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Analysis of anions in aqueous samples by ion chromatography and capillary electrophoresis
A comparative study of peak modeling and validation criteria

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Abstract

The object of this study is the comparison of two methods for the quantitative analysis of anions in aqueous samples: ion chromatography with conductimetric detection, and capillary zone electrophoresis with indirect photometric detection. The comparison includes modeling of experimental peaks as well as statistical validation criteria according to the recommendations of the International Conference on Harmonisation. In ion chromatography, peak shapes are Gaussian or exponentially modified Gaussian, and the number of theoretical plates calculated using the appropriate mathematical relations correspond well to those obtained from statistical moments. Peaks in capillary electrophoresis, however, do not follow the same models. A different model, treating the peaks as right angle triangles, has been studied. Equations corresponding to this model permit a good estimation of plate numbers. The statistical validation of these methods includes detection limits, linearity, accuracy and precision. Overall, ion chromatography yields better validation results than capillary electrophoresis. In the latter method the injection mode plays an important role, with voltage injection giving lower detection limits than hydrodynamic injection.

Keywords: Validation; Peak shape; Inorganic anions

1. Introduction

During the past decade, ion chromatography (IC), using chemically suppressed conductivity detection has proven itself as a powerful analytical tool for the simultaneous determination of inorganic ions in simple matrices such as drinking water and rainwater [1–7]. More recently, capillary zone electrophoresis (CZE) with indirect photometric detection has been successfully introduced as an alternative technique [8–12]. High efficiency [13], versatility, speed [14] and economy of analysis [15] are among the attributes that promoted the application of CZE in many real samples [16,17].

The aim of our study is to compare the performances of these two techniques for analysis of the main anions in aqueous samples, i.e., chloride, nitrite, nitrate, sulfate and phosphate. These anions were chosen because they corresponded to the species of which the analysis was required for further studies in our laboratory. For CZE experiments, hydrodynamic and electrokinetic injection modes have been used with operating conditions previously optimized in our laboratory [18]. The comparative evaluation includes separation efficiency, analysis time, validation criteria such as limit of detection

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(LOD), precision, linearity and accuracy. The study of efficiency of these two techniques has permitted an evaluation of different models of peak representation: Gaussian, exponentially modified Gaussian (EMG) and triangular. An EMG profile is defined as the convolution product of a Gaussian function with an exponential decay. It is used in chromatographic peak modeling to account for irregularity of column filling or non-linear partition isotherms due to secondary interactions. The triangular representation of the peaks obtained in CZE techniques has been tested in our laboratory to account for the deformation of the peaks due to electrophoretic dispersion.

2. Materials and methods

2.1. Apparatus

CZE experiments were performed using a Beckman P/ACE 2100 system equipped with a UV detector (Beckman Instruments, Fullerton, CA, USA). A fused-silica capillary (Beckman) of 47 cm (effective length 40 cm)×50 μm I.D. was used for the separation. Data acquisition and instrument control were carried out using Beckman Gold system (version 7.11) software. The samples were introduced into the capillary by hydrodynamic injection for 35 s at 0.5 p.s.i. or by electrokinetic injection for 5 s at 5 kV (1 p.s.i.=6894.76 Pa). The analyses were performed at −27 kV (cathode on the injector end) and 25°C, with indirect photometric detection at 254 nm due to the chromate ion added to the running buffer. Before each analysis, the capillary was equilibrated with the running buffer for 5 min and rinsed for 2 min with pure water after each analysis.

Ion chromatographic runs were performed on a Dionex Ion Chromatograph, series 2000i/SP (Sunnyvale, CA, USA), equipped with a conductivity detector (Dionex CDM-I), an IonPac AG4A-SC (50×4 mm) guard column (Dionex), an IonPac AS4A-SC (250×4 mm, 15 μm) analytical column (Dionex), and an Anion Self-Regenerating Suppressor (Dionex ASRS-I 4 mm). A Rheodyne Model 9010 (Rheodyne, Cotati, CA, USA) injector with a 50-μl injection loop was used. The eluent flow-rate was 2.0 ml/min and column effluent served for self-regenerating suppression. Data acquisition was carried out using Beckman Gold system (version 3.10) software. Data were analyzed using a Microsoft Excel 7 spreadsheet for studies on peak modeling, and using a Statgraphics (Manugistics, Rockville, MD, USA) software for statistical evaluation of the experimental data.

A Minisis 8000 pH meter equipped with a combined glass electrode (Tacussel, Radiometer Analytical, Copenhagen, Danemark) was used to measure and monitor pH values.

2.2. Reagents and procedures

Buffers and standard solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA) and were filtered through a 0.22-μm pore size membrane filter (Millex, Millipore, France). Diethylene triamine (DETA) was purchased from Aldrich (Milwaukee, WI, USA). Sodium hydroxide, potassium dichromate, sodium hydrogencarbonate, sodium carbonate and inorganic salts used as samples were analytical-reagent grade from Prolabo (Paris, France). Stock standard solutions (70 mmol/l) of individual anions were prepared by dissolving appropriate amounts of potassium or sodium salts in water. These solutions were subsequently diluted to give the multi-anion solutions for concentrations ranging from 7 to 105 μmol/l. The running electrolyte used for CZE separations contained $K_2Cr_2O_7$ and DETA at a concentration of 2 mmol/l each and was prepared from concentrated stock solutions. The final pH was between 7.5 and 7.8. In a previous work, we showed that, in these conditions, the electroosmotic flow (EOF) was diminished but still directed towards the cathode with an absolute intensity lower than the theoretical electrophoretic mobilities of the studied anions [18]. The contribution of this counter-current flow to the apparent mobility of anions allowed an improvement of resolution. For IC experiments, stock solutions of 18 mmol/l Na_2CO_3 and 17 mmol/l $NaHCO_3$ were daily mixed and diluted to provide the working concentration of mobile phase 1.8 mmol/l and 1.7 mmol/l, respectively.

2.3. Chromatographic measurements

Graphical measurements of peak height (h), width (w_r) and asymmetry factor (A_{s_r}) (at a fraction r of

peak height) were made to determine the peak shape of the chromatograms using the method of Foley [19]. The asymmetry factor was taken as the ratio of the half-width after the retention time to the half-width before the retention time at a given percentage of peak height. Peak areas were estimated with the equations of Foley by the following relation:

$$\text{Area} = ahw_r(A_{s_r})^b \quad (1)$$

where a and b are constants related to the fractional height for chromatographic measurements of A_s and w_r [19]. Four estimates of peak area were obtained related to four values, 0.1, 0.25, 0.5 and 0.75 of fractional height. A low relative standard deviation (RSD) between these four determinations (below 3%) is a sign of Gaussian peak shape if the peak is symmetrical or of gaussoexponential peak shape if the A_{s_r} factor is above 1. Significant differences between the four area determinations indicate peak shapes that cannot be described using conventional model equations. This may be the purpose for peaks obtained in CZE techniques that present a triangular profile because of a geometric deformation due to the differences of mobility between the analytes and the buffer ions. The triangle has a negative slope when the A_{s_r} ratio is below 1 (chloride, nitrite) and a positive slope when this ratio is above 1 (nitrate, sulfate, phosphate). The corresponding functions of distribution are $h=p(t_m-b)$ for a negative slope and $h=p(t_m-c)$ for a positive slope, where p is the slope of the peak, t_m the migration time of the maximum and b and c are the times related to the beginning and the end of the peak.

Efficiency of the separations was evaluated using four methods.

(1) In a first attempt, the number of theoretical plates was calculated according to the Gaussian model:

$$N = 5.54 \cdot \left(\frac{t_R}{w_{0.5}}\right)^2 \quad (2)$$

with t_R, the retention time of the maximum.

(2) Alternatively, we used two methods derived from EMG modeling to calculate plate numbers. The first one is the method of Foley who recommended the following equations to determine this parameter [20]:

$$N = \frac{41.7 \cdot (t_R/w_{0.1})^2}{A_{s_{0.1}} + 1.25} \quad (3)$$

or

$$N = \frac{1.83 \cdot (t_R/w_{0.5})^2}{A_{s_{0.5}} - 0.7} \quad (4)$$

The second method has been developed in our laboratory [21]: it involves the calculation of three universal ratios A, B and C:

$$A = \tau/\sigma \quad (5)$$

$$B = (t_R - t_G)/\sigma \quad (6)$$

$$C = w_r/\sigma \quad (7)$$

where t_G and σ are the average retention time and the standard deviation of the original Gaussian function and τ is the time constant of the exponential function. The universal ratios have values that depend on the fraction of peak height at which chromatographic measurements are carried out, namely 10% or 50% [21]. After determination of the EMG parameters – t_G, σ and τ – plate numbers are calculated as the ratio of the square of the first statistical moment to the second central statistical moment, these two moments being related to the EMG parameters according to [22]:

$$M_1 = t_G + \tau \quad (8)$$

$$M_2 = \sigma^2 + \tau^2 \quad (9)$$

$$N = \frac{(t_G + \tau)^2}{\sigma^2 + \tau^2} \quad (10)$$

(3) The third method has been developed for CZE peaks in this work. The statistical moments of a right angle triangle can be deduced from the chromatographic measurements using the following equations:

(i) Area of the peak:

$$M_0 = w_{0.5}h \quad (11)$$

where $w_{0.5}$ is the peak width at half peak height and h is the peak height.

(ii) First normalized statistical moment:

$$M_1 = t_M - \frac{2w_{0.5}}{3} \quad (12a)$$

for a negative slope and

$$M_1 = t_M + \frac{2w_{0.5}}{3} \quad (12b)$$

for a positive slope where t_M is the migration time of the peak.

(iii) Second central normalized statistical moment:

$$M_2 = \frac{2w_{0.5}^2}{9} \quad (13)$$

The number of theoretical plates can be deduced as the ratio of the square of the first statistical moment to the second central statistical moment.

(4) Finally, the different approximations above were tested by comparison with statistical moments calculated directly from ASCII data files resulting of Gold acquisitions. We used the ratio of the square of the first statistical moment to the second statistical moment to determine the number of theoretical plates.

2.4. Statistical evaluation

The validation procedure was based on the International Conference on Harmonization (ICH) requirements [23,24] for determination of the limit of detection, linear range, accuracy and precision. The limit of detection was taken either as three-times baseline noise (signal-to-noise ratio = 3, based on the height of the peak at 7 μmol/l) or as three-times the standard deviation of residual error after linear regression.

To study linearity, multi-anion solutions with concentrations ranging from 7 μ*M* to 105 μ*M* were injected onto the CZE and IC systems using standard conditions, on three consecutive days, each calibration point being analyzed three times per day. The resulting peak areas were then averaged for each day and the mean values were used in the linear regression calculation for each analyte. For CZE with electrokinetic injection, the addition of an internal standard, i.e., chlorate was necessary. The use of an internal standard for hydrodynamic injections was not necessary and calculations were proceeded without taking into account the chlorate peak although this anion was simultaneously analyzed. Linear regression was confirmed with Fisher tests studying the lack-of-fit of the least-squares estimations.

The precision was calculated using a standard solution containing each anion at a concentration of 35 μmol/l, injected three times per day for three days. The intra-day repeatability was taken as the mean of the three standard deviations of the observed concentrations determined for each day. The inter-day reproducibility was estimated after calculation of the variance related to the effect of day as recommended by the SFSTP [25]:

$$\mathrm{RSD}_{\mathrm{repeatability}} = \frac{S_{\mathrm{res}}}{m} \cdot 100 \quad (14)$$

where m denotes mean values of observed concentrations ($n = 9$) and S_{res} is the mean value of the three estimations of standard deviations calculated on each of the three days.

$$\mathrm{RSD}_{\mathrm{reproducibility}} = \frac{S_{\mathrm{repro}}}{m} \cdot 100 \quad (15)$$

and

$$S_{\mathrm{repro}} = \sqrt{S_{\mathrm{res}}^2 + S_{\mathrm{f}}^2} \quad (16)$$

where S_{f}^2 is the variance related to effect of day, calculated from the inter-group variance of analysis of variance (ANOVA):

$$S_{\mathrm{f}}^2 = \frac{S_{\mathrm{IG}}^2 - S_{\mathrm{res}}^2}{n_i} \quad (17)$$

where S_{IG}^2 is the inter-group variance and n_i is the number of repetitions (three) for each day.

The precision studied from three series of three assays each allowed an evaluation of repeatability with six degrees of freedom ($N - k = 9 - 3$). This estimation agrees with the conventional evaluation of repeatability carried out with six assays and leading to an estimation of variance with a degree of freedom of 5. In the estimation of reproducibility, the part of variation especially due to the factor is evaluated and may be negligible. This calculation differs from the evaluation of reproducibility carried out on several (5 or 6) days and taking into account the intra- and inter-day variations simultaneously.

Accuracy was studied by comparing the observed values with the theoretical value of 35 μmol/l.

3. Results

3.1. Chromatographic measurements

3.1.1. Ion chromatography

Separation of anions by ion chromatography is obtained in 12 min with the following elution order: chlorides, nitrites, nitrates, phosphates and sulfates (Fig. 1). The retention factors vary between 0.9 for chlorides and 10.2 for sulfates and remain stable whatever the concentration. The first three peaks are asymmetric with a B/A ratio at 10% around 1.5. The two others, which have migration times above 6 min, are symmetric (Table 1).

The determination of the area using the equations of Foley at four different height percentages led to homogeneous results with low scatter and mean areas close to corresponding zero-order statistical moments for almost all peaks. However for three peaks, chloride and nitrate at 7 μmol/l and nitrite at 70 μmol/l, a RSD of 4.5% between the four estimations is found and their average differs by 5% from the theoretical value. Overall agreement with the test of Foley is in favor of an EMG profile for the chloride, nitrite and nitrate peaks and of a Gaussian profile for the phosphate and sulfate peaks.

Plate numbers were calculated using the Gaussian formula and the two methods described for EMG peaks. These values were compared to the corresponding determinations using statistical moments, which were considered as true values. For the first three peaks – chloride, nitrite and nitrate – efficiency evaluated from statistical moments gives plate numbers around 2000. The two EMG methods – Foley and Universal Ratio – lead to similar results, at 10% and 50% of peak height, whereas the Gaussian method overestimates plate numbers by a factor of 1.5. For the sulfate and phosphate peaks, results obtained with EMG and Gaussian methods agree rather well with the statistical moment determination and show a twice better efficiency with plate numbers around 4000.

Table 1 and Fig. 2 show the difference between plate numbers obtained by different approximation methods (Gaussian and EMG methods) compared to statistical moments. For the Gaussian method, errors increase systematically with the asymmetry of the peak. For the two EMG methods, errors remain lower than those observed with the Gaussian method, on the average 10%, whatever the asymmetry of the peak. Higher errors are observed only for the three peaks mentioned before.

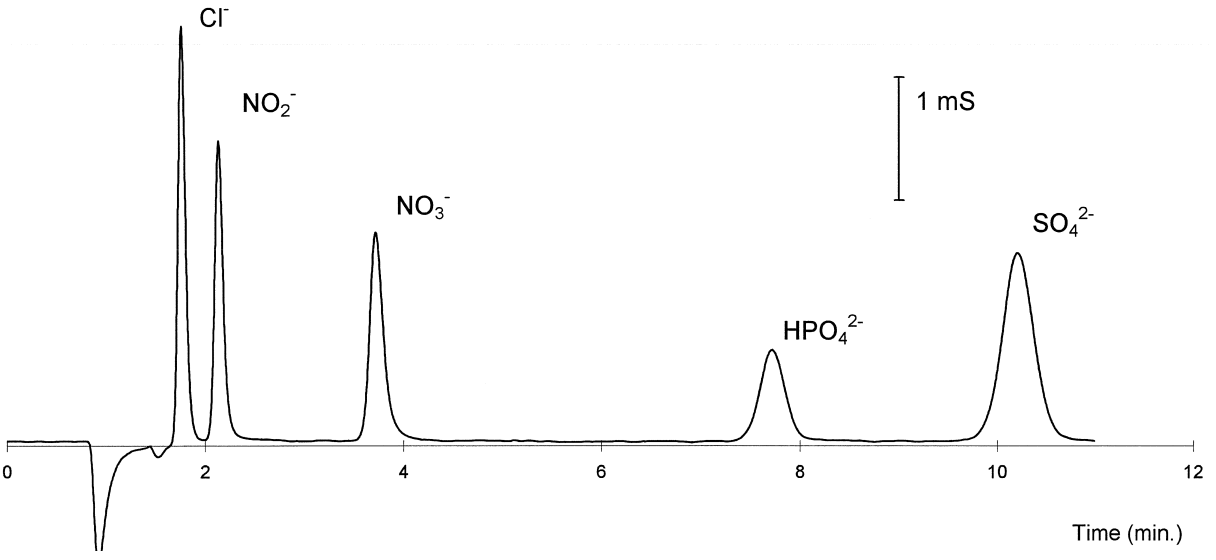

Fig. 1. Ion chromatographic analysis of a 35 μmol/l multi-anion solution. Operating conditions: IonPac AG4A-SC (50×4 mm) guard column and IonPac AS4A-SC (250×4 mm) analytical column; mobile phase containing 1.8 mmol/l Na_2CO_3 and 1.7 mmol/l $NaHCO_3$, 2 ml/min flow-rate and conductivity detection.

Table 1
Peak characteristics in ion chromatography[a]

Anion[b]	t_R[c]	Peak asymmetry		Test of Foley		Determination of plate numbers					
		$A_{s_{0.1}}$	$A_{s_{0.5}}$	RSD (%)[d]	ER (%)[e]	Moments	Gaussian	EMG 10%		EMG 50%	
								Foley	UR	Foley	UR
Cl/7	1.76	1.50	1.30	4.90	2.9	2253	2819	1929	1975	1560	1592
Cl/35	1.76	1.38	1.15	1.57	0.5	2042	2766	2066	2030	2030	2104
Cl/70	1.76	1.36	1.18	1.81	0.0	2088	2939	2019	2039	2006	2077
NO2/7	2.13	1.57	1.35	3.02	3.0	2065	2689	1758	1806	1367	1387
NO2/35	2.13	1.47	1.32	0.55	−0.7	1850	3125	2034	2078	1665	1692
NO2/70	2.14	1.39	1.17	4.08	−4.4	1903	3290	2167	2197	2307	2389
NO3/7	3.73	1.32	1.22	4.67	−5.4	1714	3424	2146	2182	2192	2260
NO3/35	3.72	1.61	1.27	3.12	−2.5	1855	3939	2251	2311	2295	2346
NO3/70	3.70	1.60	1.13	1.58	−0.7	2334	3898	2444	2507	2135	2171
PO4/7	7.72	1.09	1.04	1.29	2.6	4287	4129	3893	4016	4036	4134
PO4/35	7.72	1.03	1.04	2.49	−0.7	3734	4248	3887	3913	4164	4253
PO4/70	7.70	1.16	1.13	1.04	0.3	3827	4464	3711	3857	3430	3537
SO4/7	10.22	0.98	1.04	1.33	1.5	4547	4584	4325	4244	4493	4299
SO4/35	10.20	1.06	1.08	1.70	0.1	4769	4767	4117	4182	4122	4772
SO4/70	10.20	1.18	1.15	1.79	0.4	3946	4745	3906	3760	3491	3609

[a] Chromatographic measurements of retention time and of peak asymmetry at 10% or 50% of the peak height. Determination of the peak shape using the method of Foley (see text for explanation). Calculation of plate numbers using mathematical equations derived from Gaussian or EMG modeling and comparison of these results to the values obtained from statistical moments. For EMG modeling, two methods have been used, the first one is the method of Foley and the second one is the method using the universal ratios (UR).
[b] Anion and concentration of this anion (μmol/l) in the solution analyzed.
[c] Retention time (min).
[d] Relative standard deviation between measures at different height fractions (see text for explanations).
[e] Relative difference between mean and zero order statistical moment (see text for explanations).

3.1.2. CZE separations

CZE separation of the five anions is realized in a few minutes: chloride, nitrite, nitrate and sulfate migrate between 3 and 3.5 min, phosphate migrate in 6 min, with the internal standard, chlorate, at 4 min (Fig. 3). The electropherograms are quite similar for either injection mode but the response is more than doubled and the migration times somewhat shorter in electrokinetic mode due to solvent stacking. As migration time increases, the peak shape changes from an asymmetric profile with an A_{s_r} ratio below 1 for chlorides to an asymmetric profile with an A_{s_r} ratio above 1 for phosphates, the nitrate peak being almost symmetric (Table 2). Peak asymmetry seems more pronounced after electrokinetic injection. When the concentration of the solution analyzed increases, the A_s ratio increases whatever the mode of injection. In the same way, migration times increase with increasing concentration if the injection is hydrodynamic and decrease for electrokinetic injection.

The calculation of peak areas using the method of Foley does not lead to homogeneous results – RSD around 30% – which means that the peaks obtained cannot be described with either a Gaussian or an EMG equation. Nevertheless, we have evaluated the number of theoretical plates according to the Gaussian model [Eq. (2)] – as is currently done in CE development – and compared to the results from statistical moments (Table 3). The concentration of 7 μmol/l has not been taken into account because it is too close to the limits of detection. Plate numbers obtained are above 100 000 for symmetrical peaks and decrease when the A_{s_r} factors diverge from 1 in both directions, with 3000 plates for the phosphate peaks which are the most asymmetric. Theoretical plate numbers determined with the Gaussian equation are always higher than those calculated with the statistical moments. A correlation is observed between the Gaussian estimations and the statistical determinations: over a range of theoretical plate from 0 to 140 000, Gaussian calculations led to an over-

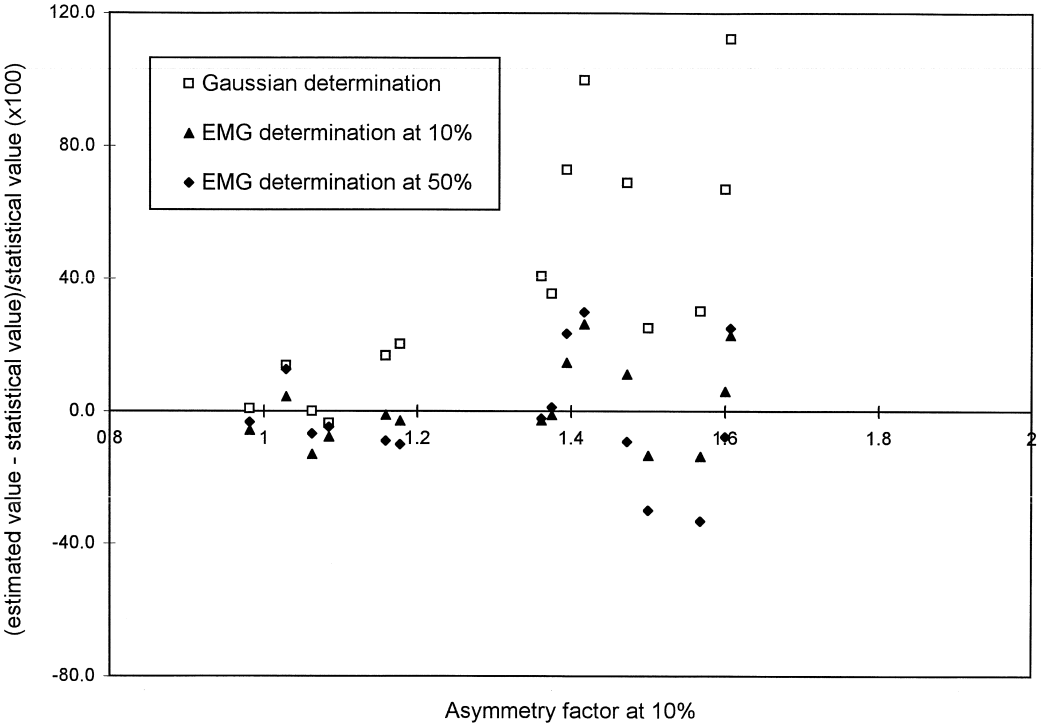

Fig. 2. Plate number determination in ion chromatography. Variation of relative errors between the estimations using Gaussian or EMG methods and the statistical values as a function of asymmetry of the peak.

estimation of 25%: Gaussian evaluation = 1.26 · statistical moments evaluation + 3276; $r^2 = 0.95$.

With the triangular method, using a negative slope for the peaks related to chloride and nitrite and a positive slope for the others, estimations of plate numbers correspond well to those from the statistical moments: triangular evaluation = 1.02 · statistical moments evaluation + 2499; $r^2 = 0.95$.

For these two regressions, the intercepts did not significantly differ from zero.

3.2. Validation criteria

Results obtained for ion chromatography or for CZE with the two injection procedures used are summarized in Table 4. The detection limits determined from the signal-to-noise ratio are around 1 ppm for IC for all peaks and 2.5 for CZE except for sulfate with values above 5 ppm. For sulfate and phosphate, limits obtained with hydrodynamic injection are twice the ones obtained with electrokinetic injection.

The limits of detection defined with the regression parameters remain lower for IC, between 1.5 and 3 ppm, than for CZE, between 3 and 9 ppm after electrokinetic injection and between 7 and 16 ppm after hydrodynamic injection. In the studies of regression, the determination coefficients for analyte concentrations in the range studied were satisfactory (between 0.991 to 0.9996), except for the calibration of chloride obtained by CZE with pressure injection. The linearity of the calibration curves was checked according to standard statistical procedures using *F* tests [25]. In any case, the existence of a significant slope and the validity of the adjustment were confirmed ($\alpha = 5\%$). Moreover, except for four calibration curves, the intercept values are not significantly different from zero.

For IC, intra-day and inter-day precisions were better than 1.4% and 2.6%, respectively. With CZE methods no significant differences are observed

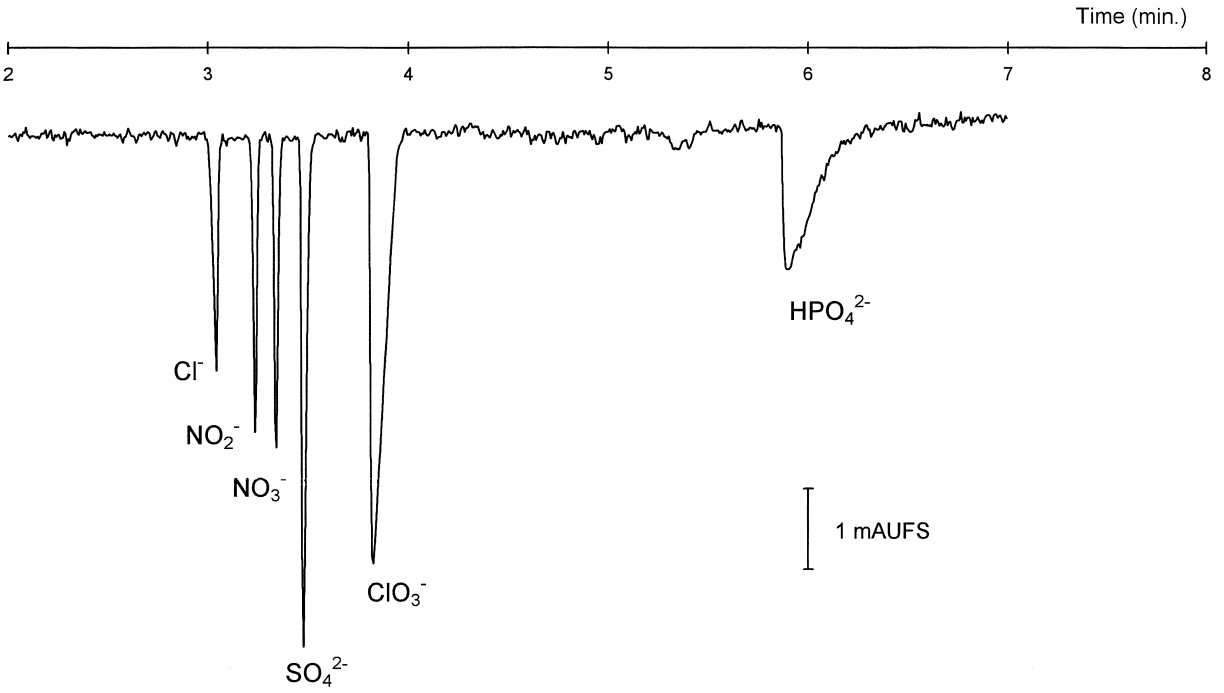

Fig. 3. CZE separation of a 35 μmol/l multi-anion solution. Operating conditions: running buffer with 2 mmol/l $K_2Cr_2O_7$ and 2 mmol/l DETA, −27 kV, 25°C, indirect detection at 254 nm. Hydrodynamic injection for 35 s.

Table 2
Peak characteristics in CZE with hydrodynamic or electrokinetic injection mode[a]

Anion[b]	Hydrodynamic injection				Electrokinetic injection			
	t_m[c]	Peak asymmetry		Foleys test RSD (%)[d]	t_m[c]	Peak asymmetry		Foleys test RSD (%)[d]
		$A_{s_{0.1}}$	$A_{s_{0.5}}$			$A_{s_{0.1}}$	$A_{s_{0.5}}$	
Cl/7	3.00	0.84	1.11	7.4	2.96	0.48	0.44	32.6
Cl/35	3.04	0.48	0.48	30.4	2.96	0.40	0.40	29.8
Cl/70	3.09	0.29	0.27	49.7	2.94	0.28	0.22	47.3
NO2/7	3.20	1.18	0.68	32.1	3.15	1.09	1.00	3.8
NO2/35	3.24	0.99	0.68	21.2	3.14	0.54	0.48	31.9
NO2/70	3.29	0.71	0.52	26.1	3.12	0.48	0.38	37.1
NO3/7	3.31	0.93	0.94	2.2	3.25	1.73	1.75	21.4
NO3/35	3.34	1.92	1.74	21.3	3.23	1.67	1.36	9.1
NO3/70	3.39	2.39	2.30	30.6	3.20	1.35	1.06	9.1
PO4/7	5.90	3.75	3.08	27.7	5.78	6.25	4.15	16.7
PO4/35	5.91	7.27	4.00	39.6	5.64	8.64	5.00	38.9
PO4/70	5.94	10.10	7.62	49.6	5.54	15.17	11.25	73.4
SO4/7	3.43	1.15	0.92	11.8	3.38	2.61	2.00	17.4
SO4/35	3.48	2.45	1.79	13.8	3.35	3.13	2.00	17.6
SO4/70	3.52	3.90	3.23	39.0	3.33	4.13	3.15	36.7

[a] Chromatographic measurements of migration time and of peak asymmetry at 10% or 50% of the peak height. Determination of the peak shape using the method of Foley (see text for explanation).

[b] Anion and concentration of this anion (μmol/l) in the solution analyzed.

[c] Migration time (min).

[d] Relative standard deviation between measures at different height fractions.

Table 3
Determination of plate numbers in CZE[a]

Anion[b]	Hydrodynamic injection					Electrokinetic injection				
	Moments	Gaussian	ER (%)	R.A.T.	ER (%)	Moments	Gaussian	ER (%)	R.A.T.	ER (%)
Cl/35	47 673	71 211	49.4	56 998	19.6	42 751	48 803	14.2	38 788	−9.3
Cl/70	32 352	42 570	31.6	33 720	4.2	33 116	37 006	11.7	29 407	−11.2
NO2/35	99 586	152 430	53.1	122 589	23.1	91 925	134 070	45.8	107 203	16.6
NO2/70	76 760	108 407	41.2	86 891	13.2	82 616	102 147	23.6	82 044	−0.7
NO3/35	93 816	143 940	53.4	118 197	26.0	133 505	162 196	21.5	132 457	−0.8
NO3/70	114 149	129 019	13.0	106 492	−6.7	134 554	159 536	18.6	130 261	−3.2
PO4/35	5469	8393	53.5	7056	29.0	3839	7706	100.7	6497	69.2
PO4/70	2308	3340	44.7	2861	24.0	3430	6466	88.5	5468	59.4
SO4/35	83 647	98 868	18.2	81 282	−2.8	43 311	68 614	58.4	56 544	30.6
SO4/70	48 686	50 863	4.5	41 950	−13.2	39 039	54 298	39.1	44 928	15.1

[a] Calculation of plate numbers using Gaussian or triangular (R.A.T.) estimations. Evaluation of the relative errors between these estimations and the statistical determinations.
[b] Anion and concentration of this anion in the solution analyzed.
R.A.T. = right angle triangle.

Table 4
Validation criteria of ion chromatography and CZE methods (P for pressure injection and E for electrokinetic injection)

	Chloride			Nitrite			Nitrate			Sulfate			Phosphate		
	CZE, P	CZE, E	IC	CZE, P	CZE, E	IC	CZE, P	CZE, E	IC	CZE, P	CZE, E	IC	CZE, P	CZE, E	IC
LOD (μmol/l) [a]	2.5	2.4	0.4	2.7	2.4	0.8	2.5	2.1	1.0	2.5	1.0	1.0	9.2	5.5	2.1
LOD (μmol/l) [b]	15.9	7.8	3.0	8.5	3.4	1.3	7.4	4.4	1.9	7.8	3.0	1.4	9.5	7.1	1.8
Slope (p)±SD (10^{-3})	2.09±0.07	6.66±0.12	8807±96	2.20±0.04	6.58±0.07	7720±37	2.44±0.04	7.09±0.09	8313±56	4.74±0.08	1.59±0.14	17 115±87	9.58±0.20	1.22±0.19	6579±43
i [c] (10^{-3})	6.53	19.36	2149	0.45	0.28	−7691	1.00	3.62	−5657	0.89	6.49	−5114	−15.72	45.19	−3241
S_{res} [d]	0.0111	0.0173	8.8379	0.0062	0.0075	3.4561	0.0060	0.0103	5.1769	0.0123	0.0158	8.0526	0.0302	0.0290	4.0213
r^2	0.97	0.994	0.998	0.993	0.998	0.9996	0.994	0.997	0.9993	0.994	0.998	0.9996	0.991	0.995	0.9993
Student test ($i=0$)	1.41 (NS) [h]	2.68 (S)	0.57 (NS)	0.17 (NS)	0.08 (NS)	5.21 (S)	0.40 (NS)	0.77 (NS)	2.56 (S)	0.17 (NS)	0.90 (NS)	1.49 (NS)	1.24 (NS)	3.74 (S)	1.89 (NS)
Fisher test ($p\neq0$)	791 (HS)	3277 (HS)	8387 (HS)	2809 (HS)	9924 (HS)	42 042 (HS)	3639 (HS)	6033 (HS)	21 757 (HS)	3305 (HS)	13 004 (HS)	38 078 (HS)	2238 (HS)	3983 (HS)	22 586 (HS)
Fisher test (lack-of-fit)	1.56 (NS)	0.31 (NS)	3.05 (NS)	0.03 (NS)	0.50 (NS)	1.86 (NS)	0.01 (NS)	0.17 (NS)	1.24 (NS)	0.07 (NS)	1.05 (NS)	0.64 (NS)	0.84 (NS)	1.09 (NS)	0.81 (NS)
Precision															
RSD (%)[e]	5.1	5.4	0.7	2.7	4.7	1.4	4.6	3.6	0.6	2.2	3.9	1.0	3.9	4.9	1.1
RSD (%)[f]	8.5	6.5	2.6	2.9	4.7	1.4	4.6	3.6	0.9	2.6	4.2	1.1	11.4	5.3	2.3
Accuracy[g]															
Mean (μmol/l)	33.8	36.6	34.0	35.6	35.5	34.7	34.9	35.1	34.8	34.5	35.5	34.7	36.5	36.7	35.1
Min (μmol/l)	30.1	35.0	33.1	33.8	33.9	34.2	33.0	34.0	34.2	33.1	34.2	34.3	31.0	33.8	34.3
Max (μmol/l)	38.9	38.9	34.8	36.8	38.4	35.4	37.8	38.1	35.2	36.0	38.7	35.5	40.6	39.5	36.5

[a] LOD = 3 Signal/Noise.
[b] LOD = 3 S_{res}/slope.
[c] Intercept.
[d] Residual standard deviation.
[e] Intra-day precision.
[f] Inter-day precision.
[g] Accuracy: mean and extreme values determined for a 35 μmol/l solution ($n=9$, three assays repeated three days).
[h] Definitions: (NS) = non-significant value, (HS) = highly significant value; (S) = significant value ($\alpha=5\%$).

between intra-day and inter-day precision, with values between 2.5 and 5% except for pressure injection, where reproducibility exceeds 8% for chloride and phosphate. For nitrite and nitrate anions, the RSDs that are observed for reproducibility are similar to the ones observed for repeatability. This mean that the variance related to the effect of day, S_f^2 [Eq. (17)], was equal to zero and led to an estimation of the variance of reproducibility equal to the variance of repeatability [Eq. (16)]. Such a case is considered as possible by the SFSTP guidelines [25].

The average concentrations observed are included in the range 35 μmol/l±5% for all the techniques studied.

4. Discussion

Peaks obtained with IC are either Gaussian or EMG. The number of theoretical plates observed (from 1500 for chloride to 4000 for sulfate) corresponds to heights equivalent to a theoretical plate (HETPs) equivalent to three- to eight-times the diameter of particles (15 μm). This is in agreement with or even better than the data of the supplier claiming 10 000 theoretical plates per meter for sulfate. The slight deformation observed for the first three peaks is related to mechanical disturbances during the injection or to scattering at the head of the column.

The efficiency noted for CZE is lower than expected especially for chloride and sulfate with plate numbers below 100 000. The results for phosphate are even worse, due to the difference of electrophoretic mobility between this anion and the electrolyte anion ($Cr_2O_7^{2-}$) leading to electrodispersion on one side of the migration zone. An increase in concentration leads to more strongly deformed peaks accompanied by changes in migration time of the peak maximum. This may also explain the greater asymmetry observed after electrokinetic injection for which more concentrated migration zones are obtained due to solvent stacking.

The main observation of this work is the fact that peaks obtained with CZE cannot be described by the usual Gaussian model: plate numbers should be determined either with the equations derived from the consideration of CZE peaks as right angle triangles or with statistical moments.

The statistical evaluation of these two techniques attempts to validate their use for quantitative determinations in real samples as environmental samples or drinking water. The Fisher tests attest the linear regression between peak areas and sample concentration. The intercepts correspond to zero in all cases but two for CZE with voltage injection and two for IC. For IC, this can be explained by a too tight precision, which raises the Student *t* value. For CZE, this deviation from zero could be explained by the scattering of the experimental areas (or ratio of areas), leading to a lower accuracy of the estimation of the slope, which has also been observed with regression estimated each day.

Furthermore, the high values of the limits of detection determined from regression parameters confirm the lower precision, especially after a pressure injection. With voltage injection, the stacking effect resulting from the difference of mobilities of the anions between the sample and the running buffer allows concentration of the analyte at the head of the capillary and, thereby, lower limits of detection than observed with hydrodynamic injection. The hydrodynamic injection protocol had been improved before validation to assure the highest injected quantities without deformation of peaks. It seems therefore impossible to get the same limits of detection for hydrodynamic injection than for electrokinetic injection because of the lack of stacking effect in the pressure mode. The precision determined for IC is better than the one observed with CZE, for which dispersion from analysis to analysis (repeatability) is high but variation between days (reproducibility) remains negligible. Thus results are more reliable with IC but the determinations obtained with CZE are still acceptable – accurate – and the lack of precision can be offset by repetition of analyses.

5. Conclusion

The results in this work are rather in favor of the use of IC instead of CZE for quantitative determinations of anions in real samples because of a better reliability, although CZE techniques have been

proven linear and the repeatability is still good especially after a voltage injection. This latter method offers the possibility of sample stacking which allows lower limit of detection than those observed after pressure injection.

The main observation of this work is that peaks obtained with CZE techniques cannot be treated as Gaussian or EMG as observed with chromatographic methods and need a different model based on the consideration of these peaks as right angle triangles.

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