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Microchip based Electrophoretic Separations with a Pressure-driven Backflow

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Abstract

It is well known that the resolving power of capillary zone electrophoretic separations may be improved with an increase in the applied electric field strength and separation time. While large electric fields may be realized in short analysis channels commonly employed in microfluidic systems, this experimental design is not suitable for achieving long separation times. In this chapter, we describe the use of a steady and/or a periodic pressure-driven backflow to increase the separation time in short microchannels thereby enabling the analysis of closely-related species on microchip devices. The reported backflow was realized in our assays using an on-chip pressure-generation capability that relied on the partial blockage of electroosmotic flow around a junction of two glass channel segments having different depths. Although the noted strategy led to additional band broadening in the system, the resolving power of our device was observed to substantially improve upon introduction of the reported steady/periodic pressure-driven backflow for analysis channels shallower than 5 μm .

Keywords

band broadening; capillary zone electrophoresis; electroosmosis; microfluidic pump; pressure-driven backflow

1. Introduction

Capillary zone electrophoresis (CZE) is an established liquid phase analysis technique that allows the separation of closely-related chemical and biological species under relatively gentle operating conditions [1]. With the emergence of microfluidic systems, further improvements in the separation performance of CZE have been possible primarily through miniaturization of the analysis column. In particular, the shorter cross-sectional dimensions of microfluidic channels have allowed the injection of narrower plugs as well as diminished mass transfer limitations and Joule heating effects in these systems, thereby minimizing the broadening of analyte bands. In addition, the ability to readily integrate other sample preparation and analysis procedures to CZE on microchip devices has enabled the realization

of complicated assays with high efficiencies and using small sample sizes. Nevertheless, the inability to accommodate long separation columns in microfluidic systems has limited the applicability of microchip based separation methods to relatively simple samples. In this situation, there is a clear need for developing strategies that would allow an improvement in the peak capacity of microfluidic separations enabling them to analyze complex mixtures of practical significance.

Over the years, a variety of approaches have been proposed to shorten the separation length of CZE assays. A key objective in these approaches has been to slow down the migration of the analyte molecules through the assay column without compromising their electrophoretic transport rates such that the sample is subjected to the electric field for the maximum possible time. To this end, the use of static and dynamic coatings to reduce electroosmotic flow (EOF) in CZE systems is well known. Such a flow is usually undesirable in electrophoretic assays as it advects all molecules with the same speed, and therefore does not contribute to the separation process. Notably, dynamic coatings are easier to apply and can withstand high pH values but they have been often reported to be less stable than their static counterpart. Moreover, these coatings usually included as additives in the separation buffer can alter the electrophoretic transport of the analyte species besides interfering with the direct integration of the CZE technique to several detection and/or downstream analysis methods. Static coatings on the other hand, are particularly unsuitable for miniaturization of the CZE method due to increasing difficulties in applying them to the channel walls as the size of the analysis column is reduced to the micro-/sub-micrometer length scale. Recently, the application of external voltages at the walls of microfluidic channels has been shown to allow local control of zeta potential, and therefore EOF, in a dynamic fashion [2]. Although this approach overcomes some of the disadvantages of static/dynamic coatings, it offers limited control over the range of electroosmotic transport rates that may be realized in the system and tends to complicate the instrumentation aspects of the CZE method. The use of a steady pressure-driven flow to counteract electroosmosis in CZE analysis presents an alternative approach to reducing the separation length of this technique [3]. The ability to periodically vary the strength of this flow field moreover, can allow the realization difficult charge based separations in short microfluidic ducts based on the Flow Counterbalanced Capillary Electrophoretic (FCCE) method as described by Culbertson and Jorgenson [4,5]. The FCCE technique relies on the use of a pressure-driven backflow to periodically reverse the direction of sample migration without inverting the electric field in it. Consequently, sample molecules can be kept in the separation field much longer than otherwise possible under normal CZE conditions, significantly increasing the effective separation length and voltage for the assay.

Unfortunately, the application of a steady or a periodic pressure-driven backflow introduces hydrodynamic dispersion of analyte bands [3–5] often compromising the resolving power of the CZE method. However, because such sample dispersion scales directly with the narrowest cross-sectional dimension of the analysis channel [6], it is possible to diminish this process by either packing CZE columns with small particles or miniaturizing their lateral dimensions. Previous studies have shown that the use of packed capillaries for CZE analyses with a pressure-driven backflow to be ineffective in reducing hydrodynamic dispersion likely due to non-uniformities in the particle packing density [7]. On the other

hand, our limited ability to dynamically control pressure-driven transport within micro-/nanofabricated structures presents a significant challenge in the miniaturization of CZE methods with counteracting pressure-gradients. It must be pointed out that while pressure-driven flows may be generated in micrometer-sized channels using an external syringe pump, the dynamic control over this flow velocity is often poor in such an arrangement as a result of the large dead volumes involved at the microchip-pump interconnect. Moreover, the hydrodynamic resistance to liquid transport increases sharply with a reduction in the lateral dimensions of a microfluidic duct which compromises our ability to realize a wide range of pressure-driven flow velocities primarily due to leakage issues at the microchip-pump junction. In this chapter, we describe the use of a simple on-chip pumping unit that allows precise introduction of a steady or a periodic pressure-driven backflow into a microfluidic separation channel enhancing the resolution of CZE assays performed in it [3, 5, 8]. The backflow in the reported devices was produced by fabricating a shallow segment (0.5 μm deep) downstream of the analysis column (5 μm deep) and applying an electric field across it. A mismatch in the electroosmotic transport rate at the interface of this segment was shown to then yield a pressure-gradient that counteracted the electrokinetic migration of the analyte bands either slowing them down or reversing their flow direction altogether without significantly compromising the separation field in the system. Although such a pressure-gradient also led to additional band broadening, the overall separation resolution of our device was observed to improve upon introduction of the reported pressure-driven backflow owing to the increased separation time in the assay.

2. Materials

Use only high purity reagents and de-ionized water for preparation of buffers and samples.

2.1 Microfluidic chip preparation for analysis

1. Microfluidic chips made from glass.
2. Cylindrical glass tubes about 4 mm i.d. and 8 mm tall.
3. A stereo microscope (e.g., Nikon SMZ).
4. Microanalytical balance (e.g., Mettler Toledo).
5. Ultrasonic bath (e.g., Branson 1/2 gallon).
6. Disposable hypodermic syringe: 1 mL, gauge 26, 1/2 inch needle.
7. Vacuum system.
8. Sodium hydroxide solution: 0.1 M NaOH. Weigh 40 mg NaOH with the analytical microbalance and dissolve in 10 mL water in a 15 mL conical tube. Disperse by sonication and filter the solution, if necessary, by using a 0.2 μm filter. Store the stock solution at room temperature.
9. Ultraviolet curable glue (e.g., Norland Products Inc.).
10. Methanol.

2.2 Fluidic flow testing and visualization

1. Nikon Eclipse TE-2000U inverted epi-fluorescence microscope with 4X, 10X or 20X objectives and appropriate fluorescence filter cubes. Other fluorescence microscope systems of similar performance can be used. An attached CCD camera and image processing software is also needed for accurate flow visualization in the microchannels.
2. LabSmith microfluidics high voltage power supply. Other microfluidics power supplies of similar performance can be used.
3. Picoammeter (e.g., Keithley 6487).
4. Digital multimeter (e.g. Fluke 179).
5. Amber glass sample vials: 20 mL.
6. Buffer solution: 1 mM sodium tetraborate. Weigh 3.81 mg of $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ with the analytical microbalance and dissolve in 10 mL deionized water in a 15 mL conical tube. Disperse by sonication and filter the solution, if necessary, by using a 0.2 μm filter. Store the stock solution at room temperature (see Note 1).
7. Rhodamine B solution: 10 μM . Weigh 47.9 mg Rhodamine B with the analytical microbalance and dissolve in 10 mL of the 1 mM sodium tetraborate solution, in an amber glass vial. Disperse by sonication and filter, if necessary, by using a 0.2 μm filter. Prepare fresh before use.

2.3 Electrophoretic separation

1. LASER, e.g., 125mW Argon ion tunable laser, Melles Griot with tunable emission wavelengths between 454 and 514 nm.
2. A single-point laser induced fluorescence (LIF) detection set-up with appropriate optical and mechanical components [9].
3. FITC labeled arginine solution: 30 μM . Prepare by adding 156.6 mg of arginine and 116.7 mg of fluorescein isothiocyanate (FITC) to a 10 mL solution of the 1 mM sodium tetraborate buffer and allow the reagents to react at 37 °C for 1 hr. Disperse by sonication and filter, if necessary, by using a 0.2 μm filter. Prepare fresh before use.
4. FITC labeled glutamic acid solution: 30 μM . Prepare by adding 132.3 mg of glutamic acid and 116.7 mg of FITC to a 10 mL solution of the 1 mM sodium tetraborate buffer and allow the reagents to react at 37 °C for 1 hr. Disperse by sonication and filter, if necessary, by using a 0.2 μm filter. Prepare fresh before use.
5. FITC labeled glutamine solution: 30 μM . Prepare by adding 131.4 mg of glutamic acid and 116.7 mg of FITC to a 10 mL solution of the 1 mM sodium tetraborate buffer and allow the reagents to react at 37 °C for 1 hr. Disperse by sonication and filter, if necessary, by using a 0.2 μm filter. Prepare fresh before use.

6. Amino acid sample 1: Mix the FITC labeled arginine, glutamic acid and glutamine solutions using a Thermoshaker (e.g., Eppendorf ThermoMixer® C) to prepare a 30 mL solution of the amino acid sample 1.
7. Amino acid sample 2: Mix the FITC labeled glutamic acid and glutamine solutions using a Thermoshaker (e.g., Eppendorf ThermoMixer® C) and add 10 mL of the 1 mM sodium tetraborate buffer to the mixture to prepare a 30 mL solution of the amino acid sample 2.

2.4 Data analysis

1. MATLAB with Curve Fitting toolbox for fitting Gaussian curves to the analyte peaks recorded on the electropherograms and subsequently estimating their migration times as well as standard deviations.

3. Methods

3.1 Microfluidic chip preparation

1. Prepare microfluidic chips in-house [3, 5] or purchase from various manufacturers, e.g., Micronit, Micralyne, Agilent, etc. We note that specialized microchip designs that allows on-chip pressure generation must be fabricated, e.g., with shallow segments at appropriate locations in the microfluidic network (see Figs. 1 and 2).
2. Attach the cylindrical glass tubes at the channel ports to serve as liquid reservoirs. Apply the ultraviolet curable glue to the bottom rim of the glass tube and then place it around the channel port. Place the microchip with the glass tubes under ultraviolet lamp (e.g., Blak-Ray UV lamp from UVP, LLC) for 30 minutes (see Note 2).
3. Clean/re-hydrate the microfluidic channels with the NaOH solution. Dispense the solution with the aid of a hypodermic syringe in one reservoir, and allow the channels to fill by capillary action. Fill the chip from only one reservoir to avoid trapping gas bubbles in the microchannels. Apply a slight vacuum from the other reservoirs to help the NaOH solution flow through the channels. Leave the NaOH solution in the channel for 15 min (see Notes 3 and 4).
4. Remove the NaOH solution from all reservoirs and channels through vacuum suction, and rinse the chip with copious amounts of de-ionized water. Next, fill the channels with water, as described above, and rinse sufficiently to completely remove the NaOH solution. Check with the pH in the chip reservoirs with pH paper.
5. Remove the water from all reservoirs and fill the reservoirs and channels with methanol. Remove the methanol from all microfluidic channels through vacuum suction and allow the chip to dry.

3.2 Fluidic flow testing and optimization

1. Fill the chip with the buffer solution, as described in section 3.1, step 3.

2. Visually check the chip under a benchtop microscope to ensure that air bubbles are not trapped in the microfluidic channels or reservoirs.
3. Secure the chip on the epi-fluorescence microscope for example, using paper tapes, and introduce Pt electrodes connected to the terminals of a high voltage power supply in all reservoirs.
4. Test for electrical continuity by applying a low electric field strength, e.g., 100–250 V/cm, between various chip reservoirs and measure the electrical current through the microfluidic channels. This can be accomplished by applying the voltage to one reservoir and grounding the opposite reservoir through the picoammeter.
5. If the current measurements are satisfactory and reproducible, increase progressively the field strength to the maximum value that will be used during analysis (e.g., ~ 1000 V/cm), repeat the above current measurements, and check reproducibility (*see* Note 6).
6. Fill the sample reservoir shown in Figs. 1 and 2 with the 10 μ M Rhodamine B solution (*see* Note 7).
7. Perform a gated injection [3, 5] of Rhodamine B by applying the appropriate high voltages to the corresponding reservoirs (*see* Figs. 1 and 2) (*see* Note 8).
8. Optimize the sample injection/analysis voltages and check again the electrical currents between different reservoirs.

3.3 Separation process

1. Fill the appropriate amino acid sample in the sample reservoir (*see* Note 9).
2. Place the microchip on the XYZ stage in the LIF detection set-up, and secure with a paper tape. Adjust the position of the stage and the optical components to maximize signal recorded by the detector, e.g., Photomultiplier tube (Hamamatsu Photonics) (*see* Note 10 and 11).
3. Apply the optimized voltages (*see* section 3.2, step 8) to the chip reservoirs and record the separation peaks on an electropherogram.
4. Perform multiple separation runs for a chosen operating voltage to estimate the reproducibility in the assay.
5. Next, raise all voltages, proportionally, and record all corresponding electropherograms to optimize the separation voltage (*see* Figs. 3 and 4).
6. Extract the electropherograms from the LIF detection set-up as an ASCII file (e.g., .txt file) and analyze the data using MATLAB.
7. Estimate the peak variance and resolution to assess separation performance.

4. Notes

1. The choice for the buffer concentration offers a balance the magnitude of the pressure-driven backflow and separation resolution. For a given buffer pH and applied voltage, while the pressure-driven flow velocity is observed to increase with a reduction in the buffer concentration, stronger adherence of the analyte molecules to the channel walls are seen under these conditions which contributes to band broadening and asymmetry compromising the separation resolution. For buffers with ion concentrations less than 1 mM, the pH also tends to drift towards that of de-ionized water, i.e., 7.
2. Any ultraviolet curable glue used for attaching the cylindrical glass tubes to the channel ports should be evaluated for possible bleeding of components that can alter electroosmotic flow rates, increase adherence of the analyte molecules to the channel walls and/or block liquid/analyte transport in the fluidic ducts.
3. Do not allow the chip to dry out while filled with the NaOH solution, as this may result in channel clogging. Replenish the reservoirs continuously with solution while the chip is treated with NaOH, and/or preserve the chip in a water-saturated chamber.
4. Alternatively, if unions are bonded to the chip, the channels can be flushed by using a 250 μ L syringe connected via a (50–100) μ m i.d. $\times$ 360 μ m o.d. fused silica capillary. The syringe can be actuated manually or by a syringe pump.
5. For channels that are $\sim$ 5 μ m deep and 100 μ m wide, the electrical current should be $\sim$ 0.5–5 μ A for a 10 mM sodium tetraborate buffer.
6. Avoid the use of high electric field strengths that produce high current, Joule heating, bubble formation and possible catastrophic failure of the experiment.
7. The concentration of the Rhodamine B solution that will be injected in the chip should be sufficient to enable visualization with the available fluorescence microscope.
8. Rhodamine B is a fluorescent dye that at neutral and high pH values has a zwitterionic structure. When neutral, it can be used as a tracer dye to determine the direction and magnitude of fluid flow. At low pH however, Rhodamine B can acquire a positive charge and, therefore, in addition to its electroosmotic transport can also display electrophoretic migration.
9. Wash the chip sequentially with methanol and the buffer solution between working with the Rhodamine B and amino acid sample in the sample reservoir.
10. It may be convenient to do a preliminary optimization of the position of the XYZ stage and optical components using a similar microchip with its channels filled with the Rhodamine B solution prior to working with the actual separation device.

11. It is recommended to visualize the separation process using the epi-fluorescence microscope first prior to operating the microchip with the single-point LIF detection set-up.

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Figure 1.

(a) Schematic of the microchip device used for performing CZE analysis with a steady pressure-driven backflow. Notice that the magnitude of the pressure-driven flow in segments A, B and C is expected to be about a third of that in segment D in this design. (b) Gated injection scheme employed for introducing sample plugs into the separation channel of the microchip device shown in (a). The fluorescence images presented above were obtained using a 10 μ M Rhodamine B sample prepared in a 1 mM sodium tetraborate buffer applying $\phi_0 = \phi_1$ 1 kV in stage III of the injection process. Reproduced with permission from reference [3], Copyright © 2012, American Chemical Society.

Figure 2. Schematic of the microchip device used for performing flow counterbalanced capillary electrophoresis with a periodic pressure-driven backflow. Notice that the magnitude of pressure-driven backflow in segment D was expected to be about 23% of that in segment F in this design. The fluorescence images presented above were obtained using a 10 μM Rhodamine B sample prepared in a 1 mM sodium tetraborate buffer applying $\phi = 1$ kV in stage III of the injection process. Reproduced from reference [5] with permission from The Royal Society of Chemistry.

Figure 3.

(a) Enhancement in the CZE analysis of a FITC labeled amino acid sample using the reported microfluidic device. The symbols R, Q and E in the electropherograms presented above refer to the amino acids arginine, glutamine and glutamic acid, respectively. **(b)** Measured separation resolution for the amino acid peaks shown in (a). The symbols ● and ◆ here refer to the separation resolutions between the FITC-R, FITC-Q and FITC-Q, FITC-E peaks respectively. The open and filled symbols here correspond to measurements made in separation channels without a shallow region and with a 0.5 μm deep one, respectively. The error bars included in figure (b) are based on 5 measurements. Reproduced with permission from reference [3], Copyright © 2012, American Chemical Society

Figure 4.

Separation of a FITC-labeled amino acid sample in the reported FCCE device. The symbols Q and E here refer to glutamine and glutamic acid, respectively. The solid lines in the separation versus pass number graph are the best fitted curves to the experimental data and can be described by the equations $R = 0.349 + 0.616\sqrt{n}$ (1 kV case) and $R = 0.522 + 0.596\sqrt{n}$ (2 kV case). The error bars for the separation resolution here represent the standard deviation in this quantity based on 5 measurements. Reproduced from reference [5] with permission from The Royal Society of Chemistry.