TWO-DIMENSIONAL CHIP-BASED PROTEIN ANALYSIS USING COUPLED ISOELECTRIC FOCUSING AND CAPILLARY ELECTROPHORESIS

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ABSTRACT

A chip-based acrylic microfluidic device that sequentially couples isoelectric focusing (IEF) and capillary electrophoresis (CE) has been designed and demonstrated. To our knowledge, this is the first time such an implementation of on-chip two-dimensional (2-D) electrophoresis has been presented. Both dimensions in this liquid-phase system were characterized using a full-field CCD imaging approach. Commercial ampholytes were used in microchannel-based IEF to sustain a stable pH gradient. Analysis of the concentrating IEF step revealed substantially reduced electroosmotic mobilities, as compared to typical mobilities for acrylic microchannels filled with standard buffer solutions. Due to this residual electroosmotic flow, IEF species were simultaneously rapidly focused and slowly mobilized into intersections. After IEF separation and 100x pre-concentration, voltage switching was used to electrokinetically inject portions of a multi-protein mixture into the second separation, which was ampholyte-based CE. Mobility information was obtained from the second dimension. Results are presented in a 2-D area plot. With optimization, this architecture has the potential to be a basis for high-throughput, high-resolution protein and peptide analysis.

INTRODUCTION

Multi-dimensional separation techniques can be used to attain ultra-high resolution. Typically, two independent separation mechanisms are employed sequentially, each providing a selective displacement along respective separation axes.[**1**] This coupling of techniques allows species that would not necessarily be resolved by either technique to become fully resolved.[**2**] Two-dimensional electrophoresis sequentially couples IEF to CE, thus taking advantage of the demonstrated independence of the two mechanisms. This technique is typically implemented, over a period of hours, using slab-gel and coupled capillary formats; wherein, an IEF step comes to completion, is halted, and a subsequent CE analysis proceeds. The sequential coupling of discrete separation mechanisms is relevant to assays with numerous sample species, as in the case of proteomic analyses[**3**], but has been described as a 'bottleneck' in such work.

During miniaturized IEF, species were simultaneously focused and mobilized, as previously demonstrated.[**4**] Species concentrations were increased by ~100x due to focusing. In chip-based 2-D electrophoresis, the focused species were subsequently electrokinetically injected into the CE dimension and separated based on mobility differences. This microfluidic system opens possibilities for further species manipulation and analysis. Additionally, the low viscosity of the ampholyte solution and the short channel lengths employed in this work substantially decreased the analysis time for both IEF and CE, as compared to a slab-gel approach. Integration of the respective dimensions on a single device is investigated and 2-D results are presented.

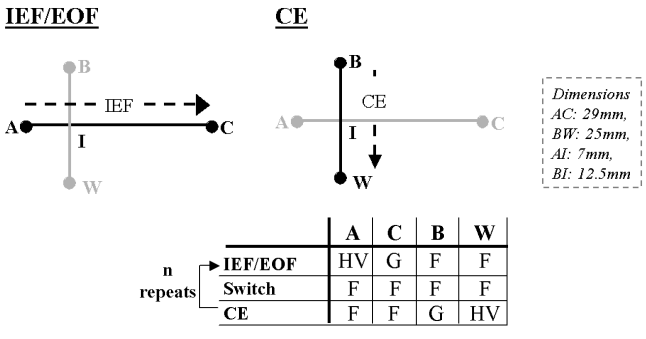
EXPERIMENTAL DETAILS

Commercial carrier ampholytes (pH 3 to 10, Bio-Rad Laboratories) were used as the separation media. Catholyte and anolyte were 40 mM sodium hydroxide and 20 mM phosphoric acid, respectively. The sample mixture consisted of green fluorescent protein (180 nM), unpurified FITC-labeled ovalbumin (100 nM), and FITC-labeled dextran (100 nM, MW 10 kDa).

The planar multi-dimensional chips were designed in-house and fabricated by ACLARA Biosciences in poly(methyl methacrylate) using an imprinting and laminating technique similar to that reported in the literature (*w* x *d*: 200 µm x 20 µm).[**5**] Channel geometry was that of a cross-t intersection (Figure 1). Custom fixturing was designed and fabricated in-house to provide wells for inserting platinum electrodes and allow for pressure cleaning of the channels. High voltage was applied and adjusted using a computer-controlled high voltage power supply (Micralyne). Species transport was monitored using standard epifluorescence techniques and a cooled CCD camera (Roper Scientific). A 0.31x demagnifier (Diagnostic Instruments Inc.) increased the field of view. Spatial electropherograms were obtained by binning each CCD image in a direction perpendicular to the particular separation axis.

INTEGRATED DEVICE OPERATION

IEF and CE were controlled by the chemistry (including pH) and applied potential at specific microchannel reservoirs. A uniform ampholyte buffer solution was used as the separation media for all separations performed using the integrated device. The device architecture is depicted in Figure 1. While the ampholytes in the IEF dimension align under an applied axial electric field to form an axial pH gradient (between *A* and *C*) the ampholytes in the CE dimension (*BW*) remain unfocused, behaving as a buffer with a measured pH of 8.



		A	C	B	W
n repeats	IEF/EOF	HV	G	F	F
	Switch	F	F	F	F
	CE	F	F	G	HV

Figure 1. IEF & CE separation modes in the multi-dimensional system. *Reservoirs: A (anolyte), B (buffer), C (catholyte), and W (waste). HV: high voltage, F: floating, G: ground.*

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The 2-D separation was conducted by repeating a three step gated injection sequence: (1) an initial IEF/EOF (mobilization) step, (2) switching, and (3) a CE step, (see Figure 1). During IEF, the species focus, separate and essentially form a 'queue' for sampling into the second dimension. Low-dispersion electroosmotic flow mobilizes the queue to the channel intersection that couples the IEF to CE. Once the species of interest arrives at the intersection, the potential is 'switched' off for 3 s, allowing relaxation of pH gradients at the intersection. This is followed by dispensing of the fluid volume at the head of the queue into the CE dimension.

RESULTS AND DISCUSSION

The protein sample was first analyzed using uncoupled, microchip-based 1D IEF and CE. Sample mixture was focused using IEF and the relative location of the peaks was observed. No absolute pI information was acquired. Sample mixture was also subjected to CE analysis and absolute mobility information was obtained. Note that the CE analysis was carried out in unfocused ampholytes. Spatial electropherograms from these analyses are presented in Figure 2.

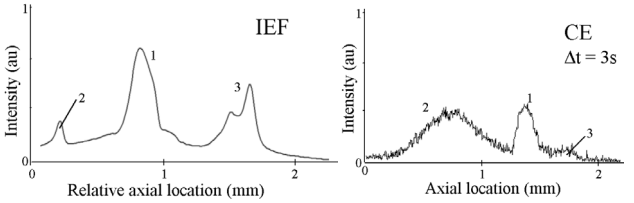

Figure 2. Spatial electropherograms of the independent 1D separations. *IEF is shown in the left image, while CE on an unconcentrated sample is presented in the right image.* $E_{IEF} = E_{CE} = 390$ *V/cm.*

The sample mixture was then analyzed using the coupled IEF-CE separation system. The voltage algorithm described in Figure 1 was employed. Figure 3, shows CCD images of the focused fluorescent species during an IEF-CE sequence. Boxes (dashed lines) highlight the queuing behavior of fluid volumes from IEF into CE.

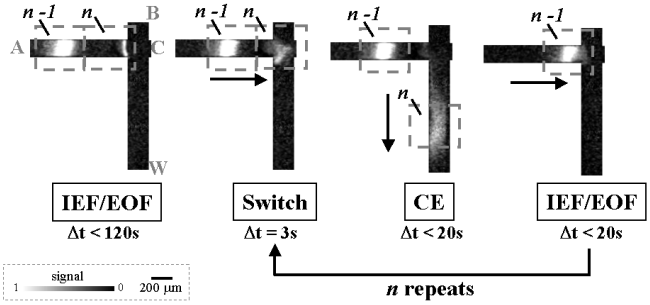

Figure 3. CCD images of queuing and sampling of fluid volumes from the IEF dimension into the CE dimension. *Fluid volume n contains focused FITC-dextran (tight band), while volume n-1 contains focused GFP (wide band). Samples are focused and mobilized during IEF/EOF, switched, and analyzed with CE. This procedure is repeated for all IEF bands. Arrows next to CCD images indicate sample motion. Channels are labeled consistent with Figure 1.* $E_{IEF} = E_{CE} = 390$ *V/cm.*

The intensity information obtained for each of the *n* CE sequences is displayed in a single 2D plot for a given time during the CE analyses (Figure 4). The axes are the spatial coordinates of the respective IEF and CE dimensions. The grayscale indicates the fluorescence intensity at a given location. Four distinct species are observed (labeled 1-4). Each of the species has a distinct pI and electrophoretic mobility. Species 1 and 4 have been oversampled, as the distributions span more than a single CE extraction. This oversampling is important for enhanced resolution. The 2-D plot also shows species (4,5) that are not resolved in the corresponding 1D IEF and CE. Analysis of 20% of the IEF fluid volume was completed in less than five minutes. This is a significant improvement over slab-gel systems that would complete such an analysis in several hours.

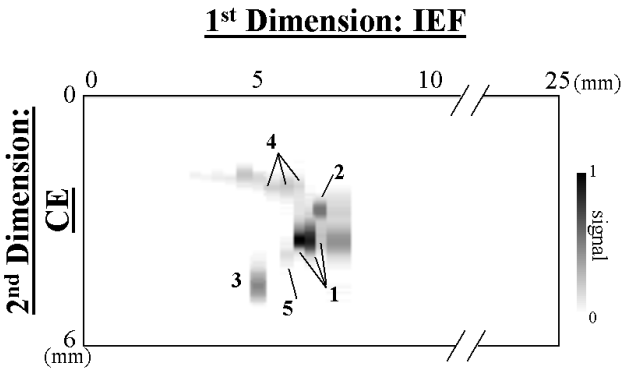

Figure 4. 2D representation of sample assay at Δt = 7s. *The multi-component sample was focused during IEF and extracted into the CE dimension using a gated injection scheme.*

CONCLUSIONS

IEF and CE were fluidically coupled and sequentially implemented using a single integrated microchip device. IEF was employed as an initial separation and pre-concentration step. The concentration increase resulting from the IEF step (100x) made possible a fluorescence-detection based CE separation of the initially dilute samples. In this system, IEF quickly reaches steady state (2 min) and exhibits residual EOF that can be used to provide a low-dispersion means of mobilizing focused sample species towards intersections for sampling into a second dimension. A mixture of proteins was analyzed using the 2-D system and results demonstrate the potential for enhanced performance through on-chip 2-D electrophoresis.

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