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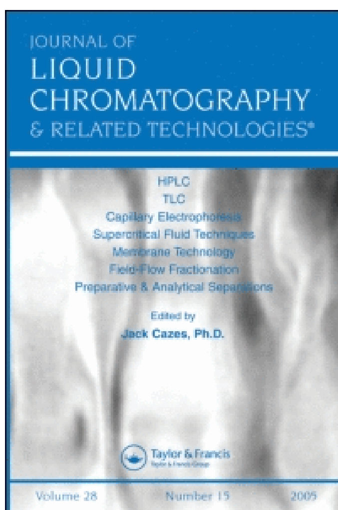

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AN EASY WAY TO ENHANCE ABSORBANCE DETECTION SENSITIVITY OF WATERS QUANTA-4000 CAPILLARY ELECTROPHORESIS SYSTEM

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ABSTRACT

Capillary electrophoresis (CE) has established itself as a powerful and widely utilized technique for routine analytical separations, characterized by short run times and high efficiencies. A major drawback to CE is its low concentration sensitivity. Because total system volume is generally only a few μl, the detector flow cell should have a small volume, which imposes a limit on detection sensitivities. In this study, we report a simple method for creating a region of extended path length for absorbance detection in capillary electrophoresis. Up to a six-fold gain in sensitivity, compared to on-column detection, was accomplished when the sleeve-cell was utilized at the detection point.

INTRODUCTION

Detection of the solute zone in CE can be achieved either while it is migrating through the capillary (on-column detection), or as it elutes from the capillary (off-column detection). In off-column detection, the detector region usually

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contributes to band broadening, while in on-column detection the detection cell is part of the separation capillary. The design of the detector cell is of great importance in ensuring high sensitivity and low background noise.

Several attempts have been made to extend the path length for UV detection in capillary separation techniques by changing the design of the detection cell or by utilizing optical fiber bundles.[1] Chervet et al.[2] and Liu et al.[3] have demonstrated the usefulness of a Z-shaped longitudinal capillary flow cell for micro separation techniques. Another possibility to increase the effective path length across the capillary is the use of an on-column multireflection absorbance cell.[4] Tsuda et al.[5] have evaluated the use of a rectangular glass capillary. Liu and Dasgupta[6] have reported a three-fold gain in sensitivity, without significant loss in efficiency (in CE), by connecting a large diameter capillary to a separation column at the measurement point. Recently introduced capillaries[7,8] with a bubble blown at the detection point (bubble cell) offer a unique approach to extending the optical path. In-depth reviews about detection techniques in CE are given elsewhere.[9-11]

In this study, we report a six-fold increase in detection sensitivity, compared to on-column detection, which was realized when a sleeve capillary (i.d. 220 μm) was utilized at the detection point. The loss of separation efficiency, about 50%, could be minimized by optimizing separation parameters and by improving the design of the sleeve cell.

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EXPERIMENTAL

The capillary electrophoresis system used was the model Quanta 4000 from Waters Chromatography Division, Millipore Corporation, Milford, MA, USA.

The capillary columns utilized in this work were an AccuSep™ (75 μm i.d., 350 μm o.d., total length 60 cm) from Waters Chromatography Division, Millipore Corporation, Milford, MA, USA, and an analytical column 75 μm i.d., and 200 μm o.d. from Scientific Glass Engineering (SGE), Weiterstadt, Germany. The capillary used as a sleeve had a 220 μm i.d. and 350 μm o.d. The polyimide coating was removed (ca. 3 mm) from the sleeve capillary to create a detection window. The sleeve cell arrangement is depicted in Figure 1. The SGE analytical column was divided into two parts. The longer section, labeled as A in Figure 1 (approximately 45 cm) was used as the separation column. One end was immersed into a buffer solution and the other end was inserted into the sleeve cell (B) up to the detector window. The other piece (C) from the 75 μm i.d. column (7.5 cm long) was inserted into the other side of the sleeve with the free end immersed in buffer. The

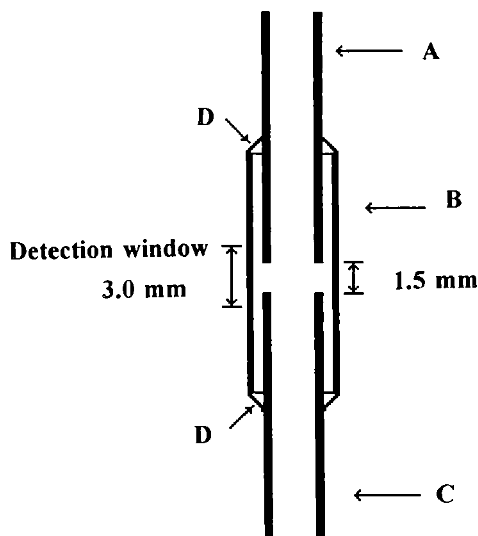

Figure 1. Experimental arrangement of the sleeve-cell (as described in the experimental section).

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distance between the two pieces (A and C) in the sleeve capillary (was approximately 1.5 mm). A detector window already built in the capillary holder for Quanta 4000 is approximately 3 mm. The capillaries A and C were sealed (point D in Figure 1) to the sleeve cell with a polyimide sealing resin, Alltech Associates, Inc., Deerfield, IL, USA. Throughout this work, hydrodynamic sample injection (by siphoning action) was utilized. The running buffer was 20 mM phosphate buffer at pH 7. The vitamin samples (thiamine hydrochloride, nicotinamide and nicotinic acid) were from Hewlett-Packard GmBH, Waldbrunn, Germany.

RESULTS AND DISCUSSION

In Figure 2 are depicted electropherograms obtained, on the one hand utilizing on-column detection with AccuSep™ column, and on the other the SGE column combined into the sleeve-cell (as described in the experimental section). A six-fold increase in normalized peak area (peak area/migration time) was observed when the sleeve-cell was utilized. A 50 % plate loss in going from a 75 μm on-column detection to a 220 μm i.d. sleeve-cell was noted. By mass balance theory, there should be peak compression when the solute z one moves from 75 μm i.d into a 220

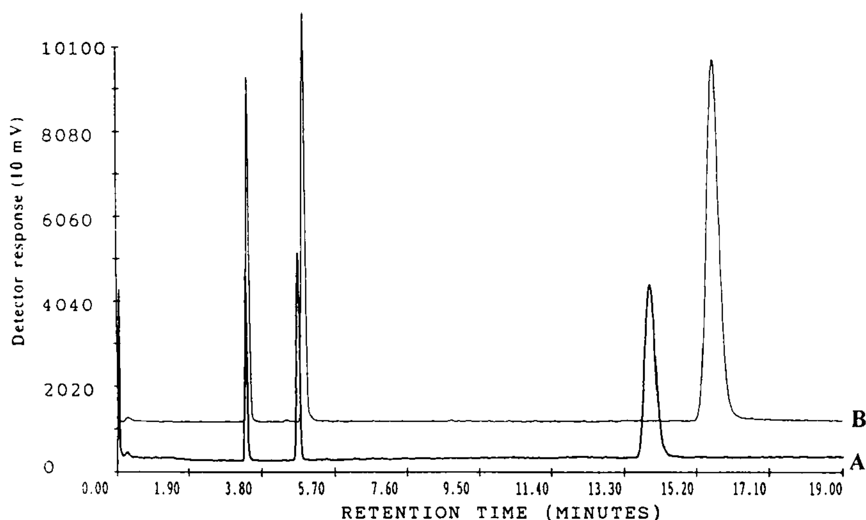

Figure 2. Electropherograms of vitamin sample (thiamine hydrochloride, nicotinamide and nicotinic acid) obtained with AccuSep™ column (A), and SGE column combined into the sleeve-cell (B). Hydrodynamic sample injection for 7 sec; Voltage 15 kV; Detection wavelength 214 nm.

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µm i.d. column, where the sample zone expands rapidly to fill the increased volume.[8] This is true if diffusion can be ignored. In the sleeve-cell, the sample was present in the 1.5 mm gap long enough to allow diffusion to take place. We were anticipating this peak broadening, and because of that, we did not extend the gap between capillaries to the full size of the detector slitwidth (3 mm). Already 1.5 mm gap was too wide and longitudinal solute diffusion in a 220 µm i.d. capillary degraded peak shape and efficiency. This effect is more pronounced for a component with a long migration time (peak 3 in Figure 2). Kuhr[12] investigated sample transfer, at different ionic strengths, across a 50-200 µm gap (without boundary) between two capillaries. There was no significant distortion in the sample zone when the gap between the capillaries was 150 µm or less. As mentioned earlier, the sample zone slows down in the sleeve cell, which explains the difference in migration times obtained in the 75 µm i.d. AccuSep™ column compared with the 75 µm i.d. combined with the 220 i.d. µm sleeve cell.

The asymmetry factors (measure of a peak shape distortion) for thiamine, nicotinamide and nicotinic acid on AccuSep™ column were 3.78, 0.45 and 0.35, respectively. The same solutes had asymmetry factors of 1.26, 1.00 and 1.34 on the

SGE column integrated into the sleeve-cell. This proves that the sample zone does slow down and that peak compression occurrs when the solute moves from the 75 μm column into the 220 μm region. In the cell, the gap between the capillaries is long (1.5 mm), and the internal diameter of the cell is large; the diffusion leads to peak broadening with an improved peak symmetry but reduced efficiency.

This cell design has to be optimized. The use of sleeve capillaries more than three times in diameter than the principal analytical column does not appear to be very practical for CE. However, for capillary analysis systems where the above degree of plate loss is tolerable, this approach may still be attractive for improving detection sensitivity. The heat effect should not be neglected. In systems where the detector itself is thermostated, the results should be better.

CONCLUSION

When the sleeve-cell was utilized at the detection point, a six-fold gain in sensitivity, compared to on-column detection, was accomplished. By optimizing the gap between the capillaries and the detector slit-width, it should be possible to minimize the loss of efficiency. We envision that the sleeve-cell arrangement can substantially increase sensitivity, especially in capillary gel electrophoresis (with cross-linked polymers), where the polyacrylamide gel interferes with the UV absorbance detection of proteins at 214 nm. Furthermore, the cell arrangement described can be produced for any type of CE instruments with any desired combination of i.d. ratio (analytical column to sleeve cell). There is almost no additional cost in producing the cell.

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