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ELECTRICALLY ACTUATED, PRESSURE-DRIVEN LIQUID CHROMATOGRAPHY SEPARATIONS IN MICROFABRICATED DEVICES

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Abstract

Electrolysis-based micropumps integrated with microfluidic channels in micromachined glass substrates are presented. Photolithography combined with wet chemical etching and thermal bonding enabled the fabrication of multi-layer devices containing electrically actuated micropumps interfaced with sample and mobile phase reservoirs. A stationary phase was deposited on the microchannel walls by coating with 10% (w/w) chlorodimethyloctadecylsilane in toluene. Pressure-balanced injection was implemented by controlling the electrolysis time and voltage applied in the two independent micropumps. Current fluctuations in the micropumps due to the stochastic formation of bubbles on the electrode surfaces were determined to be the main cause of variation between separations. On-chip electrochemical pumping enabled the loading of pL samples with no dead volume between injection and separation. A mobile phase composed of 70% acetonitrile and 30% 50 mM acetate buffer (pH 5.45) was used for the chromatographic separation of three fluorescently labeled amino acids in <40 s with an efficiency of >3000 theoretical plates in a 2.5-cm-long channel. Our results demonstrate the potential of electrochemical micropumps integrated with microchannels to perform rapid chromatographic separations in a microfabricated platform. Importantly, these devices represent a significant step toward the development of miniaturized and fully integrated liquid chromatography systems.

INTRODUCTION

Miniaturization of analytical techniques holds great potential for performing a variety of sample-limited assays, especially because of the possibility of integrating multiple analysis steps in a single substrate.¹ Since first demonstrated in 1992,² capillary electrophoresis in microfabricated devices has seen significant advances, and numerous chemical and biological applications have been reported.^{1, 3, 4} Electrically driven methods are well-suited for miniaturization, since electroosmotic flow (EOF) can be controlled without valves or external pumps. However, EOF pumping and fluid transport have inherent issues that may limit their broad application in miniaturized methods. For example, EOF typically requires high voltages (kV) and is affected by Joule heating; moreover, EOF is sensitive to the solution pH and column surface charge. Finally, electrophoretic techniques are optimal for charged analytes that can be exposed to an electric field.^{5, 6}

On the other hand, pressurized separation methods such as liquid chromatography (LC) are more general and broadly used. In 1990, Manz et al.⁷ presented advantages of the miniaturization of LC; even though no experimental data were provided, this paper showed that theoretically, the performance per unit time should be superior in microchip compared to conventional LC. Three critical elements for a fully miniaturized LC system are:^{5, 7, 8} (i)

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integration of a pumping mechanism capable of generating pressure and flow compatible with microchannel dimensions; (ii) incorporation of a separation medium inside microchannels; and (iii) a minimal dead volume interface of the separation column with the pumping and injection mechanism. Despite the challenges it presents, the miniaturization of pressure-driven separation methods is of great interest;^{5, 7, 8} however, most reports have focused on micromachining a separation column while maintaining an external pumping mechanism.^{9–17} Thus, the full advantages of LC miniaturization were not realized. Two recent studies have made important progress in the development of miniaturized LC systems with integrated pumping and injection. Lazar et al.¹⁸ fabricated a LC microdevice with integrated EOF micropumps for sample valving and separation. However, this approach required the use of relatively high electric fields (500 V/cm), was limited to solutions with low organic solvent concentrations, and was relatively slow (~40 min elution times). In other work, a hybrid silicon-parylene microfluidic chip with integrated electrochemical micropumps for sample injection and separation was used for the LC analysis of protein digests.¹⁹ This report demonstrated the advantages of having a minimal dead volume between injection and separation, but again suffered from long analysis times (~1 h).

A variety of micropumps have been constructed for microfluidic applications.^{20–23} Mechanical pumps^{24, 25} use moving parts, have relatively complex fabrication and often face challenges in integration with microfluidic systems, in terms of materials compatibility with solutions and samples. Non-mechanical pumps based on electroosmotic,²⁶ magnetohydrodynamic²⁷ or electrochemical actuation,²⁸ are thus appealing alternatives. Electroosmotic pumps, which generate pressure with EOF, are perhaps the most widely used micropumps in microfluidics applications.²⁶ However, to obtain appropriate flow rates, it is often necessary to apply high voltages (~kV) and make either packed small-diameter columns or microchannel network arrays,^{29, 30} which complicate the fabrication process. Moreover, electroosmotic pumps are only suitable for operation within a certain range of solution pH and conductance values.

Column technology for microchip LC is a key challenge.⁸ Packing microchannels with particles as in conventional LC is difficult to achieve, due to pressure constraints and difficulties in forming frits inside microchannels.³¹ In 1998, Regnier et al.³² demonstrated surface-modified micromachined posts, as a mimic of a packed microcolumn. While this approach was compatible with micromachining techniques, it was hindered by expensive fabrication protocols involving deep reactive ion etching. The possibility of performing separations in monolithic stationary phases or open tubular columns has given new opportunities for the development of miniaturized LC. The fabrication of monolithic structures inside microchannels has been demonstrated³³ and is becoming a promising approach, provided uniform monoliths in microchips with low back pressure are constructed. The use of capillaries for open tubular liquid chromatography (OTLC) was first proposed by Jorgenson et al.³⁴ A key advantage of OTLC is lower back pressure than packed or monolithic columns, leading to faster analysis times. The main disadvantages of OTLC relative to packed column LC are slower mass transfer into the stationary phase and reduced sample capacity due to lower stationary phase volume. Importantly, micromachined systems can have small channel cross sections, raising the mass transfer to the stationary phase. Theoretical work on OTLC has shown that band dispersion is lowest for microchannels with high aspect ratios.^{35, 36} Jacobson et al.³⁷ reported the use of high aspect ratio microchannels to perform open channel electrochromatography. Later, the same group determined that 5 μm channel depths were a good compromise between efficiency and ease of operation.³⁸ Although these devices were not tested for pressure-driven separations, the electrochromatography results indicate that open tubular microchannels are an attractive option for microchip chromatography.

In recent years, great interest has arisen in developing electrochemical systems for microchip pumping,³⁹ resulting in devices for valve actuation,⁴⁰ and dosing systems for applications in biology and medicine.^{41, 42} We have shown that the pressure caused by the build-up of electrolysis gases in an enclosed chamber can pump liquids in fluidic microchannels.⁴³ Our electrochemical micropumps are integrated easily with microfluidics and can pump liquids with rates as high as $\sim 10 \mu\text{L}/\text{min}$. More recently, electrochemical actuation was demonstrated for sample delivery and solvent gradient generation in electrospray ionization mass spectrometry analysis.⁴⁴ Many features of electrolysis, including simple instrumentation, rapid response time, low power consumption, and limited heat generation,⁴⁵ make it an attractive candidate for on-chip LC pumping. Moreover, a constant volume, electrolysis-based actuator can generate a maximum pressure of $\sim 200 \text{ MPa}$.⁴⁶ Surprisingly then, only one report¹⁹ has appeared on the use of electrolysis-based micropumps in LC; while this initial work demonstrated feasibility, the separation time ($\sim 1 \text{ h}$) was not different from conventional LC.

In this paper, we describe the design, fabrication and characterization of electrically actuated micropumps for pressure-driven LC in microchips. Micropumps were made in glass and integrated with microfabricated channels in the same substrate. A pressure-balanced injection approach was implemented by controlling the electrolysis time and voltage applied to the mobile phase and sample micropumps. We studied parameters affecting the reproducibility of the system, and the effects of column coating, amount of sample injected and mobile phase composition were characterized. Finally, these devices were evaluated in electrolysis-based injection and LC separation of fluorescently labeled amino acids.

EXPERIMENTAL

Chemicals

Amino acids were obtained from ICN Biomedicals (Aurora, OH). Fluorescein-5-isothiocyanate (FITC) was from Molecular Probes (Eugene, OR). Reagent-grade solvents including acetonitrile, methanol, acetone, and isopropanol were obtained from Fisher Scientific (Fair Lawn, NJ). Potassium nitrate, hydrogen peroxide and sulfuric acid were from Sigma (St. Louis, MO). Buffer solutions were prepared using deionized (DI) water ($18.3 \text{ M}\Omega \cdot \text{cm}$), which was obtained from an Easypure UV/UF purification system (Dubuque, IA).

Device fabrication

The integrated micropump-microchannel systems are composed of a three-layer glass sandwich as illustrated in Figure 1. The bottom layer contains microchannels for sample injection and separation. The middle layer contains through-holes which form the reservoirs for sample, eluent, and electrolyte solution. A pressure transfer tube (PT) on top of the middle layer connects one micropump with the sample reservoir (SR) and another micropump with the eluent reservoir (ER). Finally, access holes drilled in the top piece facilitate the introduction of sample, eluent and electrolyte solution into their respective reservoirs. Devices were made from microscope slides ($75 \times 50 \times 1 \text{ mm}$, Fisher). The fabrication process employed a combination of photolithography, wet-chemical etching, through-drilling with diamond-tipped bits (DiamondBurs.Net, Tucker, GA) and thermal bonding.

Substrate cleaning

Microscope slides were immersed in boiling piranha solution ($\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$, 3/1) for 10 min, rinsed with DI water and dried using nitrogen gas. Prior to photolithography, substrates

were cleaned with acetone and isopropanol. Slides were then dehydrated in an Ultra-Clean 100 oven (Lab-Line Instruments, Melrose Park, IL) for 5 min at 120 °C.

Photolithography

The glass slides were spin-coated with the adhesion promoter SurPass 4000 (Dischem, Ridgway, PA) at 4000 rpm for 45 s and baked on a hot plate at 90 °C for 60 s. Then, substrates were coated with AZ 3330 positive photoresist (Clariant, Germany) and baked again for 60 s at 90 °C. The photoresist was exposed to UV radiation with a 250 W mercury source for 14 s through a patterning mask using a MA150 CC aligner (Karl Suss America, Waterbury Center, VT). The photomasks were drawn using mask layout software (WieWeb) and printed onto transparency film with a 3600 dpi printer at the BYU Print and Mail Production Center. Two masks were used in microfabrication (Figure 1A). The first mask, which was used to pattern the bottom layer of the glass microdevices, contained a 3-cm-long separation column and two arms (1 cm each), one connected to the SR and the other to the ER. Channel linewidths in the mask were 100 μm . Four 1.5 $\times$ 9 mm rectangular features in this mask defined the areas for thermal deposition of the electrodes. The second mask was used to pattern the middle layer in the micropump-microchannel devices and contained two 1-cm-long $\times$ 200- μm -wide channels connecting the micropump chambers to either the SR or ER. Following exposure, the features from the photomask were developed in the photoresist by immersing the substrates in AZ 300 MIF developing solution (Clariant) for 45 s. After rinsing in DI water and drying by N_2 gas, the patterned substrates were hard baked at 115 °C for 20 min.

Etching

Unprotected areas on the glass substrates were etched isotropically by submerging the slides in 10% buffered oxide etchant (BOE, Transene, Danvers, MA). The bottom layer was immersed in BOE for 6 min, and the middle layer was exposed for up to 18 min. During the etching process, substrates were removed from the BOE bath every 3 min and immersed in 1 M HCl for 10 min to remove any insoluble fluoride products formed.⁴⁷

Electrode deposition

Gold electrodes (2500 Å atop a 200 Å Cr adhesion layer) were deposited thermally on the upper surface of the bottom layer using a CHA 600 thermal evaporator (CHA Industries, Fremont, CA). To improve metal adhesion to the glass, substrates went through an oxygen plasma cleaning step for 15 s using a Sunbird plasma enhanced chemical vapor deposition system (SHS Equipment, Milpitas, CA), followed by a 15 s dip in BOE solution and a 15 s dip in 1 M HCl. During metal deposition, the microchannels were covered to keep Cr/Au out of the separation system. A lift-off process involving immersion of the glass slides in acetone for 15 min removed the photoresist, leaving patterned electrodes.

Reservoir drilling

Reservoirs for sample, eluent, and electrolyte solutions; and holes for electrode contacts were milled in the middle glass substrate with diamond-tipped bits using a bench-top drill press (Cameron Micro Drill Presses, Sonora, CA). This process was performed with the glass devices immersed in water to avoid thermal stress and breakage. Three 2.5-mm-diameter through-holes formed the ER, SR and waste reservoir (WR). Two 1.0-cm-diameter reservoirs were made to contain the electrolyte solution in the eluent pump (EP) and injection pump (IP). Five 1.5-mm-diameter holes were drilled in the cover piece for access to load sample, eluent and electrolyte solution into their respective reservoirs. Finally, four 1/32"-diameter holes were opened in the top layer to facilitate contacting the electrodes.

Bonding

Prior to bonding, the glass slides were trimmed to 50 × 35 mm. Then, the substrates were cleaned with acetone and isopropanol to remove particles, dust and organic contamination. To increase the hydrophilicity of the glass surfaces, substrates (except the bottom layer containing gold electrodes) were immersed in boiling piranha solution for 10 min and soaked in concentrated sulfuric acid for 6 h. Finally, glass slides were rinsed with DI water under pressure for 5 min. Bonding was carried out by manually aligning the pieces as shown in Figure 1B. In some devices, we increased the volume of the micropumps and reservoirs by stacking three or four glass pieces with drilled holes as the middle layer. A few drops of water were added between the glass slides to help maintain alignment. The glass stack was sandwiched between polished quartz plates and held together using a high-temperature-alloy clamp. The assembly was placed inside a BF51800 furnace (Lindberg/Blue, Asheville, NC). The optimized bonding conditions involved ramping at 5 °C/min to 620 °C and holding for 5 h, followed by cooling to room temperature. Bonded devices had Nanoport reservoirs (Upchurch Scientific, Oak Harbor, WA) attached to the top surface, and electrical connections to the thin-film electrodes were made using Pt wires and conductive epoxy (Chemtronics, Kennesaw, GA).

Microchannel coating procedure

Octadecylsilane-coated microchannels were produced according to the method of Kutter et al.³⁸ Silanol groups were activated by pumping successively through the channels 1 M NaOH, DI water, 1 M HCl, DI water and methanol for 10 min each. Next, microdevices were dried at 110 °C overnight, and the microchannels were purged with N₂ for 20 min at room temperature. The silane solution was prepared in dry toluene and contained 10% (w/w) chlorodimethyloctadecylsilane (Aldrich) with 50 µg/mL *n*-butylamine as a catalyst. After passing through a 0.45-µm filter (Pall, East Hills, NY), the coating solution was aspirated via the WR through the separation channel for 12 h at room temperature, followed by rinses with toluene and methanol to remove any unreacted silane. Vacuum (as opposed to pressure) loading for surface derivatization prevented stationary phase deposition and analyte retention in the injection channel.

Sample preparation

FITC-labeled aspartic acid, glycine and phenylalanine were made according to a procedure described before.⁴⁸ Once prepared, aliquots of the FITC-tagged amino acids were combined and diluted to 0.5 µM in methanol or mobile phase.

Device operation

Pressure-balanced injection was performed by independently controlling the electrolysis time and voltage applied in the electrochemical micropumps (IP and EP) in the devices. A schematic diagram of the pressure-balanced injection approach is shown in Figure 2. For sample injection and separation, microchannels were filled by pipetting 20 µL of mobile phase via the Nanoports into the ER and SR, and applying vacuum to the WR. Then, 300 µL of electrolyte solution (0.1 M KNO₃) were loaded via the Nanoports into the IP and EP, after which they were capped using sealing nuts (Upchurch). Following this, the mobile phase in the SR was removed and replaced with ~10 µL of sample, and if needed, the ER was re-filled with mobile phase. Both the SR and ER Nanoports were then sealed in a manner similar to the micropumps. To equilibrate a device (Figure 2B), 20 V were applied to the EP for ~5 s until the microchannels were bubble-free and entirely filled with mobile phase. Then, for injection (Figure 2C), 20 V were applied to the IP while the voltage at the EP was turned off. After a time empirically optimized for each device (5–8 s), which allowed a plug of sample to be transferred to the microchannel intersection, the IP was

turned off and 25 V were applied to the EP to pump the injected sample through the separation column, as shown in Figure 2D.

The pressure-balanced injection mode was optimized with fluorescein and FITC-labeled glycine. Then, a 0.5 μM mixture of three FITC-labeled amino acids was injected and separated. For all experiments, laser-induced fluorescence detection was conducted in the separation channel at a 2.5-cm distance from the injection intersection. The detection system has been described elsewhere;⁴⁹ briefly, the 488 nm line from an argon ion laser was focused ~ 5 mm from the end of the separation channel. Fluorescence was collected with the same objective, spectrally and spatially filtered, and detected by a photomultiplier tube.

RESULTS AND DISCUSSION

Figure 3A shows a microchannel-reservoir intersection after etching at room temperature for 18 min in BOE. The etching rate under our experimental conditions was 0.8 $\mu\text{m}/\text{min}$, which is similar to the value of 0.9 $\mu\text{m}/\text{min}$ obtained by Lin et al.⁴⁷ The glass substrates containing the separation column were etched for 6 min to produce ~ 5 μm deep microchannels. Figure 3B shows a cross-sectional electron micrograph of a microchannel after bonding of a cover plate. Separation channel widths after wet chemical isotropic etching were 105–110 μm . As seen in Figure 3B, the thermal enclosure protocol produced well-bonded devices since there is no visible interface between the two plates. Figure 3C shows a photograph of a completed device, and a close up of a pump/reservoir (with Nanoports and electrical connections) is shown in Figure 3D.

Microdevices with one intermediate layer only pumped for short (10–15 s) periods. We thus made multilayer microdevices with larger electrolyte reservoir volumes as described in the Experimental section. The average reservoir volume in single- and four-intermediate-layer devices was determined to be 120 μL ($n = 3$) and 460 μL ($n = 6$), with relative standard deviations (RSDs) of 6% and 10%, respectively. These minor variations in volume had little effect on device-to-device injection and separation reproducibility.

To characterize pumping rates, a ruler was attached to the bottom of a microdevice and a colored solution was pumped through the microchannel from the ER to the WR by electrochemical actuation. A flow rate of 210 nL/min (7.1 mm/s) was observed for an electrolysis potential of 15 V. The RSD for the flow rate measurement was 4.2% ($n = 5$). The ability of the micropumps to operate against elevated back pressure was evaluated by connecting a gas cylinder with a pressure regulator to the WR via the Nanoport. Pumping of the colored solution was performed as above, but in this case against 100 psi. A flow rate of 86 nL/min, (RSD = 7.9%, $n = 5$) was measured for this experiment, which is comparable to published results for electrochemically driven pumping⁴⁴ and is suitable for microchip LC.⁵⁰

Due to the oxidizing character of the KNO_3 electrolyte solution and the voltage applied through the micropumps, gold electrodes typically lasted for 15–20 runs. Device lifetime could be extended with similar performance using Pt wires inserted through drilled holes in the bottom of the pump reservoirs and sealed with epoxy.

We first evaluated and optimized the pressure-balanced injection approach using 5 μM fluorescein. Multiple injections were made to find the optimal injection time, determined as the time at which the fluorescent sample plug reached the microchannel intersection. For most experiments (performed in seven different devices) this time was 5–8 s. Importantly, for this injection protocol, the amount of sample loaded can be controlled by the pumping time of the IP, which defines the length of the injected sample plug. For an injection time of 6 s, the plug length (measured at the full width at half maximum) was estimated to be 90–

100 μm , corresponding to an injection volume of ~ 50 pL, which is in the desirable range for microchip LC.⁵¹

To characterize the reproducibility of the pressure-balanced injection protocol for sample introduction, we studied the injection and detection of a single analyte. For non-optimal mobile phase compositions and pH values, we obtained broad peaks (results not shown). We optimized the mobile phase composition and pH, since these parameters have been shown to affect the efficiency of separation of similar, phenylisothiocyanate-derivatized amino acids.^{52, 53} We were able to obtain relatively narrow, well-defined peaks using 70/30 acetonitrile/50 mM acetate buffer (pH 5.45) as the mobile phase. Figure 4 shows the results obtained for repetitive injections of FITC-labeled glycine with detection at a point 2.5 cm downstream. The reproducibility of the retention time of the FITC-glycine peak is promising but not optimal (7.2% RSD). We hypothesized that stochastic bubble formation at the surface of the electrodes was causing variations in the current and hence, pressure for pumping. Thus, we recorded the current across the micropump during electrolysis of the KNO_3 solution, and Figure 5 indicates that the current varies between runs. In some experiments, the current changes by less than a few percent, while in others greater deviations are observed. Figure 5 also shows that larger current changes are seen with increased electrolysis time, and current typically decreases over time for constant voltage operation. A 24% RSD in current was observed after 120 s, whereas after only 30 s, a much smaller 5.5% RSD was measured. Importantly, this latter value is close to the retention time RSD for the FITC-glycine peak, which eluted at ~ 30 s. These results lend strong support to our hypothesis that partial current interruption by the presence of bubbles on the electrode surface is the main source of retention time variability. We also note that our pumping system is best suited for relatively fast (<1 min) microchip LC analysis.

The performance of the LC microdevices was further evaluated in separation of three FITC-labeled amino acids. For these experiments, we used the analysis conditions described before, which were optimized for the injection of a single peak. Figure 6 presents chromatograms of three replicate electrolysis-based pressure-driven LC microchip separations of aspartic acid, glycine and phenylalanine in ~ 35 s. Peak identities were determined by the elution times of individually injected amino acids and are consistent with analyte polarities. We note that the reproducibility of the retention time decreased with increasing retention time. The RSD for the retention time of the first peak was 2.2%, compared to 7.7% for the last peak. An efficiency (N) of 3350 theoretical plates was obtained for the aspartic acid peak, which corresponds to a plate height (H) of 7.5 μm for our 2.5 cm separation channel. This value improves over prior plate heights (12–50 μm) reported for microchip OTLC without integrated pumping.⁵⁴ The higher efficiency of our system is likely due to smaller injected volumes and the use of shallower channels. Microchannel geometry influences the efficiency in pressure-driven LC separations,³⁵ and large aspect ratio (e.g., shallow) channels should reduce analyte dispersion effects from less efficient mass transfer to the stationary phase.^{55, 56} Importantly, our channel dimensions are in the range predicted for optimal OTLC separations.^{7, 9} Another key advantage of our system is that it allows the injection of pL-range samples with no dead volume between the injector and column, which is critical for high-efficiency microchip LC.

The peaks in Figure 6 were either baseline or nearly baseline separated, and the resolution between aspartic acid and glycine was 1.2 (RSD = 8.0%, $n = 3$). Our resolution is lower compared to that for the same peaks in conventional HPLC (~ 24),⁵⁷ which uses both gradient elution and longer columns. We note that improved resolution could be achieved in our system by incorporating another EP to deliver a second mobile phase in the system, enabling gradient elution. Importantly, our separations are ~ 20 fold faster than those achieved by conventional LC.⁵³ Furthermore, the separation efficiency of our devices could

be improved by using microchannels with a double-etched profile near the column sidewalls to reduce band dispersion, as proposed by Dutta et al.^{55, 56}

CONCLUSIONS

Here, we demonstrate rapid microchip LC analysis with integrated electrolysis-based pumping. We have developed a straightforward microfabrication strategy for interfacing microfluidic channels with electrically actuated micropumps in a single substrate. A pressure-balanced sample injection approach was devised for microchip LC, allowing the introduction of pL-range sample volumes without valves or other components that are difficult to integrate in microdevices. On-chip LC separation of amino acids was carried out successfully in <40 s with good efficiency (3350 theoretical plates). Current stability in the micropumps represented the main limitation in analysis reproducibility. Improved electrode designs with greater surface area and feedback loop current control of the micropumps should improve results. Our approach holds great potential for the miniaturization of pressure-driven separations or other pumping applications in which nL/min - μ L/min volumes must be delivered in a simple and compact format.

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Figure 1.

Schematics of the fabrication process to integrate micropumps with microchannels for microchip LC (*drawings not to scale, specific details in the text*). (A) Two masks are used to pattern the microchannels and micropumps in the bottom and middle layers. (B) Alignment of layers. (C) The final device containing sample and eluent pumps integrated with microchannels is formed by thermally bonding all the pieces together.

Figure 2. Schematic representation of the pressure-balanced injection approach. (A) Device layout. (B) Equilibration; microchannels are filled with eluent; the EP is on and the IP is off. (C) Injection; a sample plug is transferred to the microchannel intersection with the EP off and the IP on. (D) Separation; the EP is on and the IP is off.

Figure 3. Device images. (A) Photograph of the intersection of a microchannel and reservoir etched 18 min in BOE. (B) Electron micrograph of the microchannel cross sectional area. (C) Photograph of a bonded device. (D) Image of a microchip with attached Nanoports that facilitate introduction of eluent and electrolyte solution.

Figure 4. Pressure-balanced injection of 1 μM FITC-derivatized glycine. Mobile phase: 70% acetonitrile/30% 50 mM acetate (pH 5.45). Injection: IP on for 6 s at 20 V. Separation: IP off after 6 s and EP on at 25 V.

Figure 5. Variation of the current in the electrolysis chamber as a function of time. The current in the same pump was monitored during nine consecutive runs.

Figure 6. Chromatographic separation of a mixture of 0.5 μM FITC-derivatized amino acids in an electrochemically pumped $2.5\text{ cm} \times 100\text{ }\mu\text{m} \times 5\text{ }\mu\text{m}$ LC microchip column. Peaks are: (A) aspartic acid, (B) glycine and (C) phenylalanine. Mobile phase was 70% acetonitrile/30% 50 mM acetate (pH 5.45).