Use of extremely short effective length capillaries in CE – injection of sample from the outlet end of the capillary

Technical Note

Introduction

The effective length in capillary electrophoresis, defined by the distance from the injection end to the detector, generally ranges from 40 to 75 cm. Much shorter effective lengths, below 10 cm, are also sometimes very useful. Short effective lengths have the advantage of reduced analysis times, as well as improved peak efficiency and sensitivity. With regard to the latter, when band broadening is primarily due to diffusional processes, minimization of analyte residence time will increase peak sharpness, peak height, and sensitivity. Short capillaries often yield sufficient separation capabilities, especially for simple mixtures, or when selectivity and resolution are high. One example of the latter case is the separation of double-stranded DNA by capillary gel electrophoresis. Short capillaries are also useful for initial sample screening, or for method development, even when complete resolution is not obtained. This approach can be used for the rapid determination of sensitivity requirements (that is, determining if sample concentration is sufficient), and estimation of required effective length to achieve resolution. Minimum capillary lengths are usually defined by the dimensions of the CE instrument. For the Agilent Capillary Electrophoresis system the minimum length from the inlet to the detector is 25 cm, and minimum total length is 33.5 cm. However, as the system allows random vial access to either the inlet or outlet, and as both hydrodynamic and electrokinetic injection can be made from either end, the minimum effective length can be 8.5 cm by injection at the outlet end. This method has the advantage over using capillaries with short total lengths, because injection from the longer inlet side can also be performed simply by changing the method parameters,not changing the capillary. To accomplish this, it is necessary for the system to employ a software controllable, dual-polarity power supply as used here. When injecting from the short end it is very important to have the ability to load the sample by application of pressure or vacuum in an identical fashion to that used when injection is made from the standard inlet end. With the Agilent CE system, pressure or vacuum are applied to the inlet to inject sample at the inlet and outlet, respectively. Electrokinetic injection is also applicable on both capillary ends. This injection method is mainly used when performing capillary gel electrophoresis.

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Experimental

Capillary electrophoresis experiments were performed on the Agilent Capillary Electrophoresis system with built-in diode-array detector and Agilent ChemStation software. Fused silica capillaries of 50-µm and 75-µm internal diameter (id), and an outer diameter of 375 µm were used.

Hydrodynamic injection from the short end of the capillary is achieved by placing the sample vial at the outlet, the buffer vial at the inlet, and then applying a vacuum. For electrokinetic injection the same set-up is used, however, a voltage is applied instead of a vacuum. Electrophoresis is performed with negative polarity to reverse the migration direction. This procedure results in the same reproducible injection which is accomplished when sample is pressure injected at the standard inlet. The method parameters used to generate this are given below:

Method parameters

InHome:	Buffer vial
OutHome:	Buffer vial
Injection (using Injection Table)	
Inlet:	*OutHome*
Outlet:	*InjectVial*
Pressure:	*-x mbar for x seconds*
(use negative pressure to apply vacuum)	
Electrokinetic:	*-x volts for x seconds*
Electric Polarity: Negative	

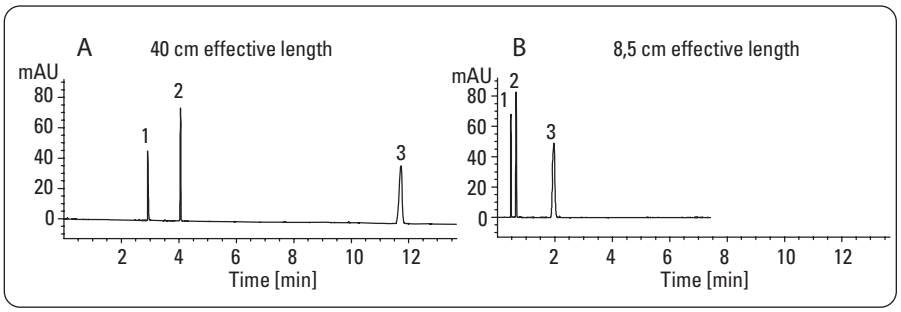

Figure 1
Decreased analysis time for water soluble vitamins by injection from the outlet end of the capillary.

Chromatographic conditions

Sample:	1 thiamine; 2 nicotinamide; 3 nicotinic acid
Buffer:	20 mM phosphate, pH 7
Capillary:	50-µm id, L = 48.5 cm
Injection:	200 mbar x s
Electric field:	412 V/cm
Temperature:	25 °C
Detection:	sig. = 215 nm, 20 nm bandwidth, ref. = 350 nm, 80 nm bandwidth

Reagents were of the highest grade possible and were purchased from either Sigma Chemical Company (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 included in the figure captions.

Results and Discussion

The first example illustrating injection from the short end of the capillary involves the CZE separation of three water soluble vitamins (figure 1). A 20-mM phosphate buffer, pH 7, was used for separation. As can be noted in figure 1a, more than adequate resolution of the three species was obtained in 12 minutes using a 40- cm effective length capillary. The identical hydrodynamic injection and electrophoresis conditions using the outlet end of the capillary is shown in figure 1b.

The short 8.5-cm effective length was sufficient to resolve all analytes, and the analysis time was reduced to about two minutes.

The second example shows the separation of DNA restriction fragments and PCR products (figure 2). For these separations a sieving buffer was employed to effect a size-dependent separation. Since electroosmotic flow was greatly reduced, the DNA eluted at the anode and reversed polarity was used.

Analysis of the restriction fragment standards, using both the 40-cm effective length when injected at the inlet, and the 8.5-cm effective length from the outlet, are shown in figures 2a and 2b. Once again, the analysis time was significantly reduced using the shorter capillary. More importantly, the slight loss of resolution was found only for the doublet at 4.3 minutes, and the last two fragments at 5.8 minutes. Resolution of the PCR sample however, was not sacrificed when analyzed from the short end. As shown in figure 3, complete resolution of the 73-bp, 198-bp, and 271-bp species was obtained.

Conclusion

This note illustrates the utility of sample injection from the outlet end of the capillary. This procedure was possible as the system offered user-definable inlet and outlet locations; the ability to perform both hydrodynamic and electrokinetic injections from either end of the capillary, and software controlled, power supply polarity.

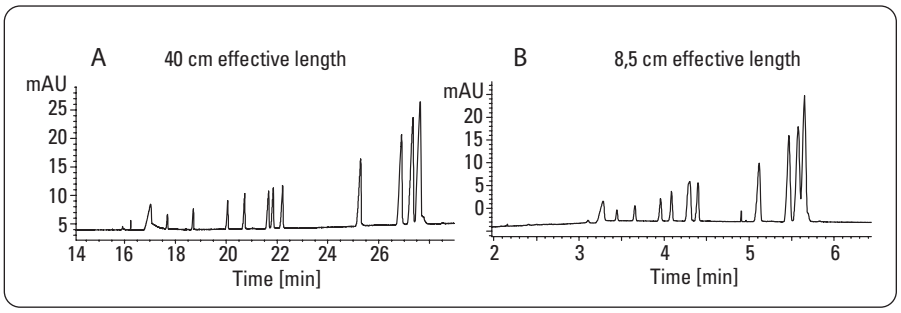

Figure 2
Decreased analysis time for the separation of dsDNA by injection from the outlet end of the capillary.

Chromatographic conditions	
Sample:	X174-Hae III (200 µg/mL), Beckman eCAP dsDNA 1000 kit
Capillary	100-µm id, L = 48.5 cm
Injection:	5 kV, 5 sec (reversed polarity for injection from standard inlet side)
Electric Field:	200 V/cm (reversed polarity for injection from standard inlet side)
Current:	19 µA
Temperature:	20 °C capillary, 10 °C carousel
Detection:	sig. = 260 nm, 15 nm bandwidth, ref. = 340 nm, 80 nm bandwidth

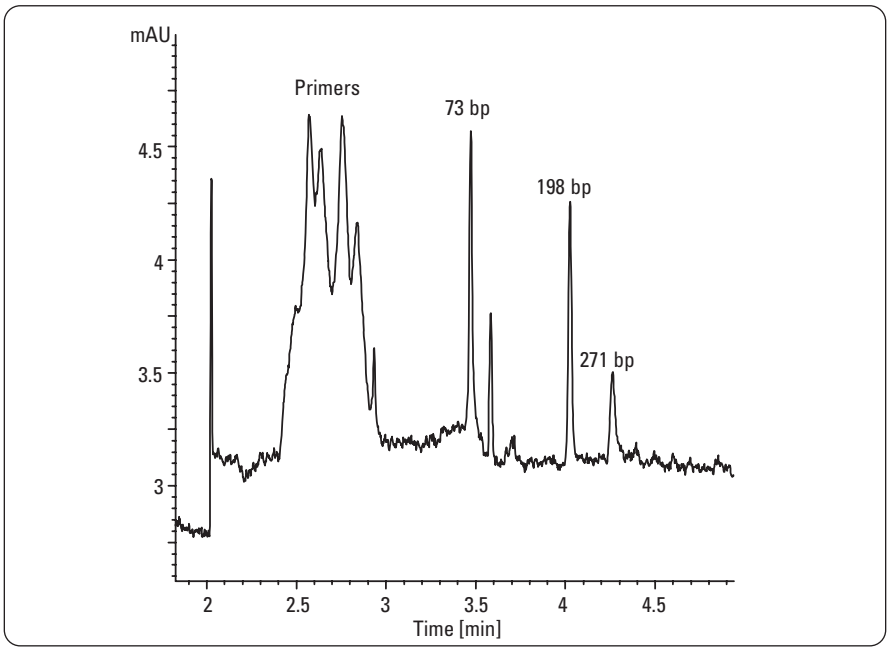

Figure 3
Analysis of PCR mixture using injection from the outlet end of the capillary.

Chromatographic conditions	
Sample:	primers, 73 bp, 198 bp, 271 bp (10 ng/Beckman eCAP dsDNA 1000 kit
Capillary:	100-µm id, l = 8.5 cm, L = 48.5 cm
Injection:	5 kV, 5 sec
Electric Field:	200 V/cm
Current:	19 µA
Temperature:	20 °C capillary, 10 °C carousel
Detection:	sig. = 260 nm, 15 nm bandwidth, ref. = 340 nm, 80 nm bandwidth

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