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Determination of phosphate in natural waters by capillary electrophoresis
Influence of solution composition on migration time and response

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Abstract

This paper deals with the determination of the more complex phosphate anion by capillary electrophoresis using indirect UV detection. First, the pH of the running electrolyte influences both the migration time and the response of the phosphate anion. Both effects could be explained well by taking into account phosphorus speciation in solution. In addition, the experimental method has been applied to three different sets of natural water systems; (i) groundwater, (ii) surface water and (iii) stemflow samples. The migration time behaviour of phosphate was different for the three sample sets and, hence, difficulties arise with respect to a clear and unique identification of the compound. Deviations herein could be minimized by applying a correction method for migration time drift. Concentrations of phosphate could be quantified in most samples and were confirmed by a colorimetric method. Average recoveries of additions of phosphate to groundwater, surface water and stemflow samples were 105, 83 and 103%, respectively. For one stemflow sample, quantitative recovery of phosphate was possible only by changing the pH of the running electrolyte solution. The latter observation might be very useful in setting up speciation-related measurement methods.

Keywords: Water analysis; Environmental analysis; Buffer composition; Phosphate; Inorganic anions

1. Introduction

Knowledge with respect to the physico-chemical behaviour of phosphorus compounds in natural aquatic and soil systems is of great importance from a biological point of view (as nutrient) as well as with respect to environmental pollution aspects [1,2]. The predominant forms of phosphorus in the natural system are the orthophosphate species, i.e. $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-} [2], which are commonly determined together by means of a colorimetric method [3,4]. From an analytical point of view, colorimetry is a time-, reagent- and sample-consuming method. Separation techniques allow the successively determination of a number of different compounds in one single run and, hence, analysis time and consumption of chemicals and sample solution are becoming rather limited. Recently, capillary electrophoresis (CE) has been introduced as a new separation technique for the determination of inorganic and small organic ions [5]. Due to an almost exponential increase in interest in this analytical tool, CE methods for the determination of anions and cations are nowadays readily available in the literature. However, these methods are mainly developed for rather "simple" salt matrices (e.g. Ref. [6]), meaning that the composition of both the running electrolyte and the standard solutions or samples are rather well

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defined. Application of CE to more complex matrices, such as natural water systems, is not a priori a straightforward procedure but should be done with care in order to interpret the results in a proper way.

Using CE, phosphate appears to be a rather difficult anion to determine, compared to other common anions such as chloride and nitrate. First, the precision of the absolute migration time is poor [7,8]. For example, in studying the influence of the type of electroosmotic flow modifier on the response (time and area) of a series of anions, Galceran et al. [7] found that the day-to-day migration time reproducibility varied from 2.5 up to 7.5% for standard phosphate solutions, whereas for nitrate, this parameter ranged from 2.5 to 3.3%. The second complicating factor is related to the quantification of phosphate, since peak area and height may vary by more than 30% [8], which is suggested to be due to adsorption phenomena of phosphate with the surface of the silica capillaries [9].

This paper deals with the effects of the pH of the running buffer on the migration time and peak area of phosphate in silica capillaries for standard solution conditions. As a comparison, the electrophoretic behaviour of fluoride has been studied simultaneously. In addition, with the obtained information, the applicability of CE for phosphate determination in natural aquatic systems has been tested using a variety of practical samples, including various European surface waters, Dutch groundwater and stemflow samples. The experimental results are discussed in the light of both CE theory and solution chemistry.

2. Experimental

2.1. Capillary electrophoresis

The CE procedure used here is based on Dionex Application Note 68 [10] and has been described in detail elsewhere [11]. Briefly, electropherograms were obtained with a Waters Quanta 4000 CE system equipped with a UV detector, a negative power supply and an automatic sample changer. Millennium 2010 (Version 2.10, Waters) software was used for data collection and treatment. Separations were carried out using an AccuSep (Waters) fused-silica capillary (60 cm×75 μm I.D.; effective length 52 cm) at a voltage of −30 kV. Injection was performed hydrostatically by elevating the sample by 10 cm at the cathodic side of the capillary. Injection time varied from 24 to 240 s depending on the matrix. Indirect UV detection at 254 nm was used. The time constant of the UV detector was 0.3 s.

The carrier electrolyte was prepared from a stock solution containing 22.5 mmol l^{-1} of 1,2,4,5-benzenetetracarboxylic acid (also known as pyromellitic acid, PMA) and 16 mmol l^{-1} triethanolamine (TEA). This solution was sonicated in an ultrasonic bath in order to dissolve the PMA completely. To a twenty-fold dilution of the carrier stock solution, an electroosmotic flow modifier solution containing hexane-1,6-bis(trimethyl)ammonium bromide (HMBr) was added using an OnGuard-A cartridge (Dionex), in order to remove ionic bromide. The resulting working carrier solution contained 1.13 mmol l^{-1} PMA, 0.8 mmol l^{-1} TEA, 2.13 mmol l^{-1} HMOH and had a pH value of 7.7.

Some of the experiments were performed using different acidities of the carrier electrolyte solution. To that end, the pH of the carrier solution was adjusted by adding various quantities of a 0.1 mol l^{-1} NaOH solution, depending on the desired pH. Prior to use, all carrier electrolyte solutions were filtered through a 0.2-μm polyethene membrane filter, after which the pH was measured.

2.2. Chemicals

Ammonium heptamolybdate, L(+) ascorbic acid, potassium antimony(III) oxide-tartrate, potassium biphosphate, sulphuric acid, methanol, potassium hydroxide, sodium fluoride, sodium hydroxide, hydrogen peroxide and triethanolamine were of analytical-reagent quality, 1,2,4,5-benzenetetracarboxylic acid was of pro synthesis quality. All chemicals were obtained from Merck (Darmstadt, Germany), except for hexane-1,6-bis(trimethyl)ammonium bromide (HMBr), Triton X-100 and FFD 6 detergent, which were purchased from Sigma (St. Louis, MO, USA), Fluka (Buchs, Switzerland) and Skalar (Breda, The Netherlands), respectively. All solutions were prepared using water purified with a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Samples

A set of samples was selected covering a large variety of matrices that are generally met in the environment including: (a) Dutch groundwater, (b) surface water from various European rivers and (c) stemflow samples, i.e. rainwater which was collected at the trunk of oak or pine trees. In our laboratory, all samples are registered in a laboratory information management system (LIMS). In this paper, the corresponding unique LIMS code will be used to identify the various sample solutions.

3. Results and discussion

3.1. Influence of pH of the running electrolyte

For a standard solution containing equal concentrations (i.e. 25 μmol l^{-1}) of phosphate and fluoride, the migration time is given as a function of the pH of the running electrolyte in Fig. 1. In the case of fluoride, a small increase in migration time is observed in the pH range from 6 to 8. Since it is not expected that the intrinsic mobility of the fluoride anion is related to the pH of the solution in the applied range of acidities, variations in migration time are the result of deviations in electroosmotic mobility, in line with other literature data (e.g. Ref. [12]). For phosphate, the migration time behaviour is quite different. Here, the migration time decreases with increasing pH, which is, in this case, the result of changes in the mobility of the phosphate ion. One can easily understand this observation by taking into account the speciation pattern of P over the applied pH range of 6 to 8. At pH 6, the phosphate ion is mainly present as $H_2PO_4^-$, whereas at increased pH values of about 8, HPO_4^{2-} is the predominant species [13]. The latter species has a higher mobility, due to an increase in charge, whereas the size of the anion remains almost equal.

In Fig. 2, the "corresponding" response in terms of time corrected peak areas are presented for fluoride and phosphate as a function of the pH of the running electrolyte solution. The change in background absorption (ΔA) is given by [14]:

$$\Delta A = c_B^S \, l \varepsilon_A \frac{z_B \mu_A [\mu_B + \mu_X]}{z_A \mu_B [\mu_A + \mu_X]} \quad (1)$$

where l is the effective path length of the detector, ε_A is the molar absorption coefficient of the chromophore, c_B^S is the concentration of the analyte in the sample zone and z is the valence. Subscripts A, X and B refer to the chromophore, the non-absorbing counterion and the analyte of interest, respectively. In the case of fluoride, the corrected peak area is constant over the studied pH range, with an average value of 5.5 (arbitrary area units) and a standard deviation of 0.3. For phosphate, the response increases from about 7 at pH 6 up to almost 12 at pH 8.3. As discussed above, the speciation pattern of P changes with increasing pH, resulting in an increased concentration of the faster HPO_4^{2-} species. Hence, according to Eq. (1), viz. a change in valence of the component of interest, the change in background absorption (ΔA) increases in line with our results.

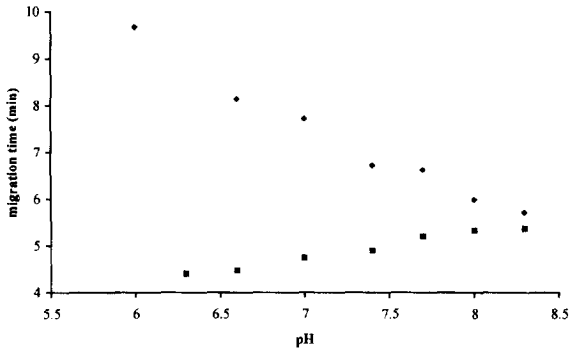

Fig. 1. Migration time of phosphate (♦) and fluoride (■) (25 μmol l^{-1}) versus pH of the running electrolyte. Applied voltage, −30 kV; hydrostatic injection, 30 s.

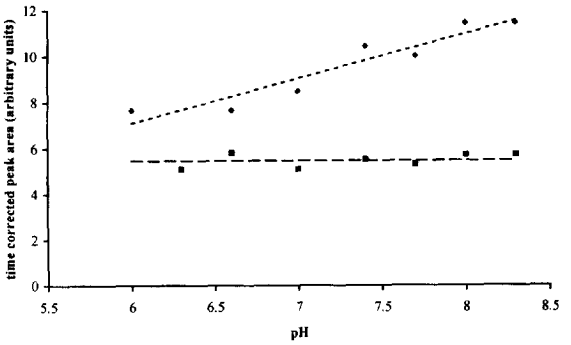

Fig. 2. Response of phosphate (♦) and fluoride (■) (25 μmol l^{-1}) versus pH of the running electrolyte. Conditions as in Fig. 1.

From an analytical point of view, the pH of the running electrolyte solution regulates both the migration time and the response of the phosphate anion in experimental set-up used here. Obviously, a constant acidity of the bulk solution is necessary in order to minimize variations in both parameters. For this study, the running electrolyte solution has been set at pH 7.7, which is a compromise to obtain a combined optimum in terms of (i) resolution for fluoride and phosphate, (ii) running time and (iii) response for phosphate.

3.2. Performance characteristics

Using reproducibility experiments, performance characteristics were obtained in terms of precision and detection limits of the analytical response. We follow a mathematical procedure as described before in detail [11]. On nine different days, various phosphate calibration solutions were measured using CE (see Section 2) and photometry [3,15]. In Table 1, the standard deviation (SD) of the response is summarized for each phosphate standard solution. The corresponding limit of detection (LOD) is defined as being three times the standard deviation at the intercept of the calibration curve, which is assumed to be linear. The physical concept of the procedure is that with maintenance of linearity, variations in response are related to (i) variations in the value of the intercept (SD_a) and (ii) variations in the slope of the calibration curve (SD_b). Values for SD_a and SD_b are obtained by linear regression analysis, as described in [11]. The resulting LOD values are 0.45 and 0.12 μmol l^{-1} for the CE (240 s hydrostatic injection) and the photometric method, respectively. Although in this case CE appears to be less sensitive than the "conventional" colorimetric method, the obtained LOD is in the lower range of

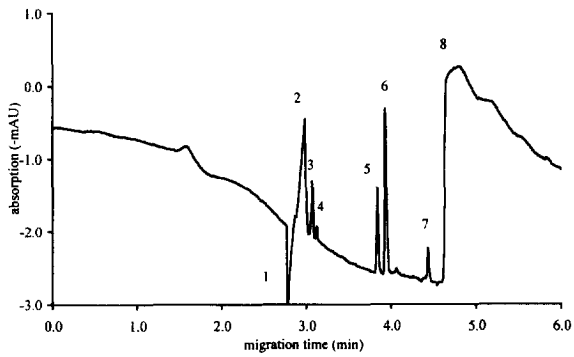

Fig. 3. Electropherogram of groundwater sample 41 188 (twenty times diluted) obtained with the running electrolyte composition: 1.13 mmol l^{-1} PMA, 0.8 mmol l^{-1} TEA, 2.13 mmol l^{-1} HMOH, pH 7.7. Hydrostatic injection: 24 s. Peaks: 1, bromide; 2, chloride; 3, sulphate; 4, nitrate; 5, fluoride; 6, unknown; 7, phosphate and 8, carbonate.

LOD values reported in the literature for hydrodynamic conditions, i.e. 0.2–20 μmol l^{-1} [9,16,17]. Smaller detection limits might be obtained by using electrokinetic injection [18], which, however, is quite difficult to apply for a series of samples with varying total ionic strength [19]. In addition, our sample set consists of natural aqueous solutions for which foreknowledge with respect to composition including ionic strength is absent. Hence, the application of electrokinetic injection techniques appears to be less opportune, taking the analysis time as one of the optimizing parameters into account.

3.3. Application to natural aquatic systems

In Figs. 3–5, typical electropherograms are presented for groundwater, surface water and stemflow samples, respectively. Although, this paper focuses on the determination of phosphate in natural aqueous samples only, one can clearly see from Figs. 3–5 that

Table 1
Precision of standard solutions in terms of standard deviation (in μmol l^{-1}) for the applied CE and photometric procedures

	[Phosphate] (μmol l^{-1})							
	0.0	0.5	1.0	2.0	4.0	6.0	8.0	10.0
CE	n.d.	0.04	0.19	0.11	0.13	0.08	0.53	0.11
Photometry	0.10	n.d.	n.d.	0.03	0.05	0.06	0.14	0.17

($n=9$).
n.d. = not determined

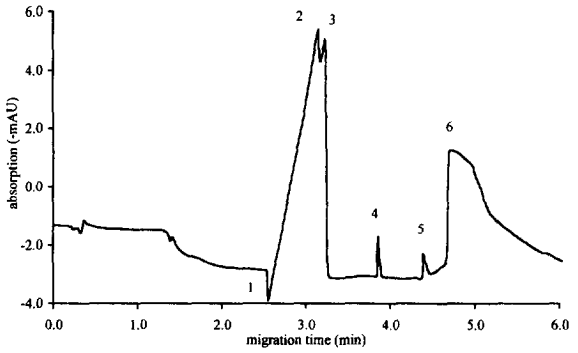

Fig. 4. Electropherogram of surface water sample 38 299 (undiluted) obtained with the same running electrolyte composition as in Fig. 3. Hydrostatic injection: 240 s. Peaks: 1, bromide; 2, chloride; 3, sulphate; 4, fluoride; 5, phosphate and 6, carbonate.

other common anions like chloride, nitrate and sulphate can be determined simultaneously by the applied experimental set-up. In all cases, the phosphate peak was confirmed by standard addition. With respect to the other anions, we note that the negative peak of bromide (number 1 in all electropherograms) is due to incomplete removal of ionic bromide originating from HMBr by the OnGuard-A cartridge. Fortunately, we are not interested in quantification of this compound and, hence, the bromide peak is very suitable to use as a reference peak for e.g. migration time correction (see below).

3.3.1. Groundwater

In Table 2, phosphate concentrations are presented for groundwater samples as obtained by the CE and

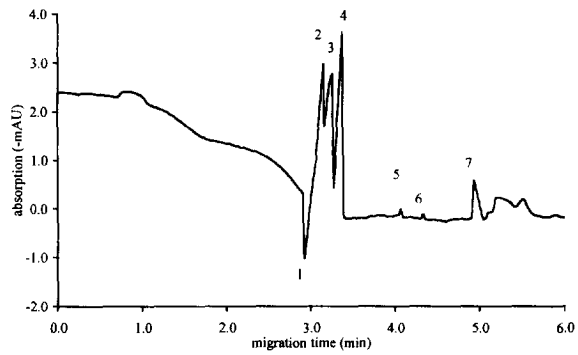

Fig. 5. Electropherogram of stemflow sample 37 295 (undiluted) obtained with the same running electrolyte composition as in Fig. 3. Hydrostatic injection: 240 s. Peaks: 1, bromide; 2, chloride; 3, sulphate; 4, nitrate; 5, fluoride; 6, phosphate and 7, carbonate.

Table 2
Phosphate concentrations (μmol l^{-1}) in groundwater samples as obtained by CE and photometry

Sample	CE	Photometry	SD/mean (%)
41 172	3.6	4.4	14
41 173	3.7	4.4	12
41 174	3.7	4.4	12
41 175	4.0	4.5	8
41 176	3.6	4.3	13
41 177	3.7	4.3	11
41 188	173.0	181.3	3
41 189	169.4	176.4	3
41 190	162.4	173.8	5
41 191	170.0	176.1	2
41 192	190.2	184.9	2
41 193	184.7	190.1	2
41 194	315.0	307.3	2
41 195	330.0	309.9	4
41 196	329.0	318.6	2

In the final column, the difference in concentration (%) between the two methods is given as the quotient of the standard deviation of the two individual results and the corresponding mean value.

the photometric procedures. In the case of CE, the samples with high phosphate concentrations (160–330 μmol l^{-1}) were diluted twenty times prior to analysis. The deviation in concentration as obtained by both techniques is expressed as the quotient of the standard deviation of the two results and the corresponding mean value. This approach seems to be justified, since it is expected that both methodologies determine the same phosphate fraction. For lower phosphate concentrations, the deviation is somewhat larger than 10%, whereas for samples with elevated phosphate concentrations, both procedures give individual results within a few percent of the mean value. Recovery experiments were performed with CE only, using additions of 10 and 200 μmol l^{-1} for low and high phosphate concentrations, respectively. The results are very satisfactory, as indicated by an average recovery and standard deviation of 105±7%.

3.3.2. Surface water

In nine of the fifteen surface water samples, phosphate was not detectable using CE, which was confirmed by the photometric procedure. In four of the remaining samples, phosphate determination was only possible (i) after removing carbonate (present at a few mmol l^{-1} level) using an Onguard-H column

or (ii) by applying shorter injection times. The average quantitative recovery of phosphate additions ranging from 2.5 up to 10 μmol l^{-1} to the whole sample set was found to be 83%, with a standard deviation of 23%. In addition, the corresponding migration time for the phosphate peak appeared to differ considerably from sample to sample (see Table 3). This observation is of great importance, since ignoring this aspect can lead to an incorrect interpretation of the electropherogram. The extent of deviation has been quantified using Δt, defined as the difference in migration time of phosphate between the standard solution and the average migration time of phosphate in a set of samples with comparable matrices. For our set of samples, Δt appears to be different for the various matrices and increases in the order of groundwater<stemflow<surface water (see Table 3). One possibility to tackle this problem is the introduction of a migration time correction procedure, which is based on the mobility of a reference compound in the standard solution and in the sample. As mentioned before, bromide might be very useful, because (i) in each analysis a small amount of bromide is present, originating from the electroosmotic flow modifier, HMBr, and (ii) bromide appears as a dip in the electropherogram and, hence, it is easy to identify. The corrected migration time is calculated according to:

$$t_{\text{correct}}^{\text{compound}} = t_{\text{sample}}^{\text{compound}} + t_{\text{standardsolution}}^{\text{bromide}} - t_{\text{sample}}^{\text{bromide}} \qquad (2)$$

For all systems, this procedure leads to a less variable value for the compound specific migration time (see Δt and $t_{\text{mean}} \pm \text{SD}$ in Table 3). In case of the surface water sample set, the remaining deviation in migration time is too large for direct peak identification and, thus, additional confirmation is necessary. This might be done by standard addition procedures or by analysis using another independent technique, leading in both cases to a considerable increase in the total analysis time. If the deviations are the result of changes in electroosmotic flow, due to interactions of solution components with the surface of the silica capillary, it is assumed that application of surface-modified capillaries will improve the constancy of the migration time. Further research along these lines is in progress.

Table 3
Average (corrected) migration times of the phosphate peak in various natural water samples

Matrix	Correction	Δt (min)	$t_{\text{mean}} \pm$SD (min)
Groundwater	None	0.03	4.60±0.03
($n=15$)	Absolute	0.01	4.62±0.01
Stemflow	None	0.19	4.80±0.12
($n=16$)	Absolute	−0.04	5.03±0.05
Surface water	None	0.57	4.49±0.22
($n=15$)	Absolute	0.08	4.98±0.16

Δt is the difference in the migration time of a standard solution and the average of the sample set (t_{mean}).

3.3.3. Stemflow samples

In the case of the stemflow samples, a detailed recovery experiment has been performed with a limited number of samples. In Table 4, the resulting phosphate concentrations are summarized for a series of stemflow samples before and after the addition of 1, 2 and 3 μmol l^{-1} phosphate, as obtained by the CE and the photometric method. The results of phosphate determination with CE are in excellent agreement with the data of the photometric method, except for samples 43 789 and 43 790. For these samples, disturbing peaks were found close to the

Table 4
Phosphate concentrations (in μmol l^{-1}) of stemflow samples before and after the addition of 1, 2 and 3 μmol l^{-1} phosphate, as obtained by the CE and the photometric method

Sample	Method	Concentration (μmol l^{-1})			
		0	1	2	3
43 367	CE	<0.45	0.88	1.94	3.07
	Photometry	<0.12	0.92	1.92	2.92
43 368	CE	<0.45	1.01	2.10	3.13
	Photometry	<0.12	1.02	2.00	3.06
43 369	CE	<0.45	0.99	1.99	3.10
	Photometry	<0.12	0.96	1.82	2.91
43 370	CE	<0.45	1.16	2.16	3.29
	Photometry	<0.12	1.10	2.11	3.12
43 371	CE	<0.45	0.98	2.08	3.15
	Photometry	<0.12	1.00	2.01	2.99
43 789	CE	<0.45	<0.45	<0.45	<0.45
	Photometry	3.51	4.49	5.51	6.46
43 790	CE	<0.45	<0.45	<0.45	<0.45
	Photometry	<0.12	0.80	1.74	2.82

expected peak of phosphate, which prevented us from determining phosphate without further pretreatment of the sample solution or changing the experimental set-up. For the remaining samples, mean recoveries were found to be 98±7% ($n=21$) and 103±6% ($n=15$) for the photometric and CE method (not taking into account samples 43 789 and 43 790), respectively.

Additional experiments were performed with sample 43 790 only, due to insufficient material from sample 43 789. The pH of the running electrolyte solution was increased to a value of 8.5 using TEA in such a way that the phosphate peak would appear earlier in the electropherogram, but outside the range of the disturbing peaks. Furthermore, phosphate was added to sample 43 790 at various concentration levels (5, 25, 50, 100 and 250 μmol l^{-1}). In Fig. 6, parts of the resulting electropherograms are presented for the non-added sample and the 25, 50 and 100 μmol l^{-1} additions, which are typical for the whole set. Surprisingly, not one, but two peaks appeared in the electropherogram. Integration of the first peak only resulted in an average recovery of approximately 40%, whereas integration of both peaks gave rise to a mean recovery of 106±22% for the five additions. Obviously, phosphate is present in different physico-chemical forms with different mobilities. Since this observation is absent in the case of the standard solution, it is assumed that phosphate reacts with components in the stemflow sample, resulting in non-labile or quasi-labile presently unknown complexes.

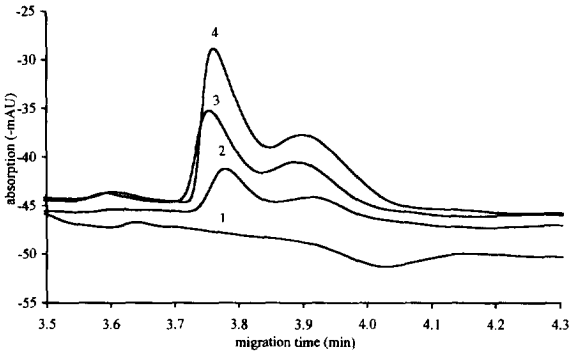

Fig. 6. Electropherogram of stemflow sample 43 790 using a running electrolyte solution with a pH of 8.5. Additions: 1, none (original sample); 2, 25; 3, 50 and 4, 100 μmol l^{-1} phosphate.

4. Conclusions

For the determination of phosphate in aqueous solutions with capillary electrophoresis, the corresponding migration time is related to bulk solution chemistry in terms of the acidity of the running electrolyte. The pH of this solution influences both the properties of the capillary surface wall, in terms of zeta-potential, and the physico-chemical form in which the phosphate anion is present in solution. In addition, due to changes in the speciation pattern of P, the response (corrected peak area) may change as well. Hence, for anions having similar chemical behaviour, a constant acidity of the running electrolyte is required to minimize variations in both qualitative and quantitative identification parameters.

Application of this method to various natural water samples is not a straightforward procedure. In some cases, additional experiments were necessary to interpret the obtained electropherograms in a proper manner. The drift in migration time was dependent on the matrix and increased in the order groundwater < stemflow < surface water. Minimization of migration drift could be established by applying a correction procedure using bromide as an internal standard. Concentrations of phosphate could be quantified well in most samples and were in good agreement with the results obtained by an independent colorimetric method.

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