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New preconditioning strategy for the determination of inorganic anions with capillary zone electrophoresis using indirect UV detection

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Abstract

It is widely accepted that preconditioning procedures are indispensable in capillary electrophoresis in order to achieve reproducibility of migration times and peak areas. Several preconditioning strategies have been employed for electrophoretic determinations of inorganic anions using indirect UV detection including simple flushing with buffer or alkaline or acid pre-rinsing followed by flushing with electrolyte. We investigated the influence of various preconditioning strategies on the reproducibility of migration times and peak areas of inorganic anions. The electrolyte systems for indirect UV detection were based on pyromellitic acid and chromic acid respectively as UV absorbing probes and hexamethonium hydroxide as electroosmatic flow modifier. Preconditioning agents under investigation were electrolyte buffer, NaOH, HCl and the free acids of the UV absorbing probes. Investigations showed that reproducibility of migration times and peak areas can be significantly improved by acid pre-rinsing using the corresponding acid of the UV absorbing probes compared to preconditioning by flushing the capillary with buffer. In contrast to acid pre-rinsing using hydrochloric acid no interfering signals within the migration time window of inorganic anions under investigation can be observed. The optimized preconditioning procedure yields relative standard deviations of migration times less than 0.25% ($n=10$). Relative standard deviations of corrected peak areas were below 5% applying acid preconditioning using pyromellitic acid.

Keywords: Inorganic anions; Indirect detection

1. Introduction

Capillary zone electrophoresis (CZE) has become a routine method for the determination of inorganic anions. For routine analysis it is indispensable to keep migration times constant in order to allow automatic peak identification by means of commercial data analysis software. Since inorganic anions have very similar electrophoretic mobilities their migration times are very close. Therefore automatic peak identification and quantification is only possible if relative standard deviations of migration times are less than 0.5%.

For the determination of anionic species by CZE the electroosmotic flow (EOF) must be reduced or reversed in order to reduce time of analysis. Various modifiers have been used for this purpose [1–4]. Modifiers are usually added to the electrolyte and adsorb dynamically to the inner wall of the capillary. The equilibrium between the free modifier in the

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electrolyte and the adsorbed modifier plays the most important role with respect to the velocity of the EOF and in consequence to the migration times of the analytes. In order to maintain a constant equilibrium during a sequence of measurements various methods of preconditioning have been applied [5–7]. Ehmann et al. performed systematic investigations dealing with the influence of pre-rinsing steps on the reproducibility of migration times of anions using various flow modifiers [4]. The authors showed that acid pre-rinsing improves reproducibility of migration times significantly. Especially for EOF modifiers based on hexamethyl alkyl diammonium salts (e.g. hexamethonium salts) acid pre-rinsing is preferable. The only disadvantage of this preconditioning method is the introduction of chloride into the capillary. Chloride ions can interfere with the determination of anions in the sample, especially if small concentrations have to be determined. The authors suggested the use of propionic acid for pre-rinsing. Propionate has a lower electrophoretic mobility and therefore it does not interfere with the determination of anions. Nevertheless system peaks can arise from cross-contaminations that may interfere with the determination of analytes of interest. The studies presented in this work start the problem from a different point of view. We tried to use the acids, which are frequently used as UV absorbing probes for acid pre-rinsing of fused silica capillaries. The advantage of acids, which are simultaneously used as UV absorbing probes, is the fact that residues of the preconditioning agent do not interfere with the determination of inorganic anions since no change in UV absorbance occurs. In our studies two electrolyte systems were investigated: pyrommelitic acid as UV absorbing probe buffered with triethanolamin and chromic acid buffered with Tris. With both electrolyte systems hexamethonium was used as EOF modifier. The aim of the studies presented here was to investigate pyromellitic acid and chromic acid as preconditioning agents. The results will be discussed in the following sections.

2. Experimental

2.1. Apparatus

All experiments were carried out using a HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany). The capillary was thermostated at 25°C. Fused silica capillaries [64.5 cm (56 cm effective length)×75 μm I.D.] were purchased from Hewlett-Packard. Samples were introduced hydrodynamically applying a pressure of 50 mbar for 4 s. The electrolyte was replenished after each measurement.

2.2. Chemicals

Chemicals were purchased from various suppliers and were of analytical grade or better. All solutions were prepared in Milli-Q water (18.2 MΩ). A stock solution containing 1 mg/ml of bromide, chloride, sulfate, nitrate and fluoride and 20 mg/ml phosphate supplied by Hewlett-Packard was used. Multi-anion standards with a concentration of 10 μg/ml of bromide, chloride, sulfate, nitrate and fluoride and 20 μg/ml phosphate were prepared daily from the stock solution by dilution with Milli-Q water. The electrolytes under investigation were prepared daily, filtered (0.2 μm, polyamide) and degassed in an ultrasonic bath for 15 min prior to use. Two electrolyte systems were investigated: the first consisted of 2.25 m*M* pyromellitic acid, 1.6 m*M* triethanolamine, 6.5 m*M* NaOH, 0.75 m*M* hexamethonium hydroxide. The pH of this buffer is 7.7. The second buffer consisted of 4 m*M* chromic acid, 5 m*M* Tris and 0.75 m*M* hexamethonium hydroxide. The pH was adjusted to 8.2 using NaOH.

New capillaries were conditioned for 30 min by flushing with 1 *M* NaOH followed by conditioning for 30 min with electrolyte by applying a pressure of 1 bar. Capillaries conditioned in that way were used for determinations without further treatment.

In order to achieve maximal reproducibility of results the electrolyte was replenished before each measurement. Preconditioning of the capillary surface was performed parallel to replenishment of the running buffer before each run. The total preconditioning time was set to 3.5 min in order to compare efficiency of preconditioning methods with respect to similar time consumption. Alkaline and acid pre-rinsing was performed for 30 s corresponding to approximately one capillary volume. Consequent flushing with electrolyte was then performed for 3 min corresponding to six capillary volumes. Between both flushing steps the inlet of the capillary was dipped into water in order to minimize contaminations of the electrolyte. Preconditioning by flushing with buffer was performed for 3.5 min corresponding to seven capillary volumes.

3. Results and discussion

Determinations of inorganic anions using capillary electrophoresis are usually performed with addition of EOF modifiers in order to reduce analysis time. Re-establishment of the equilibrium between the EOF modifier in the electrolyte and the EOF modifier at the inner capillary wall is of critical importance in order to achieve reproducibility of migration times. In fact there are three different pre-rinsing procedures published: either purging with electrolyte, alkaline pre-rinsing followed by purging with electrolyte, or acid preconditioning followed by purging with electrolyte. Most of the published procedures use alkaline pre-rinsing to re-establish the equilibrium between the EOF modifier in the electrolyte and the internal capillary surface. However study of the literature indicates that no standardized procedure for preconditioning of the capillary for the determination of anions has been evaluated to date.

During our studies we investigated various preconditioning procedures using an electrolyte based on pyromellitic acid and hexamethonium hydroxide as EOF modifier. We compared the influence of preconditioning procedures on the reproducibility of migration times and peak areas. Another topic was to eliminate possible cross-contaminations arising from the pre-rinsing step.

3.1. Purging with electrolyte

Preconditioning of the capillary surface by purging with electrolyte is one of the most frequently applied procedures for the determination of anions by capillary zone electrophoresis. The advantage of this procedure is its simplicity, the low time consumption for preconditioning and the fact that no change of pH in the capillary occurs. The disadvantage is the fact that during repetitive measurements migration times of the anions increase steadily (Fig. 1). Taking fluoride or phosphate as migration reference peaks allows automatic peak identification. Nevertheless the steady increase of migration times causes increasing time consumption for repetitive measurements. The reason is the competition between the cationic surfactant and the Na^+ ions of the electrolyte for the negatively charged silanol groups at the capillary wall. With repetitive measurements the equilibrium concentration of EOF modifier at the capillary wall decreases. As a consequence the electroosmotic flow moving in cathodic direction increases and therefore migration times of anions increase. During 10 repetitive measurements the relative standard deviation of migration times vary from 0.9% for ions with a high electrophoretic mobility (chloride, sulfate, nitrite and nitrate) to 3% for slow ions (fluoride and phosphate). The relative standard deviations of migration times and peak areas of the anionic species under investigation are summarized in Table 1.

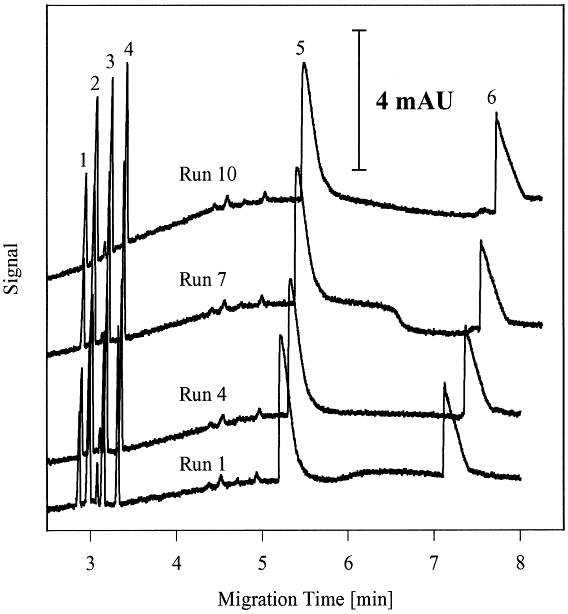

Fig. 1. Effect of preconditioning by flushing with electrolyte prior to analysis on migration times of inorganic anions. Electrolyte: 2.25 m*M* pyromellitic acid, 6.5 m*M* NaOH, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethoniumhydroxide, pH 7.9; injection: 200 mbar˙s of standard solution containing 10 μg/ml bromide (1), chloride (2), sulfate (3), nitrate (4), fluoride (5) and 20 μg/ml phosphate (6); voltage −30 kV, capillary 64.5 cm×75 μm; detection wavelength: 350 nm (signal), 245 nm (reference).

Using chromate as UV absorbing probe similar effects can be observed although the migration shift is less pronounced. The relative standard deviations of migration times are between 0.62% for highly

Table 1
Relative standard deviations of migration times and peak areas of inorganic anions applying various preconditioning techniques. Background electrolyte: 2.25 m*M* pyromellitic acid, 6.5 m*M* NaOH, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethoniumhydroxide, pH 7.9

Preconditioning method		Relative standard deviation (%) ($n=10$)					
		Bromide	Chloride	Sulfate	Nitrate	Fluoride	Phosphate
Flushing with electrolyte	Migration time	0.9	0.9	1.0	1.0	1.6	3.0
	peak area	5.6	3.9	4.4	5.3	3.3	3.1
Alkaline pre-rinsing using sodium hydroxide	Migration time	0.67	0.65	0.69	0.77	0.93	0.47
	peak area	6.2	6.1	2.6	2.1	2.5	2.5
Acid pre-rinsing using hydrochloric acid	Migration time	0.34	0.43	0.43	0.36	0.63	1.36
	peak area	13.3	8.0	3.9	2.9	1.8	3.0
Acid pre-rinsing using pyromellitic acid	Migration time	0.09	0.10	0.09	0.10	0.12	0.23
	peak area	3.0	3.0	4.2	4.3	1.6	1.8

mobile ions and 1% for slowly migrating anions. Relative standard deviations for anions under investigation determined with chromate–Tris electrolyte are summarized in Table 2.

3.2. Alkaline preconditioning using NaOH

Alkaline preconditioning of fused-silica capillaries is widely used in CZE. Typically NaOH is used in concentrations between 0.1 and 1 *M*. Alkaline conditioning of the surface can eliminate adherence of sample compounds and it re-establishes negatively charged silanol groups counterbalanced with sodium ions. During the following preconditioning step by flushing the capillary with electrolyte the original equilibrium between the EOF modifier in the electrolyte and at the capillary wall should be restored quickly. Repetitive measurements showed that the trend to longer migration times can be reduced with alkaline preconditioning (Fig. 2). The relative standard deviations of migration times can be reduced with alkaline pre-rinsing to 0.9% (Table 1). Using the chromate–Tris electrolyte as running buffer similar effects can be observed as with pyromellitic acid and alkaline preconditioning. The relative standard deviations of migration times can be reduced to

Table 2
Relative standard deviations of migration times of inorganic anions applying various preconditioning techniques Background electrolyte: 4 m*M* chromic acid, 5 m*M* Tris, 0.75 m*M* hexamethoniumhydroxide, pH adjusted to 8.2 (NaOH)

Preconditioning method	Relative standard deviation (%) ($n=10$)					
	Bromide	Chloride	Sulfate	Nitrate	Fluoride	Phosphate
Flushing with electrolyte	0.62	0.58	0.65	0.63	0.95	0.82
Alkaline pre-rinsing using sodium hydroxide	0.39	0.40	0.39	0.40	0.68	0.83
Acid pre-rinsing using hydrochloric acid	0.32	0.23	0.26	0.26	0.62	0.31
Acid pre-rinsing using chromic acid	0.19	0.18	0.28	0.30	0.57	0.57

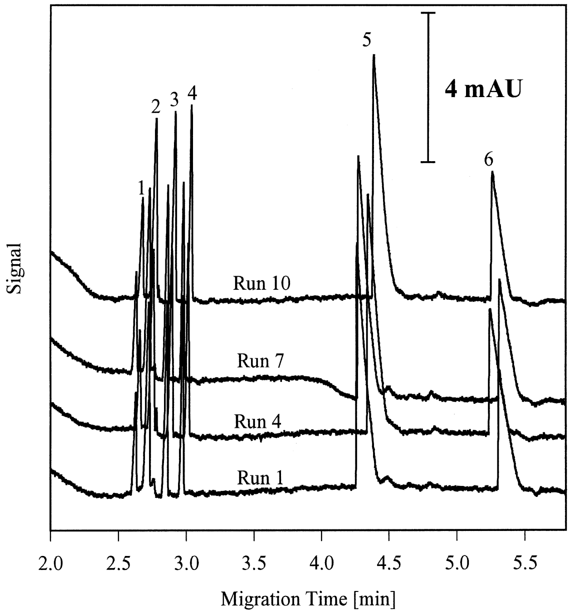

Fig. 2. Effect of alkaline preconditioning using 100 m*M* NaOH followed by flushing with electrolyte prior to analysis on migration times of inorganic anions. Electrolyte: 2.25 m*M* pyromellitic acid, 6.5 m*M* NaOH, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethoniumhydroxide, pH 7.9; injection: 200 mbar·s of standard solution containing 10 μg/ml bromide (1), chloride (2), sulfate (3), nitrate (4), fluoride (5) and 20 μg/ml phosphate (6); voltage −30 kV, capillary 64.5 cm×75 μm; detection wavelength: 350 nm (signal), 245 nm (reference).

0.4% for bromide, chloride, sulfate, nitrite and nitrate and 0.8% for fluoride and phosphate respectively (Table 2).

3.3. *Acid preconditioning using HCl*

Ehmann et al. found that acid preconditioning followed by purging with electrolyte increases reproducibility of migration times significantly, especially with hexamethyl alkyl diammonium salts as EOF modifiers [4]. The authors explained this improvement by the fact that the proton of the acid replaces all cationic species from the electrolyte at the inner capillary surface. In consequence all silanol groups are protonated and the original equilibrium can be restored quickly by flushing with electrolyte. The disadvantage of acid pre-rinsing is the fact that with introduction of anions in the electrophoretic system cross contaminations are possible. Ehmann et al. proposed either hydrochloric acid or propionic acid with concentrations of 250 mmol/l [4]. Propionic acid is advantageous since it has a lower mobility than inorganic anions and therefore system peaks arising from cross-contaminations do not co-migrate with the inorganic anions. During our studies we investigated hydrochloric acid. Investigations showed that complete removal of chloride ions is not possible by flushing the capillary with electrolyte. As a consequence the electropherograms show a baseline drop near the migration time of chloride (Fig. 3). The relative standard deviations of the migration times are between 0.5% and 0.9%, which is comparable to alkaline preconditioning using NaOH. The fact that reproducibilities of migration times are not improved significantly can be explained by the fact that due to extreme changes of pH during

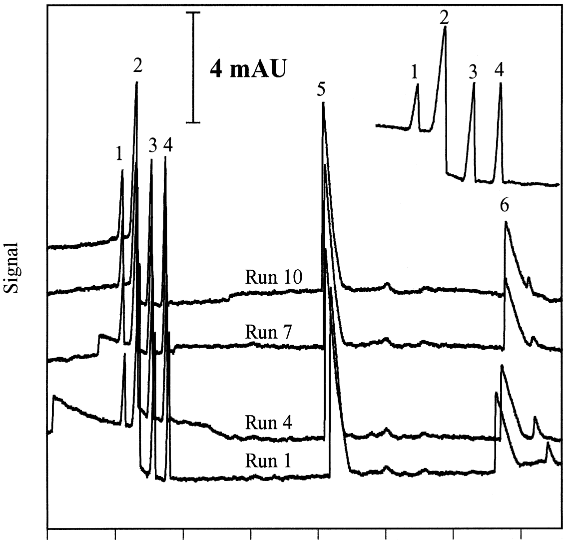

Fig. 3. Effect of acid preconditioning using 100 m*M* HCl followed by flushing with electrolyte prior to analysis on migration times of inorganic anions. Electrolyte: 2.25 m*M* pyromellitic acid, 6.5 m*M* NaOH, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethoniumhydroxide, pH 7.9; injection: 200 mbar·s of standard solution containing 10 μg/ml bromide (1), chloride (2), sulfate (3), nitrate (4), fluoride (5) and 20 μg/ml phosphate (6); voltage −30 kV, capillary 64.5 cm×75 μm; detection wavelength: 350 nm (signal), 245 nm (reference).

preconditioning steps the equilibrium between adsorbed and free EOF modifier cannot restored quickly enough. The influence on the reproducibility of peak areas is rather disastrous (Table 1). Relative standard deviations for peak areas are between 2% (for fluoride and phosphate), 13% for bromide and 8% for chloride respectively. Especially bromide and chloride are significantly influenced since their signals arise at within the baseline drop caused by cross-contaminations of HCl. As a consequence the adjustment of the baseline is quite difficult resulting in various sources of integration errors.

Applying acid preconditioning with HCl in combination with the chromate–Tris electrolyte the improvement of migration time reproducibility is similar. Standard deviations of migration times vary between 0.23 and 0.62% (Table 2). Compared to preconditioning with electrolyte the migration time reproducibility is increased by a factor of two. Cross contaminations arising from chloride ions adsorbed to the electrode cause similar interferences as mentioned above.

3.4. Acid preconditioning using pyromellitic acid

Acid pre-rinsing followed by flushing the capillary with electrolyte improves reproducibility of migration times of inorganic anions significantly as shown by Ehmann et al. [4] as well as in our studies. The problem arising from acid pre-rinsing is the introduction of co-migrating anions into the electrophoretic system. Using the corresponding acid of the electrolyte anion for acid prerinsing followed by flushing with electrolyte has the advantageous effect, that no interfering anion is introduced into the capillary. Pyromellitic acid as reagent for acid pre-rinsing was investigated in order to evaluate its effect on the reproducibility of migration times and peak areas. Pyromellitic acid is sparingly soluble in water. Therefore low concentrations of the acid can be used for preconditioning purposes. During our studies a concentration of 5 mmol/l was used. A solution containing 5 mmol/l pyromellitic acid as a pH value of 2.5 corresponding to a H^+ concentration of about 3 mmol/l. Investigations showed that the reproducibilities of migration times of inorganic anions could be improved comparable to the positive effect of HCl as pre-rinsing reagent. The relative standard deviations of migration times are between 0.06% for chloride and 0.23% for phosphate (Table 1). The electropherograms showed no interfering signal within the retention times of the inorganic anions under investigation (Fig. 4). Therefore the relative standard deviations of the peak areas could be improved significantly compared to acid prerinsing using HCl. The relative standard deviations of the peak areas vary from 1.6% for fluoride to 4.2% for sulfate and nitrate.

The acid pre-rinsing using the corresponding acid of the UV absorbing probe has two advantageous effects: on one hand the proton concentration is high enough to remove all adsorbed cations from the silanol groups in order to restore initial conditions within the capillary. On the other hand the affinity to the silanol groups of the anion of the acid used for pre-rinsing is equal to the affinity of the electrolyte

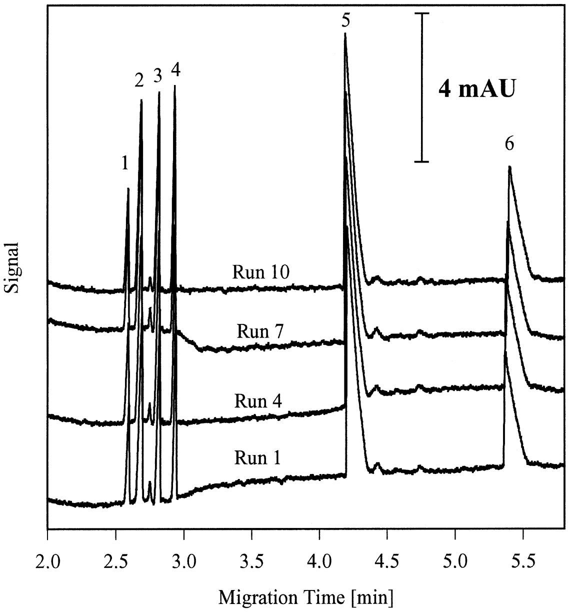

Fig. 4. Effect of acid preconditioning using 5 m*M* pyromellitic acid followed by flushing with electrolyte prior to analysis on migration times of inorganic anions. Electrolyte: 2.25 m*M* pyromellitic acid, 6.5 m*M* NaOH, 1.6 m*M* triethanolamine, 0.75 m*M* hexamethoniumhydroxide, pH 7.9; injection: 200 mbar˙s of standard solution containing 10 μg/ml bromide (1), chloride (2), sulfate (3), nitrate (4), fluoride (5) and 2 μg/ml phosphate (6); voltage −30 kV, capillary 64.5 cm×75 μm; detection wavelength: 350 nm (signal), 245 nm (reference).

anion. Therefore reproducible conditions are easy to achieve and no memory effect can be observed.

Similar strategy using the corresponding acid of the UV absorbing probe for acid preconditioning was tested with the buffer system chromic acid–TRIS. Chromic acid is easier to handle since its solubility is much higher than that of pyromellitic acid. Acid preconditioning using chromic acid again led to an improvement of migration time reproducibility without interferences from cross contaminations. The relative standard deviations of migration times vary from 0.19 to 0.57% (Table 2).

4. Conclusion

In several experiments the influence of pre-rinsing on the reproducibility of migration times and peak areas of inorganic anions was investigated. The electrolyte system consisting of pyromellitic acid as UV absorbing probe and hexamethonium as EOF modifier was studied. It was shown that acid pre-rinsing with pyromellitic acid followed by preconditioning with electrolyte has a positive influence on the reproducibility of migration times and peak areas.

In contrast to hydrochloric acid the corresponding acid of the UV absorbing probe has advantageous effects on both migration time reproducibilities and peak area reproducibilities. With the use of the optimized preconditioning technique, CZE is a reliable routine method for the determination of inorganic anions with respect to the possibility of automatic data analysis. The low risk of contaminations when using free acids of the UV absorbing probes as pre-rinsing agents additionally increases the reliability of this analytical technique.

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