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Factors influencing trace ion analysis with preconcentration by electrostacking

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Abstract

Capillary electrophoresis with indirect UV detection and electrostacking achieves low μg/l detection limits for inorganic anions and cations in various low conductivity matrices. Since this technique requires electromigration injection, parameters such as analyte electrophoretic mobility, electroosmotic flow, and sample and operating buffer ionic strength influence the amount of material that is actually injected into the capillary. These parameters are not easily controlled and are affected by such factors as injection voltage and duration, operating buffer, and operating buffer pH. The effects of these factors on sensitivity and resolution are demonstrated. The use of a novel carrier ion, dimethyldiphenylphosphonium ion, improves sensitivity for cations. Techniques for optimizing trace level determinations are also discussed.

1. Introduction

Capillary zone electrophoresis (CZE) with indirect UV detection is fast becoming a routine technique for the determination of small, inorganic ions when present at mg/l concentrations in various sample matrices. However, very few reports have dealt specifically with trace enrichment of inorganic ions to achieve μg/l detection limits [1–4]. To date, trace enrichment to enhance sensitivity in CZE has been performed using: on-line isotachophoresis (ITP) prior to CZE separation with an ITP preconcentration capillary which is then coupled to the separation capillary [1,5–8], field amplification [9,10], and electrostacking [2]. Electrostacking is an attractive technique because the isotachophoretic preconcentration step and the electrophoretic separation are performed in the same capillary [3] and can be done with unmodified, commercially available instrumentation.

To perform electrostacking, a sample of lower ionic strength than the operating buffer is injected into the capillary using an electromigration injection in which sample components migrate into the capillary under the influence of an applied electric field. The sample injected is thus biased toward ions with the highest mobilities [11,12]. When using electromigration injection, the electric field strength along the length of the capillary is not constant. The portion of the capillary filled with high resistivity, low conductance sample experiences a much higher field strength than the remainder of the capillary that contains lower resistivity, higher conductance operating buffer [11]. Since analyte velocity is directly proportional to field strength [13], analyte ions migrate rapidly to the concentration boundary between the operating buffer and the sample zone and "stack" as they slow down at the boundary interface [14]. Enrichment factors

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of several orders of magnitude from very low ionic strength samples are possible using the electrostacking technique [2,3].

To normalize the conductance of both sample and standard solutions, an isotachophoretic terminating ion, typically at low μM concentration, is added to both [3]. With normalized conductance for both sample and standard solutions, accurate quantification of analytes in low ionic strength sample matrices is possible. The terminating ion must have the same charge as the analytes of interest and must migrate slower so as not to interfere with the stacking process or the separation of the analytes. Typical terminating ions are tetrabutylammonium for cation separations and octanesulfonate for anion separations.

Since electrostacking of inorganic ions is typically performed in fused-silica capillaries, electroosmotic flow also plays a role in the trace enrichment process. For example, the movement of the concentration boundary formed by the high ionic strength operating buffer and the lower ionic strength sample is dependent on the electroosmotic flow of the entire bulk solution and is described by an average electroosmotic flow velocity [14] as opposed to a true isotachophoretic concentration boundary which moves at a constant velocity [15]. Isotachophoresis is typically performed in coated capillaries which exhibit no electroosmotic flow. Electroosmotic flow is influenced by such parameters as the nature of the operating buffer ions, operating buffer pH, and injection voltage. The extent to which these parameters affect the trace enrichment of inorganic ions using electrostacking and other factors that influence sensitivity and resolution are discussed in this paper.

2. Experimental

2.1. Equipment

The capillary electrophoresis experiments were performed with a Dionex CES-I automated system and UV detection (Dionex, Sunnyvale, CA, USA). Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50 μm I.D., 375 μm outer diameter (O.D.), and 50 cm total length were used. The detection window was located 5 cm from the end of the capillary. Data collection was with a Dionex AI-450 chromatography workstation using a 10-Hz sampling rate. Dionex OnGuard A cartridges in the hydroxide form were used to convert dimethyldiphenylphosphonium (DDP) iodide to DDP hydroxide, and hexamethonium bromide to hexamethonium hydroxide (HMOH).

2.2. Chemicals

Pyromellitic acid (PMA) and 2-(N-morpholino)ethanesulfonic acid (MES) were obtained from Sigma (St. Louis, MO, USA). Hexamethonium bromide (monohydrate), dimethyldiphenylphosphonium iodide, and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) were obtained from Aldrich (Milwaukee, WI, USA). Triethanolamine and formic acid were obtained from Fluka (Ronkonkoma, NY, USA). Sodium hydroxide, 50% aqueous solution (w/w) and phosphoric acid were obtained from Fisher Scientific (Pittsburgh, PA, USA). Copper(II) sulfate (pentahydrate) was obtained from MCB (Norwood, OH, USA). Octanesulfonic acid, 0.1 M aqueous solution, and tetrabutylammonium hydroxide, 0.1 M aqueous solution, were from Dionex. All reagents were ACS or analytical reagent grade and prepared in 18 mΩ cm resistance deionized water.

In experiments where premixed operating buffers were applicable, IonPhor Anion PMA Electrolyte Buffer and IonPhor Cation Cu Electrolyte Buffer from Dionex were used.

Cation standards were obtained as 1000 mg/l ion standard solutions from Aldrich or prepared from chloride salts obtained from Fisher Scientific. Anion standards were prepared from sodium salts obtained from Fisher Scientific.

3. Results and discussion

3.1. Operating buffer pH

During electrostacking of analytes in a low ionic strength sample matrix, the pH of the

operating electrolyte can greatly effect efficiency and resolution. Fig. 1 shows a comparison of the separation of cations using an operating buffer that contains copper(II) as the carrier ion at different pH values. A carrier ion is a buffer component that has the same electrical charge and similar electrophoretic mobility as the analytes of interest. An additional requirement for indirect UV detection is that the carrier ion must also be chromophoric. At the higher pH, electroosmotic flow is faster and analyte migration times are shorter. Higher efficiencies are observed at higher pH for the earlier migrating species (Table 1) primarily because at the higher pH, the mobility of the copper(II) carrier ion is more closely matched to the mobilities of the earlier migrating analytes and band dispersion is minimized [16].

Table 1
Efficiencies for cation standards using cupric-containing operating buffer at pH 3.0 and pH 4.8

Cation	Efficiency[a]	
	pH 3.0	pH 4.8
Ammonium	2579	4454
Potassium	8351	11 695
Sodium	8442	14 511
Calcium	24 763	26 711
Magnesium	30 000	39 624
Strontium	164 404	129 051
Lithium	132 594	77 014
Barium	68 702	48 803

[a] Values were calculated using the electropherograms in Fig. 1 and peak width at $\frac{1}{2}$ height.

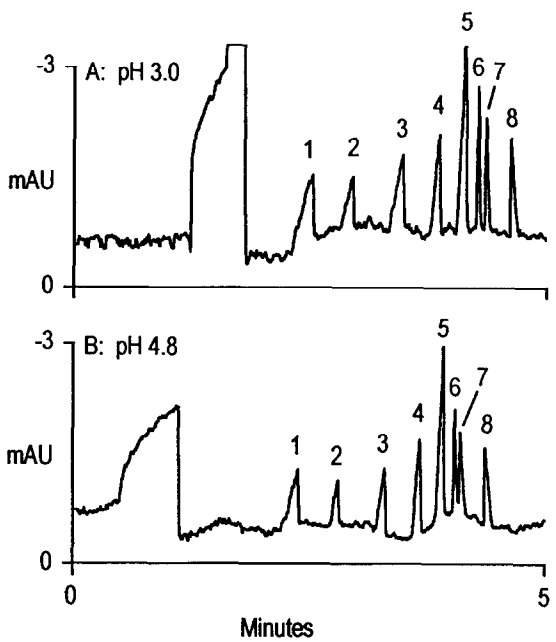

Fig. 1. Effect of operating buffer pH on efficiency. Conditions: (A) operating buffer, 4 m*M* copper(II) sulfate, 4 m*M* 18-crown-6, 4 m*M* formic acid, pH 3.0; capillary, 50 cm × 50 μm I.D. fused silica; positive polarity, detector side cathodic; constant voltage at 20 kV; injection, electromigration, 5000 V, 90 s; detection, indirect UV at 215 nm; terminating ion, 50 μ*M* tetrabutylammonium hydroxide; (B) as in A except operating buffer, 4 m*M* copper(II) sulfate, 4 m*M* 18-crown-6, pH 4.8. The large baseline disruption prior to peak 1 is likely hydronium ion. Cation standards: 1 = ammonium ion (50 μg/l); 2 = potassium (50 μg/l); 3 = sodium (50 μg/l); 4 = calcium (50 μg/l); 5 = magnesium (50 μg/l); 6 = strontium (50 μg/l); 7 = lithium (10 μg/l); 8 = barium (75 μg/l).

Another effect that may contribute to higher efficiencies of the earlier migrating analytes with the higher pH buffer is the increased electroosmotic flow as compared to the electroosmotic flow of the pH 3 buffer. With the higher electroosmotic flow, the velocity of the concentration boundary is faster and the boundary forms higher up in the capillary so that a larger area or zone of low ionic strength solution forms as compared to a lower pH buffer with slower electroosmotic velocity. The larger zone of lower conductance sample creates a larger region of higher effective electric field strength and permits more "stacking" of the analytes with higher electrophoretic mobility, since analyte velocity is increased in this region.

Hydrostatic injection of a plug of water prior to electrostacking also creates a larger, low conductance, high electric field zone [17]. In electropherogram Fig. 2A, a typical electrostacking experiment was performed in which a cation standard was injected using electromigration injection at 5000 V for 90 s. Electropherogram Fig. 2B was obtained by first injecting a plug of water using gravity injection (100 mm for 20 s) and then injecting the cation standard with electromigration injection. A significant improvement in the efficiencies of calcium through

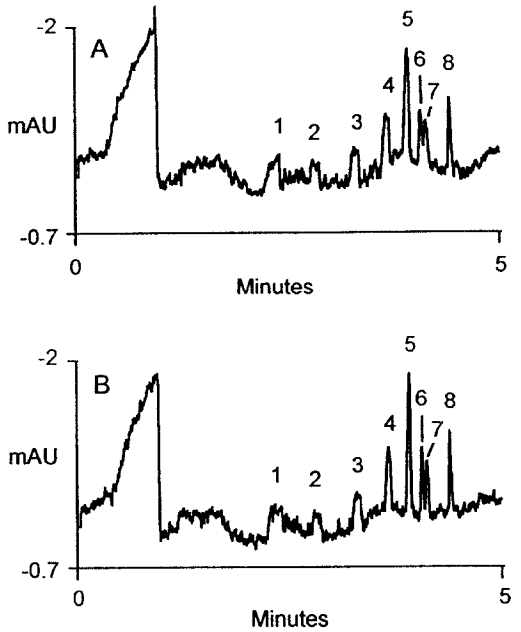

Fig. 2. Effect of water plug preinjection. Conditions: (A) as in Fig. 1A; (B) as in Fig. 1A, except first injection, water hydrostatic injection, gravity, 100 mm, 20 s; second injection, as in Fig. 1A. Cation standards: 1 = ammonium ion (5 μg/l); 2 = potassium (5 μg/l); 3 = sodium (5 μg/l); 4 = calcium (5 μg/l); 5 = magnesium (5 μg/l); 6 = strontium (5 μg/l); 7 = lithium (1 μg/l); 8 = barium (7.5 μg/l).

barium is observed. The peaks representing ammonium ion, potassium, and sodium are at or below detection limits with no improvement in efficiency. The improved efficiency has resulted in better resolution for strontium and lithium. Thus, hydrodynamic preinjection of a water plug is an effective means of improving efficiency and sensitivity for most cations. Application of this technique is obviously limited by the magnitude of the water blank.

3.2. Carrier ion

Since the carrier ion provides the UV background for indirect detection and its mobility to a large extent determines analyte peak efficiency and shape, the specific carrier ion used influences sensitivity. For example, when using dimethyldiphenylphosphonium (DDP) as the UV-absorbing carrier ion for cation separations, an improvement in detection limits is realized compared to separations obtained with a cupric carrier ion (Table 2). A representative electropherogram is shown in Fig. 3. Visual comparison of the electropherogram in Fig. 3 with the electropherograms in Fig. 1, which were ob-

Table 2
Comparison of detection limits using cupric-containing buffer and DDP-containing buffer

Cation	Detection limits (μg/l)[a]	
	Cupric-containing buffer[b]	DDP-containing buffer[c]
Ammonium	11	3
Potassium	7	2
Calcium	7	8
Sodium	9	3
Magnesium	7	1
Strontium	14	3

[a] Detection limit = (S.D.) · $t_{(s)}$ where $t_{(s)}$ for 99% single sided student's t-test distribution.
[b] Electrophoretic conditions as in Fig. 3 for pH 3.0 operating buffer.
[c] Electrophoretic conditions as in Fig. 4.

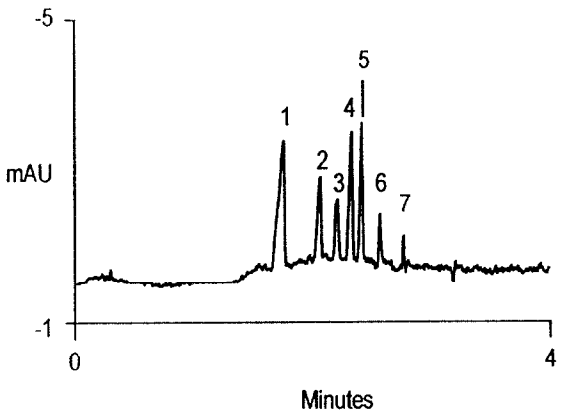

Fig. 3. Electropherogram from DDP-containing operating buffer. Conditions: operating buffer, 5 mM DDP hydroxide, 4 mM 18-crown-6, 5 mM MES, pH 6.0 adjusted with phosphoric acid; capillary, 50 cm × 50 μm I.D. fused silica; positive polarity, detector side cathodic; constant voltage at 25 kV; injection, electromigration, 2500 V, 45 s; terminating ion, 50 μM tetrabutylammonium hydroxide. Cation standards: 1 = ammonium ion (5 μg/l); 2 = potassium (10 μg/l); 3 = calcium (10 μg/l); 4 = sodium (10 μg/l); 5 = magnesium (5 μg/l); 6 = strontium (10 μg/l); 7 = barium (10 μg/l).

tained with the cupric carrier ion, shows that the mobility of DDP is more closely matched to the mobilities of the analytes than is cupric ion at either pH 3.0 or pH 4.8. More symmetric peaks are obtained with DDP as a result of the similar mobilities. In addition, the signal-to-noise ratio is much better with DDP even with analytes present at a factor of 10 less as compared to the signal-to-noise ratio for the cupric carrier ion.

Dimethyldiphenylphosphonium is commercially available as the iodide salt. In order to obtain sensitive indirect UV detection with DDP, the iodide, which absorbs strongly at low UV wavelengths, is exchanged with hydroxide by passing DDP iodide through a hydroxide-form anion-exchange resin.

3.3. Injection voltage

Electrostacking also provides low μg/l detection limits for small inorganic anions (Table 3). However, too high of an injection voltage can adversely effect resolution of some analytes. This effect is shown in Fig. 4. The electropherograms in Fig. 4A and B were obtained using identical conditions except for the injection voltage, which was 2500 V in Fig. 4A and 5000 V in Fig. 4B. In electropherogram 4B, formate and phosphate comigrate and the baseline disruption due to carbonate is significantly earlier. The reason for this has as yet not been determined, however we postulate that at the higher injection voltage, more highly mobile hydroxide ion is concentrated in the capillary creating a localized pH

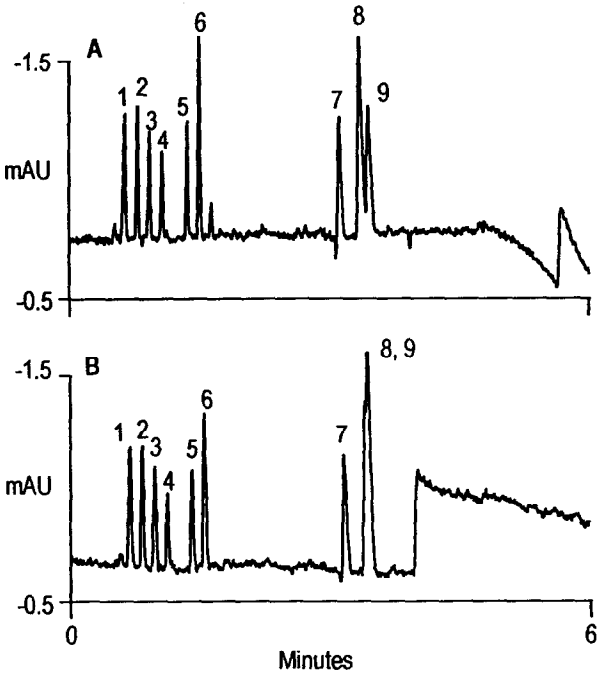

Fig. 4. Effect of injection voltage on efficiency and resolution. Conditions: (A) operating buffer, 2.25 m*M* pyromellitic acid, 6.50 m*M* sodium hydroxide, 0.75 m*M* hexamethonium hydroxide, 1.6 m*M* triethanolamine, pH 7.7; capillary, 50 cm × 50 μm I.D. fused silica; negative polarity, detector side anodic; constant voltage, 30 kV; injection, electromigration, 2500 V, 45 s; terminating ion, 50 μ*M* octanesulfonic acid; (B) as in A except injection, electromigration, 5000 V, 45 s. Anion standards: 1 = chloride (10 μg/l); 2 = sulfate (10 μg/l); 3 = nitrite (10 μg/l); 4 = nitrate (10 μg/l); 5 = molybdate (20 μg/l); 6 = azide (20 μg/l); 7 = fluoride (5 μg/l); 8 = formate (10 μg/l); 9 = phosphate (20 μg/l).

Table 3
Detection limits for anion standards using electrostacking

Anion[a]	Detection limits (μg/l)[b]
Chloride	0.5
Sulfate	0.6
Nitrate	1.5
Fluoride	0.9
Phosphate	2.0

Electrophoretic conditions as in Fig. 5A.
[a] Anion standard concentration was 5 μg/l.
[b] Detection limit = (S.D.) · $t_{(s)}$ where $t_{(s)}$ for 99% single sided student's *t* test distribution.

change that is too high to be buffered by the electrolyte. The migration of weak acids, such as formate, phosphate, and carbonate, would be the most affected by such a pH phenomenon.

An interesting and unexpected result is that the efficiencies observed for the peaks in electropherogram 4A in which sample was introduced using a 2500-V electromigration injection are better than the efficiencies observed when a 5000-V electromigration injection was used as in electropherogram 4B. Both electropherograms were obtained using the same separation conditions. The result indicates that less band dispersion occurs during separation if sample is

injected using a lower voltage. One possible explanation is that heat generated during injection is not dissipated during separation. A 5000-V electromigration injection would produce more heat than a 2500-V injection and would potentially result in more band dispersion.

3.4. Operating buffer impurities

The presence of trace ionic impurities, especially the analyte(s) of interest, in the operating buffer compromise accurate determination because subsequent to the electrostacking process, the impurities in the operating buffer zone that enters the capillary when the capillary is placed back in the buffer solution after injection migrate to and are separated with the individual analyte zones. In Fig. 5A, the electropherogram was obtained using an operating buffer to which had been added trace levels (10 μg/l) of bromide, chloride, sulfate, and nitrate. High purity, deionized water was injected using electromigration. An injection of water using identical conditions except that "uncontaminated" operating buffer was used is shown in Fig. 5B for comparison. As indicated by the electropherograms in Fig. 5, it is imperative that when performing electrostacking for trace anion analysis, the operating buffer be free of ionic impurities, most notably the ionic species of interest for the analysis. In addition, the presence of electroosmotic flow modifier counterions, such as bromide from hexamethonium bromide or tetradecyltrimethylammonium bromide (TTAB), or chloride from cetyltrimethylammonium chloride (CTAC), will prevent accurate determinations using electrostacking if the counterion is not removed from the operating buffer. Ionic impurities in the terminating ion solution added to the sample are also present as interferences.

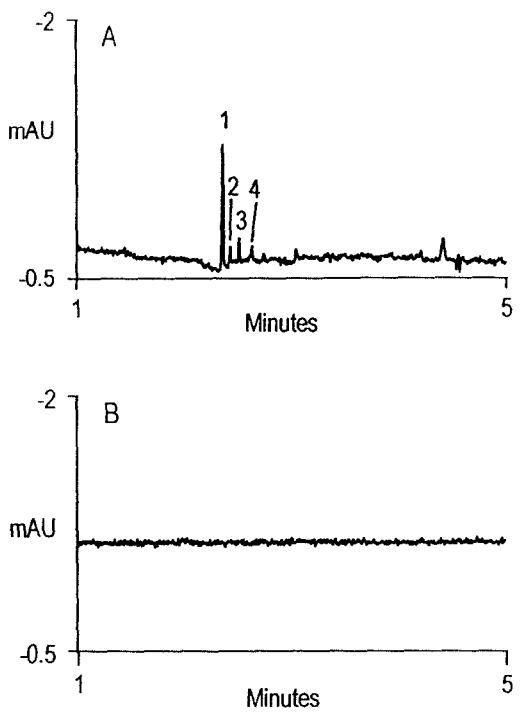

Fig. 5. Effect of trace level impurities in the operating buffer. Conditions: (A) as in Fig. 4A except 10 μg/l each of bromide, chloride, sulfate, and nitrate were added to the operating buffer; injection, water sample by electromigration, 2500 V, 45 s; No terminating ion added to the sample; (B) as in A except uncontaminated operating buffer used. Peaks: 1 = bromide; 2 = chloride; 3 = sulfate; 4 = nitrate.

4. Conclusions

Electrostacking with electromigration injection permits low μg/l level determinations of inorganic ions in low ionic strength sample matrices. Stacking can be enhanced by using conditions such as operating buffer pH, that promote formation of the concentration boundary higher up in the capillary creating a larger high electric field zone. Hydrostatic introduction of a water plug prior to electrostacking also enhances the stacking effect by creating a larger high field zone. The carrier ion, DDP, can be used for trace cation determinations as an alternative to cupric ion or chromophoric amines and provides excellent signal-to-noise ratio for trace level determinations. The resolution of weak acid anions can be adversely effected if electromigration injection voltage is too high. To ensure accurate determination when electrostacking, the operating buffer and the terminating ion solution must be free of impurities that interfere with the electrophoretic separation. By optimizing both

the electrostacking and the electrophoretic separation conditions, sensitivity for trace ions is maximized.

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