Talanta 130 (2014) 288–293

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Capillary electrophoresis coupled with automated fraction collection

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ARTICLE INFO

*Article history:*
Received 11 April 2014
Received in revised form
2 July 2014
Accepted 7 July 2014
Available online 15 July 2014

*Keywords:*
Capillary electrophoresis
Fraction collection
CE-SELEX
Aptamer

ABSTRACT

A fraction collector based on a drop-on-demand ink-jet printer was developed to interface capillary zone electrophoresis with a 96 well microtiter plate. We first evaluated the performance of the collector by using capillary zone electrophoresis to analyze a 1 mM solution of tetramethylrhodamine; a fluorescent microtiter plate reader was then used to detect the analyte and characterize fraction carryover between wells. Relative standard deviation in peak height was 20% and the relative standard deviation in migration time was 1%. The mean and standard deviation of the tetramethylrhodamine peak width was $5 \pm 1$ s and likely limited by the 4-s period between droplet deposition. We next injected a complex mixture of DNA fragments and used real-time PCR to quantify the product in a CE-SELEX experiment. The reconstructed electrophoretic peak was 27 s in duration. Finally, we repeated the experiment in the presence of a 30-μM thrombin solution under CE-SELEX conditions; fractions were collected and next-generation sequencing was used to characterize the DNA binders. Over 25,000 sequences were identified with close matches to known thrombin binding aptamers.



## 1. Introduction

Capillary electrophoresis is a useful tool for the analysis of biological samples such as polypeptides, lipids, DNA sequencing fragments, and metabolites [1–9]. Capillary electrophoresis instruments typically employ absorbance, laser-induced fluorescence, or mass spectrometry for on-line detection and analysis. While on-line detection is essential for most analyses, sample fractionation and recovery are necessary for use of capillary electrophoresis for preparative separations.

There have been few reports of capillary electrophoresis for preparative separations. Gannaro and Salas-Solano reported the use of a commercial capillary electrophoresis instrument that deposits fractions in a 96 well microtiter plate for characterization of deaminated peptide variants [10]. The technique first measures the migration time of a target molecule. In subsequent runs, the separation voltage is set to zero at the migration time of a target compound; the distal end of the capillary is then placed into a well of a microtiter plate that contains running buffer. The voltage is reapplied for a period of time corresponding to the analyte peak width to deposit the target molecule within the well. Potential is again set to zero, and the capillary tip is returned to the normal outlet buffer. Timing of subsequent fraction collection is reported to be difficult, and a separate run appears to be typically used for each fraction that is collected. This protocol requires a number of capillary manipulations, requires knowledge of the migration time of the components to be collected, and appears to allow collection of only one fraction from an injection.

It would be desirable to deposit fractions in succession into the wells of a microtiter plate without stopping the separation or careful calibration of migration time of analyte. In 1985 Hjertén and Zhu demonstrated the first fraction collection using capillary electrophoresis (CE) as the separation method [11]. Since then, fraction collection has primarily been used to couple CE with matrix assisted laser desorption/ionization (MALDI) mass spectrometry. Owing to the difficulty of performing on-line CE-MALDI-MS, off-line coupling systems are preferred [12,13]. Several approaches have been reported for off-line preparative separation and fractionation that mainly differ in the delivery of analyte to the MALDI plate [14–16].

We reported a CE-MALDI interface design that employs a drop-on-demand matrix sheath flow controlled by a high-speed ink jet printer valve [17]. That instrument placed the distal end of the separation capillary within an ink-jet nozzle, which was held at ground potential. MALDI matrix solution was introduced through a Tee fitting attached to the nozzle; the MALDI solution was pumped by nitrogen pressure. A high-speed miniaturized valve controlled deposition to the MALDI plate. In this paper, we modified our MALDI plate spotter for use as a 96 well fraction collector in preparative capillary zone electrophoresis. We demonstrate the fraction collector for analysis of a dye using a fluorescent plate

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http://dx.doi.org/10.1016/j.talanta.2014.07.018
0039-9140/

reader, for analysis of an oligonucleotide using real-time PCR, and for generation of aptamers using CE-SELEX [18,19].

## 2. Materials and methods

### 2.1. Materials and reagents

Fused silica capillary (50 μm ID and 150 μm OD) was purchased from Polymicro Technologies (Phoenix, AZ USA). The fluorescent standard 5-carboxytetramethylrhodamine SE (TAMRA) was purchased from AnaSpec (San Jose, CA USA). Other reagents were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO USA). All solutions were prepared from deionized-distilled water obtained from a Barnstead Nanopure System (Thermo-Fisher Scientific, Waltham, MA USA).

### 2.2. Laser-induced fluorescence detection

Two systems were employed. The first, described in this section, employed laser-induced fluorescence detection and was used to provide a reference separation. The instrument was similar to others reported by our group [20–22]. Briefly, fluorescence was excited using a CW 532 nm diode-pumped laser (CrystaLaser Model CL532-025), which was focused into the sample stream at the center of a sheath-flow cuvette. Fluorescence was collected at right angles and detected by a cascade of single-photon counting avalanche photodiode modules (PerkinElmer, Montreal, PQ Canada) with a sampling frequency of 50 Hz [23,24].

A bare fused silica capillary (50 μm ID, 150 μm OD, 40-cm length) was used for analysis. The first sample was 1 μM TAMRA prepared in a 15 mM sodium tetraborate running buffer. The sample was injected for 5 s at 5 kV and electrophoresis was performed at 10 kV, supplied by a Spellman High Voltage power supply (CZE1000R, Newark, NJ USA). Samples were analyzed in triplicate.

A second sample consisted of a random single-stranded DNA library (Sigma-Aldrich, St. Louis, MO USA). The 80 base sequence, 5′-AGCAGCACAGAGGTCAGATG-N(40)-CCTATGCGTGCTACCGTGAA-3′, was designed with priming regions for PCR amplification at both the 5′- and 3′-ends flanking a random region allowing up to $4^{40}$ sequences within the pool. A TAMRA fluorescent tag was incorporated at the 5′-end for laser-induced fluorescence detection. The library was injected for 5 s at 5 kV onto a preconditioned 45 cm capillary. The separation running buffer was 10 mM sodium tetraborate/HEPES (Sigma-Aldrich, St. Louis, MO USA), and the separation was performed at 15 kV.

A third sample consisted of a 10 μL aliquot of the 100 μM stock oligonucleotide library solution added to 10 μL of binding buffer (50 mM TRIS, 100 mM NaCl, 1 mM $CaCl_2$). The mixture was heated to 94 °C to destroy secondary structures that may have formed during storage. The solution was cooled by 0.5 °C/second to a final temperature of 20 °C in a thermal cycler (PTC-100, MJ Research). The heat-treated 10 μM random ssDNA library solution was incubated at room temperature with 1 mg/mL human α-thrombin protein (Hematologic Technologies, Inc., Vermont USA) for a minimum of 15 min to allow binding.

### 2.3. Fraction collector

A second system employed fraction collection and is diagramed in Fig. 1. The distal end of the separation capillary was threaded through a Tee fitting using a capillary sleeve and ferrule from Upchurch Scientific (Oak Harbor, WA USA). The valve, tee, nozzle, and inline filter were the same as used in ref 17 and were from The Lee Company (Westbrook, CT USA).

The capillary tip was positioned at the exit of the nozzle to avoid contamination within the spotting apparatus. The capillary and nozzle were secured ~2 mm above the surface of the plate to ensure proper sample delivery. The metal nozzle was held at ground potential. The deposition buffer was held under nitrogen pressure at ~5 psi. The collection plate was positioned below the instrument on a Prior Scientific microscope stage (Rockland, MA USA). The stage was mounted to an aluminum breadboard by a Plexiglas block. Instrument control was programmed in Labview (National Instruments, Austin, TX USA).

The motion of the stage was matched to a 96 well microtiter plate with 9 mm well spacing in the *X* and *Y* dimensions in a 12 × 8 pattern. The stage was moved in the *X* direction in odd rows and the $-X$ direction in even rows, creating a serpentine pattern. Fraction width, which controls time between depositions, was determined from reference data obtained by the fluorescence detector. Under typical conditions, each droplet consists of 10 μL sheath liquid and a few picoliters of solution from the capillary.

### 2.4. TAMRA fraction collection

The separation conditions and the capillary length were identical to those used for laser-induced fluorescence detection. Due to the dilution of analyte in the fraction collector and the poor sensitivity of the fluorescent plate reader, we used a very high concentration TAMRA solution for this characterization experiment. A 1 mM TAMRA solution was prepared in the 15 mM sodium tetraborate separation buffer and injected for 5 s at 5 kV.

The capillary was preconditioned for 5 min with NaOH, $ddH_2O$, and separation buffer before each analysis. Following each preconditioning step, the valve was primed for 2 s with either $ddH_2O$ or separation buffer in the valve reservoir.

Half-area, black with clear bottom, 96-well plates (Corning, Corning, NY USA) were used for collection. A fraction width of 4 s was determined from the reference data obtained by capillary electrophoresis with fluorescence detection. The valve pulse width was set to 0.05 s, dispensing 15 mM sodium tetraborate buffer through the valve. Fraction collection and electrophoresis began simultaneously. Fluorescence intensity within each of the 96 wells was measured with a SpectraMax M5 Microplate Reader (Molecular Devices, Sunnyvale, CA USA) using SoftMax Pro software (Table 1).

### 2.5. Oligonucleotide fraction collection

A random oligonucleotide library (Sigma-Aldrich, St. Louis, MO USA) served as a ssDNA pool. The 80 base sequence, 5′-AGCAGCACAGAGGTCAGATG-N(40)-CCTATGCGTGCTACCGTGAA-3′, was designed with priming regions for PCR amplification at both the 5′- and 3′-ends flanking a random region allowing up to $4^{40}$ sequences within the pool. No fluorescent tag was attached to this DNA pool.

The library was injected for 5 s at 5 kV onto a preconditioned 45 cm capillary. The separation running buffer was 10 mM sodium tetraborate/HEPES (Sigma-Aldrich, St. Louis, MO USA), and the separation was performed at 15 kV.

Hard-shell, white well, 96-well PCR plates (Bio-Rad) were used for fraction collection. A fraction width of 7 s was determined by reference data obtained by laser-induced fluorescence detection. The valve pulse width was set to 0.05 s, precisely dispensing valve buffer. To limit sample handling and sample loss, the valve buffer reservoir contained all necessary real-time PCR reagents per the manufacturers protocol; reagents and final concentrations for 10 μL reactions were iTaq Universal SYBR Green supermix (1 ×) and forward and reverse primers (300 nM each). We deviated from the recommended protocol and diluted the PCR reagents in