

# Direct Analysis of Inorganic Anions in Samples with High Salt Content by Capillary Zone Electrophoresis

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## Abstract

Trace-level inorganic anions in seawater are separated efficiently by capillary zone electrophoresis using direct UV detection. The carrier electrolyte is 50mM borate at pH 9.3 and contained 1.5M NaCl. This buffer solution is adopted to prevent interference from high concentrations of the chloride ion in seawater. No electro-osmotic flow reverser is used to shorten the analysis time. The experimental conditions such as the concentration of NaCl in the carrier electrolyte, capillary inner diameter, applied current, and temperature are optimized. Linear plots are obtained in the concentration range of 0.1 to 20  $\mu\text{g/mL}$ . The quantitation limits of the anions are in the order of 0.02 to 0.1  $\mu\text{g/mL}$ . The proposed method may be applicable to the determination of inorganic anions in other environmental samples and effluents of a power plant.

## Introduction

During the last few years, capillary zone electrophoresis (CZE) has rapidly developed as an analytical technique for the separation of inorganic ions. Thus, it has attracted considerable attention as an alternative to the well-established technique of ion chromatography (1–2). The development of inorganic anion analysis methods by CZE is still an active area of research (3–4). Only a small subset of possible applications using CZE in inorganic ion analysis has been exploited so far, and interest in this technique is likely to increase over the next few years. The analysis of inorganic anions in a real sample by CZE requires a careful optimization of operating parameters. Among other requirements, a way to control the direction and rate of the electroosmotic flow (EOF) must be found (5–7). Manipulation of the separation selectivity of CZE for a range of ions that might have similar mobilities is necessary (8–9), as is an adequate detection technique (10).

Although much of the work has been done on samples containing a relatively low total ion concentration, little has been done on samples with high salt content such as seawater or subterranean water (11–14). The problem is principally because of the interfering role of matrix constituents, such as the chloride

and sulfate ions. The direct injection of high salt samples is rarely performed without some pretreatment. Such samples normally would require either dilution (12) or removal of salts by some cleanup method (15). Dilution solves the problem of the excess ion at the expense of lowering the concentration of the trace constituent below its detection limits. In CZE it is commonly thought that even a moderately high ionic concentration in the carrier electrolyte or the sample would lead to Joule heating and serious peak distortion. Recently, Ding et al. (16) have done a theoretical and practical detailed study on the effect of Joule heating on the electrophoretic and electro-osmotic mobility of diverse inorganic anions. They concluded that Joule heating is not enough to destroy a separation; on the contrary, some heating may be helpful.

The objective of this study was to investigate the potential of CZE in the determination of anions in samples with a high concentration of salts. For this purpose, the capillary electrophoretic (CE) separation of ten anions using direct UV detection was studied. The separation can be carried out successfully in a carrier electrolyte of high salt content. EOF was greatly reduced, thus eliminating the need for a flow modifier. Other experimental parameters such as applied voltage and the inner diameter of the capillary were also investigated. The effect of a high concentration of salts in samples on the separation was also studied. Finally, the proposed method was applied to the direct determination of anions in seawater samples collected around the coastal area of the A Coruña bay. Other environmental samples (such as river water, rain water, and diverse aqueous samples of a power central) were successfully analyzed with this procedure.

## Experimental

### Chemicals

All standards and electrolytes were prepared with analytical-reagent-grade chemicals in deionized water obtained from a Milli-Q system (Millipore, Milford, MA). A 1M and 0.1M solution of sodium hydroxide and a 50mM borate buffer solution (pH 9.3) for CE were obtained from Hewlett-Packard (Waldbronn, Germany). Anion stock solutions of 1000  $\mu\text{g/mL}$  were purchased

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from Merck (Darmstadt, Germany) and prepared from the corresponding sodium or potassium salts. Working solutions were prepared by mixing the stock solutions in appropriate proportions and diluting them with deionized water. These solutions were prepared freshly every day because of the rapid degradation of thiosulfate and ferrocyanide.

The carrier electrolyte was 50mM borate at pH 9.3 and contained different concentrations of NaCl (Merck). All solutions and samples were filtered through a 0.45- $\mu$ m membrane before use.

### Apparatus and procedures

The CZE experiments were performed with an HP<sup>3</sup>DCE CE system (Hewlett-Packard, Palo Alto, CA) fitted with an on-column diode-array detector.

Separations were carried out on fused-silica capillaries having a 22-cm effective length (30-cm total length) and two different inner diameters (25 and 50  $\mu$ m). These capillaries were prepared from a large capillary (25 m) supplied by Composite Metal Service (Teknokroma, Barcelona, Spain). On-column detection windows were made by burning a small section (0.5 cm) of the external polyimide coating and removing the burned residue with methanol. Newly installed capillaries were flushed with 1.0M NaOH for 10 min, 0.1M NaOH for 10 min, deionized water for

5 min, and then finally rinsed with the buffer for 30 min.

A negative current of  $-300 \mu$ A was applied for the separation of the inorganic anion in less than 10 min. The temperature was 20°C. The direct UV absorbance was measured at 200 and 230 nm. Samples were introduced by pressure (50 mBar  $\times$  10 s), and a 3-min capillary purge of the carrier electrolyte was performed prior to each injection.

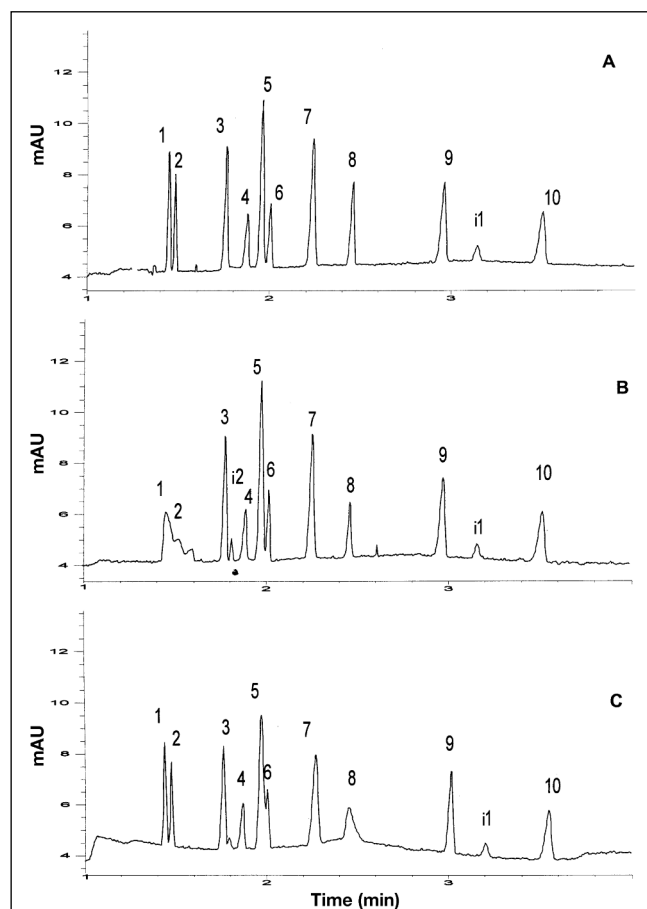
## Results and Discussion

### Effect of the high salt concentration matrix

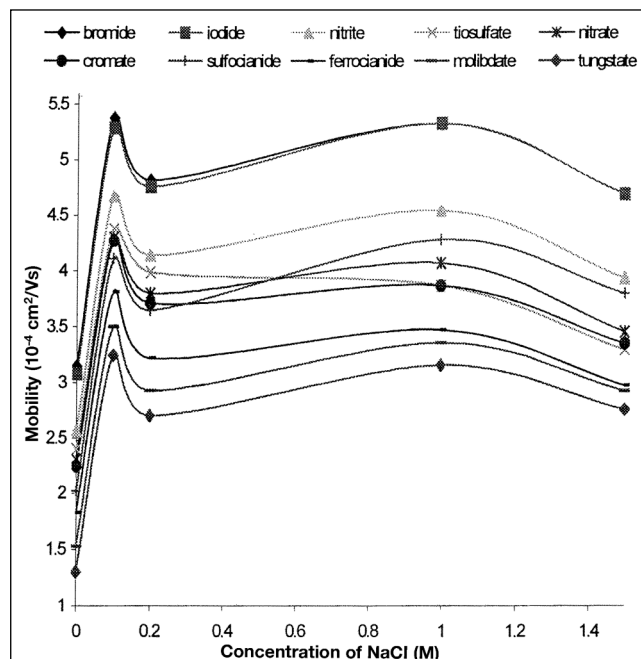
According to our preliminary experiments carried out with a borate buffer system (50mM with 5% of methanol, v/v), the analysis of ten UV absorbing anions can be performed without the need to use an external EOF modifier (17). As can be seen from Figure 1A, baseline separation of the ten studied compounds was achieved in less than 3.7 min. However, when we tried to apply the optimized method to samples with a high content of nonabsorbing anions such as  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ , the separation was adversely affected.

For this reason, a detailed study on the effects of salts on the separation was performed. The presence of chloride ions on the sample in concentrations higher than 20  $\mu$ g/mL interfered with the determination of  $\text{Br}^-$  and  $\text{I}^-$  anions (Figure 1B). The peaks were badly shaped and there was almost no resolution of individual peaks. However, the other anions could be analyzed without problems even for samples with a  $\text{Cl}^-$  content higher than 300  $\mu$ g/mL.

The separation also became significantly poorer when the sample contained a high excess of sulfate ion (Figure 1C). In this case, a baseline disturbance was observed around the  $\text{SCN}^-$  and  $\text{Fe}(\text{CN})_6^{4-}$  peaks. When the two interfering anions were added



**Figure 1.** Effect of salts in the sample on CE separation: (A) sample without salts, (B) sample with 20- $\mu$ g/mL NaCl, and (C) sample with 20- $\mu$ g/mL  $\text{Na}_2\text{SO}_4$ . Peaks:  $\text{Br}^-$ , 1;  $\text{I}^-$ , 2;  $\text{NO}_2^-$ , 3;  $\text{S}_2\text{O}_3^{2-}$ , 4;  $\text{NO}_3^-$ , 5;  $\text{CrO}_4^{2-}$ , 6;  $\text{SCN}^-$ , 7;  $\text{Fe}(\text{CN})_6^{4-}$ , 8;  $\text{MoO}_4^{2-}$ , 9;  $\text{WO}_4^{2-}$ , 10; impurity results of the degradation of  $\text{S}_2\text{O}_3^{2-}$ , i1; and impurity results of the degradation of  $\text{Fe}(\text{CN})_6^{4-}$ , i2.



**Figure 2.** Plots of electrophoretic mobility against the concentration of the salts in the carrier electrolyte.

together to the sample, no major differences were observed in regards to the interferences they produced individually.

### Effect of carrier electrolyte salt content on CE separation

Because the sodium chloride concentration in seawater is approximately 0.5M, this sample is a difficult matrix for direct CE analysis. One approach for eliminating matrix interference from the major ionic components of seawater is to perform anion separations in electrolytes containing a diverse salt concentration (16). A detailed study on the effects of salt concentration in the background electrolyte (BGE) on the separation was conducted for the ten anions. For this study the electrolytes were 50mM borate at pH 9.3 with increasing amounts of sodium chloride. Figure 2 shows the results of this analysis. When NaCl was added to the BGE, the current increased rapidly to 300  $\mu$ A. This sharp increase in current can be attributed to lesser electrical resistance. Although the most common practice with CZE is to maintain the voltage constant during the analysis, in this study the applied current was maintained constant at  $-300 \mu$ A instead and the voltage was automatically lowered as the salt concentration in the BGE continued to increase.

The electrophoretic mobilities of the sample anions increased as the concentration of NaCl in the carrier electrolyte increased from 0 to 100mM. After reaching a maximum at 100mM, further increases in salt concentration resulted in only slight decreases in

the mobility of the sample ion. The separation of the ten anions in 1.5M NaCl was also quite good but a little slower, and the separation was achieved in 10 min (Figure 3).

The results obtained showed that the resolution between the Br<sup>-</sup> and I<sup>-</sup> peaks was lost. However, this was not a very serious problem because the Br<sup>-</sup> ion did not absorb at 230 nm, thus the determination of I<sup>-</sup> could be performed at this wavelength. The migration order of the anions was changed when the concentration of NaCl was increasing. These changes were particularly important to SCN<sup>-</sup> and S<sub>2</sub>O<sub>3</sub><sup>2-</sup> anions.

This buffer system offered the possibility of preventing interference from high matrix salinity. The analysis of the studied anions could be accomplished in 10 min. Seawater samples spiked with a standard of the ten compounds were injected directly without any dilution and were successfully analyzed.

### Instrumental conditions

#### Capillary inner diameter

Another approach to minimize the effect of Joule heating is to reduce the inner diameter of the capillary. The current across the capillary decreases because of the squared dependence of the electrical current with the radius ( $I \propto r^2$ ) (18). With 25- $\mu$ m-i.d. capillaries, the current is four times lower than with 50  $\mu$ m; therefore, it is possible to use more concentrated buffers without losing resolution. Figure 4 shows the results of the analysis of the ten-anion mixture with the 50- $\mu$ m-i.d. and 25- $\mu$ m-i.d. capillary. Ferrocyanide and molybdate comigrated, but for all the other anions, good resolution was obtained. With the 25- $\mu$ m capillary, the separation was faster than with 50  $\mu$ m, but the sensitivity decreased dramatically. Therefore, a 50- $\mu$ m-i.d. capillary was selected for further investigation.

#### Effect of temperature

The influence of capillary temperature was studied at 20°C, 25°C, 30°C, and 40°C. For this specific case, temperature did not play a decisive role in the mobility of the anions. An optimum

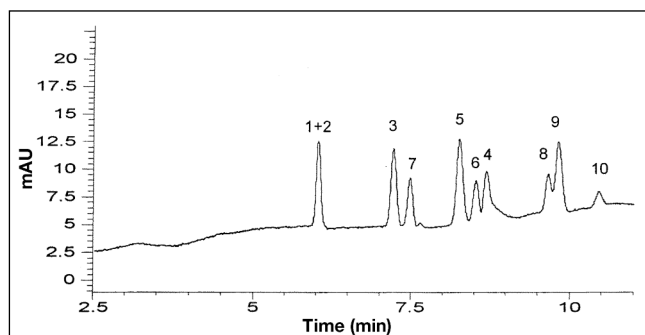


Figure 3. CE separation of ten inorganic anions (peak numbers are the same as in Figure 1).

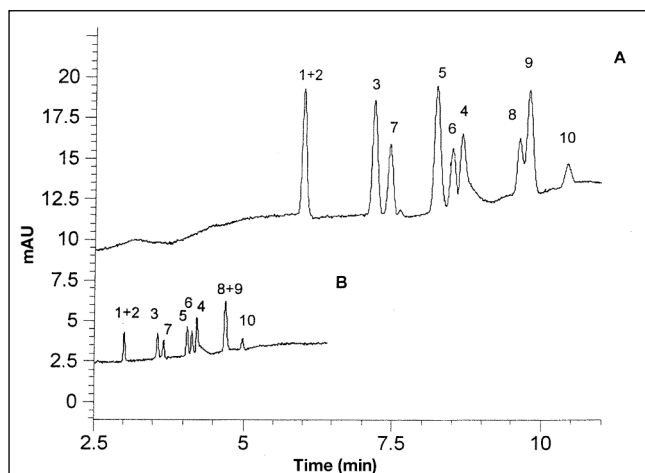


Figure 4. Effect of the capillary inner diameter on the separation: (A) 50- $\mu$ m i.d. and (B) 25- $\mu$ m i.d. Peak numbers are the same as in Figure 1.

Table I. Validation Data: Linearity, LODs, and Repeatability of the Peak Area and Migration Time Obtained with the Proposed CE Method

Anion	Linearity* Correlation ( $r^2$ )	LOQ (S/N = 10:1) ( $\mu$ g/mL)	%RSD <sup>†</sup>	
			Migration time	Peak area
Br <sup>-</sup> + I <sup>-</sup>	0.9990	0.028	0.73	1.76
I <sup>-</sup>	0.9995	0.043	0.70	2.88
NO <sub>2</sub> <sup>-</sup>	0.9992	0.027	0.74	1.49
S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	0.9980	0.058	0.64	2.85
CrO <sub>4</sub> <sup>2-</sup>	0.9966	0.091	0.66	2.25
NO <sub>3</sub> <sup>-</sup>	0.9992	0.019	0.82	1.32
Fe(CN) <sub>6</sub> <sup>4-</sup>	0.9983	0.034	0.66	2.96
SCN <sup>-</sup>	0.9996	0.037	0.89	2.55
MoO <sub>4</sub> <sup>2-</sup>	0.9987	0.025	0.92	2.70
WO <sub>4</sub> <sup>2-</sup>	0.9997	0.100	1.18	2.42

\* Linearity was tested in the range of 0.1 to 20  $\mu$ g/mL.

<sup>†</sup> Reproducibility was assayed injecting ten times a standard of 0.5  $\mu$ g/mL for each analyte.

resolution with minimal analysis time was obtained at 20°C.

### Detection

The detection wavelength was optimized by acquiring the spectrum of each inorganic anion using a diode-array detector. Detection was carried out at 200 nm because all of the anions had an absorption maximum around this wavelength. Although Br<sup>-</sup> and I<sup>-</sup> were not electrophoretically resolved, with the diode-array detector quantitative analysis could be performed because the I<sup>-</sup> ion absorbs radiation at 230 nm and Br<sup>-</sup> does not.

### Validation of the procedure

The linearity, sensitivity, and reproducibility of the method were tested, and the results are summarized in Table I.

The linearity was studied in the 0.1–20- $\mu\text{g/mL}$  range using the calibration technique with an external standard. Good linearity

was observed for all the studied compounds with correlation coefficients better than 0.9995.

The quantitation limits (LOQs) established for the signal-to-noise ratio (S/N) of 10:1 were in the range of 0.02  $\mu\text{g/mL}$  for nitrite to 0.1  $\mu\text{g/mL}$  for tungstate.

The repeatability of the migration times and peak areas was studied by injecting 10 times a standard solution of anions at a 0.5- $\mu\text{g/mL}$  concentration for each analyte. The relative standard deviation (RSD) values for the peak area were lower than 3%. The RSDs for the migration times were between 0.6 and 1.2%. Therefore, the sensitivity and repeatability achieved was adequate for the determination of absorbing anions by CZE.

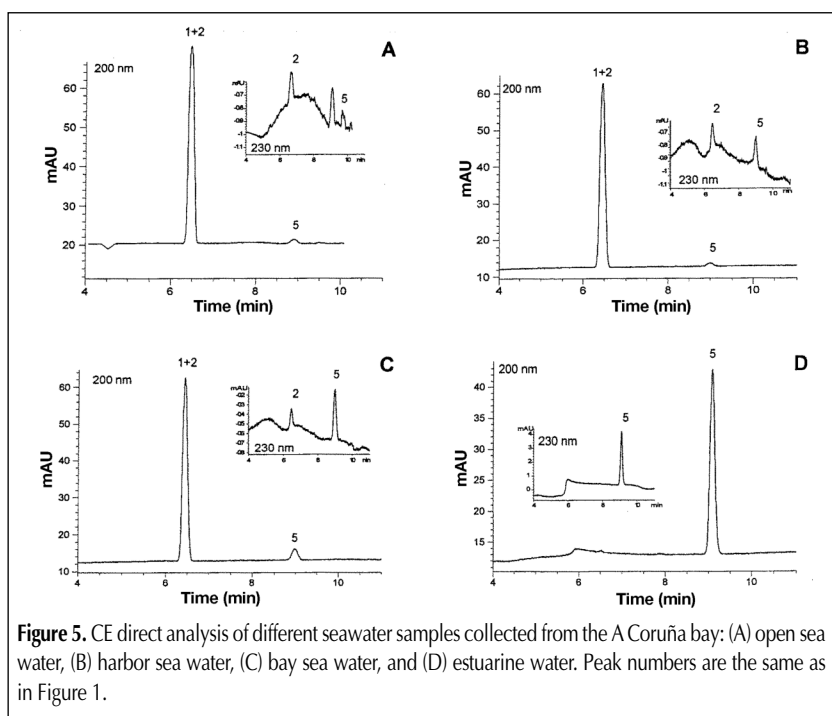
### Application to seawater

The optimized method was applied to the determination of the UV-absorbing anions in seawater samples taken from the surface and collected from around the coastal area of the A Coruña bay. The samples were injected directly without any dilution. The electropherograms are shown in Figure 5. A good bromide peak at 6.5 min was followed by a smaller nitrate peak in most of the samples (Figure 5A for open seawater and Figure 5B for harbor seawater). A standard addition method was also used to determine the amount of Br<sup>-</sup> and NO<sub>3</sub><sup>-</sup> ions in seawater. The concentration of Br<sup>-</sup> was 5–7  $\mu\text{g/mL}$  and for nitrate was lower than 0.5  $\mu\text{g/mL}$ . Traces of I<sup>-</sup> were also observed in samples from Figure 5A, 5B, and 5C. The electropherogram in Figure 5D (estuarine water) was drastically different from the other samples. There were no Br<sup>-</sup> and I<sup>-</sup> ions, and the nitrate concentration was 3.5  $\mu\text{g/mL}$ . This sample was collected from a region where the river was flowing to the sea. Figure 5C corresponded with the sample collected from a closed area of the sea (the bay), which was near the river. In this sample there were Br<sup>-</sup> and NO<sub>3</sub><sup>-</sup>. As can be seen, the concentration of NO<sub>3</sub><sup>-</sup> decreased as we went away from the river area, and alternatively the concentration of the Br<sup>-</sup> ion increased.

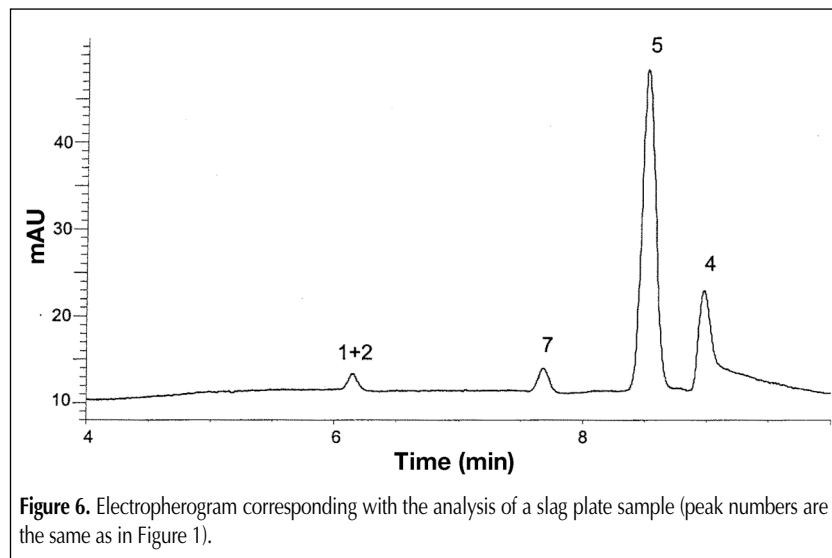
Anions in other aqueous samples such as tap water, river water, and such effluents of a power plant as the final effluent of a treatment plant and water from a slag plate (samples with a high level of sulfate, thus a high ionic strength) could also be analyzed directly with the proposed method. The nitrate ion was quantitated in all of the samples. The electropherogram corresponding with the slag plate sample showed the presence of Br<sup>-</sup>, I<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, SCN<sup>-</sup>, and S<sub>2</sub>O<sub>3</sub><sup>2-</sup> (Figure 6).

### Conclusion

The CZE method using 50mM borate with 1.5M NaCl as the carrier electrolyte is simple, rapid, and possesses sufficient precision and freedom



**Figure 5.** CE direct analysis of different seawater samples collected from the A Coruña bay: (A) open sea water, (B) harbor sea water, (C) bay sea water, and (D) estuarine water. Peak numbers are the same as in Figure 1.



**Figure 6.** Electropherogram corresponding with the analysis of a slag plate sample (peak numbers are the same as in Figure 1).

from the interference of chloride and sulfate ions in the sample. In addition, the use of an external flow modifier is unnecessary when the electrolyte contains a high concentration of salt because of the reductions in the EOF.

A rapid separation of a trace-level anion in the complex seawater matrix can be performed directly without sample dilution. The same method can also be applied to samples without a high salt content.

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