Improved reproducibility and increased sample throughput in capillary electrophoresis

Technical Note

Introduction

Analyte migration time in CE is highly dependent on the stability of running buffer composition. Electrolysis of the buffer can cause changes in composition and pH. This in turn will alter the electroosmotic flow (EOF) and migration time. In addition, the pumping action of the EOF will cause unleveled liquid depths in the buffer vials and result in laminar flow. This can degrade efficiency and resolution as well as alter migration time. To maintain consistent results, the buffer should be replaced (or replenished) often. Buffer lifetime is dependent on the capacity of the buffer system, the current generated, and the volume of the buffer reservoirs. For many buffer systems, replenishment after every analysis is necessary to maintain separation quality. It is important that both inlet and outlet reservoirs are replenished. Although the direction of EOF is usually toward the outlet (cathode), small ions migrate into the capillary from the outlet reservoir. The Agilent Capillary Electrophoresis system is equipped with a buffer replenishment system that enables replenishment to be automated. Any of the vials in the carousel can be emptied and refilled with fresh buffer from a self-contained 500-mL reservoir. Replenishment eliminates the need for multiple buffer vials in the carousel and thereby increases locations available for sample vials. This improves sample throughput and allows long term, unattended operation. The major features of the replenishment system are highlighted in table 1.

Replenishment feature	User benefit
User-defined replenishment and random vial access	Any vial in the carousel can be replenished. Typically inlet and outlet vials are replenished between runs. Replenishment frequency is determined by the user. Vials can be replenished (emptied and filled), emptied, or filled (topping-off liquid to specified height).
Only two buffer vials required	Frees vial locations in carousel for samples.
500-ml buffer reservoirs	High sample throughput and long term, unattended operation.
Automatic buffer leveling	Maintains exact liquid level in inlet and outlet buffer vials, eliminating siphoning which causes band broadening and increased migration time. This is especially important when EOF is high or when short and/or large internal diameter capillaries are used.
Parallel replenishment/ conditioning operation	Buffer replenishment and capillary conditioning can be performed simultaneously to decrease overall analysis time.

Table 1
Features of the Agilent CE automated buffer replenishment system.

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Experimental

Capillary electrophoresis experiments were performed on an Agilent CE system with diode-array detector and Agilent ChemStation. Fused silica capillaries of 50-µm, and 75-µm inner diameter (id) and an outer diameter of 375 µm were used. Reagents were of the highest grade possible and were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Scientific Resources (Eatontown, NJ, USA). Deionized water (18 Ω) was used exclusively (NANOpure, Barnstead, Dubuque, IO, USA). All buffer solutions were filtered through a 0.2-µm membrane filter prior to use. Detailed experimental conditions are listed with the figure captions.

Results and discussion

The benefits of buffer replenishment are shown for a variety of analytes and buffer systems. In the first example, migration time reproducibility for peptide analysis using a Tris-borate buffer system was examined (figure 1). Overlays of runs 5, 10, and 15 show a constant drift of migration time when the buffer is not replenished (figure 1a). This problem is eliminated by replenishing the buffer between runs (figure 1b). Buffer replenishment is especially useful for analyses that employ indirect detection buffers because these systems generally have poor buffer capacity. This is demonstrated in figure 2 which shows the analysis of inorganic anions using a pyromellitic acid buffer system. Migration time reproducibility over 20 analyses was better than 0.5 % RSD when buffer replenishment was employed. Without replenishment, migration time was unstable and constantly drifted toward longer times (figure 2b).

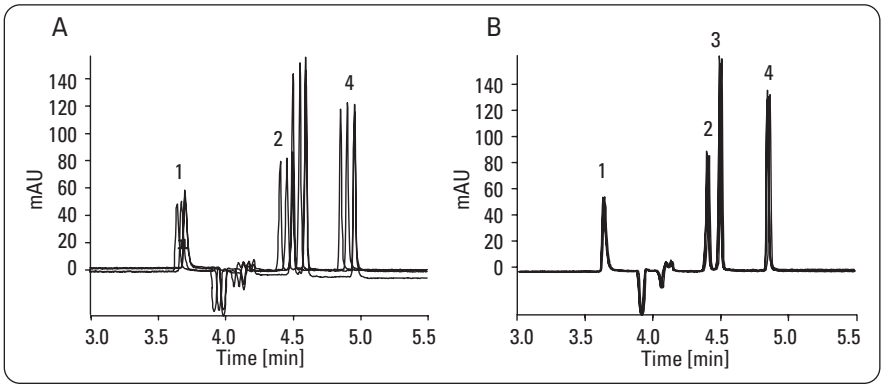

Figure 1
Improved reproducibility over 15 runs in CZE of peptides using buffer replenishment
A) without replenishment, B) with replenishment.

Chromatographic conditions

Sample:	1 neurotensin, 2 angiotensin I, 3 angiotensin II, 4 leu-enkephalen
Buffer:	89 mM Tris, 89 mM boric acid, pH 8.2
Conditioning:	5 min buffer
Capillary:	id = 50 µm; l = 56 cm; L = 64.5 cm
Injection:	100 mbar x s
Electric field:	388 V/cm
Temperature:	35 °C

Detection wavelength/bandwidth: sig. = 200/12 nm, ref. = Off

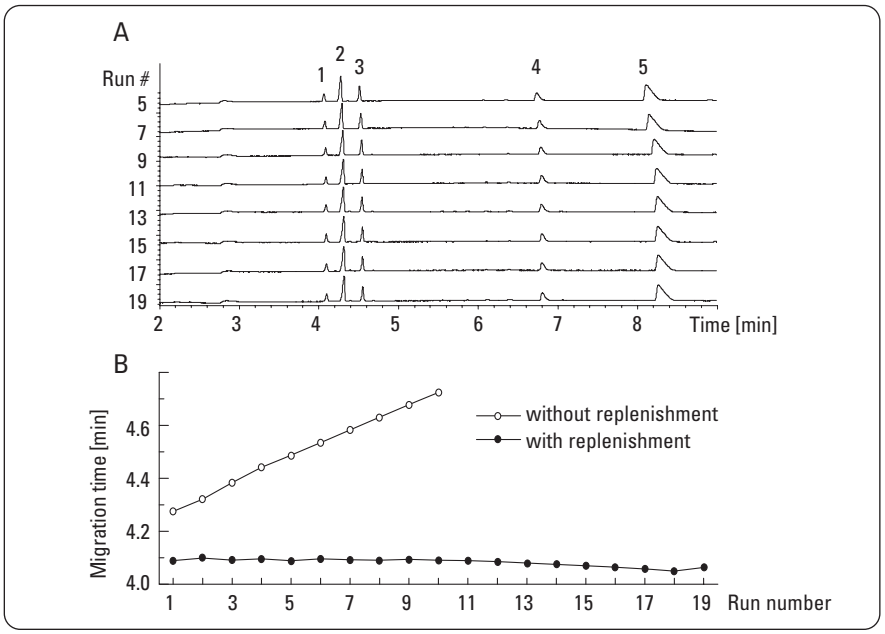

Figure 2
Use of replenishment with indirect UV-detection buffers
A) reproducibility over 19 runs with replenishment between analyses B) migration time stability of chloride with and without replenishment.

Chromatographic conditions

Sample:	1 chloride, 2 sulfate, 3 nitrate, 4 fluoride, 5 phosphate
Buffer:	2.25 mM pyromellitic acid, 6.6 mM NaOH, 0.75 mM hexamethonium hydroxide, 1.6 mM triethanolamine, pH 7.7,
Conditioning:	2 min 0.1 N NaOH, 4 min buffer
Capillary:	id = 50 µm; l = 56 cm; L = 64.5 cm
Injection:	200 mbar x s
Temperature:	25 °C
Electric field:	465 V/cm (negative polarity)
Current:	I = 9 µA

Detection wavelength/bandwidth: sig. = 350/60 nm, ref. = 245/10 nm

Separation of drug-related analytes using a borate buffer system further illustrates the benefit of replenishment (figures 3 and 4).

Again, migration time stability is highly dependent on the use of fresh buffer. The use of 75-µm id capillaries (figure 3) increased buffer degradation relative to the analysis with narrower capillaries. This was due to the higher current generated as well as to increased buffer volume transferred to the outlet vial by the EOF. Siphoning back to the inlet vial increases migration time and broadens peaks. The analysis presented in figure 3 is next used to illustrate the use of the buffer replenishment system for extended operation.

Figure 5 shows four overlaid electropherograms from a 265-run analysis. Migration time remained constant during the 1.8 days of this experiment *Note: peak heights vary since the data was extracted from a linearity study using different sample concentrations*).

Without replenishment, it would have been necessary to use numerous buffer vials and decrease the number of sample vials. However, this itself would be difficult to perform without replenishment because 265 pairs of buffer vials would be required to run the analysis constantly with fresh buffer. The maximum continuous run time of the instrument is dependent on the total analysis time of the experiment, including the time for conditioning. For this example, replenishing both buffer vials after each run with 0.5 mL and repeating the analysis every 10 minutes, more than 500 analyses were performed over 3.5 days using the 500-mL capacity of the replenishment buffer reservoirs.

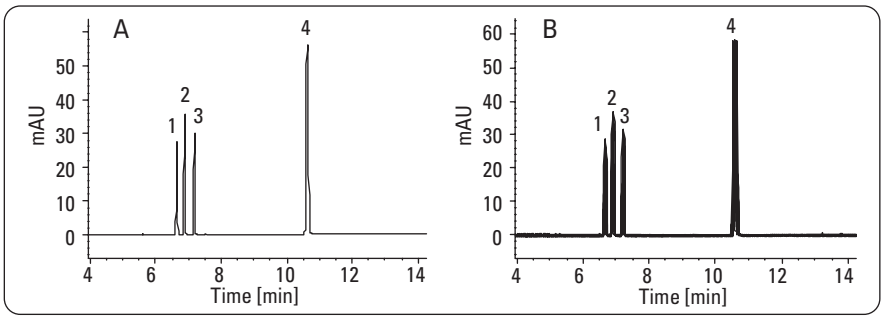

Figure 3
Buffer replenishment for pharmaceutically relevant analytes
A) with replenishment, B) without replenishment between analyses.

Chromatographic conditions

Sample:	1 acetaminophen (91 mg/mL), 2 nicotinic acid (60 mg/mL), 3 acetyl salicylic acid (150 mg/mL), 4 dihydroxybenzoic acid (120 mg/mL)
Buffer:	100 mM borate, pH 9
Capillary:	id = 75 µm; l = 56 cm; L = 64.5 cm
Injection:	175 mbar x s
Electric field:	388 V/cm
Temperature:	25 °C

Detection wavelength/bandwidth: sig. = 245/20 nm, ref. = Off

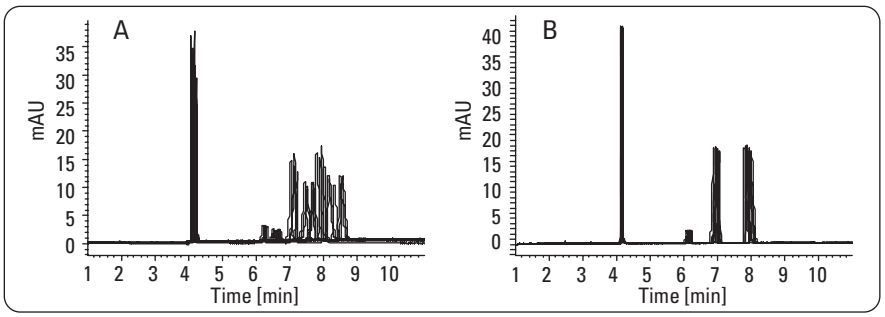

Figure 4
Buffer replenishment for paraben analyses
A) without replenishment, B) with replenishment.

Chromatographic conditions

Samples:	Four substituted parabens
Buffer:	20 mM borate, pH 10
Capillary:	id = 50 µm BF 3; l = 56 cm; L = 64.5 cm
Injection:	200 mbar x s
Electric field:	30 V/cm
Temperature:	25 °C

Detection wavelength/bandwidth: sig. = 295/20 nm, ref. = Off

For this type of work, the sample capacity of the 48-location carousel can be increased, because the system enables vials to be loaded and unloaded and sequences to be updated without interrupting the method or sequence.

Conclusion

Slight changes in buffer composition resulting from electrolysis and other processes significantly affected electroosmotic flow (EOF) and solute migration time. It was shown that frequent replenishment of buffer eliminated these effects and was found to be imperative for maintaining high migration time reproducibility. The automated replenishment system enabled both inlet and outlet buffer reservoirs to be replenished at a frequency determined by the user. Use of the system also increased the number of vial positions available for samples and allowed unattended, continuous operation for more than 250 analyses, with replenishment after each one.

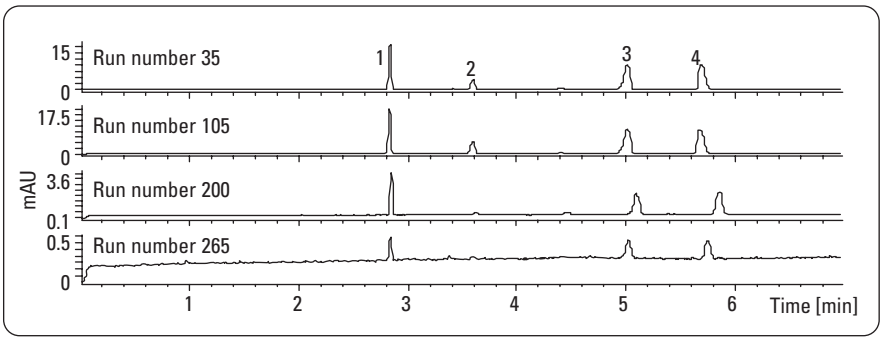

Figure 5
Use of buffer replenishment for long term, unattended operation.

Chromatographic conditions

Sample:	1 acetaminophen (91 mg/mL), 2 nicotinic acid (60 mg/mL)
	3 acetyl salicylic acid (150 mg/mL), 4 dihydroxybenzoic acid (120 mg/mL)
Buffer:	100 mM borate, pH 9
Capillary:	id = 75 µm; l = 56 cm; L = 64.5 cm
Injection:	175 mbar x s
Electric field:	388 V/cm
Temperature:	25 °C

Detection wavelength/bandwidth: sig. = 245/20 nm, ref. = Off

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