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Talanta 59 (2003) 775–783

Talanta

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Factorial designs applied to the development of a capillary electrophoresis method for the analysis of zinc, sodium, calcium and magnesium in water samples

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Received 23 May 2002; received in revised form 10 October 2002; accepted 25 November 2002

Abstract

The aim of this work was to study the influence of several both chemical and instrumental variables for the development of a new capillary electrophoresis (CE) method for the determination of zinc, sodium, calcium and magnesium in water samples by using 1,10-phenanthroline as complexing agent and UV photometric detection at 214 nm. Due to the number of parameters involved and their interactions, factorial experimental designs at two levels have been applied to investigate the influence of several experimental variables (concentration of complexing agent, concentration of a visualisation agent, pH, sample introduction, applied voltage and capillary length) in sets of several capillary electrophoretic responses. The method was applied to the simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II) in drinking water with satisfactory results and a detection limit of 32 ng ml^{-1} for Zn(II) was obtained.

Keywords: Factorial design; Capillary electrophoresis; Metalions; Water

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1. Introduction

Capillary electrophoresis (CE) has become a reliable analytical technique in different fields of environmental research. The well-known advantages of CE (i.e. high efficiency, reduced consumption of reagents, fast analysis, simultaneous determinations) have encouraged the development of analytical methodologies in order to properly determine a high number of organic and inorganic substances in environmental samples. In this area, one of the most interesting applications of environmental analysis by CE could be the determination of metal ions in water samples as a tool for the environmental monitoring of aquatic systems like, for instance, rivers and lakes [1].

Even in real samples with relatively simple matrixes like natural and drinking waters, the amount of several major metal cations can disturb

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doi:10.1016/S0039-9140(02)00618-5

the simultaneous determination of other metal cations at trace levels. In such cases, it is necessary to use separation and preconcentration methodologies in order to avoid the presence of such disturbing components. So, their applications to the simultaneous determination of metal cations in real samples depend on several factors including effects of the matrix complexity and the concentration ratios between the analytes. Both are important factors, which affect the detection limits and the possibility of simultaneous determinations.

Although the concentrations of metal cations in natural and drinking waters may depend on both natural and anthropogenic factors, it is quite usual that the concentrations of alkaline and alkaline-earth metals are at mg l^{-1} levels, whilst trace metals, are in the μg l^{-1} range. Several CE methods have been already developed to determine alkaline and alkaline-earth metal cations [2–7], but their simultaneous determination with trace metals by CE represents a difficult task mainly because of the lack of sensitivity and the existence of peaks overlapping which compromises the separation efficiency. In addition, several trace metals, zinc included, are chemical elements that plays an important role as oligoelements in several biological processes. Nevertheless, these metals may also be the origin of adverse effects on living organisms if the dosages exceed certain levels [8]. Obviously, water is a medium in which zinc can be easily assimilated by living organisms, and the uptake of zinc above certain concentration levels for a period may lead to bioaccumulation processes that could produce adverse effects or even mortality. Therefore, the control of zinc concentrations is necessary to assure good quality standards in natural and drinking waters.

In order to determine alkaline and alkaline-earth metals, as well as zinc in waters, atomic spectroscopy techniques are the most commonly used. Nevertheless, most commonly used atomic spectroscopy techniques (AAS, F-AES) are monoelemental and, consequently, they need longer analysis times. Moreover, in recent years, CE has demonstrated to be a capable technique for the determination of metal ions in aqueous solutions, including the possibility of the simultaneous analysis of metal cations in different kinds of synthetic and real samples at mg l^{-1} levels or even lower, as well as element speciation [9–17].

Due to its simplicity and reliability, the most common methodology for the determination of metal cations by CE is based on the addition of a weak complexing agent to the electrophoretic buffer in order to obtain better baseline separations and the use of UV–Vis absorbance as detection method with indirect photometric detection by adding an UV absorbing substance has been used widely for the determination of non-absorbing metal cations or their complexes [18–32]. Among others, 1,10-phenanthroline has been used by several authors to determine iron as well as other metal ions in several kinds of samples [33–44]. Despite the disadvantages of CE mainly regarding to sensitivity with UV detection, this methodology also provides several advantages (i.e. simplicity, cost, ...) that can be seized by proper studies of the effects of experimental variables that may affect resolution and sensitivity for the analysis of metal ions. However, because of the number of parameters involved in the development of new methodologies and strategies for the determination of metal cations by CE with UV absorbance as detection method, experimental designs can be considered as helpful tools for the systematic study of the effects of all these parameters in the separation and determination of the analytes [45]. These strategies make possible not only to identify the rate of influence of the variables in the system, but also to enhance the comprehension of their behaviours and trends.

This work was focused on a study based on factor designs of the behaviour of several experimental variables in metal determinations by CE. Due to these studies, sets of suitable experimental conditions have been proposed for the single determination of Zn(II) and for the simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II). Indirect UV-absorption spectroscopy at 214 nm has been used as detection method and its application to drinking waters analysis. 1,10-Phenanthroline was used as complexing agent and 4-methylbenzylamine as UV-absorbing agent. The effects of the background electrolyte composition (concentration of complexing and visualisation agents, pH) and instrumental parameters as capillary length, run voltage and sample introduction time were studied by factorial experimental designs at two levels (2^6) to find those suitable experimental conditions for the separation of these metal cations.

2. Experimental

2.1. Apparatus

A Waters Quanta 4000 CE system (Waters, Milford, USA), equipped with a positive power supply and two fused-silica capillaries of 0.60 and 1.00 m total length (75 μm I.D.; Accusep, Waters) were used. Indirect UV detection was achieved at 214 nm with a mercury lamp and an UV filter assembly. Polyethylene vials were used as containers for the background electrolyte and for all the standards and samples. A PC with Millennium Chromatography Manager Software (WATERS) was used to control the CE system and evaluate the electropherograms.

2.2. Reagents and solutions

All solutions, electrolytes and standards were prepared using deionised 18 MΩ water from a Milli-Q system (Millipore, Bedford, USA). All glassware and plastic ware was washed carefully with 2 M nitric acid and deionised water. The following reagents were used: 4-methylbenzylamine (Aldrich, Steinheim, Germany, 97%), 1,10-phenanthroline (Merck, Darmstadt, Germany, p.a), methanol (Merck, Darmstadt, Germany, p.a) and acetic acid (Merck, Darmstadt, Germany, p.a). Standard solutions of 1000 mg l^{-1} of calcium, sodium, magnesium and zinc in 0.5 M HNO_3 were purchased from Merck (Darmstadt, Germany).

2.3. Procedure

The composition of the background electrolyte (BGE) solutions for CE was established according to the conditions required for each experiment in the experimental design. The BGE solutions were prepared by dissolving certain amounts of 1,10-phenanthroline and 4-methylbenzylamine in methanol (10% final concentration) and deionised water. The pH was adjusted with 0.5 M acetic acid solution. Electrolytes and samples were filtered through 0.45 μm pore size mixed cellulose–esters filters (MF-Millipore). The BGE was homogenised and the dissolved O_2 was removed by using ultrasounds.

The capillary was conditioned with 1 M KOH solution and then purged with BGE for 2.0 min between runs. Sample solutions were introduced into the capillary using hydrostatic injections, from a height of 9.8 cm. Indirect UV detection was used at 214 nm. The peaks were quantified (peak height, peak area and migration time) using the Millennium Chromatography Manager Software.

2.4. Selection of the variables and experimental design

Two-level factorial designs are screening designs that combine the low and high levels of each factor with two levels of every other factor. These designs require only a reduced number of experiments to find those trends that are helpful to understand the behaviour of the system. In addition, they can be easily upgraded to surface response designs in order to perform further optimisations.

In this work, six design variables as well as their levels were chosen according to the previous experience on the system and by considering limitations imposed by the equipment and reasonable analysis conditions, i.e. not excessive long analysis times. These design variables were concentration of UV absorbing agent (4-methylbenzylamine), sample introduction time, run voltage, concentration of complexing agent (1,10-phenanthroline), pH and capillary length. Then, these variables were codified (X_i; $i = 1–6$) and normalised (-1, $+1$) as shown in Table 1 and a 2^6 (64 experiments) factorial design was performed.

Firstly, those circumstances that may affect only to the Zn(II)-1,10-phenanthroline peak were studied. Zinc peak enhancement as well as speed of analysis was studied by considering three responses: peak high, peak area and migration time

Table 1
Codification of the variables and level values in the 2^6 factorial design

Variables	Code	Lowest level (-1)	Highest level ($+1$)
Concentration of 4-methylbenzylamine	X_1	3 mM	7 mM
Sample introduction time	X_2	10 s	40 s
Run voltage	X_3	15 kV	30 kV
Concentration of 1,10 phenanthroline	X_4	2 mM	6 mM
pH	X_5	3.7	5.3
Capillary length	X_6	0.60 m	1.00 m

for the Zn(II)-1,10-phenanthroline complex. Then, a 2^6 factorial design was used to investigate trends and to find a set of suitable experimental conditions for the single Zn(II) determination. Therefore, those conditions that led to the maximisation of the Zn(II)-1,10-phenanthroline peak and the minimisation of the migration time were set as main objectives for this study.

On the other hand, the simultaneous determination of Zn(II), Na(I), Ca(II) and Mg(II) was investigated by considering a compromise between sensitivity and resolution. For this purpose, three new responses were studied. Peak height and peak area of the shortest and smallest peak were used as responses related to sensitivity, whilst minimum difference between two neighbouring peaks was used to study the separation efficiency. Then, the maximisation of these responses was set as the main objective in this study in order to enhance the performance of the methodology.

In order to avoid problems related to quantification of the peaks, the concentrations of sodium, calcium, magnesium and zinc were 2 mg l^{-1} for all the experiments, which were performed in random order to avoid any interactions among successive experiments.

The results were analysed using the software program STATISTICA 4.0 [46]. Interactions between parameters up to second order have been studied.

3. Results and discussion

3.1. Single determination of Zn(II)

A negative peak for Zn(II) caused by the spectrophotometric absorption of the positively charged Zn(II)-1,10-phenanthroline complex was found. Peak height (absolute value) and peak area for the Zn(II)-1,10 phenanthroline complex were considered as responses in the study of sensitivity, whilst migration time as response for the study of speed of analysis. According to the results of an ANOVA for main effects and two-way interactions, the following response equations with the significant ($P < 0.05$) normalised ($-1, +1$) variables and interactions up to second order can be used as a model to determine the influence of the variables related to the peak enhancement: R_h (peak height, μV) $= 2.521 - 0.362X_1 + 1.288X_2 - 0.190X_3 - 1.321X_4 - 0.783X_6 - 0.197X_1X_2 + 0.126X_1X_4 - 0.673X_2X_4 + 0.092X_3X_4 + 0.117X_4X_5 - 0.323X_2X_3 + 0.380X_4X_6$, R_a (peak area, μV s) $= 10.500 - 1.653X_1 + 5.909X_2 - 4.034X_3 - 4.912X_4 + 0.650X_5 - 0.925X_1X_2 + 0.622X_1X_3 - 2.334X_2X_3 - 2.582X_2X_4 + 1.913X_3X_4$ and R_s (migration time, min) $= 12.302 + 0.436X_1 - 4.783X_3 + 1.048X_4 - 0.854X_5 + 5.799X_6 - 0.443X_1X_3 - 0.410X_3X_4 + 0.296X_1X_6$.

Significant single variables and observed trends are summarised in Table 2. According to the results, a set of suitable experimental conditions for the single Zn(II) determination was obtained.

Results have shown that sensitivity is affected by all the variables except for pH (X_5), when considering peak heights and for capillary length (X_6) when considering peak areas. In both cases, and related to the rest of the variables, the peak enhancement followed the same trends. Decreases in concentration of 4-methylbenzylamine (X_1), applied voltage (X_3) and concentration of 1,10-phenanthroline (X_4), and the increase in the introduction time (X_2) produced peak enhancement. Therefore, a set of suitable experimental

Table 2
Tendencies of the variables for the improvement of the selected responses in the single determination of Zn(II) and the simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II)

	Response	X_1	X_2	X_3	X_4	X_5	X_6
Single	Peak height (R_h)	−	+	−	−	N.S.	−
	Peak area (R_a)	−	+	−	−	+	N.S.
	Speed of analysis (R_s)	−	N.S.	+	−	+	−
Simultaneous	Peak height (R_h')	−	+	N.S.	−	N.S.	−
	Peak area (R_a')	−	+	N.S.	N.S.	N.S.	N.S.
	Resolution (R_r')	−	+	−	N.S.	N.S.	+

−, A decrease produces an increase in the response; +, an increase produces an increase in the response; N.S., not significant ($P > 0.05$).

Table 3
Suitable conditions for the single determination of Zn(II) and the simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II)

Variables	Units	Single	Simultaneous
Concentration of 4-methylbenzylamine	nM	3	3
Sample introduction time	s	40	40
Run voltage	kV	15/30[a]	15
Concentration of 1,10-phenanthroline	nM	2	2
pH		5.3	3.7/5.3[b]
Capillary length	m	0.60	0.60/1.00[c]

[a] Considering only speed of analysis.
[b] Not significant, both are valid.
[c] It depends if selectivity or sensitivity is required.

conditions related to sensitivity are shown in Table 3. In those samples with enough amount of zinc, whenever sensitivity is not a priority and considering only the speed of analysis, the analysis time can be reduced by using a set of experimental conditions also shown in Table 3.

3.2. Simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II)

The simultaneous determination of the analytes at different concentration ranges in a single analysis exhibits the additional problem of peaks overlapping for those at higher concentration rates and the lack of sensitivity for those at lower concentration levels. Therefore, a compromise between concentrations of analytes and experimental variables need to be considered in order to establish a suitable set of experimental conditions for the analysis. Firstly, factorial experimental designs have been used for a systematic study of the separation between neighbouring peaks and then, sensitivity in terms of peak height and peak area was also evaluated. Although electropherograms showed three positive peaks for Na(I), Ca(II) and Mg(II), and a negative peak for the Zn(II)-1,10-phenanthroline complex (Fig. 1), all peak heights have been considered in absolute value.

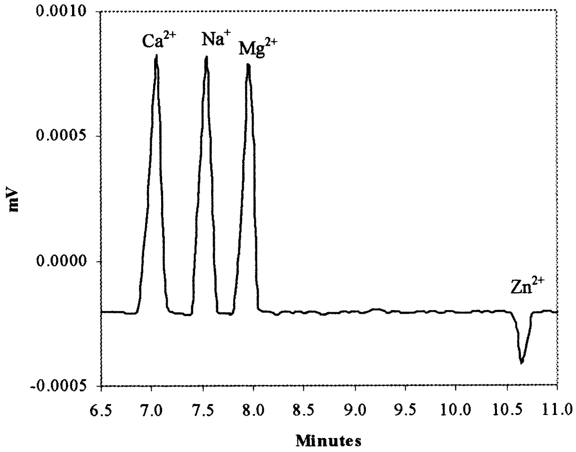

Fig. 1. Electropherogram of a solution of 1 mg l^{-1} Na(I), 1 mg l^{-1} Ca(II), 0.5 mg l^{-1} Mg(II) and 0.1 mg l^{-1} Zn(II) at suitable experimental conditions. Electrolyte: 2 mM 1,10-phenanthroline, 3 mM 4-methylbenzylamine, pH 3.7. Indirect UV detection at 214 nm. Hydrodynamic injection for 40 s, run voltage of 15 kV and capillary length of 0.60 m.

3.2.1. Separation efficiency: selectivity

As peaks resolution is closely related to the distance between two neighbouring peaks, the study was performed with the minimum differences in migration times between neighbouring peaks as response element. Thus, the maximisation of such minimum differences between the two nearest neighbouring peaks would improve the resolution and separation efficiency of these peaks. As a result, the following response equation with normalised $(-1,+1)$ variables was obtained for the significant variables $(P<0.05)$: R'_r (minimum migration time difference between neighbouring peaks, min) $= 0.137 - 0.099X_1 + 0.121X_2 - 0.038X_3 + 0.049X_6 - 0.083X_1X_2 - 0.054X_2X_3 - 0.070X_1X_6 + 0.040X_2X_6$.

According to the effect estimate values (coefficients of the equation), the most significant single variables are the concentration of UV-absorbing agent (X_1) and the sample introduction time (X_2). By decreasing the concentration of 4-methylbenzylamine the response (R'_r) can be improved and by increasing the sample introduction time, the response for separation efficiency can be also improved. Related to the other variables, by decreasing the run voltage (X_3) and by increasing the capillary length (X_6), the separation efficiency can also be improved but their influences have shown to be much lower than the other variables. The most important second order interactions are between the concentration of 4-methylbenzylamine (X_1) and the sample introduction time (X_2) and between the concentration of 4-methylbenzylamine (X_1) and the capillary length (X_6).

3.2.2. Sensitivity

Once the significant variables for the separation efficiency had been established, the six parameters were studied again in order to determine their influence on the response for the sensitivity. Peak height and peak areas were considered as response elements for the evaluation of the sensitivity of the system. Then, a two level factorial design (2^6, 64 experiments) was performed with the aim of maximising the sensitivity of the smallest peak in the experiments, which made possible to enhance the sensitivity for the whole system. Therefore, peak height and peak area of the smallest peak in each experiment were chosen as response elements to achieve better sensitivity. According to the results, two response equations with normalised variables were obtained for the significant variables $(P<0.05)$: R'_h (peak height, nV) $= 65.25 - 35.00X_1 + 53.88X_2 - 23.03X_4 - 22.50X_6 - 23.63X_1X_2 - 20.00X_2X_6$ and R'_a (peak area, nV s) $= 142.47 - 88.65X_1 + 128.8X_2 - 74.94X_1X_2 - 54.63X_2X_3 + 33.82X_5X_6$.

Considering first the peak height, there are four significant parameters $(P<0.05)$: the concentration of 4-methylbenzylamine (X_1), the sample introduction time (X_2), the concentration of 1,10-phenanthroline (X_4) and the capillary length (X_6). An increase in the sample introduction time causes an increase in the response (R'_h), being the effects of the other significant variables just the opposite: decreases in the concentration of 4-methylbenzylamine, concentration of 1,10-phenanthroline and capillary length produce the increase of the peak height. Related to the peak area (R'_a) only two variables, the concentration of 4-methylbenzylamine (X_1) and the sample introduction time (X_2) were significant. Obviously, in both cases, an increase in the sample introduction time produces an increase in the peak size.

The capillary length (X_6) has shown two opposite tendencies related to selectivity and sensitivity. An increase in the capillary length origins the improvement in the separation efficiency, while a decrease produces higher peaks. The effect on the separation efficiency can be explained by considering that the increase in the capillary length means an increase in the number of theoretical plates in the separation and obviously an improvement in the resolution of the peaks. However, as the capillary length increases, the peak height decreases because diffusion effects of the metals along the capillary.

3.2.3. Suitable experimental conditions

Although factorial experimental designs at two levels do not provide the optimal conditions, it has been possible to establish a set of suitable conditions for the simultaneous determination of the four metals, considering first good separation efficiency and then a good sensitivity for the peaks with the exception of capillary length (X_6). Due to capillary length (X_6) is the only variable that shows an opposite behaviour for the optimisation of selectivity and sensitivity, the lower capillary length was chosen in order to achieve a better sensitivity for the simultaneous determination of main and trace metals in natural waters. This selection would also make the analysis times shorter.

These conditions are shown in Table 3. Concentration of 1,10-phenanthroline and pH of the BGE have found to be not significant in the studied ranges. Therefore, 2 mM phenanthroline concentration and pH 3.7 were chosen because of their convenience for the analysis in terms of economy of reagent and a favourable pH value for metal ions in aqueous solution.

As shown, the most suitable experimental conditions for the simultaneous determination of the metals agree with those for the single determination of Zn(II), except for the run voltage, which is limited to 15 kV, and pH and capillary length, which are extended to both levels for each variable.

3.2.4. *Metal concentration ranges and method specifications*

Several combinations of metal concentrations were studied to find the concentration intervals within the four metals can be determined simultaneously in natural water. The metal concentration ranges were chosen from previously reported bibliographical data related to metal concentration ranges and averaged metal concentration ranges in natural and drinking waters for Na(I), Ca(II), Mg(II) and Zn(II) [47].

Problems to quantify the peak heights because of peaks overlapping for Na(I), Ca(II) and Mg(II) can be observed only when the concentrations exceed at the same time the upper limits of linear concentration ranges showed in Table 4. No problems of peak overlapping were found in the determination of Zn(II) even at concentrations above the averaged zinc concentrations in natural and drinking waters. Therefore, in order to apply this method to determine simultaneously concentrations of Na(I), Ca(II), Mg(II) and Zn(II) in natural and drinking water above showed in Table 4, it is necessary to dilute the samples according to their concentration levels.

Due to the concentration ratio between the studied alkaline and alkaline earth metals and Zn(II) is in average about 50 times or even higher, the detection limit for Zn(II) was determined in order to establish the appropriated dilution ratios to determine simultaneously all the metals. The detection limit defined as three times the standard deviation of the blank divided by the slope of the calibration curve, was found to be 32 μg l^{-1} for Zn(II). No problems for detection were found for the alkaline and alkaline earth metals in natural water samples.

Linearity in the determination of the four metals was obtained by using the improved experimental conditions. The linear regression coefficients are shown in Table 4. The linear range was found to cover, at least, one order of magnitude. Zn(II) showed good linearity at these experimental conditions (y (nV) = 1508 × (mg l^{-1}) + 91; $r^2 = 0.998$).

Precision (for five determinations and with 95% confidence level) was evaluated for each metal and the results were: 3.32% for 15 mg l^{-1} Na(I), 4.66% for 10 mg l^{-1} Ca(II), 3.48% for 2 mg l^{-1} of Mg(II) and 3.74% for 0.1 mg l^{-1} Zn(II).

3.3. *Application to real samples*

Once a set of suitable experimental conditions had been established and the analytical parameters of the method had been studied, a sample of drinking water from the laboratory was analysed in order to determine simultaneously the concentrations of Na(I), Ca(II), Mg(II) and Zn(II) (Fig. 2). The sample had to be diluted to avoid the peaks overlapping of the alkaline and alkaline earth metal ions. Calibration curves were used to quantify the metals. Concentrations of 27 ± 0.5 mg l^{-1} Na(I), 52 ± 0.5 mg l^{-1} Ca(II), 9.6 ± 0.07 mg l^{-1} Mg(II) and 92 ± 4 μg l^{-1} Zn(II) ($n = 5$) were found. Results showed good agreement with those obtained by ICP–AES and DP–ASV for Zn in such samples (error $< 3\%$).

Table 4
Usual concentration ranges in natural and drinking waters, linear concentration ranges for the proposed simultaneous determination of the four metals and linear regression coefficients

Element	Usual concentration ranges	Linear concentration ranges	Number of std[a]	r^2
Sodium	10–300	1–30	6	0.986
Calcium	10–200	1–30	6	0.996
Magnesium	5–50	0.5–5	6	0.993
Zinc	0.1–5	0.01–5	11	0.998

Concentrations are expressed in mg l^{-1}.

[a] Number of standards used in the linearity study.

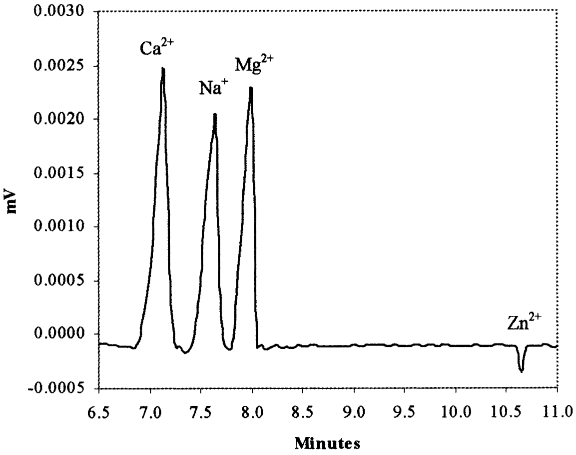

Fig. 2. Electropherogram of a diluted sample of laboratory drinking water at suitable experimental conditions. Electrolyte: 2 mM 1,10-phenanthroline, 3 mM 4-methylbenzylamine, pH 3.7. Indirect UV detection at 214 nm. Hydrodynamic injection for 40 s, run voltage of 15 kV and capillary length of 0.60 m.

4. Conclusion

The development of analytical methodologies can be further improved by the assistance of experimental design procedures. Even, the use of factorial experimental designs represents a key help in understanding not only the influence of several variables on analytical systems, but also to find their trends and behaviour. In this work, a study of the determination of Zn(II) individually and the simultaneous determination of Na(I), Ca(II), Mg(II) and Zn(II) in waters by CE have been achieved by using factor analysis.

In addition to the well known characteristics of CE methodologies, this method exhibits major advantages compared with other techniques for metal analysis, including the possibility of simultaneous and fast determinations, relatively low costs, easy automation, simplicity and the use of UV photometric detection, the most extended commercially available detection method for CE. Thus, a set of suitable experimental conditions has been proposed and the method has been applied to the simultaneous determination of such metals in tap water with satisfactory results.

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