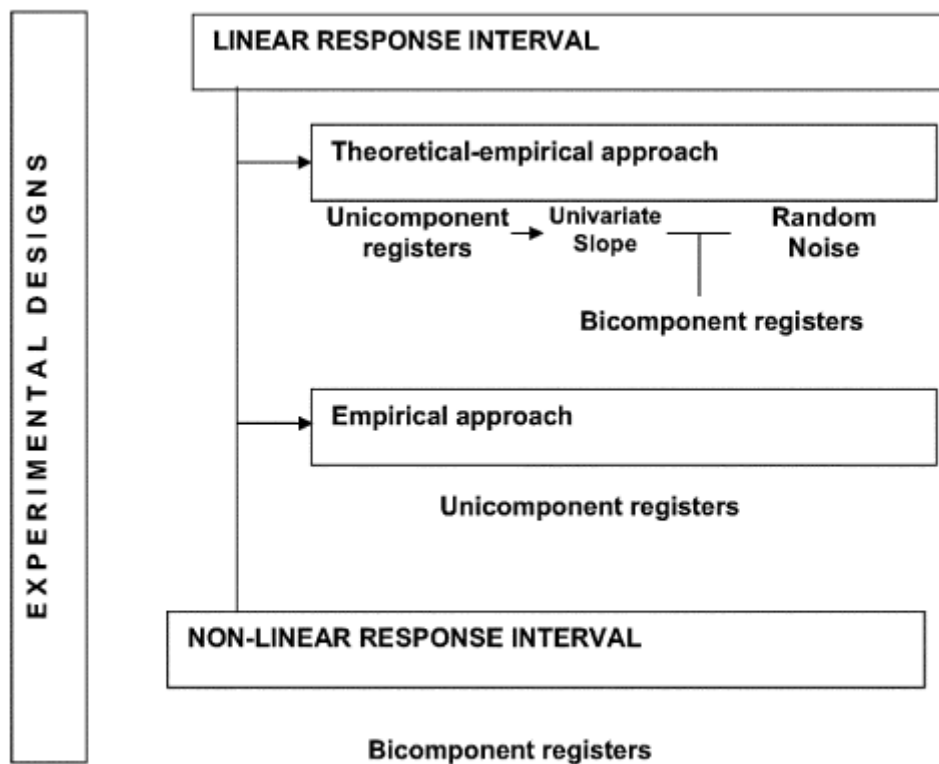


Fig.2 Schedule of calibration study

Linear response

A calibration set in the concentration interval with linear response was studied. Unicomponent standard solutions were composed of chromium (0–20 $\mu\text{g/L}$) or cobalt (0–80 $\mu\text{g/L}$). The aim is modelling the response for simultaneous prediction in samples where both metals are present. Two approaches were studied in order to assess the additivity of signal produced for both metals. Empirical-theoretical approach Theoretical calibration set is composed of bicomponent registers obtained from experimental unicomponent registers. Empirical approach Calibration set is composed of experimental unicomponent registers. In the empirical-theoretical approach, univariate linear regression (chemiluminescence signal vs metal concentration) was performed for each time. The set of slope values for each time was the chemiluminescence register of each metal. The calibration set (bicomponent registers) was built from the numeric sum of the corresponding response

Heteroscedastic error was added in order to avoid model overfitting. This simulated error was generated as random noise with the same magnitude to signal reproducibility. Figure 3 shows slope registers calculated for

chromium and for cobalt. The theoretical profile, including heteroscedastic error, calculated for a mixture of 10 $\mu\text{g/L}$ Cr and 10 $\mu\text{g/L}$ Co is also plotted.

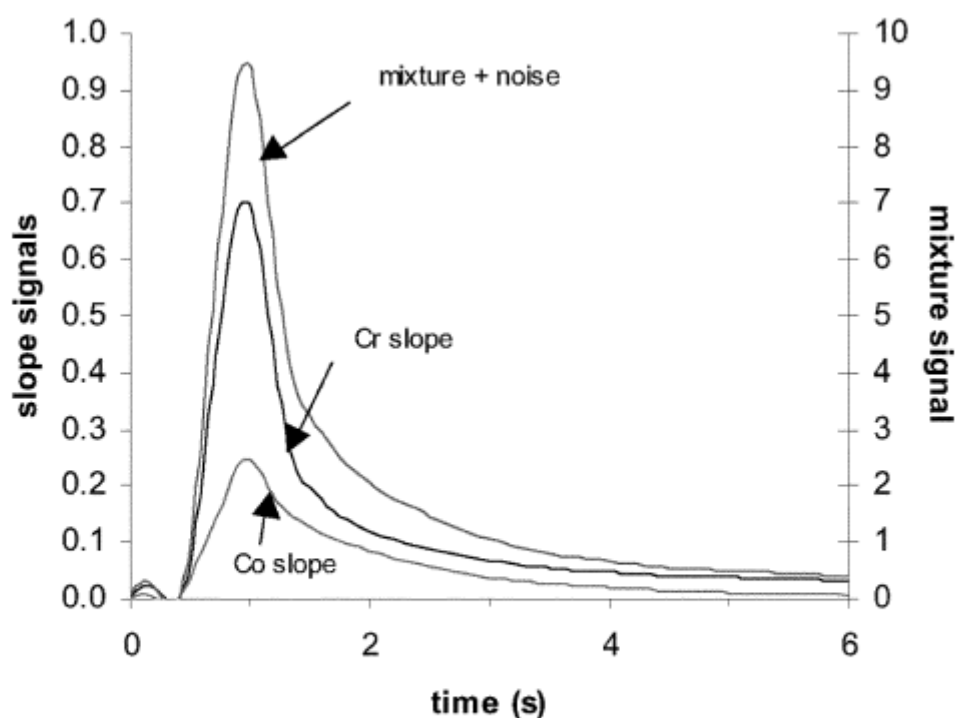


Fig.3 Profiles calculated from slopes of univariate linear regression (Cr and Co) and theoretical profile of a mixture 10 $\mu\text{g/L}$ Cr and 10 $\mu\text{g/L}$ Co including heteroscedastic error

For empirical-theoretical and empirical approaches, PLS-1 and PLS-2 models were obtained using as X-block the data (column centred, 61 or 46 variables). The percentage of explained variance, the number of factors and root mean squared errors of prediction (SEP) are given in Table 3. SEP values were calculated in order to evaluate the prediction ability of the models. SEP of calibration was obtained using all standard solutions employed in the model. SEP of validation was calculated using a prediction set composed of unicomponent and bicomponent standard solutions. Some conclusions can be established from the results. Mainly, the additivity of chemiluminescence signals from simultaneous presence of chromium and cobalt was confirmed. Therefore, any synergism effects of metal interactions were observed. Data were modelled by two factors corresponding to the number of species present in the solution and the relationship between chemiluminescence signal and the concentration is linear. The robustness of the models can be checked by the similarity

between calibration and validation errors (SEP values). Finally, both approaches (empirical-theoretical and empirical) provided similar results. Then, the calibration set could be composed of registers of unicomponent standard solutions or simulate bicomponent registers from univariate slopes. In both cases, the prediction of binary mixtures is possible because of signal additivity.

Table 3 PLS models for calibration sets in linear interval

		Variables	factors	% Explained variance			SEP calibration		SEP validation	
				X-block	Y-block Cr	Y-block Co	Cr	Co	Cr	Co
Empirical-theoretical approach	PLS-1	1-61	2	98.70	99.88	99.90	0.8	2.7	1.7	4.9
		16-61	2	99.92	94.22	99.71	5.9	7.6	6.4	11.4
	PLS-2	1-61	2	98.70	99.89		0.8	2.7	1.7	4.9
		16-61	2	99.92	99.36		6.0	7.5	6.7	11.2
Empirical approach	PLS-1	1-61	2	99.98	99.98	99.99	1.5	4.3	2.7	7.7
		16-61	2	99.99	95.65	99.58	6.2	9.3	7.7	12.5
	PLS-2	1-61	2	99.98	99.99		1.5	4.3	2.7	7.7
		16-61	2	99.99	99.35		6.2	9.3	7.7	12.4

Non-linear response

A 52 design was studied, see Table 1. Cr(III) and Co(II) concentrations of unicomponent and bicomponent solutions were in the range 0–80 mg/L. Chemiluminescence registers of the calibration set were treated with PLS-1 and PLS-2 algorithm. For this purpose, Cr and Co concentrations of standard solutions were used as the Y-block. The X-block consisted of the data (column centred) from 0 to 7.5 s every 0.1 s, so 61 variables were introduced. The percentage of explained variance and the number of factors are given in Table 4.

Table 4 PLS models using standard solutions or calibration slope

Variables		PLS-1		PLS-2	
		1-61	16-61	1-61	16-61
Factors		4	3	4	3
% Explained variance	X-block	99.93	99.94	99.93	99.92
	Y-block Cr	92.81	95.97	85.14	95.46
	Y-block Co	78.62	96.24		
SEP calibration	Cr	7.6	5.7	7.5	6.5
	Co	13.1	5.5	13.5	5.5
SEP validation	Cr	10.6	7.4	10.5	7.4
	Co	18.0	7.5	17.8	8.4

As explained in the previous section, the most significant differences between both metals are obtained in the chemiluminescence SEP decay profile. PLS-1 and PLS-2 models were obtained using as X-block the data (column

centred) from 1.5 s to 7.5 s, so 46 variables were introduced. The percentage of explained variance, the number of factors and the squared sum of prediction residual errors are given in Table 4. Figure 4 shows the score plot for two first factors. The distribution of the sample was similar to experimental design but the quadratic shape has been lost due to the second factor, also described the non-linear contribution. The first factor was related to the concentration of cobalt and the second one to the concentration of chromium. The method required more than two factors in order to describe the non-linear relationship between metal concentration and signal [24] as can be seen in Table 4. SEP of calibration was obtained using all standards as calibration samples. SEP of validation was evaluated by leave-one-out cross validation. The robustness of the models can be checked comparing the SEP values, see Table 4. Comparing the different options, the following conclusions were obtained. The previous selection of the variables with more selective information has provided PLS models with lower number factors but similar prediction ability. Moreover, the simultaneous determination of chromium and cobalt was demonstrated for the studied interval.

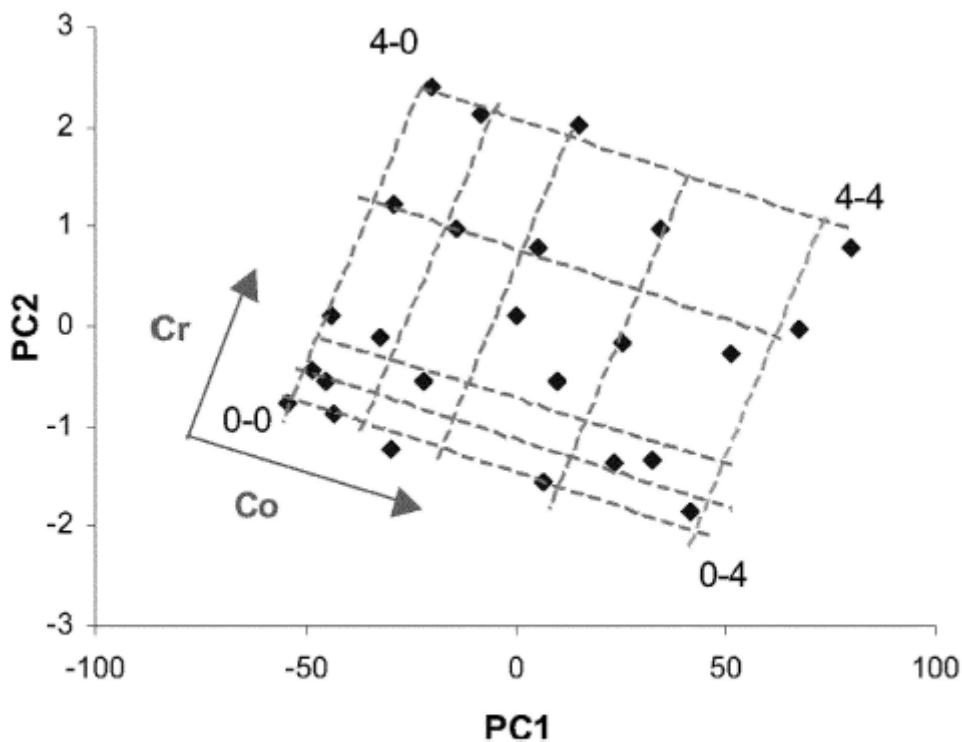


Fig.4 Score plot of PLS-2 model (centred data, 3 factors and 46 variables) for 5^2 design calibration set

Validation

For model validation, a certified reference material was analysed. SRM 1640 is a fresh water sample with 17 trace metal elements (Table 2). The concentration of trace metals has been certified by using different techniques and laboratories. In order to build the calibration model, unicomponent solutions were prepared under the same conditions as reference material. PLS models were built using chemiluminescence registers (column centred) as X-block data. Tables 5 and 6 include the percentage of variance explained for X-block and Y-block and SEP values for the different models. SEP validation values were calculated using leave-one-out cross validation procedure.

Table 5 PLS models for calibration in nitric acid (Part 1)

Model	Variables	Factors	% Explained variance			SEP calibration		
			X-block	Y- Cr	Y- Co	Cr	Co	Total
PLS-1	1-61	4	99.94	99.94	99.80	0.7	1.2	1.0
	16-61	3	99.93	99.94	96.63	0.7	5.1	2.1
PLS-2	1-61	4	99.94	99.47		1.7	2.3	3.7
	16-61	3	99.94	99.47		0.9	5.6	4.6

Table 6 PLS models for calibration in nitric acid (Part 2)

Model	SEP validation			Predicted vs real			
	Cr	Co	Total	a	b	R ²	s _{yx}
PLS-1	2.6	4.6	3.6	0±2	0.98±0.07	1.0	3.3
	4.1	10.3	4.0	-2±6	0.9 ±0.2	0.9	8.8
PLS-2	3.4	5.6	7.9	0±2	0.98±0.07	1.0	3.4
	4.4	10.1	7.8	0±4	0.94±0.15	0.9	6.5

Additionally, these models were applied to predict the standard Cr(III)-Co(II) mixture solutions prepared under the same conditions as reference material. The parameters of the linear regression between predicted concentration and real concentration are also included in Table 6. It can be seen that the unit was included in the confidence interval of the slope and the zero was included in the confidence interval of the intercept; therefore the results were acceptable.

The predicted concentration for standard reference material can be seen in Table 7. A variance test provided precision of the proposed strategy and was worse than the standard deviation of certificate value; but it is suitable

for water analysis. The precision obtained by us is similar to that obtained by other researchers for the analysis of real water samples. A t-test was performed in order to compare the predicted and certified values at the significance level of 95%. The best results were obtained using the complete register (61 variables). Therefore, it has been demonstrated that the proposed method predicts the cobalt and chromium concentration, avoiding the interference of other elements. Therefore, this study establishes that the chemiluminescence signals are robust.

Table 7 Prediction for standard reference material ($\mu\text{g/L}$, $n=5$)

	Variables	Factors	Concentration ($\mu\text{g/L}$)		<i>t</i> value	
			Cr	Co	Cr	Co
Reference	–	–	38.6 \pm 1.6	20.28 \pm 0.31	–	–
PLS-1	1–61	4	32 \pm 7	21 \pm 7	–2.4	0.3
	16–61	4	(35 \pm 2)	(11 \pm 6)	(5.7)	(–3.1)
PLS-2	1–61	3	31 \pm 7	25 \pm 9	–2.1	1.0
	16–61	3	(36 \pm 2)	(12 \pm 5)	2.5	(–3.8)

Conclusions

In this paper, PLS algorithms have been employed to model the chemiluminescence time response. The simultaneous determination of two metals (chromium and cobalt) has been possible in water samples. Different experimental designs have been tested in order to evaluate the influence of addition of signals provided by the two metals and the non-linearity relationship between response and concentration. The calibration set has been selected based on two criteria: unicomponent and/or bicomponent standard solutions and slope calculated from linear univariate calibration. The selection of variables has also been studied. In all cases, good results were obtained, although the best results were achieved using the entire register (increase and decay of chemiluminescence signal). Robust results have been obtained for different concentrations of analytes. This condition is critical for simultaneous analysis of both species. The proposed method has been validated using test standard solutions and standard reference material. The main advantages of this method are the low cost, rapid response and that it does not require qualified personnel.

These characteristics are useful for monitoring water analysis. The novelties of the paper in reference to published ones are: We have demonstrated the robustness of the intensity vs time profiles. To our knowledge, it is the first

application of the intensity vs time profiles in multivariate calibration for simultaneously chemiluminescence analysis of two species. Other papers employ intensity vs wavelength registers obtained from special devices (DDC) in order to determine an analyte [15, 16] We validated our findings with a reference material

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