Journal of Chromatography, 640 (1993) 481–487
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2750

Optimization of injection technique in capillary ion electrophoresis for the determination of trace level anions in environmental samples

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ABSTRACT

Capillary ion electrophoresis (Waters' trade name: Capillary Ion Analysis) offers several advantages compared to ion chromatography for the analysis of ionic solutes, primarily simplicity, matrix independence, and a different separation selectivity. The use of electromigration sample introduction leads to on-capillary enrichment of ionic analytes at the sample–buffer interface, permitting the determination of low ng/ml levels of anions in environmental samples of moderate ionic strength. This injection method allows improved sensitivity compared to hydrostatic injection; and is significantly more rapid than precolumn concentration ion chromatography. The analyte enrichment rate, hence peak response, is strongly dependent upon ionic strength and appropriate measures, such as standard addition or internal standard techniques, must be used to account for differences in standard and sample conductance.

INTRODUCTION

Capillary ion electrophoresis (CIE) (Waters' trade name: Capillary Ion Analysis, CIA) is emerging as an alternative analytical technique to ion chromatography (IC) for the determination of inorganic anions, cations and organic acids [1]. There are a number of advantages of using capillary zone electrophoresis (CZE) for ion analysis when compared to IC, primarily simplicity, matrix independence and the fact that the selectivity differs from conventional ion exchange separations [2]. Sample introduction in CZE differs from IC or HPLC in that an injection valve is not typically used. Injection modes in CZE include hydrostatic (or gravity), application of either pressure or vacuum and electromigration injection [3,4]. Electromigration injection involves applying a voltage to the sample in order to force the ions to migrate into the capillary. This injection mode is selectivity biased toward ions of the opposite charge to that of the detection electrode [4], with the bias being proportional to the total mobility of each ion [5]. When electromigration injections of low ionic strength samples are carried out, (electro)stacking of the sample ions occurs [1]. When electromigrating such samples, the voltage drop is not constant over the entire length of the capillary. There is very little voltage drop over the length of the capillary filled with conductive (low resistivity) buffer as almost all of the voltage drop occurs over the low conductance (high resistivity) volume of the sample injection [4]. This effectively leads to on-capillary concentration of the analyte at the sample–buffer interface [6–10].

Applications of on-line sample preconcentration in IC are typically restricted to the analysis of ultratrace (ng/ml) level anions and cations in low ionic

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0021-9673/93/$06.00

strength samples, such as boiler, steam condenstate and deionized waters [11–13]. Similarly, electromigration (concentration) injection has been applied to the analysis of ng/ml levels of anions in ultrapure water samples using CIE [10]. It has been demonstrated that quantitative on-column preconcentration of high ionic strength samples is possible in IC for certain anions through appropriate selection of system configuration and operating parameters [14]. In this paper, both qualitative and quantitative aspects of the application of CIE with electromigration concentration injection for the analysis of trace anionic solutes in moderate ionic strength environmental samples, such as estuary waters and soil extracts, are discussed.

EXPERIMENTAL

Instrumentation

The capillary electrophoresis instrument used was a Waters Quanta 4000 with a Waters 820 data station. Data were collected at 20 points per second for CIE. Separations were carried out using a conventional fused-silica capillary (60 cm × 75 μm I.D.) obtained from Waters. Detection was achieved using either indirect spectrophotometry at 254 nm or direct spectrophotometry at 214 nm.

Reagents and procedures

Water (18 MΩ) purified using a Millipore Milli-Q water purification system (Bedford, MA, USA) was used for all solutions. Sodium chromate tetrahydrate was obtained from Aldrich (Milwaukee, WI, USA). Sodium tetraborate [analytical reagent (AR) grade], glycerin [laboratory reagent (LR) grade] and boric acid (AR) were obtained from Ajax Chemicals (Sydney, Australia), as were the analytical grade sodium salts used for the preparation of all the anion standards. Sodium gluconate (LR) was obtained from Fluka (Buchs, Switzerland). Acetonitrile and *n*-butanol (HPLC grade) were obtained from Waters. The electroosmotic flow modifier, CIA-Pak OFM anion-BT is a proprietary chemical obtained from Waters. Eluents and electrolytes were prepared daily, filtered and degassed with a Waters solvent clarification kit. Specific operating conditions are provided as captions to the figures.

RESULTS AND DISCUSSION

Enhancement of sensitivity using electromigration injection

Electromigration injection is frequently used as a sample introduction technique in conventional CZE as it is possible to very accurately control both voltage and time of injection, resulting in good precision. Also, electromigration is the only possible mode of injection when using gel-filled capillaries. However, as this mode is biased toward ions of the opposite charge to that of the detection electrode [4], hydrostatic (or pressure/vacuum) sample introduction is most commonly used in CIE in order to introduce a representative sample into the capillary [2,15]. Fig. 1 shows an electropherogram obtained using CIE with a 30-s hydrostatic injection of a low μg/ml level anion standard using a chromate electrolyte and indirect UV detection at 254 nm and Fig. 2 shows an electropherogram of estuarine water sampled near a sewage effluent outlet obtained using a 15-s hydrostatic injection. Chloride, sulfate, nitrate and carbonate were detected in the water sample at low μg/ml levels. The duration of the hydrostatic injection was then increased in order to introduce more sample into the capillary and investigate the possibility that sub-μg/ml levels of other anions might also be present in this sample. Detection limits improved with longer sampling times, however, sampling times longer than 60 s, which corresponded to the injection of approximately 75 nl on the capillary [4], resulted in poor peak shape due to disturbance of the sample–buffer interface zone; and no additional sensitivity was obtained beyond this point. In this respect, CE is similar to HPLC, where large injection volumes typically lead to distorted peaks [16].

Analysis of the same estuarine water by CIE using a 3-kV electromigration injection for 10 s allowed low ng/ml levels of fluoride (and formate) to be detected in the sample. Increasing the injection time to 30 s further increased the detection sensitivity and allowed phosphate to be detected in the sample, as shown in close-up view in Fig. 3. Using single point, external calibration, the phosphate-P was determined to be present at *ca.* 13 ng/ml in the sample by CIE, compared to *ca.* 8 ng/ml when using a flow injection analysis technique. Table I details the detection limits (2 times the signal-to-noise ra-

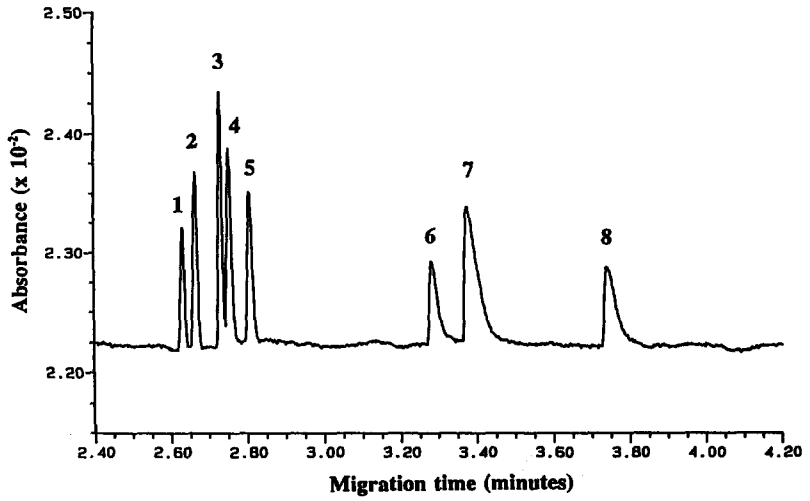

Fig. 1. Electropherogram of standard ions using hydrostatic injection. Conditions: capillary, 60 cm × 75 μm I.D. fused-silica; power supply, negative at 20 kV; electrolyte, 5 m*M* chromate with 0.5 m*M* CIA-Pak OFM Anion-BT at pH 8.0; injection, hydrostatic for 30 s; detection, indirect UV at 254 nm. Solutes: 1 = bromide (4.0 μg/ml); 2 = chloride (2.0 μg/ml); 3 = sulfate-S (1.3 μg/ml); 4 = nitrite-N (1.2 μg/ml); 5 = nitrate-N (0.9 μg/ml); 6 = fluoride (1.0 μg/ml); 7 = phosphate-P (1.9 μg/ml); 8 = carbonate (not quantitated).

tio) obtained using both hydrostatic and electromigration injection modes for the anions present in the estuarine water sample. Overall, CIE with electromigration injection resulted in an approximately 10 times improvement in sensitivity for the common anions for this particular sample when compared to hydrostatic injection. Generally, the lower the ionic strength of the sample, the greater the enhancement of sensitivity and detection limits in the sub-ng/ml range have been achieved for high purity waters with CIE [10], as the electrostacking of the analyte at the sample–buffer interface is very large for high resistivity samples.

Nitrite could not be detected in the estuarine wa-

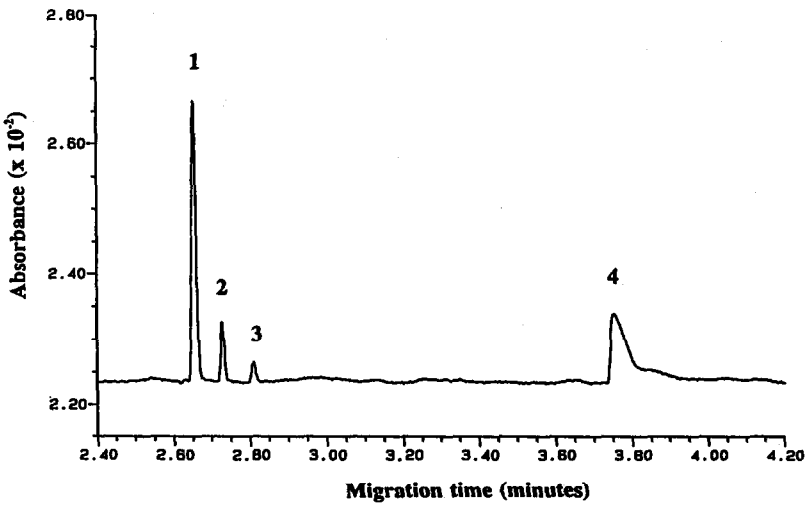

Fig. 2. Electropherogram of estuarine water sample using hydrostatic injection. Conditions as for Fig. 1 except: injection, hydrostatic for 15 s. Solutes: 1 = chloride (12.4 μg/ml); 2 = sulfate-S (1.1 μg/ml); 3 = nitrate-N (0.4 μg/ml); 4 = carbonate.

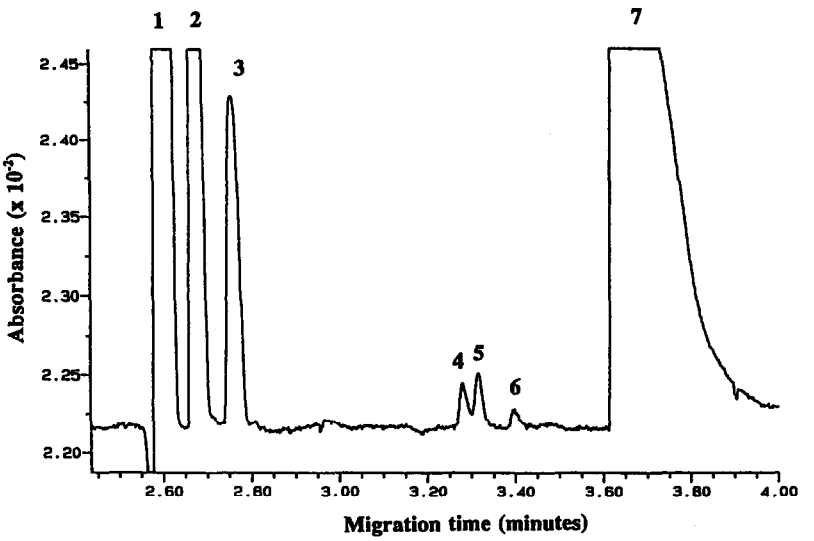

Fig. 3. Electropherogram of estuarine water sample using electromigration injection. Conditions as for Fig. 1 except: injection, electromigration, 3 kV for 30 s. Solutes: 1 = chloride (12.4 μg/ml); 2 = sulfate-S (1.1 μg/ml); 3 = nitrate-N (0.4 μg/ml); 4 = fluoride (0.031 μg/ml); 5 = formate (not quantitated); 6 = phosphate-P (0.013 μg/ml); 7 = carbonate.

ter sample with electromigration injection when using indirect UV detection, as the sulfate peak response increased as higher sampling times (or voltages) and interfered with the detection of nitrite. As is the case with IC [17], increased detection sensitivity and selectivity can be achieved with capillary electrophoresis for UV absorbing anions, such as nitrite and nitrate, by using direct UV detection. Fig. 4 shows an electropherogram of the estuarine water sample obtained with a 3-kV electromigration injection for 3 s using a sulfate electrolyte and direct UV detection at 214 nm. The combination of electromigration injection and direct UV detection allowed the determination of nitrite-N (at a concentration of *ca.* 9 ng/ml) and nitrate-N in the sample. The disturbance in the early portion of the electro-

TABLE I

DETECTION LIMITS (ng/ml) FOR ANIONS IN ESTUARINE WATER SAMPLE BY CIE USING HYDROSTATIC AND ELECTROMIGRATION INJECTION

Anion	Injection method			
	Chromate electrolyte 30 s hydrostatic	Chromate electrolyte 3 kV, 30 s electromigration	Sulfate electrolyte 30 s hydrostatic	Sulfate electrolyte 3 kV, 30 s electromigration
Chloride	157	[b]	[c]	[c]
Sulfate	79	[b]	[c]	[c]
Nitrite-N	31[a]	[b]	19	2.5
Nitrate-N	30	[b]	15	1.6
Fluoride	61[a]	7.1	[c]	[c]
Phosphate-P	74[a]	6.3	[c]	[c]

[a] Detection limit obtained from standard.
[b] Not determined.
[c] Anion not detectable using direct UV absorption at 214 nm.

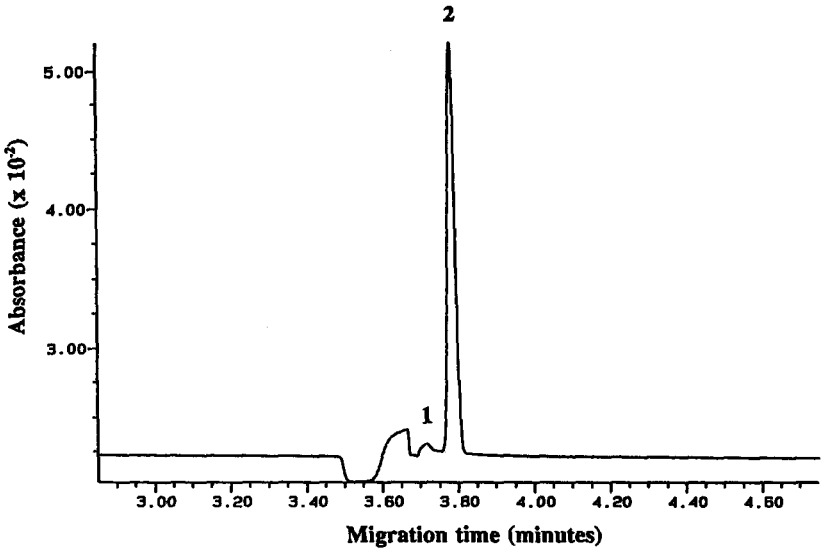

Fig. 4. Electropherogram of estuarine water sample using electromigration injection and direct UV detection. Conditions as for Fig. 2 except: power supply, negative at 14 kV; electrolyte, 10 m*M* sulfate with 0.5 m*M* CIA-Pak OFM Anion-BT; injection, electromigration, 3 kV for 10 s; detection, direct UV at 214 nm. Solutes: 1 = nitrite-N (0.4 μg/ml); 2 = nitrate-N (0.009 μg/ml).

pherogram was due to the migration of the UV transparent chloride and sulfate anions. Table I also details the detection limits obtained by hydrostatic and electromigration injection when using the sulfate electrolyte and direct UV detection at 214 nm.

Quantitation using electromigration injection

CIE offers a distinct advantage over IC for ultratrace analysis in terms of the time taken for the sample 'enrichment' step. In IC, a typical sample volume (10–100 ml) is loaded onto a concentrator column via an HPLC pump at flow-rates of 2–4 ml/min, hence the sample loading step usually takes 5–25 min, or perhaps longer [18]. Large enrichment factors can be achieved in CIE in a matter of seconds using electromigration injection. However, the major disadvantage of using CIE with electromigration injection for ultratrace analysis is that considerable care must be taken in order to achieve accurate quantitation. Initial attempts to use CIE with electromigration injection for the determination of sub-μg/ml levels of fluoride in a low salinity bore water consistently resulted in low values compared to IC when using an anion HR column, a borate–gluconate eluent and conductivity detection [19]. The difference in ionic strength of the standard and sample led to different enrichment factors, resulting in low recoveries for the sample fluoride. This was confirmed by determining the peak response for a fluoride standard prepared containing increasing concentrations of chloride using CIE with both hydrostatic and electromigration injection, shown in Fig. 5. As the ionic strength of the sample increased the fluoride response decreased significantly when using electromigration sample introduction with CIE.

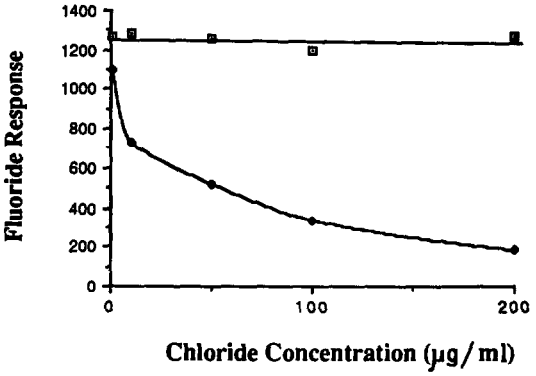

Fig. 5. Effect of chloride concentration on fluoride response using hydrostatic (□) and electromigration (◆) injection. Conditions as for Fig. 1 except: injection, hydrostatic for 30 s and electromigration, 3 kV for 10 s. Solutes: fluoride (1.0 μg/ml); chloride as indicated.

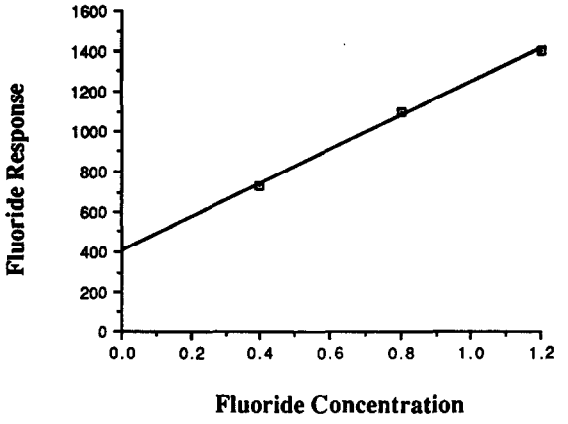

Fig. 6. Standard addition calibration plot for fluoride in bore water using electromigration injection. Conditions as for Fig. 3 except: injection, electromigration, 3 kV for 10 s. Solutes: fluoride spiked at 0.4, 0.8 and 1.2 μg/ml; original fluoride sample concentration calculated to be 0.47 μg/ml.

The use of CIE with electromigration injection has been shown to given accurate quantitation for the analysis of anions in ultrapure waters, after the addition of a constant amount (low μ*M* concentrations) of a non-intefering anion (such as octanesulfonate) to normalize the ionic strength of both standards and samples [10]. This approach is less attractive for samples of higher ionic strength, as the amount of added buffer required to ensure a constant ionic strength would be high, therefore the enrichment factors achieved using electromigration injection would be reduced. When using conventional CZE with electromigration sample introduction, typically, one, or possibly two, internal standards are often added in order to improve quantitation [20]. Another option suggested has been to monitor the current generated during sample introduction and correct for variations, in a similar fashion to the use of an internal standard [20].

Perhaps the simplest approach to achieve accurate quantitation when using electromigration injection is to use standard addition techniques. Fluoride could be accurately determined in the low salinity bore water with electromigration injection and standard addition. The bore water sample was spiked with 0.4, 0.8 and 1.2 μg/ml fluoride and from the calibration plot, shown in Fig. 6, fluoride was calculated to be present at 0.47 μg/ml in the original sample. This compared to a value of 0.45 μg/ml when using IC with an anion HR column, borate–gluconate eluent and conducticity detection. However, there are practical limitations when using standard addition techniques, particularly the accurate preparation and addition of sub-μg/ml standard spikes.

Generally, the use of an internal standard proved to be the most versatile approach for accurate quantitation when performing electromigration sample introduction. A wide range of environmen-

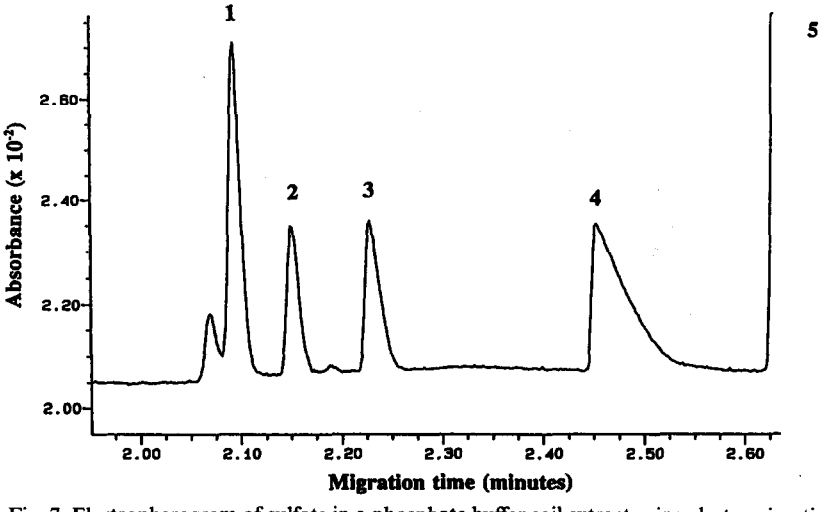

Fig. 7. Electropherogram of sulfate in a phosphate buffer soil extract using electromigration injection. Conditions as for Fig. 3 except: injections, electromigration, 3 kV for 10 s; sample, 4.0 g soil in 20 ml 0.02 *M* potassium dihydrogenphosphate. Solutes: 1 = chloride; 2 = sulfate-S (3.06 μg/ml); 3 = nitrate; 4 = citrate (internal standard added at 40 μg/ml); 5 = phosphate.

tal samples, including sewage effluents, run-off waters, tailings dam waters, river and lake waters, were successfully analyzed by CIE with electromigration injection. An important example is the determination of sulfate in soil extracts, as this analysis is widely used as an indication of soil fertility. Fig. 7 shows a close-up view of an electropherogram obtained using CIE with a 3-kV, 10-s electromigration injection of a phosphate buffer extract of a soil sample. Citrate was added as the internal standard and sulfate-S was determined to be 3.06 μg/ml in this case, compared to 3.28 μg/ml by IC. A precision of 2.0% R.S.D. was obtained for four consecutive injections of the sample with a detection limit of 21 ng/ml at 2 times the signal-to-noise ratio.

CONCLUSIONS

The use of electromigration sample injection with CIE leads to on-capillary concentration of the analyte at the sample–buffer interface, permitting the determination of low ng/ml levels of anions in samples of moderate ionic strength. This technique allows significantly improved sensitivity compared to hydrostatic injection; and offers a distinct advantage over precolumn concentration IC, as large enrichment factors can be achieved in a matter of seconds. Analyte enrichment rate, hence peak response, is strongly dependent upon ionic strength and appropriate measures, such as the use of standard addition or internal standard techniques, must be used to account for differences in standard and sample conductance.

ACKNOWLEDGEMENTS

The authors wish to thank the Ministery of Agriculture and Fisheries (Hamilton, New Zealand), Auckland Regional Council (Auckland, New Zealand) and Western Australian Water Authority (Perth, Australia) for supplying the various water samples.

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