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Sample stacking of fast-moving anions in capillary zone electrophoresis with pH-suppressed electroosmotic flow

Joselito P. Quirino*, Shigeru Terabe

Faculty of Science, Himeji Institute of Technology, Kamigori, Hyogo 678-1297, Japan

Abstract

On-line sample concentration of fast moving inorganic anions by large volume sample stacking (LVSS) and field enhanced sample injection (FESI) with a water plug under acidic conditions is presented. Detection sensitivity enhancements were around 100 and 1000-fold for LVSS and FESI, respectively. However, reproducibility and linearity of response in the LVSS approach is superior compared to the FESI approach. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Sample stacking; Field enhanced sample injection; Injection methods; Inorganic anions

1. Introduction

The short optical pathlength of capillaries and small injection volumes in capillary zone electrophoresis (CZE) hinders the direct photometric detection of very dilute samples. Chien and Burgi had exploited on-line concentration by sample stacking to solve this problem [1]. Sample stacking arises from the abrupt change in electrophoretic velocities of sample ions across a concentration boundary. Samples are generally prepared in a lower conductivity matrix relative to the separation buffer to bring out an enhanced field in the sample zone upon application of high voltages. The enhanced field causes a marked increase in electrophoretic velocities of sample ions in the sample zone compared to the separation zone. Hydrodynamic or electrokinetic injection modes can be performed. The major rationale is to increase the amount of samples introduced into the capillary without impeding the high efficiencies that can be obtained with CZE.

In order to maximize the potential of sample stacking with hydrodynamic injection, long injections must be performed (large volume sample stacking, LVSS) and is often associated with a polarity-switching step, which is not always available in commercial instrumentation. Polarity switching is done in order to remove the large volume of sample matrix introduced, thus providing higher stacking efficiencies. Burgi demonstrated the use of diethylenetriamine an electroosmotic flow modifier as a pump to remove the sample matrix without polarity reversal for the stacking of anions [2]. Albert et al. evaluated tetradecyltrimethylammonium bromide that provided greater sensitivity enhancements compared to that used by Burgi [3].

Among the sample stacking techniques, field enhanced sample injection provides the highest possible sensitivity enhancements. A water zone prior to electrokinetic injection improves the focusing effect [1]. Another modification is injection of third zone (a high viscosity and high conductivity liquid) prior to the water zone, which had been shown to improve the detection sensitivity of closely related opioids by CZE in a binary system [4].

*Corresponding author. Fax: +81-791580132.
E-mail address: jquirino@sci.himeji-tech.ac.jp (J.P. Quirino)

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Here, we developed and evaluated LVSS and FESI (with a water plug) using low pH buffers to restrain the electroosmotic flow for the on-line concentration of fast moving anions. Although the approaches will be limited for fast moving anions, it is straightforward, provides better or comparable sensitivity enhancements, and simpler (i.e. does not necessitate a polarity-switching step or a buffer additive) compared to those already reported.

2. Experimental

2.1. Apparatus

Capillary electrophoresis and stacking were performed with a Hewlett Packard 3D capillary electrophoresis system (Waldbronn, Germany), equipped with fused-silica capillaries of 64.5 cm (effective length 56 cm)×50 μm I.D. obtained from Polymicro Technologies (Phoenix, AZ, USA). Capillaries were thermostated at 20°C. Detection was performed at variable wavelengths. Conductivity of sample and separation solutions was measured using a Horiba ES-12 conductivity meter (Kyoto, Japan).

2.2. Reagents and solutions

All chemicals were obtained in the finest grade available from Nacalai Tesque (Kyoto, Japan). Background solutions (BGS) were prepared from 100 m*M* sodium dihydrogenphosphate and 0.5 *M* phosphoric acid stock solutions. Anion standard stock solutions were 2306 ppm potassium bromide (KBr), 2296 ppm potassium nitrate (KNO_3), and 2374 ppm potassium bromate ($KBrO_3$). Anion stock solutions were mixed and diluted with water to concentrations providing comparable peak heights to make the sample solutions (S). Electroosmotic flow was measured using mesityl oxide as neutral marker at an effective length of 8.5 cm. All solutions were prepared from water purified with a Milli-Q system (Millipore, Bedford, MA, USA) and were filtered through 0.45 μm filters (Toyo Roshi, Japan) prior to use.

2.3. Procedure

Newly installed capillaries were flushed with 1 *M* NaOH (30 min), followed by methanol (30 min), purified water (30 min), and finally with the electrophoresis buffer (30 min). To ensure repeatability, capillaries were flushed in between runs with 1 *M* NaOH (1 min), followed by methanol (1 min), 0.1 *M* NaOH (1 min), purified water (2 min), and finally with the electrophoresis buffer (3 min). For LVSS, S were injected at 50 mbar at different intervals with the S in the inlet position (far from detector end) and voltages applied at negative polarity. For FESI, water plugs were injected at 50 mbar at different intervals prior to injection of S electrokinetically (voltage at negative polarity). Injections were stopped at certain percentages of original current followed by negative voltage with the BGS at both ends of the capillary.

3. Results and discussion

3.1. The stacking processes under acidic conditions

3.1.1. LVSS

Fig. 1 illustrates the steps involved in LVSS. From sample introduction and focusing (A), removal of the sample matrix and separation (B), and consequent detection of focused zones (C). The electroosmotic mobility is very low at acidic pH as a consequence of the decreased ζ potential due to the suppressed ionization of the silanol groups. Anions with high mobility would therefore migrate towards the anode, and in order to detect these analytes voltages should be applied at negative polarity. In a sample zone of lower conductivity, the electrophoretic velocity of sample ions would even be higher.

Contrary to that reported by Chien and Helmer [5], stacked analyte zones will leave the concentration boundary after a while and before total removal of the sample matrix (see Fig. 1B). This is proven in Fig. 2A and B. Fig. 2A shows the detection and separation of the test anions before the stabilization of current. A stable current is indication that the capillary is filled with the separation solution only. Fig. 2B shows the experimental profile of the zones in Fig. 1B, done by cutting the voltage and

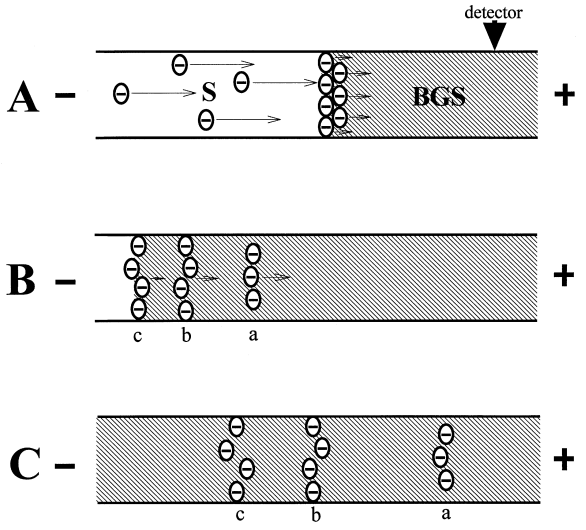

Fig. 1. Evolution of anionic species in the sample solution S (unshaded) and separation solution BGS (shaded) zones under LVSS at acidic pH. A, starting situation (injection of S in water and application of voltage); B, stacked zones separate prior to the complete removal of the sample matrix; C, zones migrate toward the detector and continue to separate; magnitude of electrophoretic mobilities, $a>b>c$.

pushing the zones by pressure. Similar results were reported in the sample stacking of neutral analytes in micellar electrokinetic chromatography (MEKC) [6]. The velocity of the boundary is toward the negative electrode while the electrophoretic velocities of anions are toward the positive electrode. Naturally, anions with higher electrophoretic mobilities leave the concentration boundary earlier than anions with lower electrophoretic mobilities. The velocity of the concentration boundary is equal to the averaged electroosmotic velocity of the two liquids inside the capillary while each electrophoretic velocity is equal to the product of the field strength in each zone and the anion's electrophoretic mobility [7]. We have shown evidence of the mixing of zones when the capillary is filled with liquids of different conductivity, providing the S zone higher conductivity than expected [7,8]. Thus, separation and stacking can be said to occur synchronously.

Fig. 1C depicts the further separation and latter detection of analyte zones, also the sample matrix was completely removed from the column. Note that removal of the sample matrix is facilitated by the electroosmotic flow that moves in the direction of the cathode. If very long injections are made, it may be possible that the focused zones will be detected prior to complete removal of sample matrix.

3.1.2. FESI with a water plug

Fig. 3 illustrates the steps involved in FESI with a water plug. After conditioning the capillary with the BGS, a plug of water is injected. With the S in the cathodic vial and the BGS in the anodic vial, voltage is applied to affect introduction of anions into the water plug. Besides, removal of the water plug and subsequent focusing in the concentration boundary occur (Fig. 3A). The magnitude of current increases starting the minimum when the voltage was applied due to removal of the water zone by electroosmosis. When the optimum percentage of original current (observed when the whole capillary is filled with BGS) is reached, the voltage is cut (Fig. 3B) and the S vial is replaced by a BGS vial. Percentage of original current is directly related to electrokinetic injection time. Voltage is then applied to affect complete removal of the water plug, focusing of the analyte ions introduced (see Fig. 3B), separation, and detection of zones (Fig. 3C). The length of the water plug and the percentage of original current are very crucial parameters. Long water plugs and/or high percentages of original current produced undesirable peak shapes (Fig. 4A and C) due to broadening caused by the continuous injection of anions and overloading. Broadening is serious for the faster moving anion (bromide) because it reaches the concentration boundary earlier. The electropherograms in Fig. 4 also suggest that there should be a compromise between peak shapes and sensitivity enhancements. Greater enhancements were obtained with longer water plugs (A) or higher percentages of original current (C) while better peak shapes were obtained with B.

Hydrodynamic injection of the S after injection of the water plug was also conducted. The water zone in this case acts like a second enhanced field zone similar to that described for MEKC [9]. However, these experiments produced peak shapes similar to those without the injection of water plug (LVSS). Therefore, no benefit can be gained.

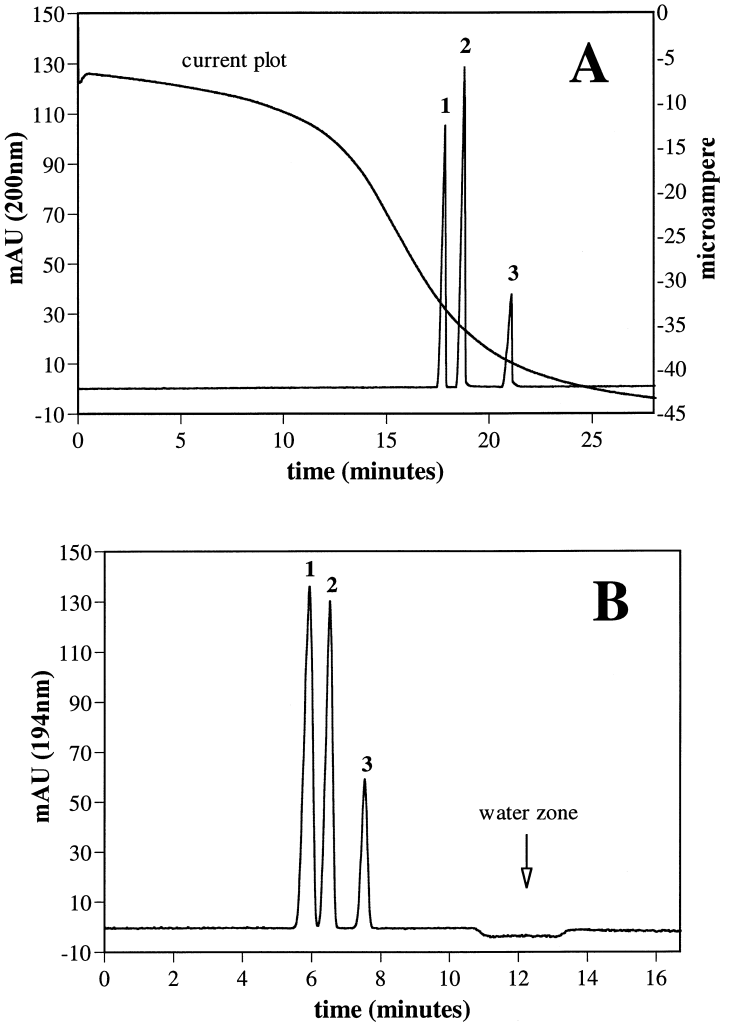

Fig. 2. Evidences of the separation of anions prior to the total removal of sample matrix. BGS, 100 m*M* sodium dihydrogenphosphate – 0.5 *M* phosphoric acid (pH 2.5). Peaks: 1=bromide, 2=nitrate, 3=bromate. S, Samples in water; injection, 200 s; stacking and separation regimen, −16 kV throughout the run (A), −16 kV for 10 min followed by pressure at 50 mbar until all the peaks are detected (B); data collection, from the application of voltage (A), from the application of pressure (B).

3.2. Stacking enhancement factors obtainable

Sample electropherograms obtained from a 1 s or usual injection (A), LVSS (B), and FESI (C) schemes are shown in Fig. 5. The sample solution in B and C is a 100 and 1000-fold dilution of A, respectively. The peak shapes therefore suggest around 100-fold improvement in detection sensitivity using LVSS. Around 1000-fold improvement is apparent using FESI. However, reproducibility of electropherograms and linearity of responses were obstacles in the FESI approach. Use of internal standard technique might be useful. Some reasons include variation in the amounts of anions injected and electrophoresis in the BGS during sample focusing. Variation in the amounts injected is primarily caused by the irreproducible electroosmotic flow, which causes variations in the time of injection. Note that during injection, the capillary is filled with two liquids having different properties (i.e. pH and

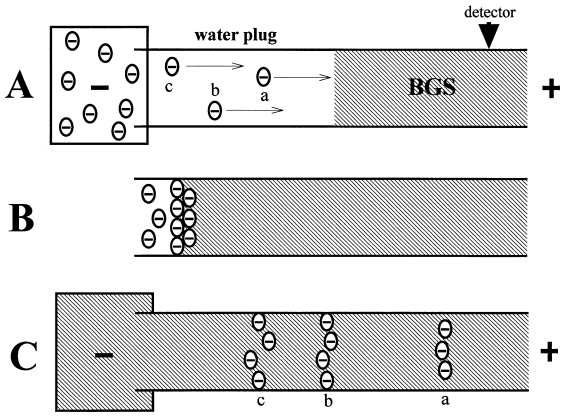

Fig. 3. Evolution of anionic species in the water plug and BGS zones under FESI with a water plug at acidic pH. (A) Starting situation (injection of a water plug) then application of voltage at negative polarity with the S in the inlet position (anions are injected electrokinetically into the water plug); (B) the voltage is shut at a certain percentage of actual current (shows anions focused in the concentration boundary and anions found in the water plug, and partial removal of the water plug); (C) separation and later detection of focused zones

conductivity). Automation of the FESI technique, that is setting a constant time for electrokinetic injection, improved reproducibility.

3.3. Applicability to quantitative analysis

Table 1 lists the limit of detection values and relative standard deviations of migration times, peak heights, and corrected peak areas for the test anions using the LVSS approach. These values are fairly acceptable. Current FESI approach data are not acceptable and thus not included in the table. More studies are essential.

In conclusion, we have conferred two simple approaches for the on-line concentration of fast moving anions in CZE, which can provide two to three orders of magnitude increase in concentration sensitivity. A variety of compounds have been separated in acidic buffers with CZE [10–12], application will simply be employing water or low conductivity matrices to prepare the samples and injecting using the suggested techniques.

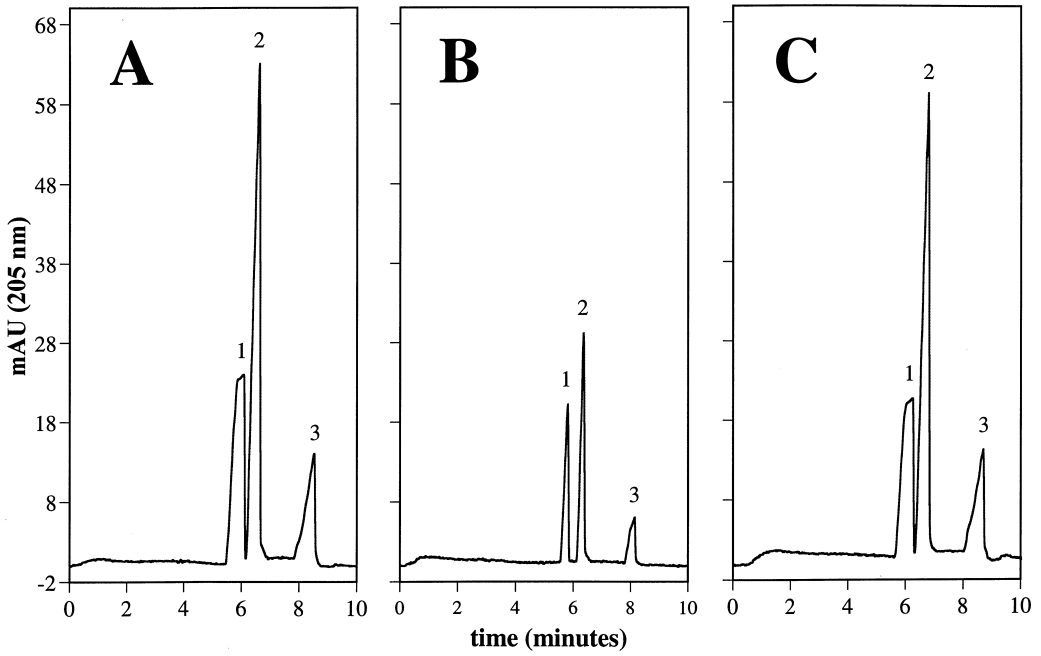

Fig. 4. Effect of the length of the water plug and percentage of original current on peak shapes. BGS, 75 m*M* phosphoric acid (pH 1.9); S, samples in water; concentration, 6.3 ppm (bromide), 4.2 ppm (nitrate), 13.0 ppm (bromate); identity of peaks, same as those in Fig. 2; applied voltage, −15 kV (injection and separation); water plug injection times, 30 s (A), 20 s (B), 60 s (C); percentage of original current, 75% (A), 60% (B, C).

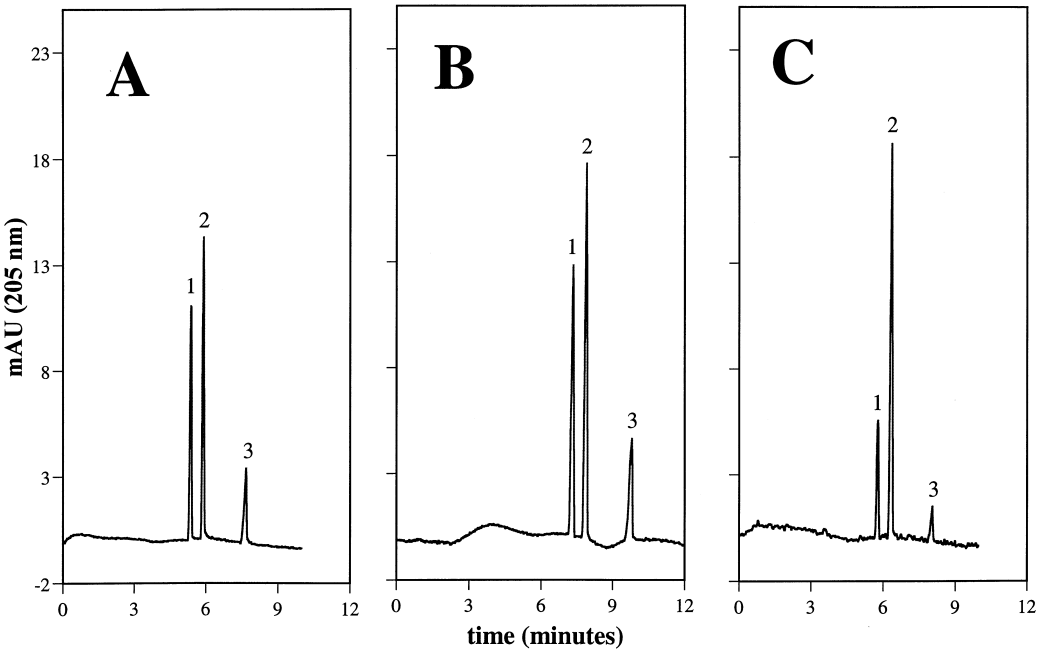

Fig. 5. Sample electropherograms obtained with LVSS and FESI with a water plug under acidic pH. Conditions. usual injection (A), LVSS (B), FESI with a water plug (C); BGS, 75 m*M* phosphoric acid (pH 1.9); S, samples in water; concentration of samples, 629 ppm (bromide), 418 ppm (nitrate), 1295 ppm (bromate) (A), 100-fold dilution of A (B), 1000-fold dilution of A (C); identification of peaks, same as those in Fig. 2; applied voltage (also for electrokinetic injection), −15 kV; injection regimen, 1s (A), 100 s (B), 20 s water plug and 60% of original current (C).

Table 1
Limits of detection and relative standard deviations (RSD, %)[a]

	Bromide	Nitrate	Bromate
Limit of detection (S/N=3) (ppb)	2.22	1.83	6.51
RSD (%)			
Migration time (n=15)	4.03	3.82	11.00
Height (n=3)	12.04	4.99	4.54
Corrected area (n=3)	12.69	9.19	11.79

[a] Conditions: S, samples in water; BGS, 75 m*M* phosphoric acid (pH 1.9); concentration range, 0.4–13 ppm; detection, 200 nm; voltage, −20 kV.

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