GLASS CHANNELS AND CAPILLARY INJECTORS FOR CAPILLARY ZONE ELECTROPHORESIS

Yolanda Fintschenko[1], Vincent Spiering[2],Johannes van der Moolen[3], Gert-Jan Burger[2], Albert van den Berg[1]

[1] MESA Research Institute, University of Twente
PO Box 217, 7500 AE Enschede, The Netherlands

[2] Twente MicroProducts, PO Box 217, NL-7500 AE Enschede; tmp@eltn.utwente.nl

[3] University of Amsterdam, Nieuwe Achtergracht 166, NL-1018 WV Amsterdam

0. Abstract

Novel glass and silicon microstructures and their application in chemical analysis are presented. The micro technologies comprise (deep) dry etching, thin layer growth and anodic bonding. With this combination it is possible to create high resolution electrically isolating silicon dioxide structures with aspect ratio's similar to those possible in silicon. Main applications are chemical separation methods such as High Performance Liquid Chromatography (HPLC) or Capillary Zone Electrophoresis (CZE). Beside these channel structures, a capillary connector with very low dead and mixing volume has been designed and fabricated for use in correlation CZE, and demonstrated by determining the precision of consecutive single injections.

Keywords: Microchannels, microfabrication, capillary zone electrophoresis (CZE), high performance liquid chromatography (HPLC)

1. Introduction

In the field of chemical analysis, especially in the area of separation techniques, planar microstructures have a large potential, because they offer a number of advantages as compared to fused silica capillary-based systems. Microfabrication techniques allow for the (i) realization of microchannels with variable cross-sections and depths, (ii) easy manipulation of the dimensions and cross-sectional geometry of microchannels, (iii) integration of detection components into the fluid channels, and (iv) minimization of the dead volume associated with interconnections. Also, miniaturization leads to improved separation efficiencies and higher resolution in shorter times than conventional sized methods. Thus, a generic separation method when used on the seconds time scale becomes a generic multianalyte sensor capable of quasi-continuous monitoring. For this reason, the miniaturization of capillary zone electrophoresis (CZE) and high performance liquid chromatography (HPLC) using microfabrication is of interest.

Conventional CZE typically uses fused silica columnar capillaries of varying inner diameters (10 μm - 100 μm) [1]. It is possible to pump a solution through narrow bore capillaries using an electric field rather than moving parts and differential pressure as in high pressure liquid chromatography (HPLC). This is due to the electroosmotic flow or EOF. EOF results from the movement of buffer cations organized at the negatively charged capillary wall as they migrate towards the cathode, dragging the bulk solution in the same direction. The resulting flow has a plug profile instead of the parabolic profile associated with pressure driven flow at

A. Berg et al. (eds.), *Sensor Technology in the Netherlands: State of the Art*

similar Reynolds numbers. As a result, ideally with CZE there is no band broadening due to solution movement.

Several groups have been working on the fabrication of planar structures for capillary electrophoresis and liquid chromatography. Recently, Colyer et. al. published a minireview of microchip capillary electrophoresis systems, or CZE "on-a-chip"[2]. Manz *et al.* [3] was the first to use anisotropically etched channels in silicon for liquid chromatography, later followed by work from Ramsey *et al.* [4] and Cowen *et al.* [5]. Simultaneously, work was concentrated on CZE, where Manz and Harrison [6,7] were the first to use planar etched glass structures for this purpose. By etching small bore capillary channels in planar glass substrates, Manz *et al.* were able to integrate the injector with the separation channel on one structure [6,8]. Rapid improvements of instrumentation led to the demonstration of this technology in the separation of amino acids with 100,000 to 160,000 theoretical plates and mostly baseline resolution in 2 minutes [9] to 15 seconds [10]. Other structures described by Jacobson *et al.* are capable of making entire separations in a total of 150 ms [10]. Thus, both the integration of two aspects of the CE system were effected and the analytical figures of merit were improved while decreasing total analysis time. The impact of CZE "on a chip" is the elevation of a separation technique to a quasi-continuous, generic chemical sensor.

Despite these contributions, planar structures have several problems in practice. The microchannels frequently need to be connected to conventional system components such as an injector, sample (micro)vial, detector and waste. Thus, the interconnection techniques and system concept are of crucial importance. Our planar Micro System Fluid (MFS) concept was described earlier [11], and in this paper a capillary connection to microchannels is described. This technique can also be used to connect two capillaries with very low dead volume. This can facilitate the use of capillary interconnects as a standard interconnect for hybrid miniaturized systems.

Since the quality and speed of separations for both LC and CZE applications depends on channel geometry, it is important to employ a technique that enables the fabrication of microchannels with a virtually arbitrary cross-section. It is also interesting to see if separation channels for capillary electrophoresis, where high voltages on the order of several kV's are used, can be fabricated using silicon etching techniques. The conventional micro system technology (MST) way to fabricate glass microchannels is to etch one or two wafers in HF and then bond these on top of each other [7,12]. This method has the disadvantage that in glass only isotropically etched channels are feasible to make. This severely limits the geometry's available for channel design. For this, the Black Silicon Method (BSM) [13,14] is applied. With the BSM technology, deep high aspect ratio channels can be dry etched. The cross-sectional shape can be varied by means of process variable control (type and flow of gasses, pressure). In this way channels shapes from square to elliptical or round can be fabricated. Particular emphasis is laid on a technology that enables the realization of an enclosed, fully insulated microchannel with very thin walls for efficient heat transfer.

Correlation CZE, which uses a repeating pseudo-randomly generated pattern of injections in rapid succession, also requires rapid switching of the injection potential and separation potential [15]. The result is a signal over a wide frequency range, and it is possible to gain the same signal-to-noise increase typically associated with Fourier transform. However, it is important that each injection is reproducible, and that the separation voltage is not interrupted to make each injection. For this reason it is of interest to apply microfabrication to make an

electrically isolated connection from the run buffer and the sample to the separation capillary for repetitive injections.

2. Experimental

Transparent glass microchannel fabrication

The main process steps are as follows (Fig. 1.):
1. Channel etching in silicon
2. Channel material deposition on silicon
3. Machining of the Pyrex™ wafer
4. Anodic bonding of the Pyrex™ wafer on top of the silicon wafer
5. Etching the back of the silicon in KOH
6. Addition of a protection/planarization layer over the channels

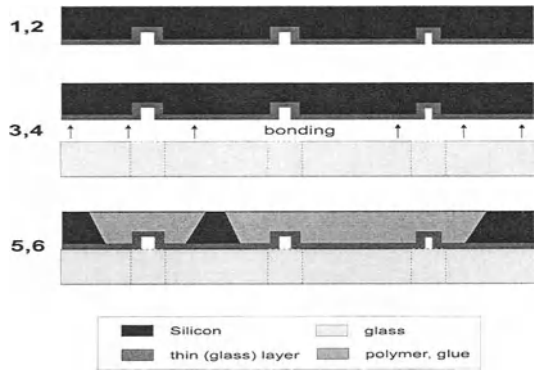

Fig. 1. Process scheme for glass microchannels.

1. Channel etching in silicon
With the BSM technology, deep channels with a variable cross-sectional area can be etched.

2. Channel material deposition on silicon
Materials applied for structures are usually low stress LPCVD silicon-nitride, and/or TEOS oxide, sometimes combined with a thin layer of polysilicon. These layers are required for mechanical strength, electrical isolation, and to stop the etch process for backside KOH-etching. The polysilicon prevents local release at <111> faces of silicon during the KOH etch back process. The total thickness of the layers is restricted by the requirements for successful anodic bonding.

3. Machining of the Pyrex™ wafer
This is accomplished by means of conventional precision machining techniques. The inlet/outlet holes are drilled in the glass wafer.

4. Anodic bonding of the Pyrex™ wafer on top of the silicon wafer
The machined glass wafer is bonded to the silicon wafer with an intermediate layer of oxide and/or nitride.

5. Etching the back of the silicon in KOH
This step can be done without patterns (a complete etch of the back of the silicon wafer) but also with a pattern in silicon to acquire more mechanical strength of the total structure.

Especially in combination with the next step, this will prevent breaking of the tiny glass channels walls.

6. Addition of the protection/planarization layer over the channels
Several layers/materials such as thin films, polymers, and glue can be applied for this purpose. If a transparent material is used, the whole structure remains transparent from the top and the bottom side, which allows for optical detection methods.

Relevant steps are the thin layer deposition followed by an anodic bonding process. Critical to the success of the final structure are the mechanical *and* electrically isolating properties of the thin layers [16] as well as the heat transfer coefficient and absorption spectrum of the various substrates.

Figure 2 shows the outside of a complete glass covered channel on a glass substrate (step 5 carried out without mask). These structures are of great interest in the field of electrophoresis and other applications where electrical isolation of the structure is essential. Currently, specially shaped glass microchannels are to be tested for their fluid resistance and electrical isolation in concentration experiments for pre-separation applications.

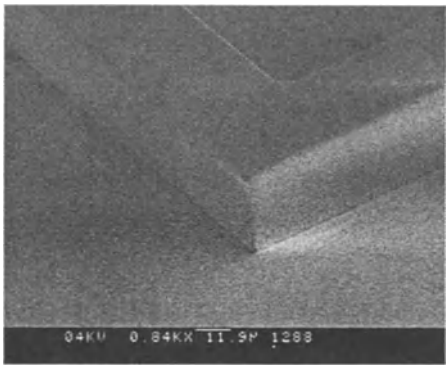

Fig. 2. Complete closed glass microchannel on a glass substrate.

Capillary connector fabrication

Connections between the capillary and the sample injector or between several capillary's are realized via micro channels in silicon. The capillary connection chips were designed and fabricated by Twente MicroProducts [17]. The sample injection holes, channels, and wafer-through holes are realized by a combination of dry and wet etching steps in a silicon substrate. The channel dimensions are matched to the inner diameter of the capillary, thus minimizing the dead and mixing volume of the connection. The channels are electrically isolated with a thin layer of silicon oxide and they are covered by a glass plate. The electrically isolated connectors are fabricated in a very reproducible way. Essential for a suitable connection are the application of high performance dry etch techniques, the design of a pre-aligned connection, and the gluing technique.

3. Results and Discussion

Figures 3 and 4 show a diagram and a SEM of a cross-section of a glued capillary connection with a microchannel. Figure 5 shows a photograph of the microconnector incorporated into

the correlation CZE apparatus. The dead volume was less than 0.5 nL, and the alignment was within 5 μm. The structure was able to sustain voltages up to 250V/cm across the channel, and pressures up to 120 bar.

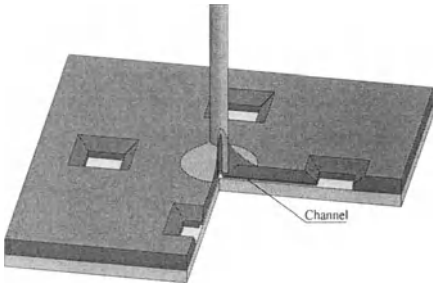

Fig. 3. Capillary connector combined on-chip with sample injector holes.

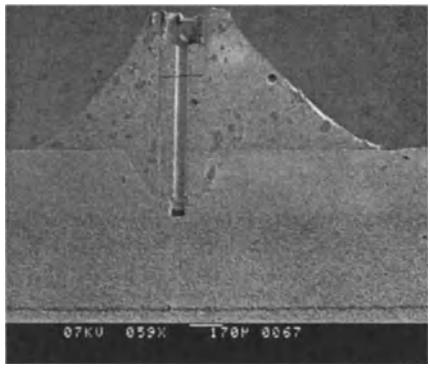

Fig. 4. Cross section of glued 280/75 μm capillary

The connector has been tested by the Laboratory of Analytical Chemistry at the University of Amsterdam with a set-up as given in Figure 5 [15]. A photograph of the injection device using the microchannel connector with a fused silicon capillary is seen in Figure 6.

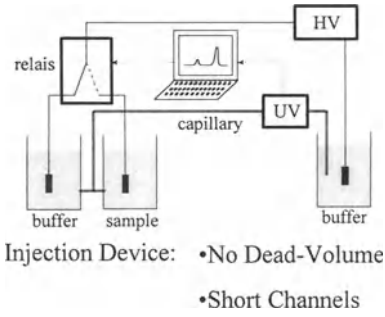

Fig. 5. Correlation CZE system

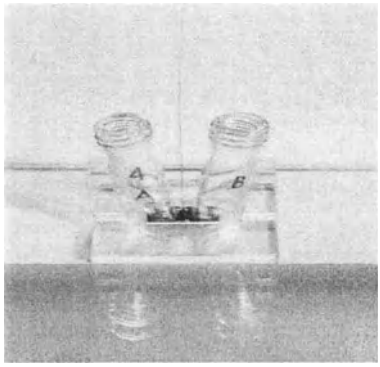

Fig. 6. Photograph of capillary and two sample injector connected via the chip in a correlation CZE system

After proven precision of eight consecutive injections (see Fig. 7) the so-called correlation capillary zone electrophoresis (CCZE) technique was applied [18]. The capillary connector showed reproducible behavior (RSD's <1 %).

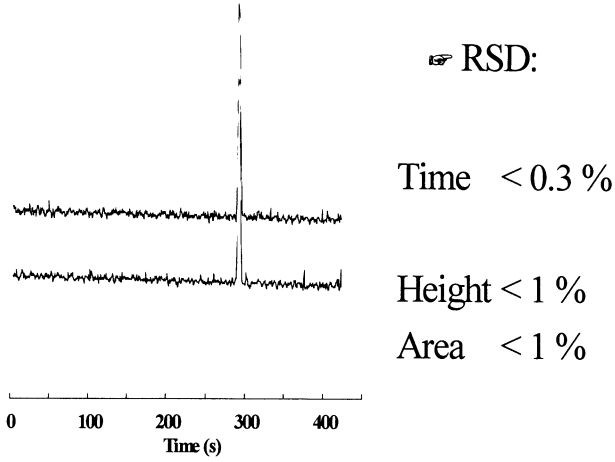

Fig. 7. Precision of eight consecutive injections

The results in Figure 8 show the improvement of the limit of detection as a result of the CCZE technique. A comparison of the typical electropherogram to the correlogram shows the signal to noise improves eight-fold in the latter case.

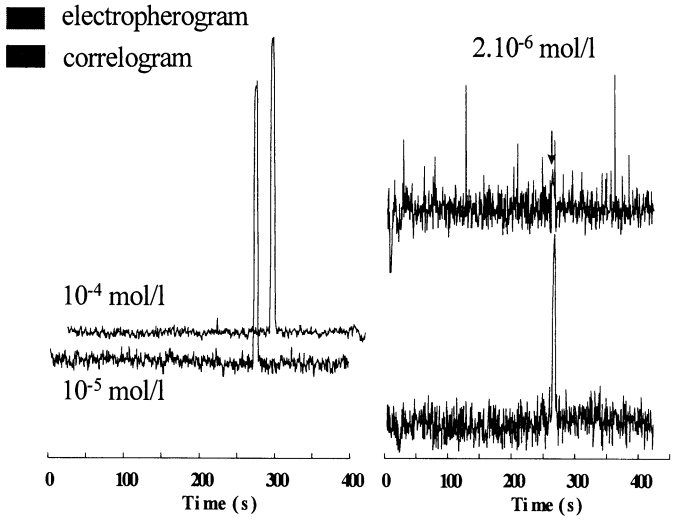

Fig. 8. Electropherogram vs. Correlogram

4. Conclusions

Microchip technology is very well suited for accurate fabrication of complex structures. Beside the fabrication of glass micro channels, it is demonstrated that connecting a microchip injection device to a fused silica capillary can be done quit easily. SEM photographs proved that the connection is excellent, with extremely low dead-volume. The reproducibility in peak

height and peak area of the electrokinetic injection was very good, with RSD-values better than 1.1 %. An improved reproducibility with the microchip injection device compared to conventional injections has been demonstrated. With correlation CZE, the injection reproducibility was even better than the reproducibility with conventional CZE with the microchip injection device. Correlation experiments showed a considerable improvement in S/N ratio at high as well as low concentration. An 8-fold improvement in the detection limit has been demonstrated.

Acknowledgements

The authors recognize that (in alphabetical order) Erwin Berenschot, Meint de Boer, and Henri Jansen are behind most of the technological ideas and tricks presented in this paper. We greatly appreciate their useful contributions.

References

1. J.W. Jorgenson, K.D. Lukacs, Zone electrophoresis in open-tubular glass capillaries, Anal. Chem., 53 (1981) 1298.
2. C.L. Colyer, T. Tang, N. Chiem, D.J. Harrison, Clinical potential of microchip capillary electrophoresis systems, Electrophoresis, 18 (1997) 1733-1741.
3. A. Manz, Y. Miyahara, J. Miura, Y. Watanabe, H. Miyagi, and K. Sato, Design of an open-tubular column liquid chromatograph using silicon chip technology, Sensors and Actuators, B1 (1990) 249-255.
4. S.C. Jacobson,. R. Hergenroeder, L.B. Koutny, J.M. Ramsey, Open channel electrochromatography on a microchip, Anal. Chem., 66 (1994) 2369-2373
5. S. Cowen , D.H. Craston, An on-chip miniature liquid chromatography system: design, construction, and characterization, in 'Micro Total Analysis Systems', Kluwer Academic Publishers, Dordrecht NL , 1992, p. 295.
6. A. Manz, D.J. Harrison, E.J.M. Verpoorte, J.G. Fettinger, H. Lüdi, H.M. Widmer, Planar chips technology for miniaturization and integration of separation techniques into monitoring systems. Capillary electrophoresis on a chip. J. Chromatogr., 593 (1992) 253-258.
7. D.J. Harrison, A. Manz, Z.Fan, H. Ludi, J.M. Widmer, Capillary electrophoresis and sample injection systems integrated on a planar glass chip, Anal. Chem., 64 (1992) 1926-1932.
8. K. Seiler, D.J. Harrison, A. Manz, Planar glass chips for capillary electrophoresis: repetitive sample injection, quantitation, and separation efficiency, Anal. Chem., 65 (1993) 1481-1488.
9. C.S. Effenhauser, A. Manz, H.M. Widmer, Glass chips for high-speed capillary electrophoresis separations with submicrometer plate heights, Anal. Chem., 65 (1993) 2637-2642.
10. S.C. Jacobson, R. Hergenroeder, L.B. Koutny, J.M. Ramsey, High-speed separations on a microchip, Anal. Chem., 66 (1994) 1114-1118.
11. T.S.J. Lammerink, V.L. Spiering, M. Elwenspoek, J.H.J. Fluitman, and A. van den Berg, Modular concept for fluid handling systems: a demonstrator micro analysis system, 9th IEEE Workshop on Micro Electro Mechanical Systems (MEMS'96), San Diego, USA, Feb. 11-15,1996, pp. 389-394.
12. M.-A Grétillat, F. Paoletti, P. Thiébaud, S. Roth, M. Koudelka-Hep, N.F. de Rooij, A new fabrication method of borosilicate glass capillary tubes with lateral inlets and outlets,

Proc. Eurosensors X The 10th European Conference on Solid-State Transducers, 1996, 1 (1996) 259-262.
13. H.V. Jansen, Plasma etching in microtechnology, Thesis, The University of Twente, MESA Research Institute, Enschede, The Netherlands, 1996.
14. R.W. Tjerkstra, M. de Boer, E. Berenschot, J.G.E. Gardeniers, A. van den Berg, M. Elwenspoek, Etching technology for microchannels, 10th IEEE Workshop on Micro Electro Mechanical Systems (MEMS 97), Nagoya, Japan., Jan. 26-30 1997, pp. 147-152.
15. J.N. van der Moolen, D.J. Louwerse, H. Poppe, H.C. Smit, Correlation capillary zone electrophoresis: a novel technique to decrease detection limits, Chromatographia, 40 (1995) 368.
16. V.L. Spiering, T.S.J. Lammerink, H. Jansen, J.H.J. Fluitman, A. van den Berg, Technologies and microstructures for separation techniques in chemical analysis, Proc. SPIE - The International Society for Optical Engineering, 2882 (1996) 91-100.
17. Twente MicroProducts, PO Box 217, 7500 AE Enschede, The Netherlands, tel +3153 4894264 fax +3153 4358259, tmp@eltn.utwente.nl.
18. J.N. van der Moolen, H.F.M. Boelens, H. Poppe, H.C. Smit, Origin and correction of bias caused by sample injection and detection in capillary zone electrophoresis, J. Chromatogr. A, 744 (1996) 103-113.