MULTI-STAGE FREE-FLOW ISOELECTRIC FOCUSING FOR ENHANCED SEPARATION SPEED AND RESOLUTION

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Abstract

This work presents the first implementation of tandem stages for free-flow isoelectric focusing. Both simple analytical and rigorous computational models for IEF suggest device performance can be improved by utilizing multiple stages to reduce the time required for high resolution separations. A device to test this principle was designed and tested, with fraction analysis by SDS-PAGE. We report the initial results of device characterization, showing separation of four model proteins.

Keywords: Free flow electrophoresis, isoelectric focusing, prefractionation

1. Introduction

Isoelectric focusing (IEF) is often used to separate proteins in order to reduce sample complexity or as part of a 2-D separation, and has been performed in micro free flow electrophoresis format (FF-IEF) using isolated electrodes [1-3]. However in these systems, resolution is limited by high diffusive fluxes. To increase resolution, higher electric fields may be applied, but precipitation, Joule heating, and electrodynamic dispersion become limiting. A new technique to circumvent these limits to resolution involves designing a free-flow IEF chamber with outlet fractions that enter a second IEF stage.

2. Theory

Scaling arguments imply that the resolution of FF-IEF increases roughly with the square root of the channel width, but that the time to reach this resolution increases proportionally with the channel width. This suggests that by performing a separation in a narrow channel prior to a wider channel, the resolution can be reached in less time. Analogous to mechanical power, a free flow IEF stage can be "geared" for either speed or resolution; "shifting" enhances the overall performance of the device.

To examine this phenomena, a model developed previously [2] was expanded to include dynamic changes in focusing length and applied voltage. Figure 1 shows the simulated focusing (normalized to the channel width) of BSA under identical field strengths and residence times. As shown by the three cases presented in Figure 1, focusing within a 1mm wide channel (solid line) reaches steady state rapidly, but the high diffusive flux

prevents sharp focusing. Focusing within a 3mm channel (dotted line) has a higher final resolution, but requires approximately three-fold more time to reach steady state. By shifting from a 1mm channel at steady state to a 3mm channel (dashed line), the greater resolution of the wider channel is realized in 40% less time.

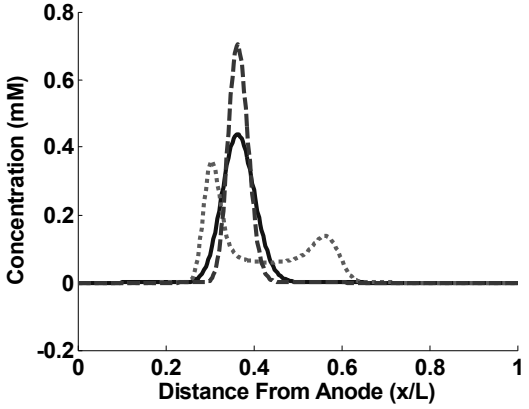

Figure 1: Simulation of BSA focusing with different channel geometries

3. Results and Discussion

A schematic of a device with two tandem stages is shown in Figure 2. The body of the device (a) is made of PDMS and is designed for a maximum of 3 inlets and 9 outlets. Post structures (b) define the polyacrylamide used to apply the electric field to the open sample channel. These UV-polymerized gels can be patterned without a photomask, and allow for arbitrary electric fields in each stage. The first stage is designed to perform a rapid prefractionation step with a steep pH gradient, while each of the three secondary stages are designed to perform a higher-resolution focusing step with a shallower pH gradient. The channel geometry at the splits has been optimized to ensure equal fluidic resistance for each outlet. A photo of the device is shown in Figure 3. Here, ten fluidic and six electrical connections are held in place by the PDMS without the need for epoxy.

An additional feature of the multistage devices is the ability to collect fractions for analysis, or orthogonal separations such as SDS-PAGE. A stained SDS-PAGE gel (Figure 4) of prefractionated amylosglocosidase, carbonic anhydrase II, trypsinogen, and trypsin inhibitor shows basic separation at a relatively low applied voltage (18V across each section). Amyloglucosidase focuses more tightly than the other three proteins due to its lower diffusive flux.

4. Conclusions

This work presents a two stage free-flow IEF device that is theoretically capable of fractionating proteins more efficiently than previous FF-IEF designs. We demonstrate in our initial tests the prefractionation of four model proteins prior to separation and

detection by SDS-PAGE, making these devices a potentially attractive alternative to more laborious isoelectric separation schemes.

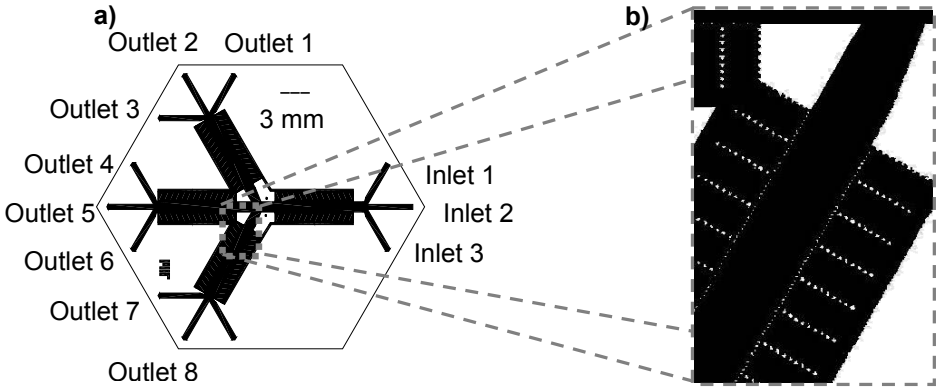

Figure 2: Schematic of multi-stage FF-IEF device.

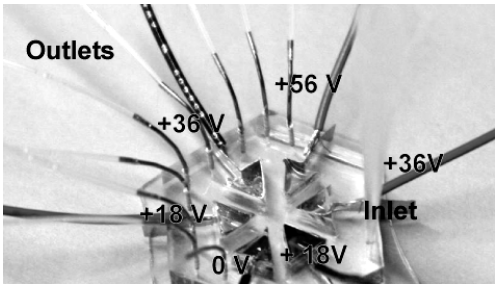

Figure 3: Photo of device in operation.

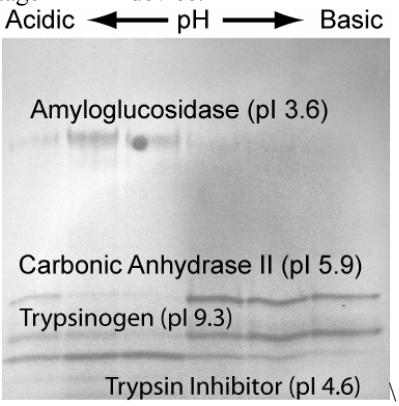

Figure 4: SDS-PAGE of six outlet fractions

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References

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