Micromachining a Miniaturized Capillary Electrophoresis-Based Chemical Analysis System on a Chip

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Micromachining technology was used to prepare chemical analysis systems on glass chips (1 centimeter by 2 centimeters or larger) that utilize electroosmotic pumping to drive fluid flow and electrophoretic separation to distinguish sample components. Capillaries 1 to 10 centimeters long etched in the glass (cross section, 10 micrometers by 30 micrometers) allow for capillary electrophoresis-based separations of amino acids with up to 75,000 theoretical plates in about 15 seconds, and separations of about 600 plates can be effected within 4 seconds. Sample treatment steps within a manifold of intersecting capillaries were demonstrated for a simple sample dilution process. Manipulation of the applied voltages controlled the directions of fluid flow within the manifold. The principles demonstrated in this study can be used to develop a miniaturized system for sample handling and separation with no moving parts.

Photolithography and chemical etching techniques have been combined to create the field of micromachining (*1*, *2*), in which three-dimensional microstructures such as motors, tweezers, beams, pumps, and valves have been fabricated on the micrometer scale (*3*). This technology has attracted considerable interest in the development of both sensors and actuators, and sensors for physical forces are becoming well developed (*1*). However, the use of micromachining to fabricate chemical sensors, chemical analysis systems, or even laboratories on the scale of a silicon chip remains in its infancy, despite the considerable promise this approach appears to offer (*4*–*9*). Compared to conventional systems, such devices could reduce solvent and sample consumption or decrease analysis times because of their decreased dimensions (*4*, *5*). Integration of several different processes on a single chip to form a system for sample pretreatment, separation, and detection may allow for new overall processes, improve efficiency, make possible automation, and reduce manufacturing costs (*5*).

Electrokinetic effects could be used to advantage in a miniaturized analysis system (*5*). Electroosmotic flow provides a pumping method that is convenient for small capillaries that develop high back pressures with conventional pumps, and electrophoretic separation has been shown to be an extremely useful chemical separation technique (*10*–*12*). We have recently shown that capillary electrophoresis (CE) can be performed in capillary channels etched in a glass substrate (*13*, *14*). In this report we show that very rapid separations (<5 s) of fluorescently labeled amino acid mixtures can be effected within such devices. Furthermore, we were able to control the flow rate and flow direction of the solvent within a manifold of intersecting channels by applying voltages to each of the capillary channels simultaneously (*5*, *14*). Both sample dilution and injection can be effected with this technique, as described below. Taken in combination, these results show that it will be possible to develop a complete, miniaturized, integrated system with sample pretreatment, separation, and detection on a "chip." The use of electroosmotic pumping offers an additional advantage compared to micromachined pumps and valves (*1*) in that there need be no moving parts, and reliable performance can be achieved with present technology.

We fabricated devices by using standard microphotolithographic techniques (*1*, *2*), either at a commercial facility (Baumer IMT, Zurich, Switzerland) or at the Alberta Microelectronic Centre (AMC) (Edmonton, Canada). Devices made at AMC used Corning 7740 Pyrex, polished and then fine-annealed. A solution of HF: HNO_3 [200 ml (49% HF) and 140 ml (70% HNO_3) diluted to 1 liter] was used to etch the glass to a depth of 10 to 20 μm through a Au and Cr metal mask. An electron micrograph of an intersection between two channels is shown in Fig. 1. The characteristic curvature of the walls associated with isotropic etching as well as the relative smoothness are clearly apparent. With a photomask feature width of 10 μm and an etch depth of 10 μm, the channels were about 30 μm wide at the surface. The yield of devices was about 70% and was apparently limited by defects in the glass. A top plate of Corning 7740 glass with access holes drilled into it was thermally bonded to the etched plate at 650° to 660°C for 4 to 6 hours. This process was repeated two to three times to eliminate bonding defects (*14*). The glass devices prepared at the commercial facility were bonded at 620°C for 4 hours. The remaining figures illustrate various channel layouts that were fabricated. Plastic pipette tips inserted in the holes in the cover plate acted as reservoirs into which buffer and Pt electrodes were placed.

The rapid separation of six amino acids labeled with γ-fluorescein isothiocyanate (FITC), along with the hydrolysis products of the fluorescent label, is shown in Fig. 2. The individual 1 mM amino acids were allowed to react with 0.1 mM FITC in pH

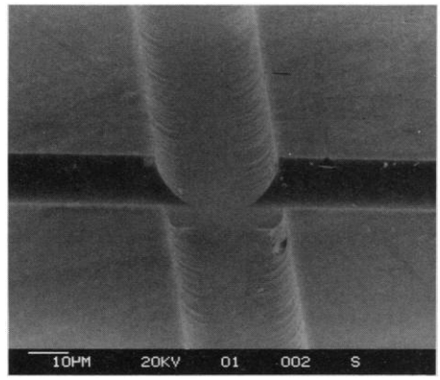

Fig. 1. Electron micrograph of capillary channels etched into Corning 7740 glass to a depth of 10 μm.

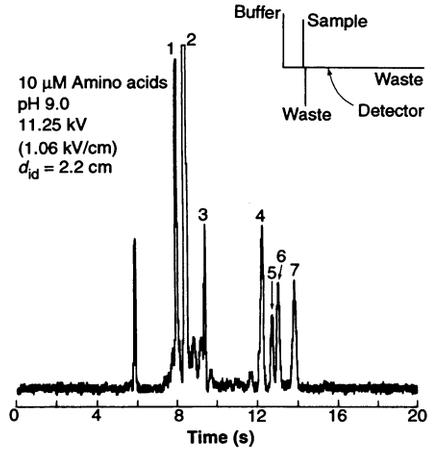

Fig. 2. Electropherogram of six FITC-labeled amino acids in pH 9.0 buffer with 2330 V applied between the injection and detection points and a potential applied to the side channels to reduce leakage of the sample. The peaks were identified by the separate injection of each component and are as follows: 1, Arg; 2, FITC hydrolysis product; 3, Gln; 4, Phe; 5, Asn; 6, Ser; and 7, Gly. The inset shows the approximate layout of the device, with a buffer to separation channel–waste distance of 10.6 cm.

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9 buffer (20 mM boric acid and 100 mM tris) for 16 hours. These samples were diluted to 10 μM in formal concentration of the amino acids. Excitation of the fluorescent labels was effected with the 488-nm line of an Ar ion laser (*14–17*). For the data in Fig. 2, we injected a sample plug by applying 2 kV (1.2 kV/cm) for 10 s between the sample reservoir and the waste reservoir shown at the bottom of the inset in Fig. 2. We then separated the plug while applying 11.25 kV (1.06 kV/cm) between the buffer reservoir and the waste reservoir on the right of the inset in Fig. 2; the total analysis time was 25 s. The injection to detection distance was 2.2 cm, corresponding to 2.3 kV across the region of separation, and the number of theoretical plates obtained ranged from 40,000 to 75,000 for the different amino acids. This corresponds to 17 to 32 plates per volt, which is equivalent to results in conventional fused silica capillaries (*15*). The results show that the planar glass structure is capable of resolving many components of a complex sample mixture with an efficiency equal to that of conventional CE for a similar applied potential.

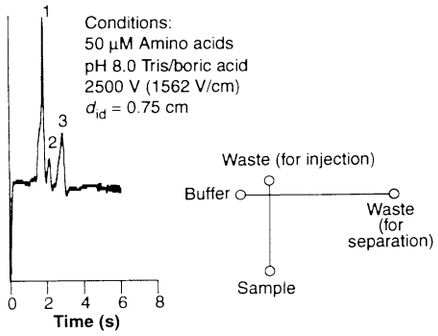

Fig. 3. Electropherogram of 10 μM FITC-labeled Arg, Phe, and Glu (peaks 1, 2, and 3, respectively) in pH 8.0 buffer in a device 1 cm by 2 cm, with an injection to detection distance of 0.75 cm.

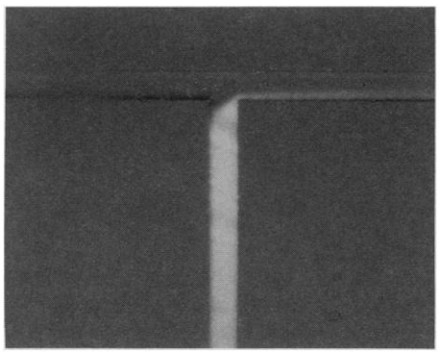

Fig. 4. Photomicrograph of a device with 10 kV (1 kV/cm) applied along the horizontal channel, while the side channel (vertical in the photo) was left floating. The potential drove buffer solution (pH 9.1) from left to right and caused a 100 μM fluorescein dye solution in the side channel (vertical in the figure) to be pulled in the direction of flow. The channels are 30 μm wide.

A very rapid separation within a smaller device with a separation distance of 0.75 cm is illustrated in Fig. 3. Three amino acids of differing charge were separated within 3 s, with 2.5 kV applied (1.56 kV/cm), corresponding to 1.17 kV across the separation region. A 1-s injection time was used with 500 V applied between sample and injection-waste reservoirs. The separation efficiency corresponded to about 600 plates but may be improved if the injection parameters are optimized. This rapid cycle time illustrates that the response time of such a device can compete with the response time of many chemical sensors. Furthermore, rapid separations are required if CE is to be considered for the second stage of a two-dimensional separation, where CE is combined with a technique such as liquid chromatography (LC) as the first stage (*18*). Integration of both CE and LC manifolds on a single chip provides an attractive means of circumventing problems with dead volumes. This work shows that a CE system on a chip is indeed feasible. Fabrication of an LC system in Si has been reported (*19*).

We have been able to use this system to perform quantitative analysis of the amino acids by varying the size of the injection plugs or the sample concentration. The labeled amino acids were prepared by reaction of 1.7 mM FITC with 8.3 mM amino acids in pH 9.1 carbonate buffer (60 mM carbonate) with 0.001% pyridine for 16 hours and were then diluted with pH 9.1 buffer. The peak areas varied linearly with labeled Arg and Tyr concentrations between 0.05 and 20 μM (assuming complete reaction with FITC). A precision of ±2 to 4% was obtained for both amino acids, based on the error in slope and intercept of the calibration curve. Similarly, increasing the sample plug size at constant concentration from 200 to 1800 μm gave a linear increase in peak area, with ±2% precision on the basis of the error in slope of a plot of peak area versus plug length.

When the potential of a side channel is left uncontrolled, solution may leak into the active channel. For the device used in Fig. 3, control of the potential at only the buffer and waste reservoirs during separation led to ~3 to 4% leakage from the sample channel. The increase in the background fluorescence signal this causes depends on the layout of the device and can reach 20 to 30%.

A photomicrograph of a device taken while two of the three channels were actively under potential control is shown in Fig. 4. The device was flooded with 488-nm light while positioned on an Olympus BH-2 microscope stage (20:1 magnification). The applied potential drove buffer along the horizontal channel, while the channel running vertically in Fig. 4 was filled with fluorescent dye and left floating. There is convective flow of the dye out of the side channel, and the dye mixes with the buffer downstream of the intersection point. The magnitude of the effect is more pronounced at high applied potentials, and mixing occurs over an extended distance downstream. It is significant that the leakage arises from a convective effect and is not due to diffusion alone, as it indicates that the resistance to flow of side channels may be manipulated to reduce leakage.

In principle, convective flow at intersecting channels could be controlled by controlling the potential of all reservoirs that contact the intersection. The result of such an experiment for a T intersection is shown in Fig. 5. The applied potentials caused flow from the buffer and fluorescein sample reservoirs toward the waste reservoir. These two solutions mixed downstream of the T intersection point as they flowed toward the detector, diluting the fluorescein dye. An increase in the potential of the buffer reservoir increased the flow of buffer and further diluted the sample solution downstream of the intersection. The fluorescence intensity decreased as the potential of the buffer channel was increased as a function of time, illustrating the dilution effect. This result also shows that control of the potential of all of the channels could be used to control the leakage phenomenon. More importantly, a

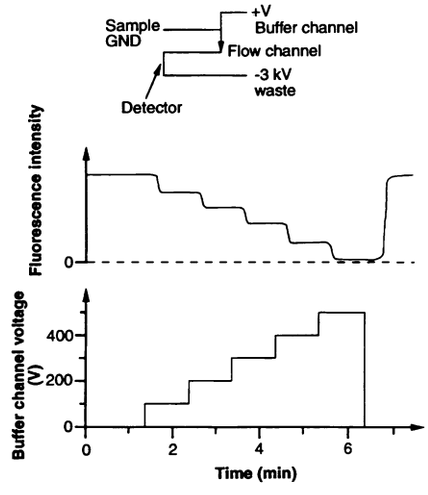

Fig. 5. Fluorescence intensity downstream of the T intersection is shown as a function of time, while the potential on the buffer channel was increased with time. The flow of buffer dilutes the 10 μM fluorescein dye coming from the sample channel; the extent of dilution is controlled by the potential of the buffer channel relative to the sample channel.

common sample pretreatment step, dilution, can be effected within the capillary channel manifold. Typical sample preparation steps performed within a conventional flow injection analysis system could also be effected within these devices by electroosmotic pumping of the fluid phase.

Taken together, our results show that the micromachining of capillary channels on planar substrates provides a route to fabricating miniaturized chemical systems on a chip capable of quantitative analysis. Rapid separation of complex sample mixtures combined with sample handling steps such as dilution and injection provide a basis for more complex, miniaturized analysis systems. The application of micromachining techniques to the miniaturization of chemical analysis is very promising and should lead to the development of analytical laboratories on a chip.

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17. The Uniphase/Cyonics laser was operated at an output power of 4 to 5 mW (*15*). Either the beam was directed onto the sample through a 600-μm optical fiber or mirrors and a lens were used to focus the beam to about 40 μm. Emission was collected with a 10:1 or 25:1 microscope objective and then directed into a photomultiplier tube after filtering with an Omega 508- to 533-nm bandpass filter. The signal was electronically filtered and digitally collected with a Macintosh computer equipped with a National Instruments NB-MIO-16 analog-to-digital converter and Labview software or an 8-bit digital LeCroy 9310 oscilloscope (*14, 16*). The potential programs were generated in the computer and applied to the device reservoirs through high-voltage relays and FUG model HCN 12500 power supplies.
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High-Density Nanosecond Charge Trapping in Thin Films of the Photoconductor ZnODEP

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An electrooptical memory effect is observed with solid thin films of the photoconductor zinc-octakis(β-decoxyethyl) porphyrin (ZnODEP) sandwiched between two optically transparent electrodes. Upon irradiation with the simultaneous application of an electric field, electron-hole pairs are generated and separated within the photoconductive layer. These electron-hole pairs become "frozen" within the films when the irradiation is interrupted. These trapped charges can be released by irradiation of the cell, resulting in a transient short-circuit photocurrent. No cross talk between adjacent memory elements separated by ~0.2 micrometer (a density of 3 gigabits per square centimeter) was detected. The charge storage system is robust and nonvolatile. The response time for the write-read beam is in the subnanosecond range, and no refreshing is required for long-term retention of trapped charges.

A nonvolatile, rewritable electrooptical method of data storage has been developed that is based on charge trapping in thin films of a photoconductive material, in this case, ZnODEP, with which high densities and high speeds can be achieved. This information storage method requires a material with a high resistance in the absence of light, good photoconductivity, and the capacity to inject stored charge upon simultaneous application of light and an electric field. Information, as trapped charge, can be written, read, and erased by simultaneous application of an electric field and a light pulse. We also used a scanning tunneling microscope (STM) for charge storage (writing) and charge measurement (reading) within elements as small as 40 nm in diameter.

Photoconductivity has been widely investigated (*1*), and the displacement of charge in photoelectrets upon application of an electric field and light, a phenomenon called persistent internal polarization, has been known for more than 35 years (*2, 3*). Analogous effects are also important in electrophotography (*4*).

Our interest in this approach grew from our investigations of solid-state photocells composed of a solid film of the liquid crystal porphyrin ZnODEP (Fig. 1) held between transparent indium-tin-oxide (ITO) electrodes, first described by Gregg *et al.* (*5*). Such cells produce steady-state short-circuit photocurrents by preferential injection of electrons into the irradiated electrode. While investigating the behavior of such cells under a constant bias with pulsed irradiation or with a pulsed bias under steady illumination, we observed charge trapping.

Cells (Fig. 2) were constructed as previously described (*5*) by capillary filling of molten ZnODEP (*6, 7*) into the 1- to 2-μm gap between two ITO electrodes (area, 0.5 cm^2). Upon solidification, the film was illuminated with a write beam (wavelength, 550 nm) to produce a cathodic current when a negative potential was applied to the irradiated ITO electrode (we choose the sign of the applied potential to be that of the irradiated, or front, electrode with respect to the back electrode). In this writing step, initially vacant traps within the film are filled with electrons (Fig. 3). Because the resistivity of ZnODEP in the dark is very high ($\geq 10^{14}$ ohm·cm), electron movement "freezes" when the light is switched off; the trapped charge remains stored in the

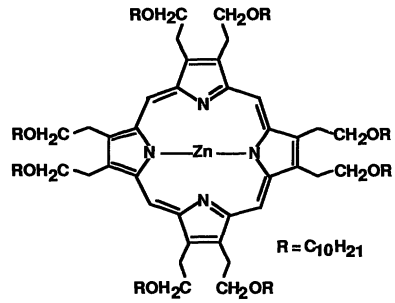

Fig. 1. Structure of the photoconductor ZnODEP.

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