A Microchip Capillary Electrophoresis Experiment for the Instrumental Analysis Laboratory

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Abstract

We have set up and evaluated a microchip capillary electrophoresis (CE) laboratory experiment for the instrumental analysis laboratory class at Brigham Young University (BYU). In this experiment the students learn to load liquids into microfluidic channels, to align polymer microchips in a laser-induced fluorescence (LIF) detection microscope, and to perform microchip CE of fluorescently tagged amino acids. Moreover, the students study how the injection time duration and distance to detection in a CE microchip affect the separation. In this manuscript, we describe what is needed to set up and carry out this laboratory. In addition, we discuss the experimental outcomes from various student groups who have completed this experiment. This new laboratory module provides students with valuable training in the fields of microfluidics and separations, and should help the next generation of chemists learn to use microscale methods.

Introduction

Although microchip CE was introduced in 1992,¹ and many publications have followed since (as indicated by recent reviews),²⁻⁴ such experiments have not yet become a part of the analytical chemistry curriculum. A microchip CE laboratory experiment would provide students valuable exposure to miniaturized methods for chemical analysis, which are growing in importance due to their potential to facilitate rapid assays, while reducing chemical waste generation and reagent consumption. Why has microchip CE not yet become a part of the chemistry laboratory curriculum? The answer is most likely tied to the direct correlation between the separation performance and the cost/fabrication complexity of CE microdevices. At one extreme, expensive and difficult-to-fabricate photolithographically patterned glass microfluidic systems offer the best CE performance and reliability,⁵,⁶ while on the other hand, rapidly prototyped and easy-to-make polydimethylsiloxane⁷⁻¹⁰ microchips offer inferior separation performance and reproducibility. The only published description of an undergraduate microscale CE laboratory experiment, which utilized mechanically patterned glass substrates, also followed this cost/performance correlation (both were low).¹¹ Alternatively, commercial microchip CE instrumentation (e.g., from Agilent) can be used,¹² but again, the cost/performance correlation (in
this case, high) is a deterrent to large-scale implementation. Importantly, my group has developed polymer microdevice fabrication methods that allow rapid and simple construction of high-performance CE microchips, thus breaking the cost/performance correlation and providing an ideal platform for a microchip CE laboratory experiment. We anticipate that our development of this experiment will modernize the chemistry laboratory curriculum and enhance the teaching of separations in analytical chemistry courses.

Our focus was to develop a broadly applicable platform for microchip CE experiments in chemistry laboratories, and this work accomplishes two main purposes. First, the microchip CE experiment updates the analytical chemistry laboratory curriculum to include more current techniques. Second, the microchip CE experiment should provide a more in-depth exposure to separations. In this manuscript, we describe the requirements for setting up and carrying out a microchip CE experiment in instrumental analysis laboratories. In addition, we discuss the experimental outcomes from various student groups who have completed this laboratory module. This experiment provides students with valuable experience in the fields of microfluidics and separations, and helps train chemists in the use of microscale methods.

**Experimental Section**

*Instrumental setup.* The microchip CE and LIF detection setup has been described previously in the literature. A listing of the components that are needed to assemble the microchip CE and LIF detection setup are included as part of the electronic materials accompanying this document. A schematic diagram of the instrumentation is shown in Figure 1, and a photograph of the setup is given in Figure 2. In this setup a solid-state laser is directed through a 10X beam expander into the optical input of a fluorescence microscope. The excitation source goes through a 488 nm excitation filter and reflects off of a dichroic filter before being focused via a 20X 0.45 NA microscope objective into the separation channel. Fluorescent light is collected via the same objective, and then passes through the dichroic and longpass filters before being spatially filtered via a confocal pinhole prior to photomultiplier tube (PMT) detection. The PMT signal is converted to voltage, filtered with a pre-amplifier and digitized with an analog-to-digital converter (ADC) to enable collection as detector signal vs. time using a designed LabView virtual instrument. This LabView program is included as part of the electronic materials accompanying this document. For researchers lacking access to lasers, a UV source coupled with a filter cube can also be used for fluorescence detection.

Microchip CE is carried out using a “pinched” injection scheme, wherein sample is placed into reservoir 1 (see Figure 1), and buffer solution is in the other three reservoirs. Applying a positive potential to reservoir 3 while grounding the other three reservoirs for a time interval causes sample loading into the injection intersection. Electrophoretic separation is then carried out with reservoir 2 grounded, reservoirs 1 and 3 at an intermediate positive potential, and a higher voltage applied to reservoir 4. Voltages are switched using relays built by the BYU Chemistry and Biochemistry Department Instrument Shop. Because the microchip is on a microscope stage, the focused laser can be positioned anywhere within the channels, but for optimal performance, the laser is usually aimed near reservoir 4 in the separation channel.
Figure 1. Schematic diagram of the microchip CE and LIF detection setup.
Microchip fabrication. Microchip CE devices are fabricated using a hot embossing and thermal bonding approach that has been described in the literature. The channel design is transferred (via photolithography) to a silicon wafer; the pattern is wet etched into the silicon using 40% potassium hydroxide, to provide hot embossing templates. Hot embossing of the poly(methyl methacrylate) (PMMA) devices occurs at 140 °C under C-clamp pressure, and thermal bonding of the embossed channels to a blank PMMA substrate with laser cut reservoir openings is done at 110 °C in a manner similar to the hot embossing.
We had an experienced graduate student carry out device fabrication for the experiment. However, we note that it is feasible for a graduate student teaching assistant or even an undergraduate student (see the Results and Discussion) to be trained in a short time to reliably carry out the thermal bonding of devices from the hot embossed and drilled PMMA pieces. Moreover, we are willing to provide (at cost) hot-embossed PMMA microchannel substrates and laser-cut cover plates to educational institutions that are interested in developing this microchip CE experiment. We further note that PMMA microchip CE devices are available commercially (e.g., from Microfluidic ChipShop, Jena, Germany) at a cost of ~$10/device if purchased in bulk.

Sample preparation. The amino acids are labeled fluorescently using fluorescein-5-isothiocyanate (FITC; Molecular Probes, Eugene, OR), as we have reported in a prior publication. We combined 200 µL of 6 mM FITC in dimethyl sulfoxide with 600 µL of a 3 mM solution of the amino acids. The solutions are left to react at room temperature in the dark for >24 h (even longer times led to more complete reaction and elimination of the unconjugated FITC peak).

Laboratory write-up. The experimental instructions that are given to the students are included as part of the electronic materials accompanying this document.

Results and Discussion

Device fabrication. In the first implementation of the microchip CE experiment in 2008, we had the students bond their own devices using a PMMA piece having hot-embossed channels and another PMMA substrate with laser-cut reservoir holes. Of the three laboratory groups that carried out the experiment that year, one team succeeded in making a working CE microchip on their initial bonding attempt on the first day of the experiment (see Figure 3), one team had an operable CE microdevice after their second bonding try late in day one of the experiment, and a final team was unable to fabricate a working CE microchip during the full three laboratory periods allotted. We were very much encouraged by the two groups that succeeded in constructing usable devices in one laboratory period, because they demonstrated that undergraduates and first year graduate students could readily make workable microfluidic systems, even if they have fairly limited training. However, we found that the time expended in making devices left less than the desired amount of time to study and learn about microfluidic phenomena using these microchips. Thus, the focus in subsequent years was shifted from device fabrication to device usage. Instructions to students regarding device bonding (from an older version of the laboratory writeup) are included as part of the electronic materials accompanying this document.
Figure 3. Photograph of a CE microchip fabricated by students in the instrumental analysis laboratory. The device is about 2 cm x 5 cm. The black marker “1” in the middle of the device indicates that this was their first device.

We had also initially planned to have students evaluate the fabrication of CE microchips using a solvent bonding approach we developed. As we tried to adapt this procedure for the laboratory experiment, we found it to be more time consuming and less reliable (in terms of device fabrication success), than the thermal bonding approach we ended up using. We note that the solvent-bonded devices allow higher voltages to be applied than thermally bonded microchips, so they could potentially be used in this experiment if the fabrication can be streamlined appropriately.

Experimental data. The microchip CE laboratory provides students with considerable opportunity to obtain and evaluate data from these microdevices. Figure 4 shows a microchip electropherogram from a student team that did the experiment in 2008. Although the amino acid peaks are clearly visible in the plot, the students also detected several impurity peaks. Because the purity of the FITC-labeled samples was less than desired, in subsequent experiments, the sample quality was evaluated carefully before students attempted the laboratory. Indeed, the data from a 2009 team’s laboratory work on the identification of the amino acid components of an unknown mixture (Figure 5) showcases both the much improved sample purity and the separation reproducibility. These electropherograms demonstrate the type of data that can be obtained in a classroom setting using this laboratory experiment.
Figure 4. Microchip electropherogram of an amino acid mixture obtained by a student group in the instrumental analysis laboratory, using the device shown in Figure 3.
Figure 5. Student microchip electropherograms used in identifying an amino acid mixture. (A) Individual runs of FITC-Asp, FITC-Gly, FITC-Phe and FITC. (B) Electropherogram of the unknown mixture; peaks are (in elution order) FITC-Asp, FITC-Gly and FITC.

In addition to obtaining electropherograms, students can also use the experimental data to determine numbers of theoretical plates and gain information about separation performance from those results. For example, Figure 6 shows student data plotting the number of theoretical plates in a separation as a function of “pinched” injection time. The “pinched” injection scheme is designed to provide a time-independent injection volume. The student data agree with expectations, as the number of theoretical plates appears to plateau after ~60 seconds injection
Furthermore, students can use the flexibility of the experimental platform to study the effect of microchip CE separation distance on performance (at constant overall applied voltage). Figure 7 shows student data that indicate a linear correlation between separation distance and number of theoretical plates in microchip CE. We note that for standard CE the number of theoretical plates depends only on the separation voltage, because the detection position is fixed, usually at the end of the column. With our platform, we can position the detector anywhere along the separation channel, and the number of theoretical plates (at constant overall applied potential) is expected to depend linearly with the distance to detection, in accord with student results. Thus, the microchip CE experiment offers a more in-depth exposure to separation phenomena.

**Figure 6.** Student plot of number of theoretical plates (N) vs. “pinched” injection time.
Figure 7. Student plot of number of theoretical plates as a function of distance to detection in microchip CE at constant separation voltage. The data follow a linear trend, in accord with expectations.

Conclusions

We have designed, set up, implemented and evaluated a microchip CE experiment for the instrumental analysis laboratory. The device fabrication procedures are sufficiently straightforward that minimally trained undergraduates or graduate students can bond embossed polymer substrates to create CE microchips. However, device fabrication consumes laboratory time that could be spent on studying and evaluating microfluidic and separation phenomena, such that we chose to provide students with fabricated CE microchips. In this laboratory, students can readily obtain microchip CE data, and they are capable of performing additional analyses to understand microfluidic and separation phenomena. The material covered in the lecture portion of the class needs to be well correlated with the laboratory module for maximal impact. We feel that this new experiment provides an excellent opportunity to introduce students to the fundamentals of microfluidics, and also reinforces key concepts in separations, microscopy and fluorescence detection. As such, this microchip CE experiment forms an excellent addition to the instrumental analysis laboratory.
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References