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Capillary Electrophoresis: Analysis of Trace Inorganic Anions in Ultrapure Water – Methods and Procedures

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REPORT SUMMARY

This project studied and optimized procedures for baseline drift, resolution, electrode polymerization, and separation reproducibility for capillary electrophoresis (CE) of trace inorganic anions in ultrapure water.

Background

Capillary electrophoresis is an alternative to ion chromatography for analyzing trace inorganic impurities in ultrapure water systems (for example, the condensate of boiling water reactors [BWRs]). However, baseline drift, resolution limitations, electrode contamination, and poor separation reproducibility have hindered successful application of the method.

Objectives

- To report on the resolution of CE analysis issues related to baseline drift, reproducibility, polymerization of probe chemicals, sample injection, and quantification of anion separations.
- To provide a generic anion procedure for CE analysis.

Approach

The project team analyzed the problems related to baseline drift, reproducibility, polymerization of probe chemicals on the electrodes, sample injection, and quantification of anion separations by studying each issue separately until the optimum methodology was identified. CE anion analyses were done using a Groton Biosystems GPA100 CE instrument equipped with a Bischoff variable UV/Vis wavelength detector. An 80.5-cm bare silica capillary was installed in the instrument. When the capillary was coated, rinsed, and ready to analyze anions, a buffer solution carried out the separations in a constant pH and conductivity media. Anion CE analyses are then performed at a constant temperature and -20,000 kV is applied to force migration of anions through the detector. Data were collected and analyzed with DAx 8.0 software.

The six anions studied were Br, Cl, SO_4, NO_3, PO_4, and F.

Results

The report suggests steps to optimize baseline drift, reproducibility, polymerization of probe chemicals, sample injection, and quantification of anion separations during CE anion analysis. A generic anion procedure is outlined based on these results.

EPRI Perspective

CE—with proper optimization—is capable of very sensitive, rapid analysis of sub-ppb ions in power plant process streams. Requirements on primary water chemistry are becoming increasingly strict, and operators of nuclear power plants need to quickly detect ever-lower concentrations of water-borne impurities. Today's water chemistry limits for anions, such as chloride and sulfate, are important for the control of intergranular stress corrosion cracking (IGSCC), common to all BWRs.

Keywords
Anions
BWR chemistry
Capillary electrophoresis
Cations
Ion chromatography

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1
INTRODUCTION

Capillary electrophoresis (CE) is a well known analytical method capable of rapid detection of very low concentrations of simple metallic and non-metal ions. With proper optimization, the method is capable of very sensitive, rapid analysis of sub-ppb ions in power plant process streams. Requirements on primary water chemistry are becoming increasingly strict, and operators of nuclear power plants need to quickly detect ever lower concentrations of water-borne impurities. Today's water chemistry limits for anions, such as chloride and sulfate, are important for the control of intergranular stress corrosion cracking (IGSCC), common to all boiling water reactors (BWRs)[1].

EPRI's extensive experience with this detection technique has been detailed in previous reports [2-6]. It is the purpose of this document to report on the resolution of CE analysis issues related to baseline drift, reproducibility, polymerization of probe chemicals, sample injection and quantification of the anion separations as well as provide a generic anion procedure for those interested in pursuing this technology.

2
EXPERIMENTAL

Instrumental

The Groton Biosystems GPA100 CE instrument equipped with a Bischoff variable UV/Vis wavelength detector were controlled by a Dell Computer and data collected and analyzed with DAx 8.0 software.

The pH meter with a glass electrode from Hanna instruments, Model 209, the conductivity-meter was from Oakton, Con5 Acorn series. A Setra Model S1-4105 and a Mettler model H51AR balances were used.

Chemicals

All the chemicals were of analytical grade and solutions prepared with TraceSelect ultra pure water (Fluka) with anions and cations content <0.1 ppb. The PDDMAC- 20% solution (poly(diallyldimethylammonium chloride)), Squaric acid (3,4-dihydroxy-3cyclobutene-1,2-dione), TRIS (Tris-hydroxymethyl aminomethane), ultra pure water and 10 ppm anion standard were purchased from Sigma- Aldrich.

Materials and solutions

The 75µm internal diameter LPA coated capillaries were from Microsolv, Bio-Rad and some coated in-house. The 75µm internal diameter silica capillaries were from Polymicro. For all the experiments, the total length of the capillaries was 80.5 cm with the window at 49 cm from the inlet side. The capillary windows were made by electrically burning the polyimide coating and cleaning with methanol prior to installation.

The final 0.5% PDDMAC solution was prepared by diluting 250µl of the compound in 10 ml of ultra pure water. The buffer was prepared daily just before experiments.

All the solutions were prepared, stored and analyzed in plastic tubes, vials, bottles, caps, tips and containers in order to avoid contamination. The vials, caps and tubes for sample preparation were previously rinsed with hydrochloric acid 0.1M and ultrapure water.

3
RESULTS

Electrode polymerization

It has been observed that squaric acid in contact with Pt electrodes under high voltage creates degradation in current. This is due to squaric acid polymerization on Pt electrodes surface. Therefore, several studies were performed to avoid the polymerization of the main buffer component. The polymerization can not be avoided even when using lower voltage, gold electrodes or by varying the squaric concentrations, but the best results can be obtained by cleaning the electrodes in concentrated HNO_3 for10 minutes after about 3 hours of use.

Baseline drift and resolution

The previous studies have shown a baseline drift when using commercial or homemade LPA coated capillaries (Fig. 3-1) after more than 1 hour of use. In an attempt to stabilize the baseline various modifiers were added to the buffer (Fig. 3-2) when using bare silica capillaries.

Several modifiers were added individually to the buffer (not all shown). None of the modifiers provided the “flat” baseline that was needed. The next step in these efforts to get well-resolved peaks was to coat the capillaries in the laboratory (LPA) and recoat them when instability was observed. The coating showed the same magnitude of EOF as the commercial capillaries, but unfortunately the baseline was not stable; it was identical to the previous cases even when re-coating before each run.

Additional experiments were performed adding modifiers to the buffer and using commercial or in-house LPA coated capillaries (Fig. 3-3). In this figure it can be observed that the EOF (peak 20-30 minutes) was changing when doing multiple experiments, because of modification of the coating from run to run. This was observed even if the separation of anions was good (peaks before 5 minutes); the EOF still changed depending on the reproducibility of the coating. In this case, the coating may be a combination of both coatings (LPA and PDDMAC).

Since it was observed that coating with PDDMAC influenced EOF, a set of experiments using just silica capillaries and coating and re-coating before each run with PDDMAC was done (Fig. 3-4). The results obtained when re-coating before each run showed that the EOF was increasing in magnitude and decreasing in time. This meant that an increasing coating was obtained progressively. Therefore, it was decided to leave the capillary standing overnight filled with coating solution in order to reach equilibrium, and then re-coating before each run in order to obtain stable, reproducible anion separations. The resulting improvement is clearly shown in electropherogram B of Fig 3-5.

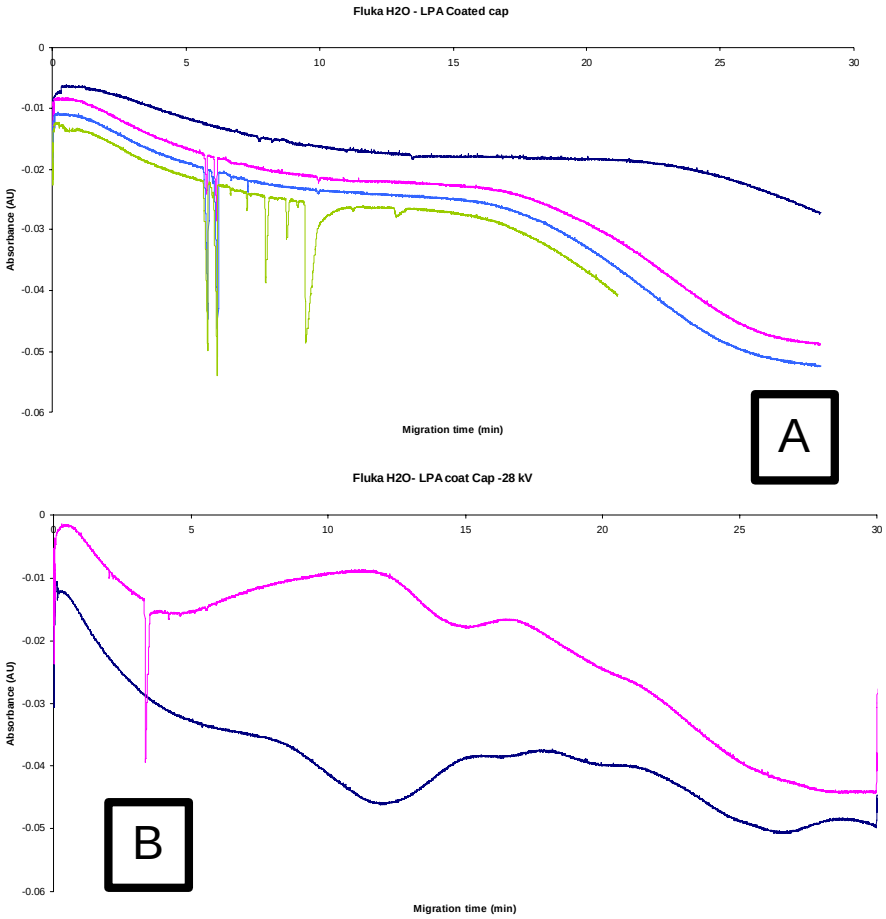

Figure 3-1
Commercial Microsolv LPA coated capillaries (A) analysis with new capillary at -20 kV (B) analysis with capillary after 2 hrs use at -28 kV

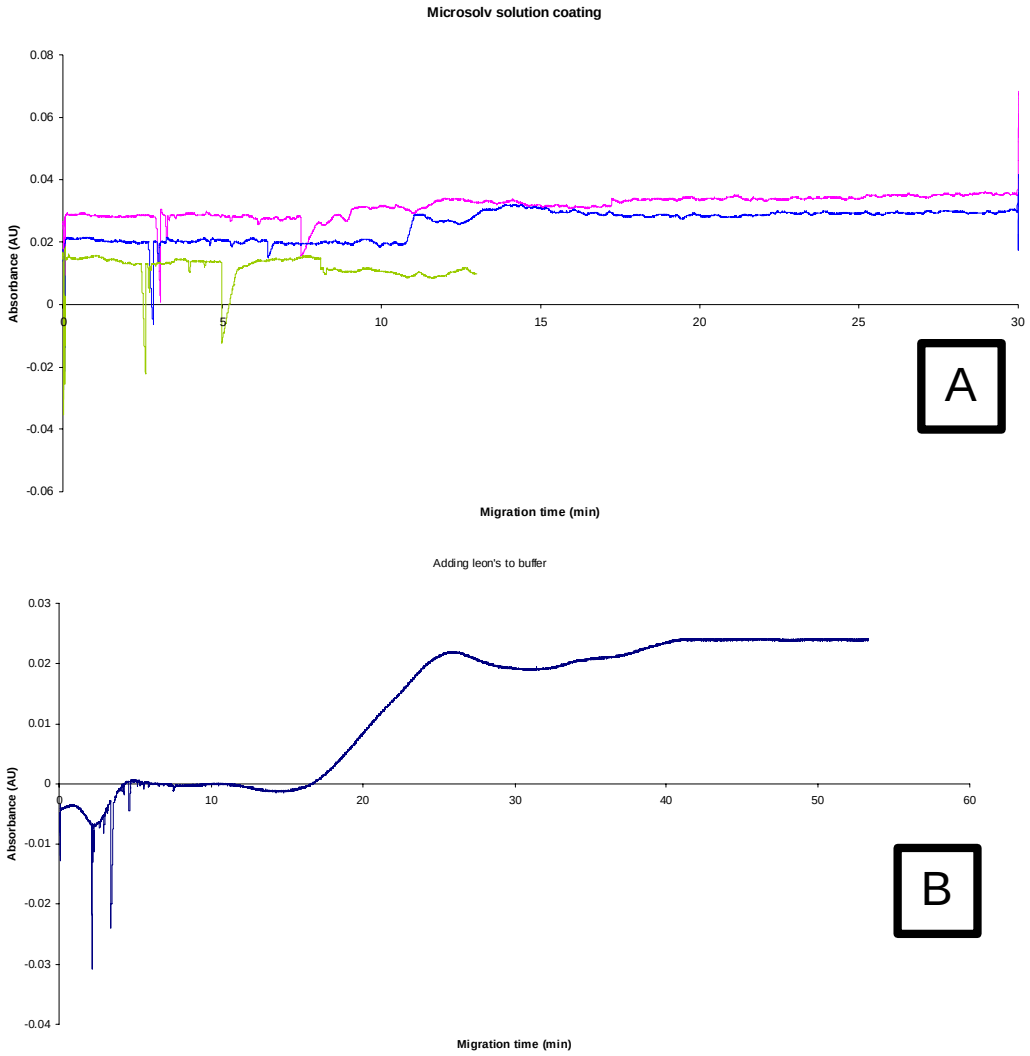

Figure 3-2
Bare silica capillaries with coating agents added to the buffer (A) adding commercial LPA solution to the buffer (B) concentrated PDDMAC commercial coating solution

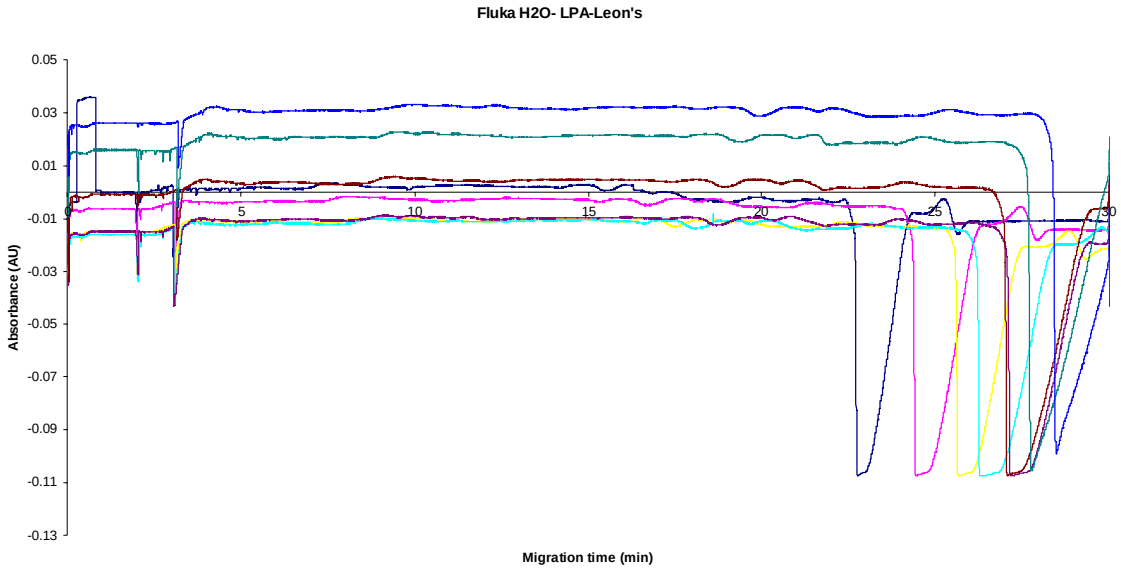

Figure 3-3
Microsolv LPA coated capillary and adding PDDMAC solution to the buffer

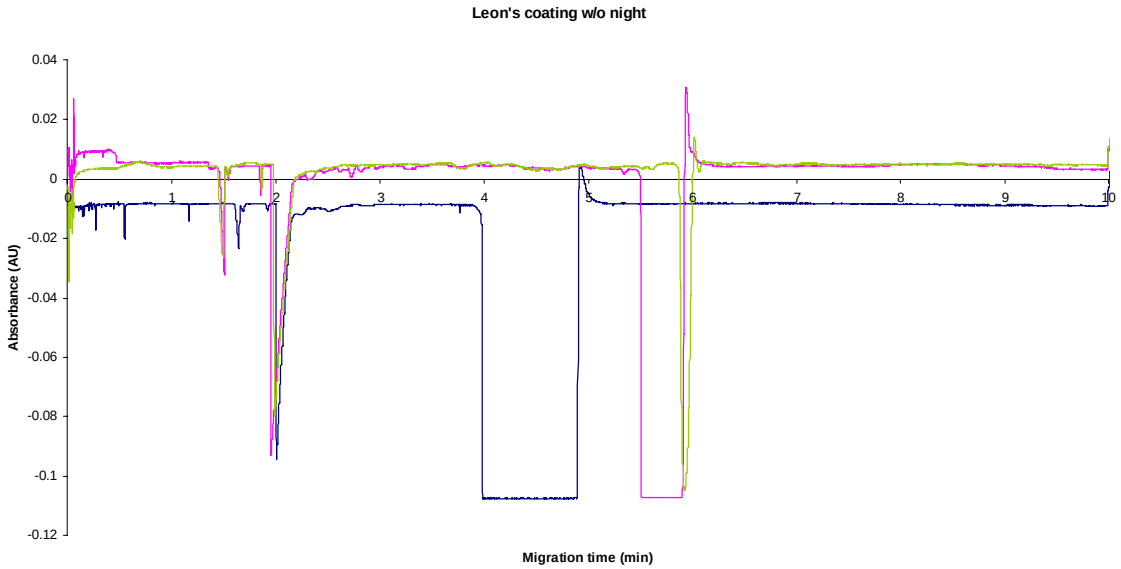

Figure 3-4
Bare silica capillary coating before each run with a solution of PDDMAC

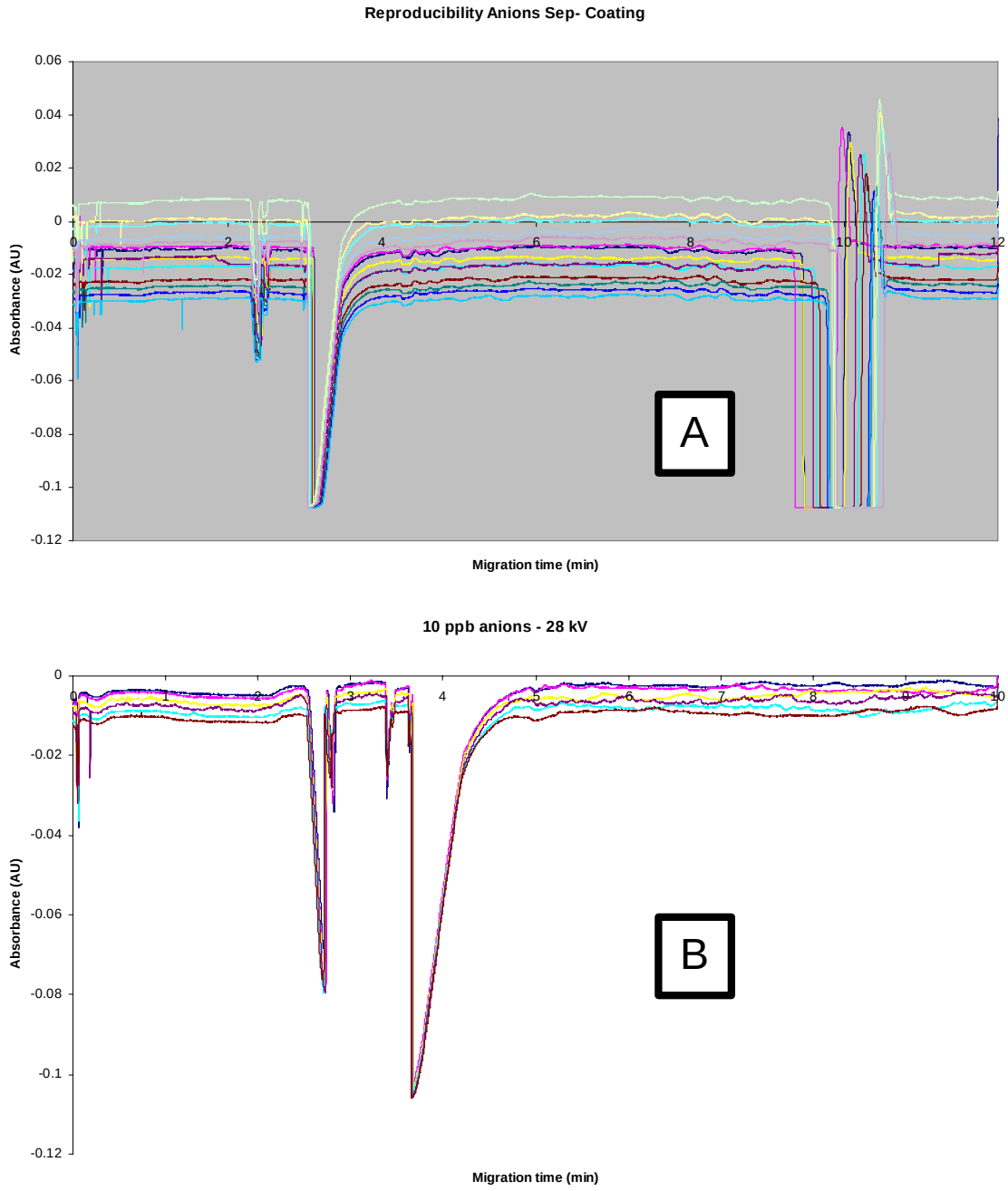

Figure 3-5
(A) bare silica capillary coated before each run with a solution of PDDMAC (B) bare silica capillary coated overnight and before each run

At this point of experimentation, the results were very encouraging as the baseline was very stable, the polymerization, injection, intensity of signals and reproducibility issues were under control, but the resolution was noticeably decreased (Fig 3-6 B) compared to the very first experiments (Fig 3-6 A). The main factors possibly influencing resolution are: buffer viscosity, separation voltage and/or capillary coating. Taking the best conditions for baseline stability, the next experiments were focused on adding gels to the buffer (viscosity modification) looking for

improved resolution (Fig. 3-7). As the resolution didn't increase by adding modifiers to the PDDMAC coated capillaries, the original buffer was then used (5 mM SQ + 20 mM Tris, pH 8.2) but different voltages were tried (Fig. 3-8). The conclusions were that the peak resolution was strongly affected by the type of coating and was not improved by varying voltage or using buffer modifiers. Therefore, a study focused on the PDDMAC was performed.

Initially a PDDMAC coated capillary was rinsed with NaOH 1M in order to have a clean internal surface to continue with experimentation, and then was rinsed with buffer and then anion analysis was performed. These results were surprising as the resolution was extremely high and the first peaks (Cl and SO_4 ions) appeared after 12-15 minutes and the carbonate peak was appearing after more than 30 minutes. Poor reproducibility was due to the NaOH rinse. A rinse with HCl acid was then used in an effort to neutralize the hydroxide excess. Surprisingly, the separations were very reproducible and stable. Several runs were done and the RSD of the migration time of the peaks was about 99%.

Despite the baseline stability, and that NaOH and HCl acid rinses were necessary to get reproducible separations, higher resolution was still needed. A set of experiments was then performed by modifying the PDDMAC coating concentration (Fig. 3-9) as it seemed to be the next step for resolution improvement. From the above figures, and several additional experiments not shown in this report, it was observed that the coating concentration and type of acid used to rinse were the main factors to achieve proper resolution and reproducible electropherograms. Other acids were then used for rinsing before each experiment (Fig. 3-10).

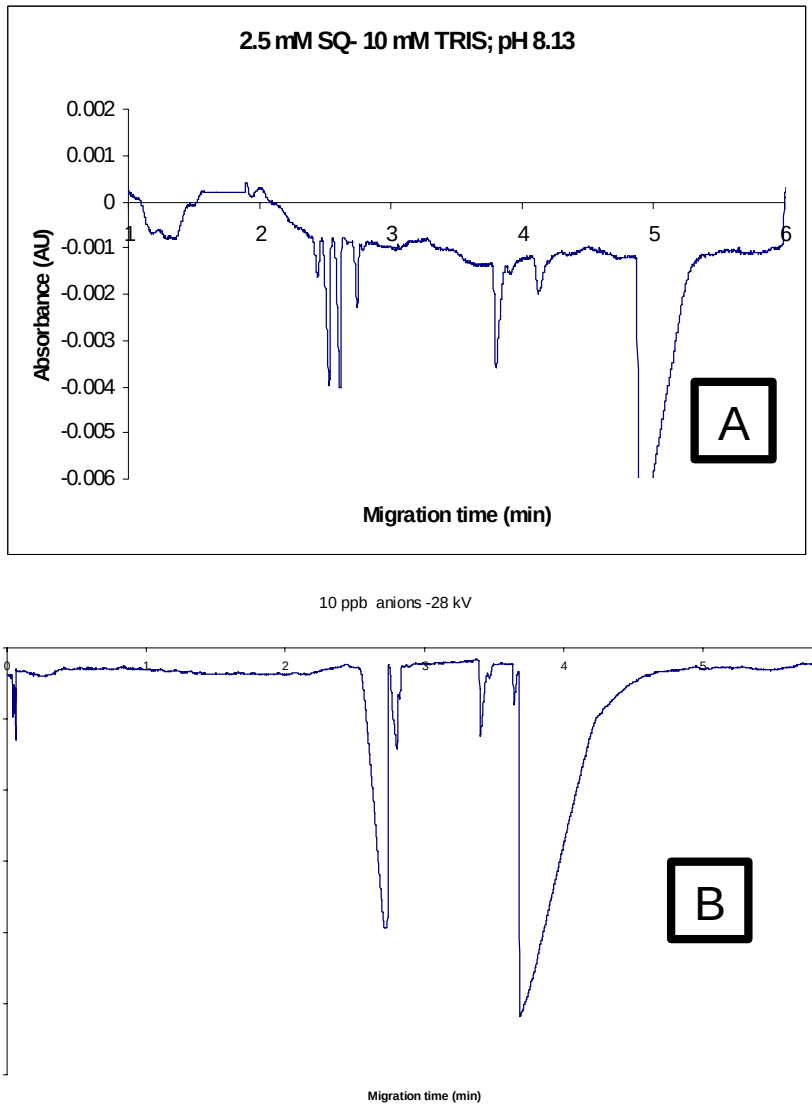

Figure 3-6
CE anion separations at -28 kV. Sample: 10 ppb (A) Earlier electropherogram using BioRad LPA coated capillary (B) Coating overnight and before each run with a solution of PDDMAC

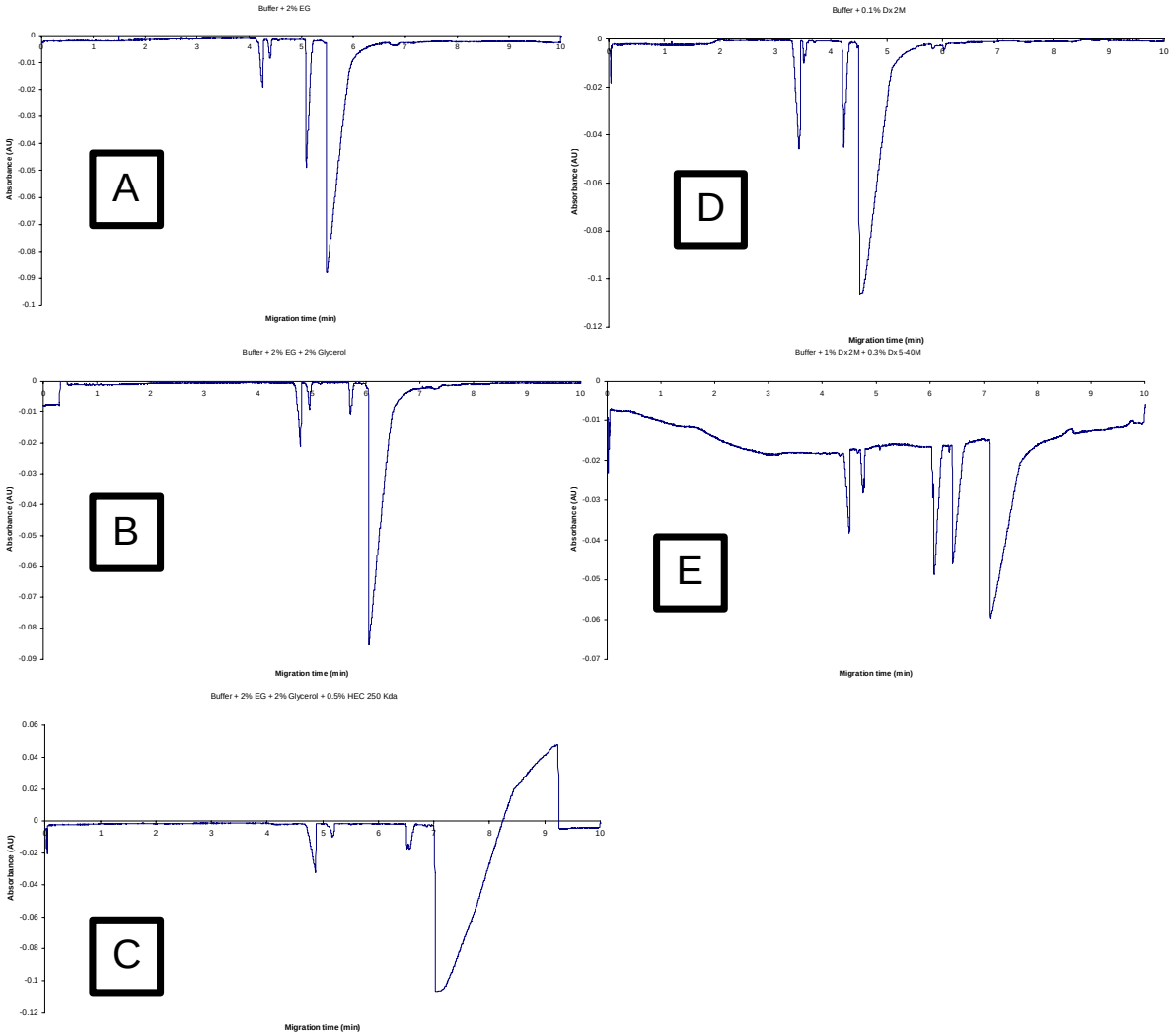

Figure 3-7
Resolution study in PDDMAC coated capillaries. Addition of different buffer modifiers in order to increase resolution A: 2% EG, B: 2% EG + 2% Glycerol, C: 2% EG + 2% Glycerol + 0.5% HEC 250kDa, D: 0.1% Dx 2M and E: 1% Dx 2M + 0.3% Dx 5-40M

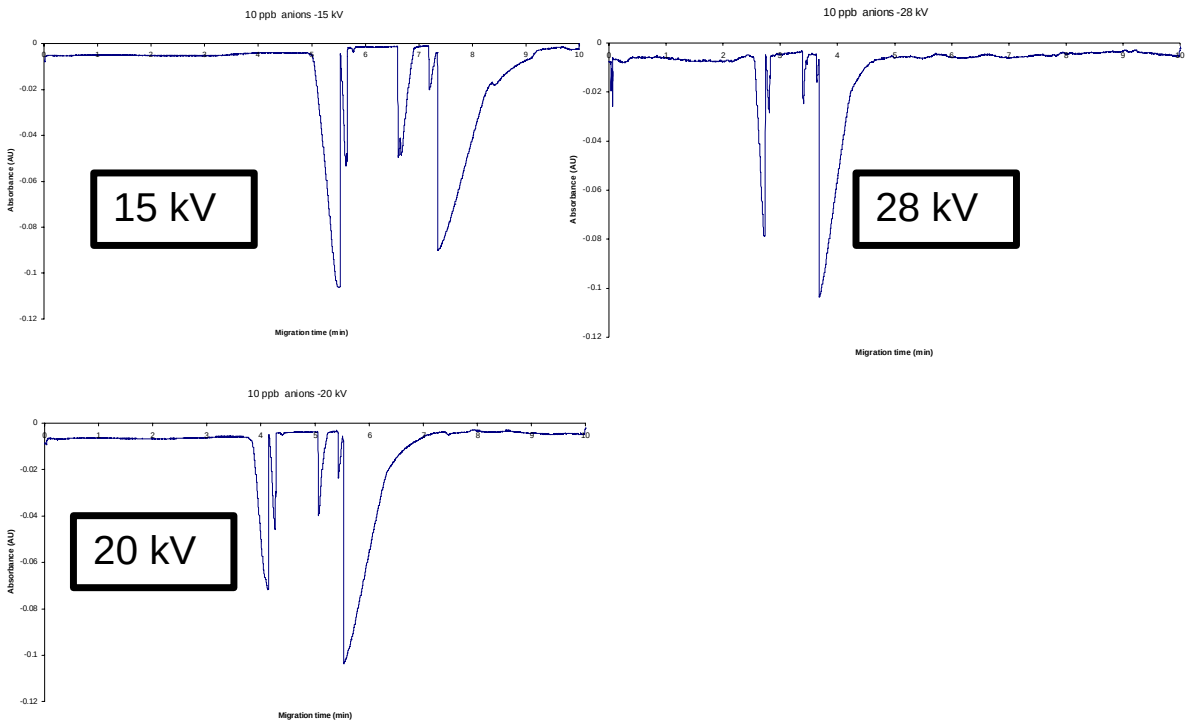

Figure 3-8
Resolution study
Different separation voltages using SQ-TRIS buffer pH 8.2, 10 ppb anions standard

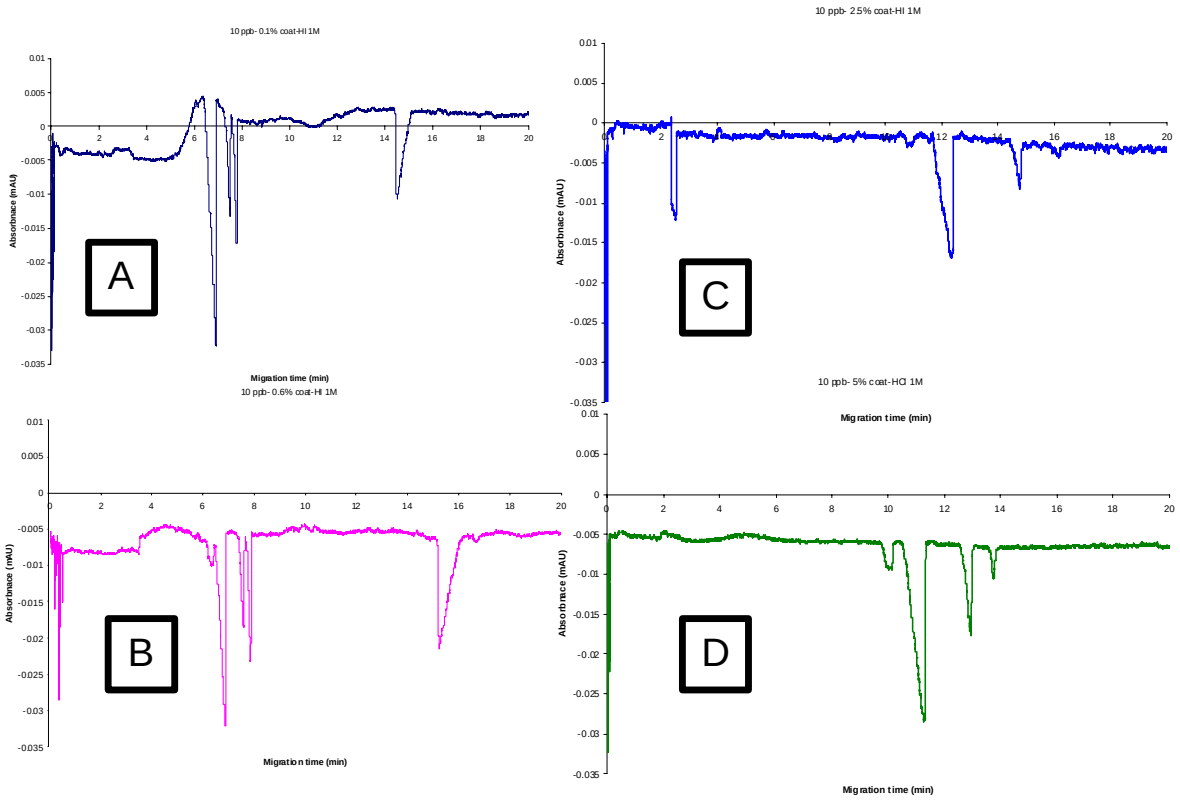

Figure 3-9
Coating (PDAAC) concentration study (A) 0.1% and HIO_3 rinse, (B) 0.6% and HIO_3 rinse, C: 2.5% and HIO_3 rinse, D: 5% and HCl rinse. SQ-TRIS buffer, pH 8.2; 10 ppb anions standard

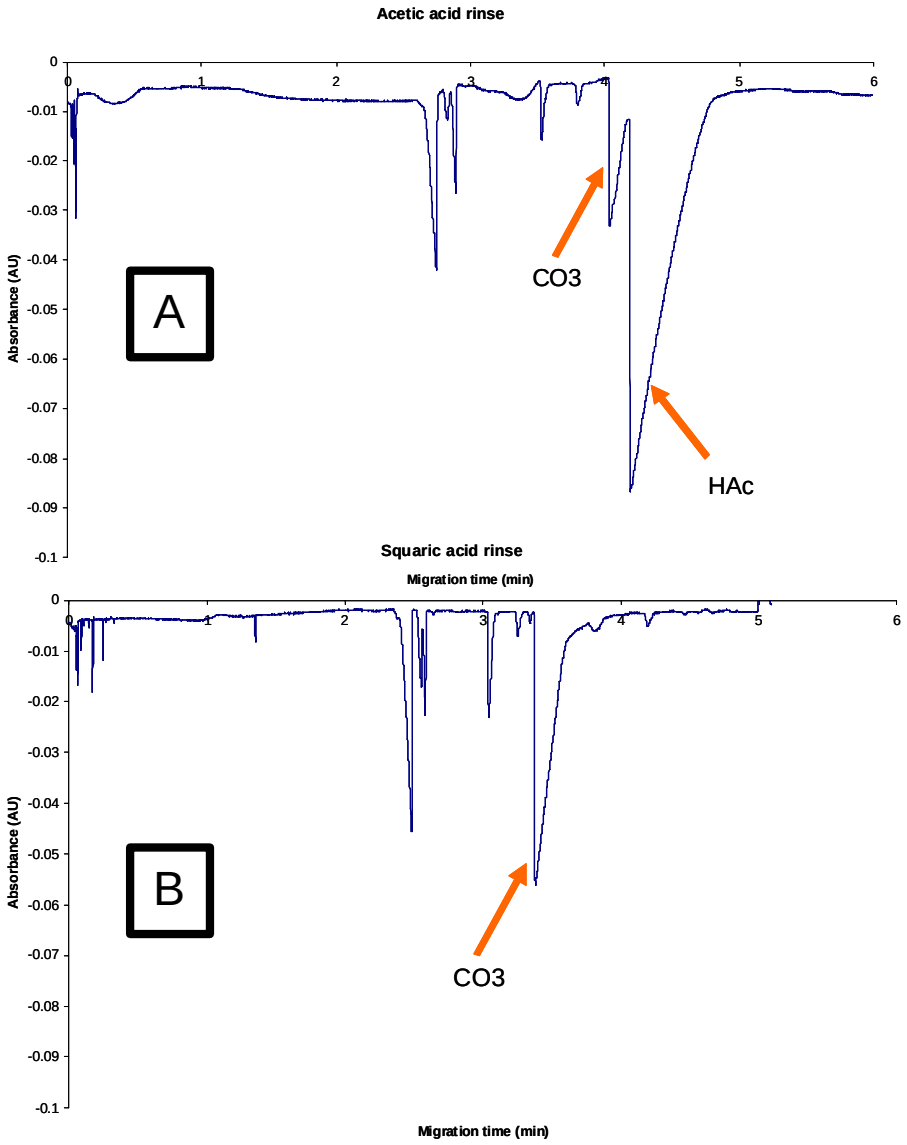

Figure 3-10
Post-coating acid rinse study
(A) Acetic acid 1M rinse and (B) Squaric acid saturated sol'n rinse SQ-TRIS buffer, pH 8.2; 10 ppb anions standard

In general it was observed that the baseline stability and signal intensities were directly affected by the acid used. Also, a peak of the counter-ion of the acid used appeared in the electropherogram; sometimes overlapping other peaks.

During the acid-base rinsing optimization, some experiments were also done varying the coating time, the acid and base concentrations, rinsing time, rinsing the coated capillary with only acid or with only base, etc. The optimum results were achieved using the methods detailed in Tables 3-1 through 3-3.

Table 3-1
New capillary activation

Solution	Pressure/Voltage	Time (min)
NaOH 1M	1500 mbar	15
Water	1500 mbar	10
Coating 0.5%	1500 mbar	15
Coating 0.5%	0 mbar	15

Table 3-2
Capillary conditioning (recommend running twice)

Solution	Pressure/Voltage	Time (min)
Water	1500 mbar	5
HIO_3 0.1M	1500 mbar	10
Water	1500 mbar	5
Buffer	1500 mbar	15
Separation	-20 kV	15

Table 3-3
Electrophoretic anion analysis

Solution	Pressure/Voltage	Time (min)
HIO_3 0.1M	1500 mbar	3
Buffer	1500 mbar	1
Buffer	1500 mbar	2
Sample Injection	-5 kV	1
Separation	-20 kV	7

When setting up a new bare silica capillary in the CE instrument, the **"Activation" and "Conditioning"** methods are always used. For day-to-day analyses, only the **"Electrophoretic anion analysis"** method is used.

It is important to keep a record on the total time the electrodes have been used. The electrodes must be rinsed in concentrated nitric acid for 10 minutes after each 3 hours of use. After cleaning the electrodes it is recommended that the **"Capillary Conditioning"** method should be run two times.

At the end of the workday, the capillary should be rinsed for 5 minutes with water, and then the capillary should be filled with 0.5% PDDMAC coating solution and let to stand overnight. At the

beginning of the day the **"Capillary conditioning method"** should be done once before the first analysis.

Under these optimal conditions the electropherograms shown in Fig. 3-11 were obtained. Reproducibility of the electropherogram of a 1 ppb anion solution under these optimum conditions is shown in Fig. 3-12.

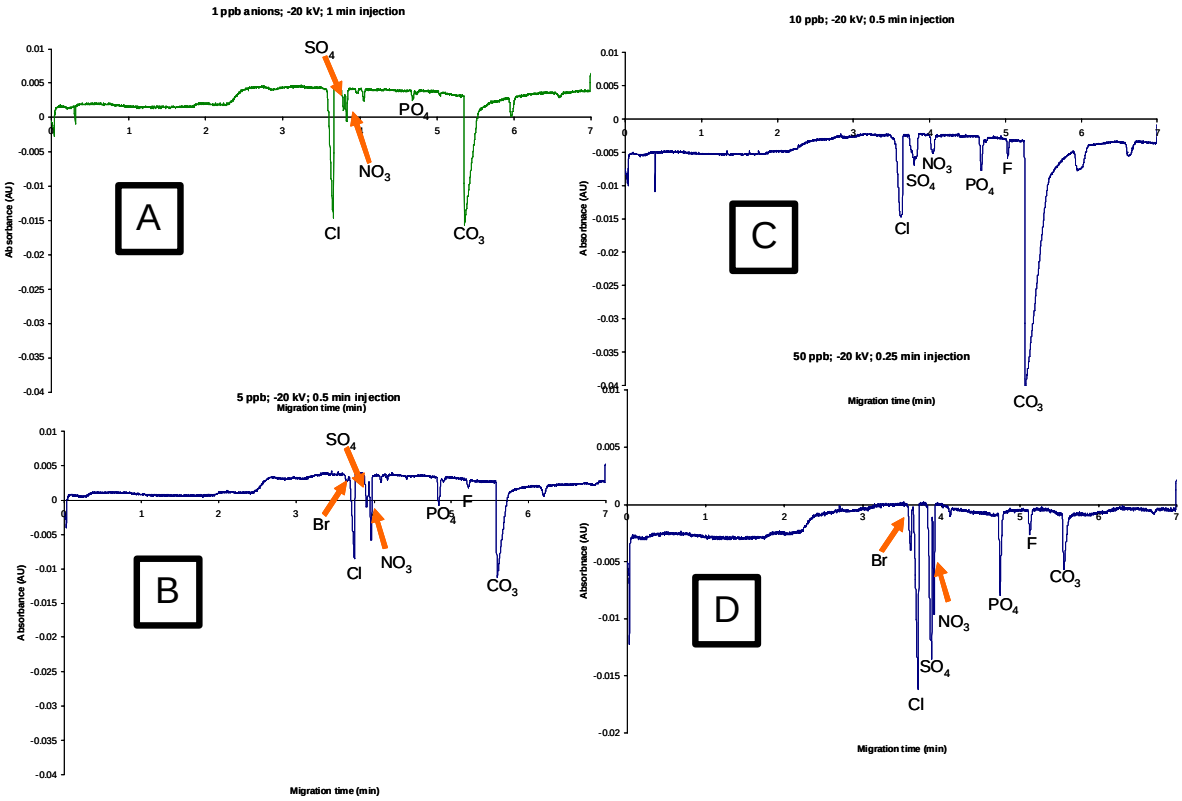

Figure 3-11
Anion CE analysis. All experimental conditions were the same except for the time for sample injection. A: 1 ppb, B: 5 ppb, C: 10 ppb, and D: 50 ppb

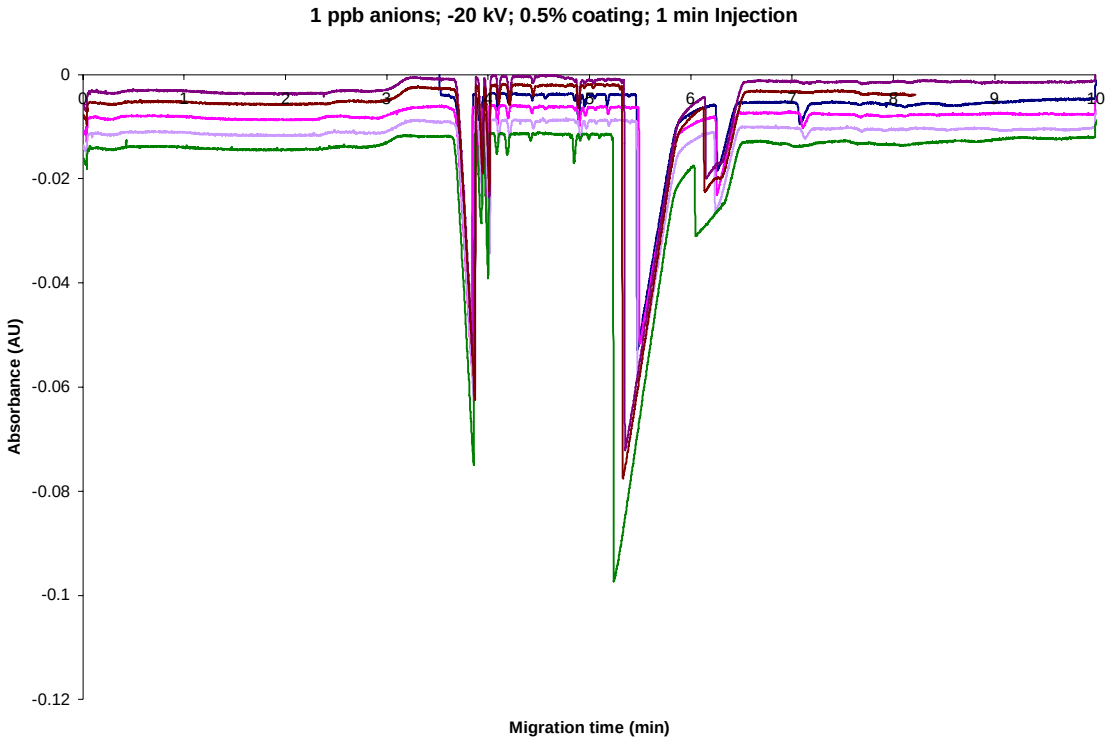

Figure 3-12
Reproducibility study. Conditions: 1 ppb anions std, 0.5% coating (PDAAC) and HIO_3 rinse, 1 min injection at -5 kV, -20 kV separation, SQ-TRIS buffer, pH 8.2

When running very pure samples some extraneous peaks were always observed. These interferences were especially troublesome when analyzing for chloride and sulfate. In fact, when running the baseline (by injecting buffer only) a peak with migration time similar to chloride was always present. Therefore, some studies were done to find the source of these peaks. After several attempts, the only consistent way to get rid of this extraneous signal was to avoid coating the capillary before each run and rinsing for a longer period of time with iodic acid after a new capillary had been coated. The optimum methods for activation, conditioning, and anion analysis were therefore slightly modified to reflect these changes. The sensitivity of the electropherograms for chloride and sulfate using these modified analysis conditions is shown in Fig. 3-13.

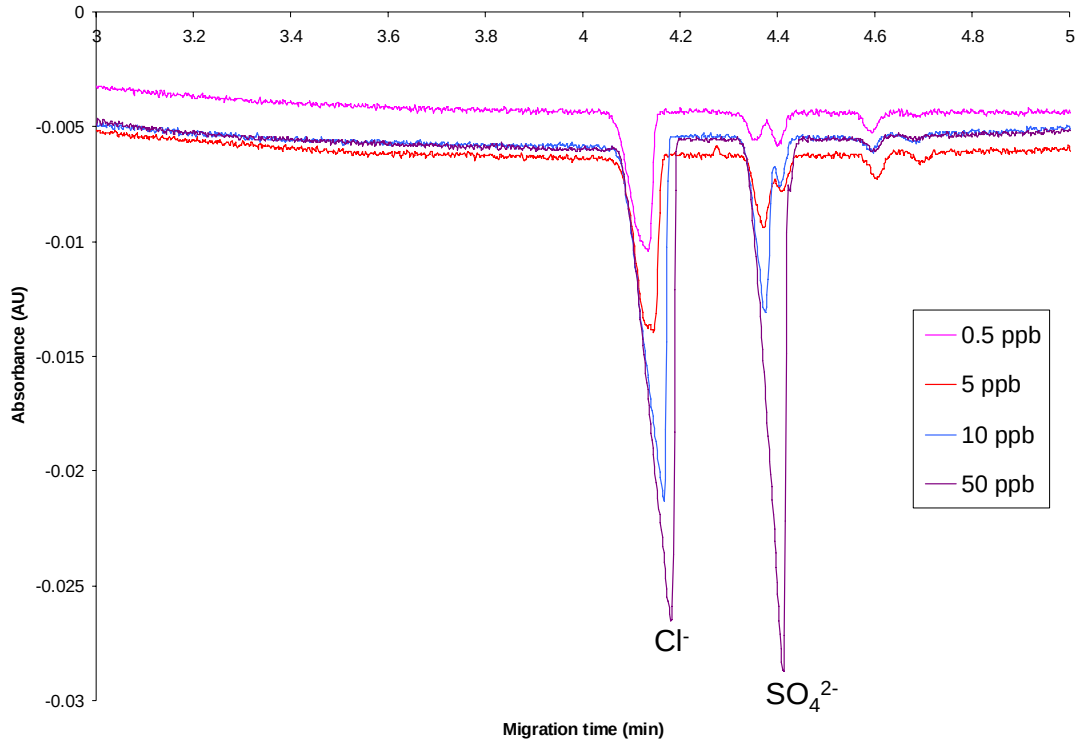

Figure 3-13
Concentration dependence of chloride and sulfate peaks

Quantification

The calibration curves for each anion of interest resulting from these studies are shown in the next series of graphs, Fig. 3-14 through Fig. 3-19. Each data point on these graphs is the average of three determinations. The limit of detection (LOD) and limit of quantification (LOQ) for each anion were estimated from these graphs, and from the Fig. 3-11 and Fig. 3-13 electropherograms that were made under the best experimental conditions. The LOQ has been arbitrarily defined as 10x the LOD. The LOD and LOQ values are summarized in Table 3-4.

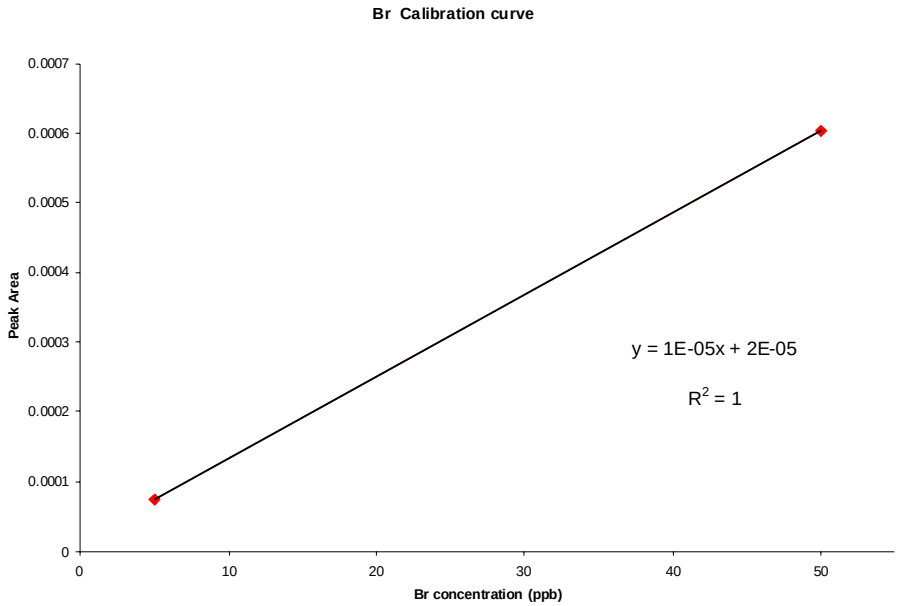

Figure 3-14
Calibration curve for bromide

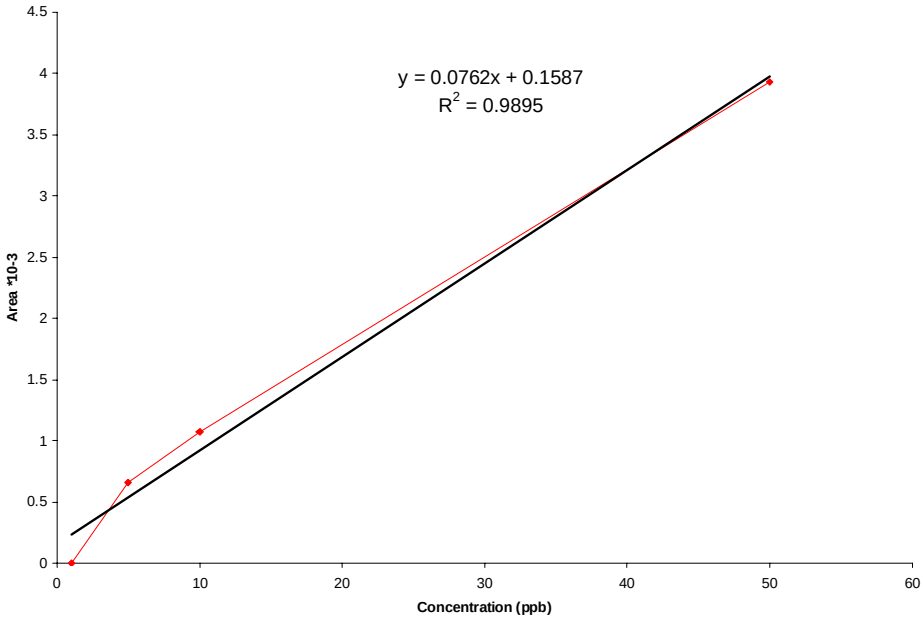

Figure 3-15
Calibration curve for chloride

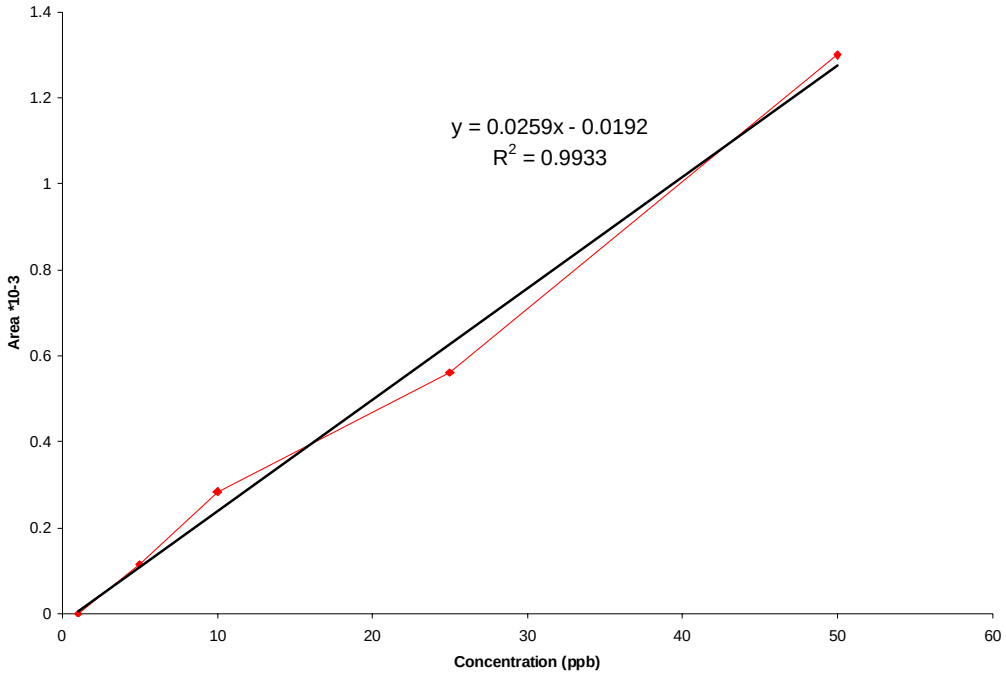

Figure 3-16
Calibration curve for sulfate

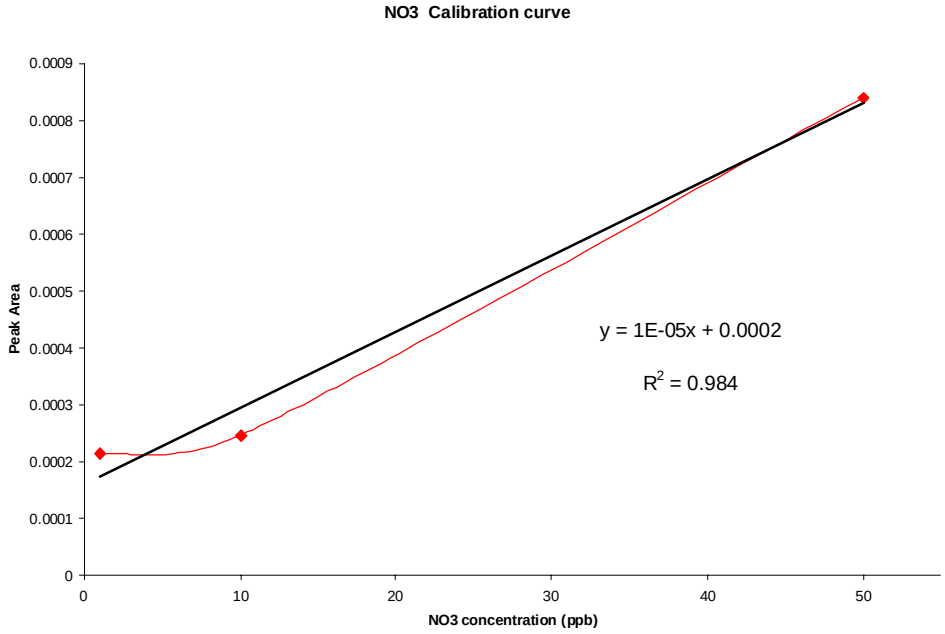

Figure 3-17
Calibration curve for nitrate

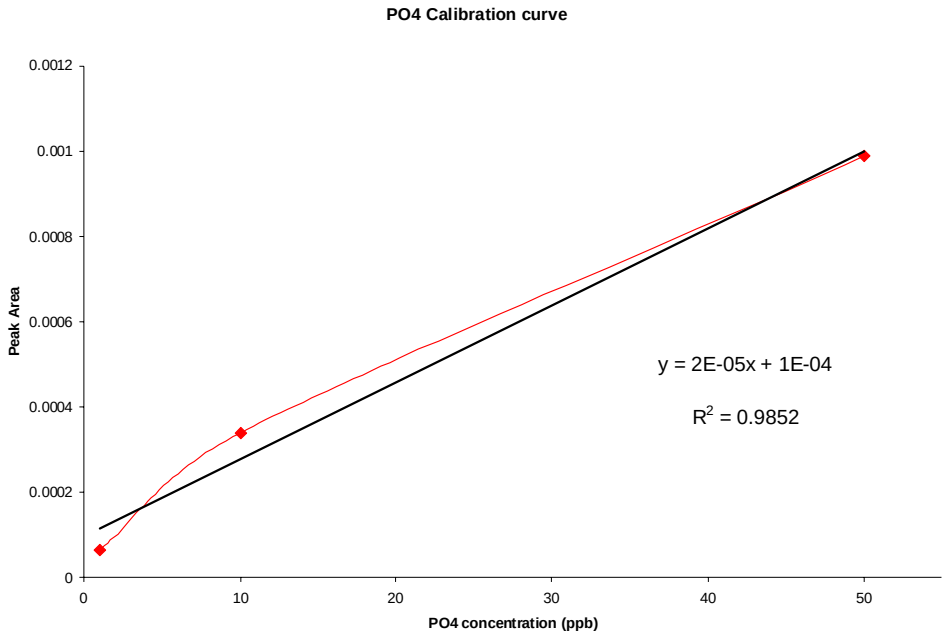

Figure 3-18
Calibration curve for phosphate

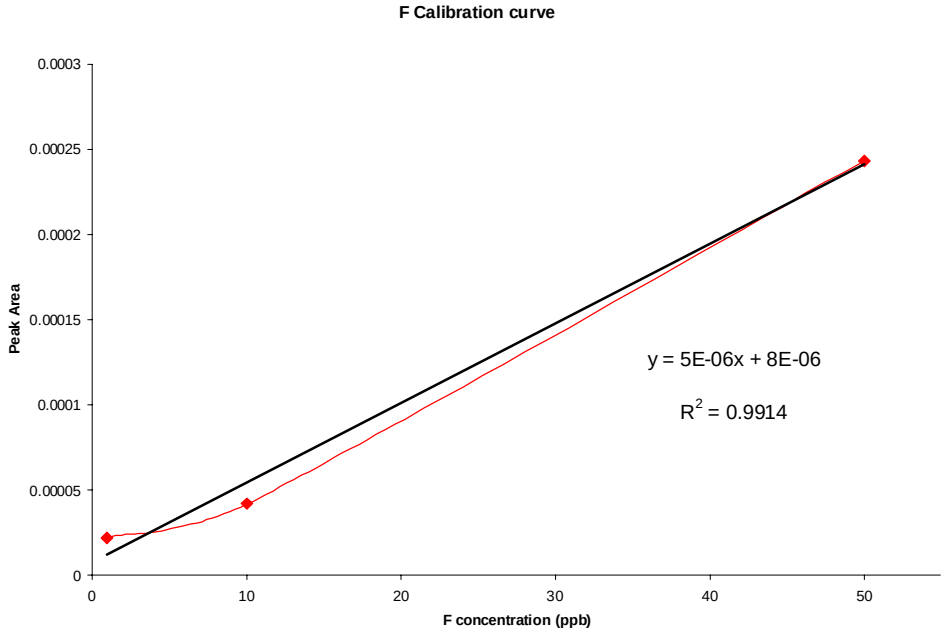

Figure 3-19
Calibration curve for fluoride

Table 3-4
Optimum detection and quantification limits for selected anions

Anion	LOD (ppb)	LOQ (ppb)
Bromide	2.0	20
Chloride	0.05	0.5
Sulfate	0.75	7.5
Nitrate	1.0	10
Phosphate	5.0	50
Fluoride	1.6	16

4
CONCLUSIONS

Cleaning electrodes

The electrodes should be cleaned for ten minutes with concentrated nitric acid after approximately every 3 hours of use. If this is not done, the deposits of polymerized probe chemical will cause the current to fluctuate, the injections will be non-reproducible, and even the baseline will be altered and non-reproducible.

Baseline drift

After testing several modifiers and coatings, the best stable and "flat" baselines are obtained by coating with 0.5% PDDMAC before each run and overnight. The optimal methods are described in this report and these procedures give reproducible injections (with 99%) even for 1 ppb anions solutions.

Resolution

After coating and re-coating the capillary, it must be rinsed with acid solution in order to get reproducibility and resolution of the anion peaks. Iodic acid was chosen as the best acid for this purpose because the acid used for rinsing interacts strongly with the coating. If the rinse is done with nitric acid, then the nitrate peak increases and overlaps the other peaks. If hydrochloric acid is used, then the chloride peak increases and overlaps the other peaks. The same effect was observed with other acids such as acetic, squaric, etc. Without acid rinsing there is neither stability nor reproducibility in the separations.

While doing these experiments it was observed that if the capillary is rinsed with NaOH after the coating and before the acid rinse, then the resolution is greatly increases and the 7 anions migrate between 20-50 minutes. The option of rinsing with NaOH was eliminated however, because reproducibility was poor even though resolution was improved.

Sample carbonation

It was observed that when analyzing samples that were prepared the pervious day or earlier, the injections were always reproducible, even for the first run. Since these samples would be saturated with CO_2 from the atmosphere, is may be concluded that samples having sufficient conductivity through carbonate absorption, or saturated with carbonate, allow reproducible injections.

Peak identification

Single-ion spiking was done to confirm peak identification. The migration order of these experiments was the same as had been observed before, namely: Br, Cl, SO_4, NO_3, PO_4, F and carbonate.

Water quality

Experiments done with commercial ultrapure water gave different intensities/peaks when compared to runs using other water of different quality. Therefore, the analysis is dependent on the quality of the water and the anions present and a "water standard" will be necessary for routine analysis.

Limits of detection and quantification

Anions solutions were injected at 1, 5, 10 and 50 ppb in order to estimate the limits of detection (LOD) and quantification (LOQ). The LOD for the six anions of interest are: Br: 2 ppb, Cl: 0.05 ppb, SO_4: 0.75 ppb, NO_3: 1 ppb, PO_4: 5 ppb and F: 1.6 ppb. The linearity of the six calibration curves was between 98-99%. The limits of quantification were arbitrarily assigned as ten times the LOD's, so these values are: Br: 20 ppb, Cl: 0.5 ppb, SO_4: 7.5 ppb, NO_3: 10 ppb, PO_4: 50 ppb and F: 16 ppb. It should be noted, however, that significant quantitative information can still be gained for sample concentrations below these LOQ values.

Generic anion procedure

Capillary Electrophoretic anion analyses are done using a Groton Biosystems GPA100 CE instrument equipped with a Bischoff variable UV/Vis wavelength detector and data collected and analyzed with DAx 8.0 software.

An 80.5 cm bare silica capillary is installed in the instrument with the window (burning the coating) at 49 cm from the inlet side. The internal diameter of the capillary should be 75 um. Prior to CE analysis, the capillary must be activated, conditioned and internally coated. The coating is necessary as it reverses the electro osmotic flow allowing the anions separation.

When the capillary is coated, rinsed and ready to analyze anions, then a buffer solution is prepared with squaric acid and TRIS salts (pH 8.2). This buffer highly absorbs at 270 nm and is used to carry out the separations in a constant pH and conductivity media.

The anion CE analyses are then performed at constant temperature (25°C controlled by the instrument) and applying -20,000 kV to force migration of anions and pass through the detector. All this procedure is automated as well as the data collection by a computer.

All the solutions need to be prepared, stored and analyzed in plastic tubes, vials, bottles, caps, tips and containers in order to avoid contamination. The vials, caps and tubes for sample preparation should be previously rinsed with hydrochloric acid 0.1M and ultrapure water.

5
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