Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 85 (1994)

Heft: 1

Artikel: Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip

Autor: Seiler, Kurt / Fluri, Karl / Harrison, D.Jed.

DOI: https://doi.org/10.5169/seals-982747

Mitt. Gebiete Lebensm. Hyg. **85**, 59–68 (1994)

Kurt Seiler, Karl Fluri and *D.Jed Harrison,* Department of Chemistry, University of Alberta, Edmonton, Canada

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

Key words: Capillary electrophoresis, Micromachining technology, Amino acids, Electroosmotic pumping, Microtechnology

Introduction

Most analysis in the laboratory involve a complete system of sample treatment that is often time consuming and labor intensive. With conventional instrumentation it is possible to couple techniques such as flow injection analysis (FIA) to chromatographic systems or other techniques. This allows continuous sample monitoring in applications such as process control. Miniaturization of such systems using micromachining should provide an exciting route to complex analysis systems on the scale of a microchip (1). Such a device would be able to measure the concentrations of several components in a complex sample matrix and provide rapid, repetitive, and quantitative output. Miniaturization also offers advantages for many separation methods, as has been well established, and integration of capillaries, detectors and injectors could increase separation efficiency by reduction of dead volumes. Micromachining of a chemical analysis system on a silicon substrate was pioneered by *Terry* et al. in developing the components of a gas chromatograph on a silicon wafer (2).

Practical benefits of such devices include considerable reductions in solvent and reagent use, smaller sample volumes, and increased speed of analyses (1, 3, 4).

It has been proposed that multiple streams of fluid could be handled in a complex flow-manifold of capillaries etched in a planar substrate, allowing processes such as sample dilution and chemical derivatization to be combined with a sensor or detector to form a miniaturized analytical system (1, 5). The miniaturized analysis systems presented are fabricated on planar glass substrates using bulk micromachining methods to form a complex manifold of intersecting capillaries. They are based on the technique of capillary electrophoresis (CE) (6). Electroosmotic pumping is

utilized to drive solution flow with valveless switching between channels and electrophoresis for separation of sample components. It has recently been shown that separations of different fluorescent dyes (3) and fluorescein isothiocyanate-labelled amino acids (7) can be effected in such planar structures.

Further, it has been shown that flow injection analysis style sample handling is also possible within a manifold of capillary channels by the application of voltages to each of the capillary channels simultaneously (8). The direction of flow in a given channel is determined by the electric field applied. This manifold could be coupled on the same chip to a capillary intended for electrophoretic separations of the sample components. The resultant device can be described as a miniaturized total chemical analysis system (μ-TAS).

Experimental

Device fabrication

Devices were fabricated using standard microphotolithographic techniques at the Alberta Microelectronic Centre (AMC, Edmonton, Canada) (9). The patterned glass plate was bonded to a cover glass plate by melting at 650 °C. Holes in the cover plate were ultrasonically drilled before bonding, and were aligned with the capillaries to provide access for sample and mobile phase introduction, as well as the access for electrodes with which to apply potentials.

Reagents, solutions

Two buffer solutions were used: a pH 9.2 and a pH 9.1 buffer (30 and ,10 mM in carbonate, respectively). The fluorescein sodium salt, the L-amino acids and fluorescein 5-isothiocyanate (FITC) were obtained from Sigma (St. Louis) and were used as received. FITC labelled amino acids were prepared as described elsewhere (10), and then diluted with the mobile phase buffer. Before use all solutions were passed through a 0.22 μm filter (Millex-GV, Millipore, Bedford, MA) to remove particles.

Procedures

The manifold of channels was initially flushed with the buffer by applying pressure to one of the reservoirs with a syringe. It was very important to clean the surface of the glass carefully with distilled water to remove conductivity paths arising from excess electrolyte solution on the surface. Fine Pt wires were inserted in the reservoirs to supply the electrophoresis voltage. Except where indicated,

sample and buffer solutions were driven between various reservoirs by the application of a voltage to the two reservoirs in question.

Instrumentation and signal processing

The power supplies, high voltage relays, computer and programing used have been described elsewhere, as has the fluorescence detector we utilized (3, 7). After the digitally acquiring of data, the electropherogram peak parameters (center of gravity, peak area, variance) were obtained by statistical moment analysis. The time of migration of the center of gravity is used as the peak migration time. The number of plates was calculated after baseline correction for each peak. This was done either from analyzing peak height and area or from calculating the second statistical moment.

For the valveless injection and separation of a sample, the number of plates can be calculated on the basis of the following equation (7):

$$N = d_{id}^{\,2}\,[\,2DLd_{id}/(\mu V_{ap}) + w_{inj}^{\,2}/12 + w_{det}^{\,2}/12 + 2\,Dt_d\,]^{-1} \qquad [1]$$

In this expression D is the diffusion coefficient of the sample component, μ is its observed electroosmotic mobility, L is the total channel length across which the voltage is applied, d_{id} is the distance from the injection point to the detector, V_{ap} is the applied potential, w_{inj} and w_{det} are the length of the injected sample plug and the length of the channel sampled by the detector. The time t_d is the delay time between two separations. It accounts for diffusion of the sample plug between two separation steps. The form of this equation assumes that the sample plug and detector geometry can be described as rectangular plugs within the capillaries. The expression does not incorporate any possible effects from adsorption of components on the walls of the channels.

Results and discussion

A typical layout of one of the realized capillary electrophoresis TAS devices is illustrated in figure 1. The channels were etched into one glass plate, and a second was then bonded on top. This second piece has holes drilled into it to contact the channels.

Electrical characteristics

By applying a voltage between any pair of reservoirs in a device, the channel resistances could be evaluated from the current-voltage responses. The agreement with the channel geometry indicated that the current path was through the chan-

nels. The bonded bulk glass has been shown to have good insulating ability in sustaining electric fields of up to $7.8 \cdot 10^4$ V/cm (7). It has been found that the I/V curves for most devices are linear up to electric fields of 2.3 kV/cm, which can be compared to the value of 0.3 kV/cm frequently used in CE in conventional fused-silica capillaries. The linearity of the I/V curves indicates that Joule heat is effectively dissipated at an energy density of at least 1.8 W/cm (7). This is larger than the value of 1 W/cm that has been suggested as the limit for CE in uncooled fused-silica capillaries. These high electrical fields mean fast analysis times and high separation efficiencies can be achieved, according to the theory of capillary zone electrophoresis (9).

In some cases, e.g. for a simple injection and subsequent separation, the potential of a side channel does not always have to be controlled and therefore is left floating (see figures 1a and 1b). In other cases, e.g. for the mixing of two solutions, the controlling of the potentials of more than the two reservoirs in question is required (see figure 1c). Figure 2 shows the current between the intersection and ground as a function of the two applied potentials at the sample and waste reservoirs. The I/V curves are linear and the channel system behaves perfectly like a system of resistors, where one is in series with two parallel resistors (8).

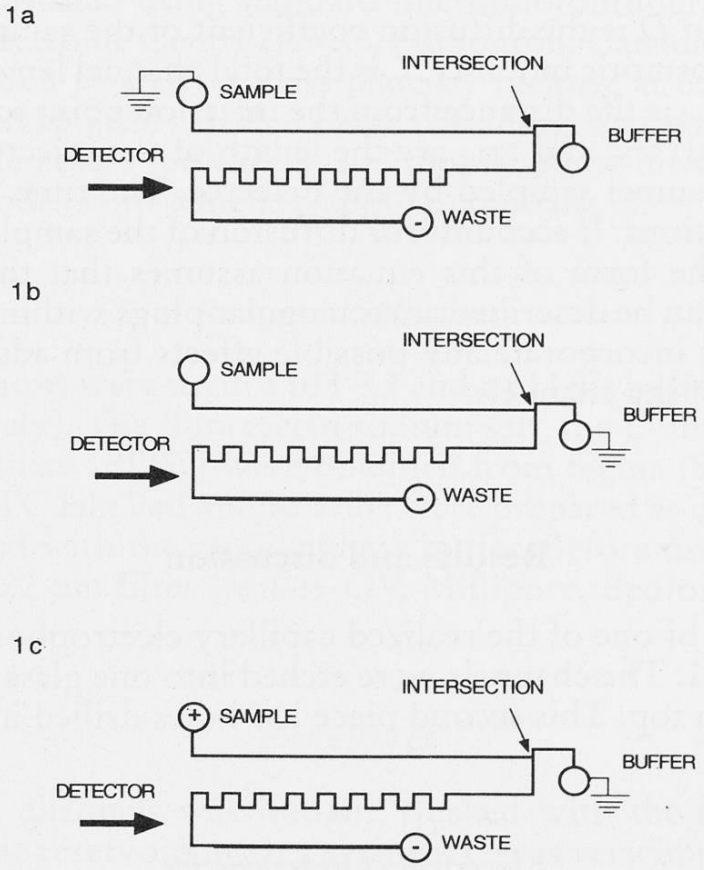

Fig. 1. Layout of a glass device with different potential settings: for injection (a), for separation (b), and for mixing of two solutions (c). Most channels are 10 µm deep. The channel lengths (width) are as follows: sample reservoir to intersection, 4.5 cm (30 µm wide); buffer reservoir to intersection, 0.8 cm (30 µm wide); intersection to waste reservoir, 9.0 cm (30 µm wide) and 2.3 cm (220 µm wide)

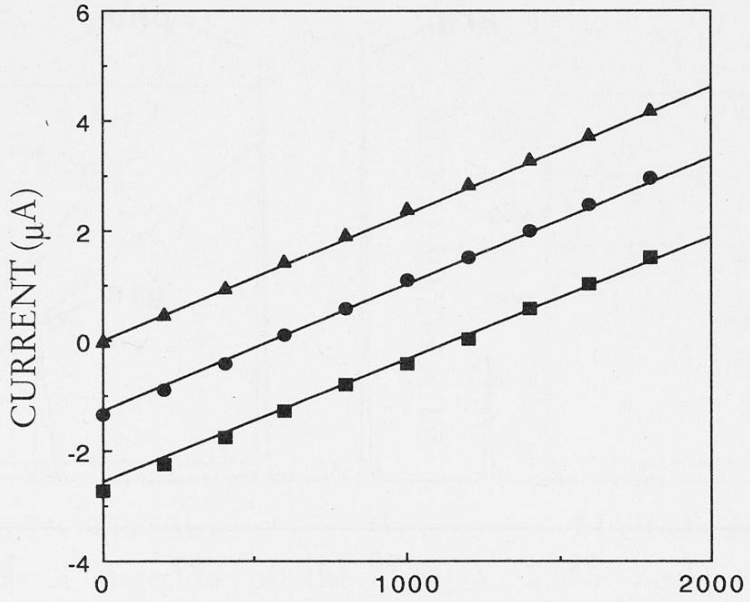

Fig. 2. Current vs the applied potential at the sample reservoir, and as a function of different potentials at the waste reservoir: (▲): 0 V, (●): –1250 V, and (■): –2500 V (for layout see figure 1c)

Separations

Sample injection was effected by driving sample between the sample and the waste reservoirs (see figure 1a). The plug formed at the channel intersection was then driven towards the detector with a potential applied between the buffer and waste reservoirs (see figure 1b). In order to show the ability to effect separation of components in a mixture several amino acids were studied: phenylalanine (Phe), glutamine (Gln) and arginine (Arg). The electropherogram of the three amino acids in a pH 9.2 carbonate buffer was obtained on a similar device (3) and is shown in figure 3. The separation was performed over a distance of 9.6 cm and with an electric field of 655 V/cm applied, while the injected plug lengths were 1193, 953, and 670 μm for Arg, Phe, and Gln, respectively.

The size of injected sample plugs can be controlled by both the period of application and the magnitude of the injection voltage used during the injection stage, as given in eq 2.

$$l_i = \mu\, t_{inj}\, V_{ap}/L_{inj} \qquad [2]$$

The calculated length of the plug is l_i, t_{inj} is the time the potential V_{ap} is applied to the injection channel, with length L_{inj}. There should be a linear relationship between l_i and the area of an injected peak, since the cross section of the channel is a constant and the fluorescence detector responds linearly to the amount of sample present. Figure 4 shows that this is true for injection of labelled Phe and Arg for plug lengths greater than 200 μm. However, shorter plugs introduce more material

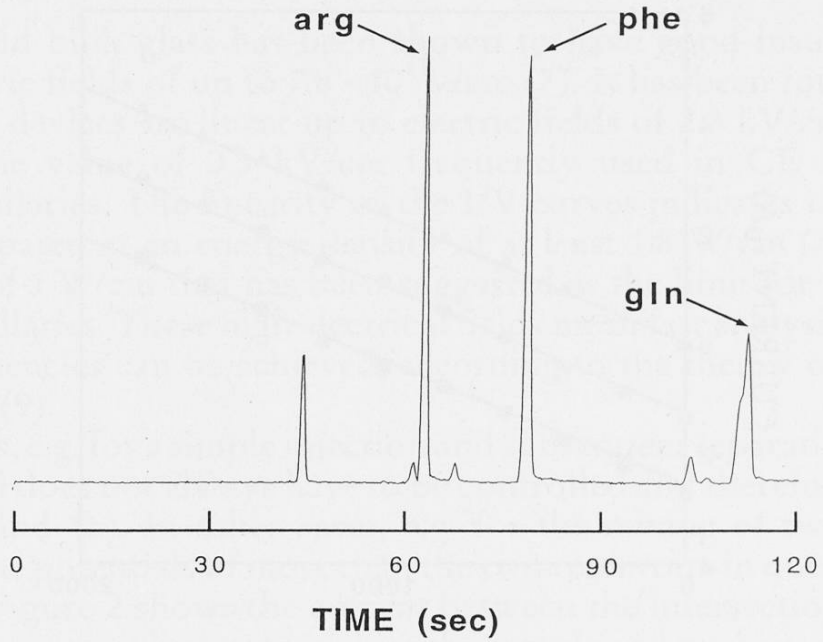

Fig. 3. Electropherogram of 3 FITC-labelled amino acids in pH 9.2 carbonate buffer. The peaks were identified by injecting each component separately. (Reprinted with permission from (7). © 1993 American Chemical Society)

than predicted by eq 2. The shortest plugs that can be injected are about 50 µm in length, calculated on the basis of their peak areas. This study was performed in a device with 30 µm wide channels, so that the minimum sample plug injected is about the size of the intersection of the channels.

Since the size of the sample plug can be estimated from the injection conditions it is possible to analyze the number of plates that can be achieved as a function of sample plug size and compare the results to the prediction of eq 1. Figure 5 shows the experimental and calculated results for the separation of two FITC labelled amino acids in pH 9.2 buffer. The diffusion coefficients for the labelled amino acids were 3.8 and $4.3 \cdot 10^{-6}$ cm^2/s for Arg and Phe, respectively. The separation distance, d_{id} was 9.6 cm, w_{det} was 10 µm, the delay time t_d was 70 sec, V_{ap} was 6 kV, and w_{inj} was assumed to be equal to l_i, calculated from eq 2 with $L_{inj} = 29.5$ cm and mobilities of 2.35 and $1.88 \cdot 10^{-4}$ cm^2/V s for Arg and Phe, respectively (the device layout was that of ref. 3). The good match between the experimental and calculated results shows that separations can be performed within these devices in a nearly ideal fashion. Figure 4 shows that for plug sizes below 200 µm the calculated lengths are not meaningful, and nominal values below 50 µm are actually about 50 µm long. The fact the separation efficiency reaches a plateau before these lengths are reached indicates that the band broadening is controlled only by the diffusion terms, so that with more rapid separations higher efficiencies could be obtained. In fact with a shorter delay time it was possible to achieve more than 100 000 theoretical plates with $d_{id} = 9.6$ cm within these devices.

The ability to inject and separate a sample plug by control of the potential at two different reservoirs also demonstrates that the concept of manipulation of flow patterns selectively within the channel manifold is possible. However, the sample

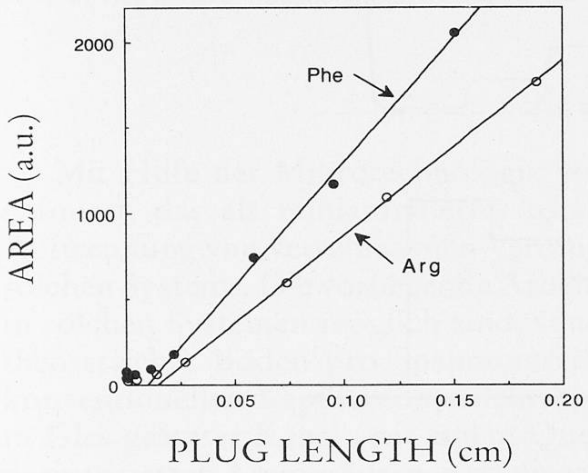

Fig. 4. Peak area as a function of the calculated plug length for Arg and Phe. (Reprinted with permission from (7). © 1993 American Chemical Society)

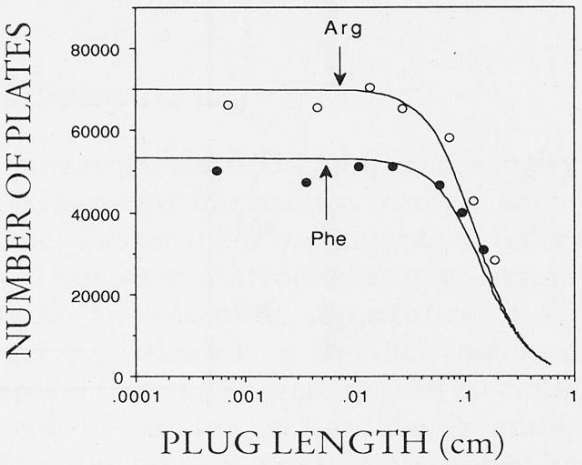

Fig. 5. Theoretical number of plates versus the calculated plug length. The solid lines were calculated with eq 1. (Reprinted with permission from (7). © 1993 American Chemical Society)

in the sample channel is free to diffuse into the intersection volume, or be pulled in by convective flow along the separation channel during separation. We have shown that this effect increases the background fluorescence by 3% or more depending on the design and layout of the capillary channels (3). The principal factor affecting the extent of leakage from side channels appears to be the resistance to fluid flow of the channels due to their geometry.

Mixing of two solutions

A leakage of fluid from a side channel may be perfectly acceptable in some applications, but not others. It will certainly decrease the accessible dynamic range of detection for complex mixtures. In principle, convective flow at intersecting channels could be controlled by actively controlling all reservoir potentials that are in contact with the intersection (see figure 1c). Figure 6 illustrates the result of such an experiment for a T-intersection, with ground potential on the sample (10 μ*M* fluorescein) reservoir, –3.0 kV applied to the waste reservoir and potentials of 0 to +500 V applied to the buffer reservoir. The initial potentials applied caused flow from the buffer and sample reservoirs towards the waste. The two solutions mixed downstream of the T-intersection point as they flowed towards 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. Figure 6 shows the decrease in the fluorescence intensity as the potential of the buffer channel was increased as a function of time, illustrating the dilution effect. This result also clearly shows that control of the potential of all of

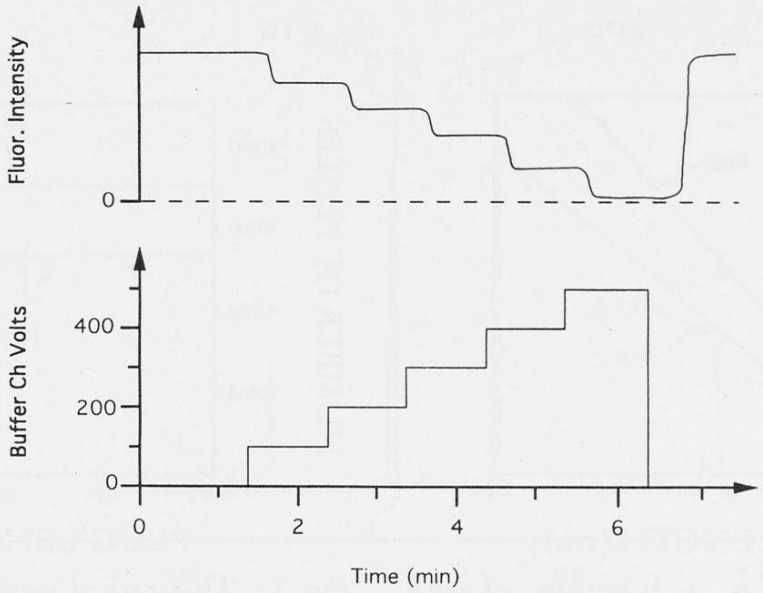

Fig. 6. Fluorescence intensity downstream of the T-intersection is shown as a function of time, while the potential on the buffer channel was increased with time

the channels could be used to control the leakage phenomenon we have observed previously. More importantly, these results show that a common sample pretreatment step, dilution, can be effected within the capillary channel manifold. This demonstrates that typical sample preparation steps performed within a conventional flow injection analysis system could also be effected within these devices, utilizing electroosmotic pumping of the fluid phase.

Acknowledgment

We thank the Natural Sciences and Engineering Research Council of Canada, the University of Alberta, and Ciba-Geigy for support.

Summary

Micromachining technology has been used to prepare miniaturized total analysis systems by defining a manifold of channels on planar glass devices. Separation of sample components is an important aspect, and the present work shows that electrophoretic separation can be achieved on such planar substrates. In fact, the efficiency expressed as the number of plates per volt is similar to that achieved with conventional open tubular, fused silica capillaries. A 10 cm long capillary etched in glass (10 x 30 µm cross section) allows for separations of derivatized amino acids with more than 100 000 theoretical plates in about 3 minutes.

This study has also demonstrated that electroosmotic pumping to control flow in a manifold of channels without the use of valves is feasible. By a careful control of the potential of several channels a simple sample dilution step has been demonstrated. Its realization demonstrates that more complex sample treatment steps such as those used in flow injection analysis can be achieved with this approach.

Overall, glass appears to be a satisfactory substrate for development of planar structures. It is compatible with both micromachining methods and electrophoresis.

Zusammenfassung

Mit Hilfe der Mikrotechnologie wurde auf einem planaren Glaschip ein Kanalsystem definiert, das als miniaturisiertes totales Analysensystem bezeichnet werden kann. Die Auftrennung von verschiedenen Verbindungen einer Probe ist ein wichtiger Eckpfeiler eines solchen Systems. Die vorliegende Arbeit zeigt nicht nur, dass elektrophoretische Trennungen in solchen Systemen möglich sind, sondern dass die Trennleistung, ausgedrückt in Anzahl theoretischer Böden pro Spannungseinheit, sogar vergleichbar ist mit derjenigen, die in konventionellen Kapillarzonenelektrophoresekapillaren erreicht werden. Ein 10 cm langer, in Glas geätzter Kanal (mit einem Querschnitt von 10 x 30 µm) erlaubt die Trennung von derivatisierten Aminosäuren in weniger als drei Minuten und mit einer theoretischen Bodenzahl, die über 100 000 liegt.

Die präsentierten Resultate zeigen ebenfalls, dass das elektroosmotische Pumpen gebraucht werden kann, um den Fluss in einem einzelnen Kanal, der Teil eines grösseren Kanalsystems ist, ohne die Verwendung von Ventilen zu kontrollieren. Mittels Potentialkontrolle von mehreren Kanälen konnte eine einfache Verdünnung einer Probe realisiert werden. Das zeigt, dass kompliziertere Probenbehandlungsschritte möglich sein sollten, wie sie etwa bei der Fliessinjektionsanalyse zur Anwendung kommen.

Zusammenfassend kann gesagt werden, dass Glas für die Entwicklung von solchen planaren Systemen geeignet zu sein scheint. Es ist kompatibel mit mikrotechnologischen Verfahren und den elektrophoretischen Effekten.

Résumé

La microtechnologie a été utilisée pour préparer des systèmes miniaturisés d'analyse totale constitués d'une multitude de canaux sur du verre planaire. Un des aspects les plus importants d'un tel système est la séparation de différents composants d'un échantillon d'analyse. L'étude présentée montre non seulement que des séparations électrophorétiques sont réalisables dans de tels systèmes, mais aussi que le pouvoir séparateur, exprimé en nombre de plateaux théoriques par unité de tension, est comparable à celui de tubes capillaires conventionels pour électrophorèse en «fused silica». Un canal, d'une longueur de 10 cm et d'une section de 10 x 30 µm, gravé dans du verre, est capable de séparer des derivés d'acides aminés en moins de trois minutes, avec un nombre théorique de plateaux dépassant 100 000.

En outre, les résultats obtenus montrent qu'il est possible de contrôler le flux dans l'une des branches d'un système de canaux au moyen de la pompe électro-osmotique sans utilisation de valves. En contrôlant le potentiel de plusieurs canaux, on peut obtenir une dilution simple d'un échantillon. Cette technologie démontre que des procédés plus complexes de traitement d'échantillons peuvent être ainsi réalisés, comme par exemple ceux utilisés pour l'analyse automatisée de type «flow injection».

En conclusion, on peut dire que le verre constitue une matière appropriée pour la fabrication de systèmes planaires. Il est compatible avec les méthodes microtechnologiques et suffit aux exigences de l'électrophorèse.

Literature

1. *Manz, A., Fettinger, J.C., Verpoorte, E., Lüdi, H., Widmer, H.M.* and *Harrison, D.J.:* Micromachining of monocrystalline silicon and glass for chemical analysis systems. A look into next century's technology or just a fashionable craze? Trends Anal. Chem. **10,** 144–149 (1991).
2. *Terry, S.C., Jerman, J.H.* and *Angell, J.B.:* A gas chromatograph on a silicon wafer. IEEE Trans. Elec. Dev. **ED-26,** 1880–1886 (1979).
3. *Harrison, D.J., Manz, A., Lüdi, H.* and *Widmer, H.M.:* Capillary electrophoresis and sample injection systems integrated on a planar glass chip. Anal. Chem. **64,** 1926–1932 (1992).
4. *Manz, A., Harrison, D.J., Verpoorte, E.M.J., Fettinger, J.C., Paulus, A., Lüdi, H.* and *Widmer, H.M.:* Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip. J. Chromatogr. **593,** 253–258 (1992).
5. *Ruzicka, J.* and *Hansen, E.H.:* Integrated microconduits for flow injection analysis. Anal. Chim. Acta **161,** 1–25 (1984).
6. *Jorgenson, J.W.* and *Lukacs, K.D.:* Zone Electrophoresis in open-tubular glass capillaries. Anal. Chem. **53,** 1298–1302 (1981).
7. *Seiler, K., Harrison, D.J.* and *Manz, A.:* Planar glass chips for capillary electrophoresis: Repetitive sample injection, quantitation, and separation efficiency. Anal. Chem. **65,** 1481–1488 (1993).
8. *Seiler, K., Fan, Z., Fluri, K.* and *Harrison, D.J.:* Anal. Chem., submitted 1993.
9. *Harrison, D.J., Fluri, K., Seiler, K., Fan, Z., Effenhauser, C.S.* and *Manz, A.:* Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. Science **261,** 895–897 (1993).
10. *Monnig, C.A.* and *Jorgenson, J.W.:* On-column sample gating for high-speed capillary zone electrophoresis. Anal. Chem. **63,** 802–807 (1991).

Dr. Kurt Seiler*
Dr. Karl Fluri
Prof. Dr. D.Jed Harrison
Department of Chemistry
University of Alberta
Edmonton, Alberta, Canada T6G 2G2

*Present address:
Kantonales Laboratorium Schaffhausen
Postfach 322
CH-8201 Schaffhausen