Electrically Driven Separations on a Microchip

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Summary

We have fabricated glass microchips using standard photolithographic, etching and deposition techniques. Following the fabrication of the channels, a direct bonding technique is used to join the substrate and cover plate to form a network of closed channels used in the experiments. Control of the electroosmotically driven flow of the buffer, analyte and reagent makes possible the precise control of the fluids within the channel manifold. The microchip can be operated under a continuous or discrete sampling mode, and analyte plugs can be injected onto the column independent of time, with no analyte bias and high reproducibility. Several microchip column geometries for capillary electrophoresis and open channel electrochromatography have been produced and studied using laser induced fluorescence. Also, chemical reactions for pre- and post-column derivatization have been performed on-microchip.

Introduction

The microfabrication of analytical instrumentation will potentially enable the laboratory to be transported to the samples rather than vice versa. The concepts of miniaturization can be based on conventional laboratory approaches to chemical measurement problems and would benefit from this large, well-established knowledge base. In addition, micromachined chemical instruments as opposed to single analyte chemical sensors would be able to identify and quantify the individual members of the desired class of compounds using a single device. Microinstrumentation could well derive benefits similar to microelectronics, i.e., low cost, compact size, high speed, integration of several functions and highly parallel analyses. The approach taken is to micromachine a monolithic device and on this microchip implement chemical separation techniques, e.g. capillary electrophoresis[1,2,3,4,5,6] and open channel electrochromatography[7]. Also, on-microchip pre- and post-separation derivatization[8,9] have been coupled to capillary electrophoresis separations.

Experimental

The microchips were fabricated using standard photolithographic, wet chemical etching, and bonding techniques. A photomask was fabricated by sputtering chrome (50 nm) onto a glass slide and ablating the column design into the chrome film using a CAD/CAM laser machining system (Resonetics, Inc.). The column design was then transferred onto the substrates using a positive photoresist (Shipley 1811). The channels were etched into the substrate in a dilute HF/NH_4F bath. To form the separation column, a coverplate was bonded to the substrate over the etched channels using a direct bonding technique. Cylindrical glass reservoirs were affixed on the substrate using RTV silicone (General Electric). Platinum electrodes provided electrical contact from the power supply (Spellman CZE1000R) to the solutions in the reservoirs.

Column performance and separations were monitored on-microchip via laser induced fluorescence (LIF) using an argon ion laser (514.5 nm for sulforhodamine B, dichlorofluorescein, and fluorescein, 20 mW; 351.1 nm for amino acid/OPA products, 15 mW; Coherent Innova 90) for excitation. The fluorescence signal was collected with a photomultiplier tube (PMT; Oriel 77340) for point detection. The data acquisition/ voltage switching apparatus is computer controlled using programs written in-house in Labview 3.0 (National Instruments). The compounds

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used for the experiments were sulforhodamine B, disodium fluorescein and dichlorofluorescein (Exciton Chemical Co., Inc.), arginine, phenylalanine, glycine, and o-phthaldialdehyde (Sigma Chemical Co.).

Results and Discussion

Capillary electrophoresis appears to be the most promising separation technique because of the experimental simplicity. However, microchips have a limited surface area on which to fabricate a device. One approach is to design and construct a column geometry such as a serpentine which enables a long separation column to be fabricated in a compact area. The schematic in Figure 1 shows the microchip with the serpentine separation column which is 165 mm long in an 8 mm by 8 mm area.

The sample is loaded into the injection cross via a frontal electropherogram travelling from the analyte reservoir to the analyte waste reservoirs. Once the front of the slowest analyte passes through the injection cross, the sample is ready to be injected. The analyte plug is confined to the injection cross by flows from the buffer and waste reservoirs, thereby pinching the plug at the injection cross. The size of the sample plug can be controlled by the relative potentials applied to the buffer, analyte, and waste reservoirs. Here, the relative potentials applied to the reservoirs for the sample loading mode at the buffer, analyte, analyte waste, and waste reservoirs are 0.6, 0.8, 0.0, and 1.0, respectively. To inject the sample into the separation column, the potentials applied to the reservoirs are reconfigured. The primary flow path for the separation is from the buffer reservoir to the waste reservoir. To prevent bleeding of excess analyte into the separation column, the analyte and analyte reservoirs are maintained at a fraction of the potential applied to buffer reservoir. The relative potentials for the separation mode at the buffer, analyte, analyte waste, and waste reservoirs are 1.0, 0.5, 0.5,

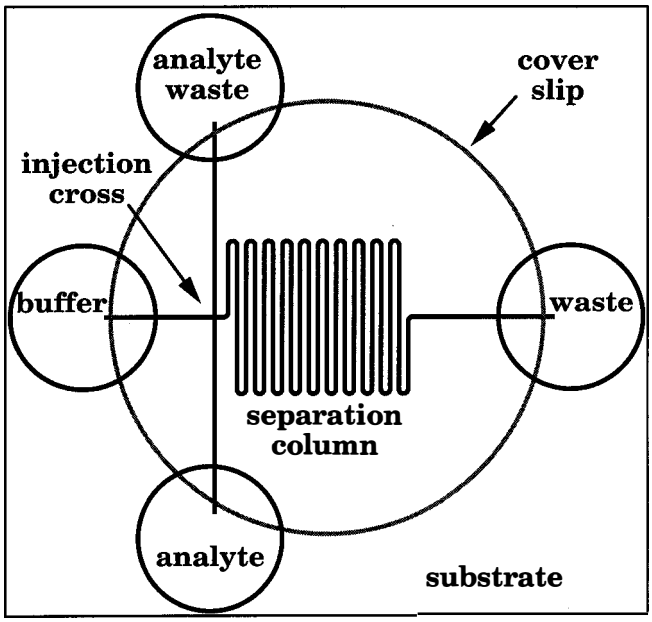

Figure 1. Schematic of the microchip with serpentine channel geometry. Reservoirs are affixed at the terminus of each channel and are labeled by the solution present. The separation column is 165 mm long. The channels are 80 μm wide and 5 μm deep.

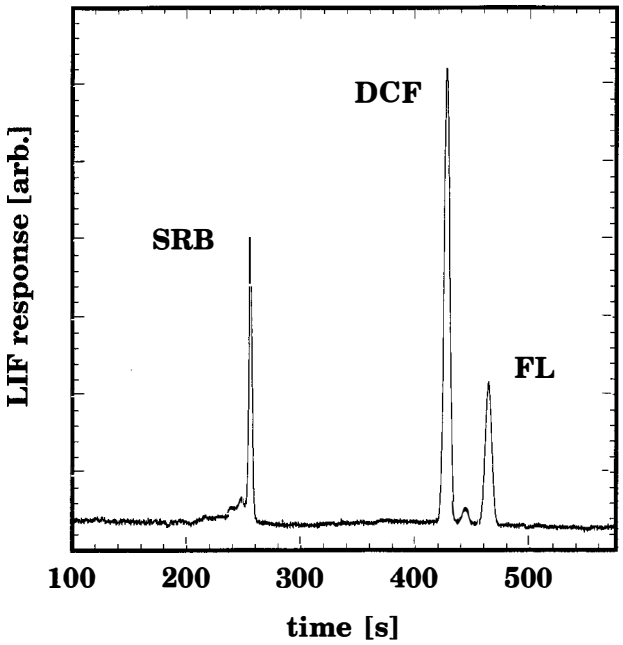

Figure 2: Electropherogram of sulforhodamine B (SRB), dichlorofluorescein (DCF), and fluorescein (FL) separated on the serpentine microchip with E_{sep} = 160 V/cm and L_{sep} = 165 mm.

and 0.0, respectively.

The electropherogram in Figure 2 demonstrates a separation detected at the end of the serpentine geometry. The efficiencies for sulforhodamine B, dichlorofluorescein, and fluorescein are 42400, 34600, and 31400 plates, respectively. The primary contributions toward band broadening on this microchip are from axial diffusion[10], injection plug length, detector observation length[11], and column geometry[5]. The contribution of the column geometry to the total plate height is:

(1) $H_{geo} = n (w \theta)^2/(12 L_{sep})$

where n, w, θ, and L_{sep} are the number of turns, the width of the turns, the angle of the turns, and the separation length, respectively. For this microchip, H_{geo} equals 0.85 μm for twenty 180° turns, one 90° turn and a 165 mm separation length. In the case of sulforhodamine B, H_{geo} constitutes 22% of the total band dispersion.

Another device that has been fabricated on a microchip is a pre-column reactor coupled to capillary electrophoresis for analysis. The reaction studied is o-phthaldialdehyde (OPA) with amino acids in the presence of a reducing agent, 2-mercaptoethanol[12]. The reaction is moderately fast (typical half-time of reaction with amino acids of 4 s [13]), yet for several amino acids the fluorescent product is short lived, ≈ 10 minutes[14]. A microchip with a pre-column reactor addresses both problems by having sufficient time for the reaction and the continuous preparation of new product. In Figure 3 the schematic for the microchip with a pre-column reactor is shown.

The microchip was operated in a continuous reacting/ separation mode. The sample and reagent are continuously pumped in a volumetric ratio of ≈ 1:1 through the reaction chamber toward the analyte waste reservoir. Buffer was simultaneously pumped from the buffer reservoir toward the waste and analyte waste reservoirs. The buffer stream prevents the analyte from leaking into the

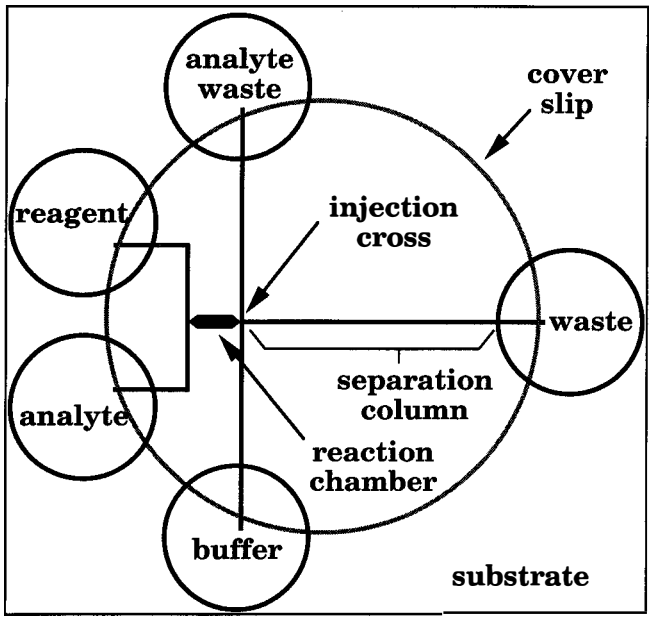

Figure 3. Schematic of the microchip with pre-column reactor. Reservoirs are affixed at the terminus of each channel and are labeled by the solution present. The reaction chamber and separation column are 2 and 15.5 mm long, respectively. The channel in the reaction chamber is 96 μm wide and 6 μm deep. The channel in the separation column is 31 μm wide and 6 μm deep.

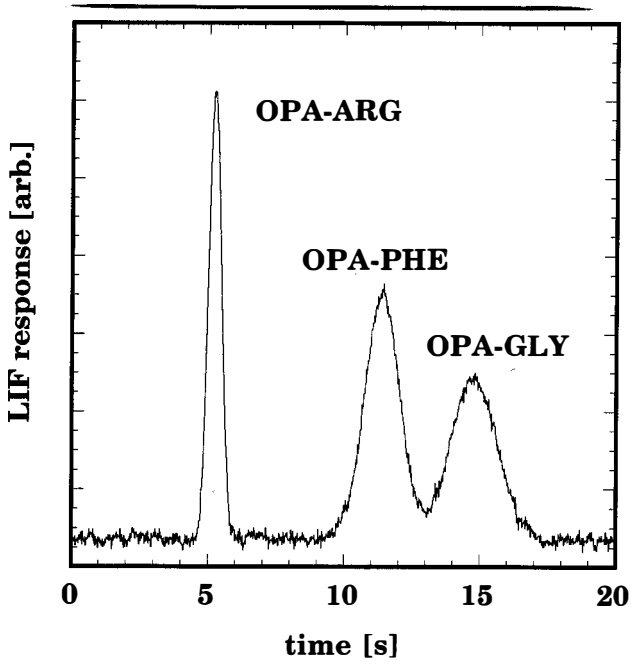

Figure 4. Electropherogram of arginine (ARG), phenylalanine (PHE) and glycine (GLY) using pre-column derivatization with o-phthaldialdehyde (OPA) with E_{sep} = 800 V/cm and L_{sep} = 10 mm.

separation column. To make an injection of the reaction chamber effluent, the potential at the buffer reservoir was simply floated for a brief period of time (0.1 to 1.0 s), which allowed sample to migrate into the separation column as in an electrokinetic injection[15]. To break off the injection plug, the potential at the buffer reservoir is reapplied. The length of the injection plug is a function of the time of the injection and the electric field strength in the column. To obtain these flow patterns, one configuration for the relative potentials at the buffer, analyte, reagent, analyte waste, and waste reservoirs is 1.0, 0.5, 0.5, 0.2 and 0.0, respectively.

Figure 4 shows an electrophoretic separation of arginine, phenylalanine and glycine after on-microchip pre-column derivatization with OPA. The microchip used here offers the unique capability of reacting a continuously flowing stream of the analyte and a reagent of choice prior to analysis. Because the flow patterns are controlled using potentials applied to the reservoirs, the field strengths in the reaction chamber and separation column are interconnected. To have a long reaction time for the derivatization, the field strength in the reaction chamber should be minimized, but for capillary electrophoresis, the resolution between compounds increases with increasing electric field strength. With the current design, a compromise must be reached so that the OPA has a sufficient time to react without destroying the quality of the separation. The ideal arrangement would be to have an electrode placed at the intersection to control the potentials in the reaction chamber and separation column independently.

Conclusion

Microfabrication of electrically driven separations has many advantages. The small dimensions of the microchips enables the use of relatively high electric field strengths which corresponds to faster analysis times. Electroosmotically driven flow enables buffer, analyte and reagent streams to be controlled precisely without valves or pumps. Well-defined plugs of sample can be reproducibly injected onto the separation column. Low dead volume connections between channels can be easily fabricated allowing pre- and post-separation reactions to be incorporated on-microchip.

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