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Microchip isoelectric focusing using a miniature scanning detection system

A miniature scanning fluorescent detector has been developed for plastic microchannel isoelectric focusing (mIEF) analysis. The detector, comprised of a lamp and photomultiplier tube (PMT) on a moving stage, measured the real-time distribution of fluorescently labeled peptides subjected to gel-free mIEF. During the run, the effective length of the 6-cm channel was scanned every 9 s. Analysis was completed within 5 min while still obtaining high resolution and sensitivity. In addition, the scanning detector was used to characterize peptide migration properties within the channel by providing simultaneous temporal and spatial measurements.

Keywords: Microchip isoelectric focusing / Miniature scanning detector EL 4464

1 Introduction

Capillary isoelectric focusing (CIEF) is routinely used to separate and characterize peptides [1]. Typical applications utilize a fused-silica capillary with an optical fixed window for laser induced fluorescence detection (LIF). However, microchip platforms, typically consisting of one or more microchannels etched in glass, are becoming an attractive alternative to capillaries since run times can be significantly decreased and integration with other channels in a microfluidic circuit is more feasible [1, 2]. Most published reports describe channels etched in glass. Unfortunately, glass is a relatively expensive material for fabricating complex microfluidic structures. Since our goal is to develop automated, disposable cartridge systems for peptide analysis using an inexpensive instrument, we have explored, based on previous studies of several groups [1–9], the combined use of a plastic microchannel and a miniature scanning detection system to perform rapid, gel-free microchannel IEF (mIEF) analysis. In this report, a lamp-induced fluorescence detection system is described that incorporates scanning capability to monitor, real-time, peptide movement along the length of a microchannel during mIEF. In addition, the ability to simultaneously collect time and spatial measurements of the fluorescent signal in the microchannel permitted the characterization of electroosmotic flow, residual pressure-driven flow and diffusion within the microchannel.

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Abbreviations: mIEF, microchannel isoelectric focusing; **PMMA**, poly(methyl methacrylate); **PMT**, photomultiplier tube; **RG**, rhodamine green

2 Materials and methods

2.1 The scanning detection system

A plastic microchip containing a microchannel (see below) was placed in a plastic holder. Wells at each end of the microchannel accommodated anolyte and catholyte, sample, and platinum electrodes. The platinum electrodes were connected to a programmable high-voltage power supply (model PS350; Stanford Research Systems, Sunnyvale, CA, USA). The plastic holder was fitted on a support platform suspended above the scanning detection system. A longitudinal side of the microchip was illuminated with excitation light from a linear fiber optic array (model BFX936; Dolan-Jenner, Lawrence, MA, USA) originating from 150 W quartz-halogen lamp (model PL-900, Dolan-Jenner) and filtered by a bandpass filter at 485 nm (model XM-485; Corion, Franklin, MA, USA). The excitation light uniformly excited the microchannel. The signal from the microchannel was detected with confocal optics and a photomultiplier tube (PMT) (Fig. 1). The confocal optical system collected the light from the channel, sending the light through a bandpass filter (model XM-530; Corion), and imaged the light through a spatial filter with a 50 μm wide slit to the PMT (model C944; Perkin-Elmer Optoelectronics, Santa Clara, CA, USA). The PMT had a standard bialkali photocathode, and a very low dark current due to the channel photomultiplier geometry. The PMT was driven with ~2000 V from a high-voltage power supply, and the output from the PMT was amplified and detected with an A/D and DIO card (model Daq2000; Iotech, Cleveland, OH, USA).

The confocal optical system and PMT were mounted on a manual translation stage to provide a focus for the optical system onto the channel. The manual translation stage was mounted on a motorized translation stage that had a maximum scan length of 6.3 cm. The motor for the stage was a standard two phase stepper motor controlled by a

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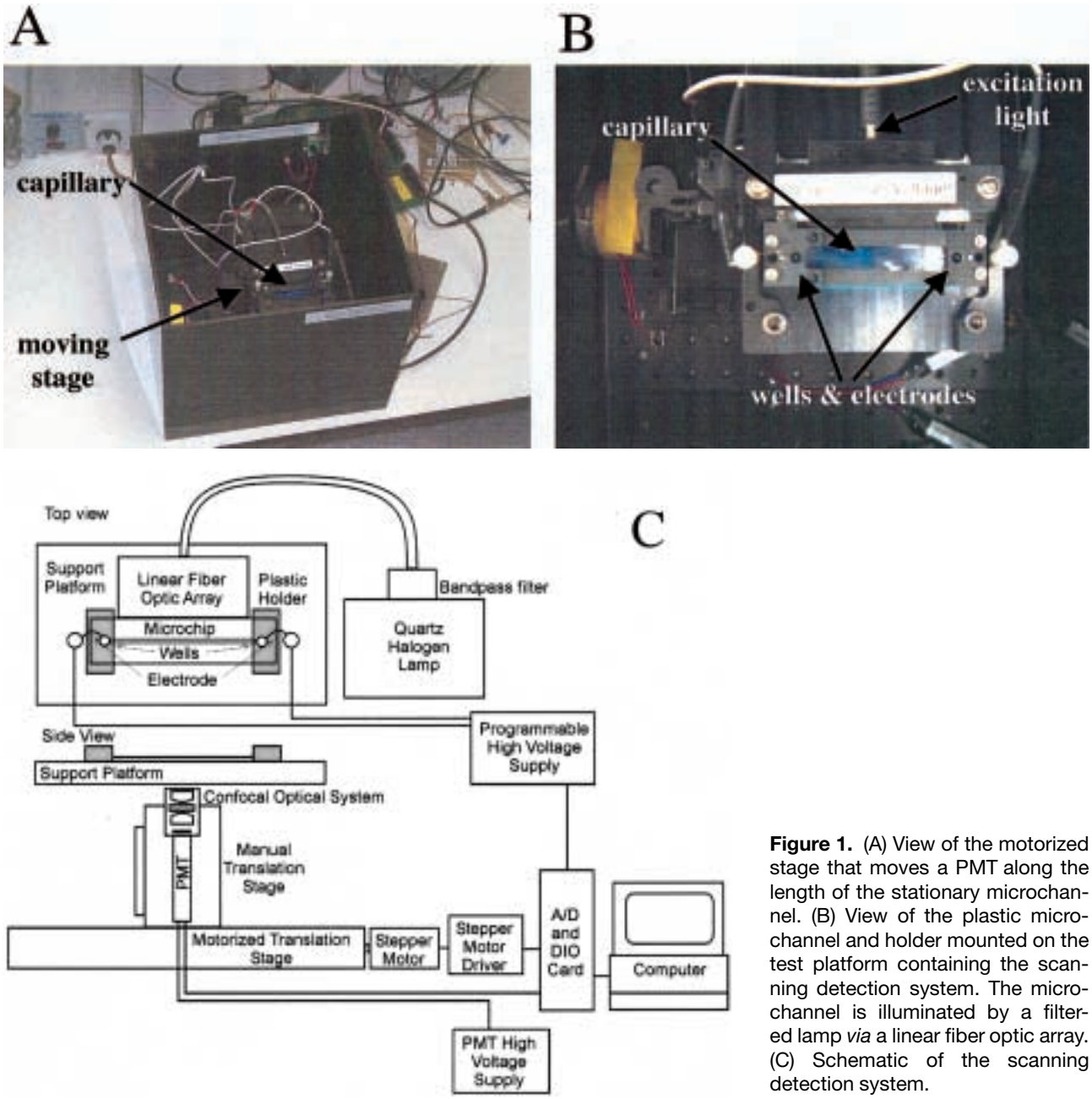

Figure 1. (A) View of the motorized stage that moves a PMT along the length of the stationary microchannel. (B) View of the plastic microchannel and holder mounted on the test platform containing the scanning detection system. The microchannel is illuminated by a filtered lamp *via* a linear fiber optic array. (C) Schematic of the scanning detection system.

driver (model RD-021M8; Semix, Fremont, CA, USA). The mechanical resolution of the stage was less than 10 μm. For the experiments, the resolution of the system was limited by optical resolution of the system as determined by the spatial filter. The A/D and DIO card also interfaced to the stepper motor diver and the programmable high-voltage supply. A computer with customized software controlled the scanning and data collection. To test the effects of possible background fluorescence due to absorption of excitation light in the microchip material, a comparison was made between a 1-mm thick sample of the cast poly (methyl methacrylate) (PMMA) material used in the microchip material and a 1-mm thick sample of soda-lime glass. The comparison tests showed that the background fluorescence absorbance of the acrylic was 50% of that of glass material. Typical backgrounds for the microchips were less than 5% of the dynamic range of the system.

2.2 Microchannel fabrication

Microchannels (100 μm × 100 μm × 6 cm) were created in PMMA by laser ablation using either an Excimer (248 nm) or a TEA-CO_2 laser. The capillary passage was formed by

bonding the PMMA piece containing the channel to a PMMA piece with 0.4-mm holes that matched with the ends of the channel. Bonding between the etched substrate and the cover piece was accomplished by incubating at 105°C for 60 min with the pieces held firmly together [7, 8, 10–12]. The effective scan length of the microchannel was 4 cm.

2.3 IEF conditions

For mIEF, the channel was primed with a solution of 400 μL H_2O, 30 μL Bio-Rad carrier ampholytes (pH range 3–10), and 10 μL of a mixture of three peptides (20 pmol each). The catholyte was 20 mM NaOH and the anolyte was 91 mM H_3PO_4. Focusing and detection was achieved at 1.5 kV for 5 min. Peptide RG1-HHHKG (basic), peptide RG1-VHLTPVEK (intermediate) and marker RGX (acidic), where RG = rhodamine green, were provided by Vysis, Dowers Grove, JL, USA [9, 13]. The Beckman MDQ capillary system (Palo Alto, CA, USA) was used to perform conventional CIEF analysis. Ten μL of the three labeled analytes (45 nmol each), 200 μL eCAP-CIEF gel and 4 μL ampholytes (pH range 3–10) (Beckman) were injected for 1 min at 138 kPa in a 27 cm × 50 μm ID coated eCAP neutral capillary. The catholyte was 20 mM NaOH and the anolyte was 91 mM H_3PO_4 in CIEF gel. Focusing and detection was accomplished at 15 kV (555 V/cm) and 0.5 psi for 55 min. All runs were performed in the normal polarity mode.

3 Results and discussion

The aim of the study was to demonstrate that a relatively inexpensive scanning detector could be used for plastic mIEF analysis (Fig. 1) without using a gel to stabilize the focused peptides [7, 9, 11, 13]. The system is capable of both fixed-point or scanning detection modes. However, since standard CIEF using a fixed-point detector requires gel addition to the sample to minimize analyte diffusion as the focused sample is pneumatically driven past the detector, scan mode was employed to eliminate the gel and decrease the run time. Scan mode enabled real-time data acquisition as the peptides focused when voltage was applied. The scan rate could be adjusted for optimal speed or sensitivity. Slow scans increased sensitivity by allowing greater signal acquisition, whereas fast scans in conjunction with higher run voltages decreased run times. For this study, an 8-s scan was performed every 9 s.

Three analytes labeled with rhodamine green (RG) were used for system testing [2]. The analytes consisted of two peptides, RG1-HHHKG (theoretical p*I* 8.77) and RG1-VHLTPVEK (theoretical p*I* 6.72), and marker RGX (experimental p*I* 5.4). A mixture of the two peptides and the acidic marker was subjected to mIEF using a 6-cm channel with an effective scan length of 4 cm. The short length of the microchannel and the real-time data acquisition in scan mode allowed simultaneous separation and detection. mIEF analysis was completed within 5 min compared to 40 min using the Beckman MDQ system (Fig. 2). Despite using only a 6-cm microchannel and an inexpensive detection system, ample resolution and sensitivity were achieved to detect and discriminate the three analytes. Nevertheless, additional optimization and testing is required to obtain similar resolution to the commercial CIEF system.

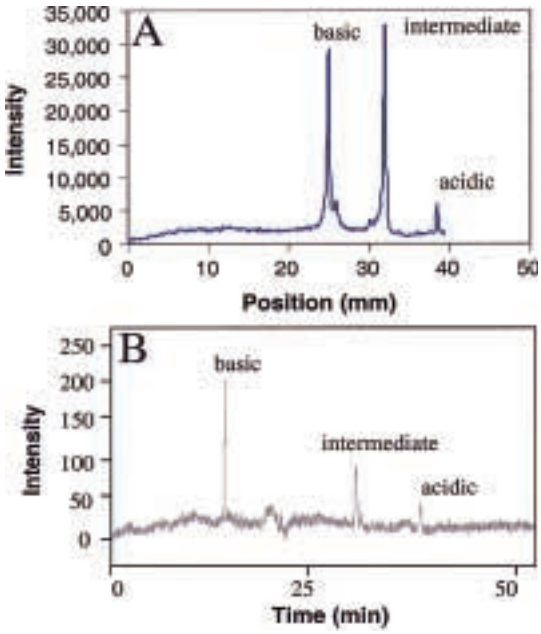

Figure 2. Analysis of peptides (RG1-HHHKG and RG1-VHLTPVEK) and an acidic marker (RGX) by (A) mIEF using a TEA-CO2 laser-ablated PMMA channel and (B) CIEF using the Beckman MDQ system.

The real-time imaging of the microchannel allowed for the determination of EOF and pressure-driven flow rates (Fig. 3). To accomplish this, the peptide RG1-HHHKG was focused, the peak movement was monitored for 2 min with the voltage on and then for 10 min with the voltage off. The peak velocity with the voltage on was a function of residual pressure-driven flow and EOF, and was determined to be 0.36 cm/min (Fig. 3B). When the electric field was discontinued, the peak velocity, affected exclusively by pressure-driven flow, was calculated to be 0.23 cm/min (Fig. 3C). Therefore, an EOF rate of 0.13 cm/min was obtained from the difference in peak velocity in

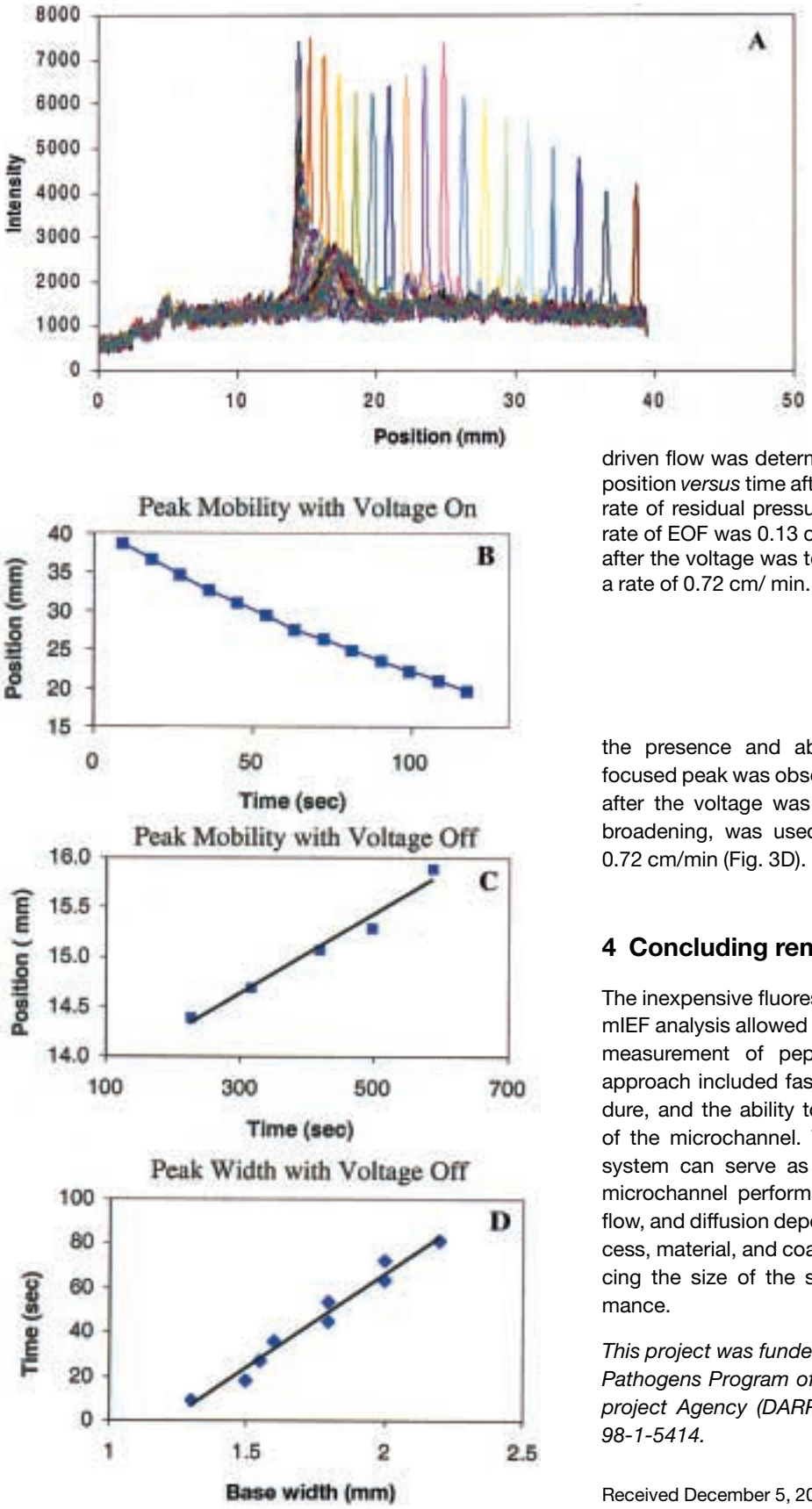

Figure 3. (A) EOF and pressure-driven flow measurements of peptide RG1-VHLTPVEK subjected to mIEF (The figure contains successive scans captured every 9 s, indicating the movement of the peak from an initial position at 39 min travelling to the left and defocusing at 15–17 mm). The focused peak mobility was monitored at 375 V/cm for 2 min followed by 0 V/cm for 10 mm. (B) The focused peak position in the microchannel as a function of time when voltage was applied. Peak migration as a result of combined EOF and pressure-driven flow was determined to be 0.36 cm/min. (C) Peak position *versus* time after the voltage was terminated. The rate of residual pressure flow was 0.23 cm/min and the rate of EOF was 0.13 cm/min. (D) Peak width *versus* time after the voltage was terminated. The peptide diffused at a rate of 0.72 cm/ min.

the presence and absence of an electric field. The focused peak was observed to rapidly decay immediately after the voltage was terminated. This decay, or peak broadening, was used to calculate a diffusion rate of 0.72 cm/min (Fig. 3D).

4 Concluding remarks

The inexpensive fluorescent scanning detector for plastic mIEF analysis allowed simultaneous temporal and spatial measurement of peptide migration. Benefits of this approach included fast analysis times, a gel-free procedure, and the ability to quantitate fluidic characteristics of the microchannel. The latter demonstrates how the system can serve as a tool to evaluate and optimize microchannel performance, since EOF, pressure-driven flow, and diffusion depend on the channel fabrication process, material, and coating. Future work will involve reducing the size of the system and improving the performance.

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