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Planar chips technology for miniaturization and integration of separation techniques into monitoring systems

Capillary electrophoresis on a chip

Andreas Manz*, D. Jed Harrison☆, Elisabeth M. J. Verpoorte, James C. Fettinger, Aran Paulus, Hans Lüdi☆☆ and H. Michael Widmer

Central Analytical Research, Ciba-Geigy Ltd., CH-4002 Basle (Switzerland)

ABSTRACT

Miniaturization of already existing techniques in on-line analytical chemistry is an alternative to compound-selective chemical sensors. Theory on separation science predicts higher efficiency, faster analysis time and lower reagent consumption for microsystems. Micromachining, a well known photolithographic technique for structures in the micrometer range, is introduced. A first capillary electrophoresis experiment using a chip-like structure is presented.

INTRODUCTION

The continuous monitoring of a chemical parameter, usually the concentration of a chemical species, is gaining increasing attention in biotechnology, process control, and the environmental and medical sciences. Chemical sensors exhibit only a minimal number of applications for measurements of combustion gases, certain ions and enzyme substrates. The state-of-the-art strategy is called "total chemical analysis system" (TAS), which periodically transforms chemical information into electronic information. In such a system, sampling, sample transport, necessary chemical reactions, chromatographic or electrophoretic separations and detection are performed automatically. Some examples of TAS, such as a gas chromatographic monitor [1] and an on-line glucose analyser [2], have been reported. Recently, we proposed a general concept for a miniaturized TAS [3–6].

As far as separation techniques are concerned, miniaturization has been heavily discussed for many years. Improved separation performance at shorter retention times is predicted by theory. Miniaturization has been experimentally realized using small-diameter particles or open capillaries. Deviations from theoretical predictions have usually been caused by inhomogeneity in column packings or capillary diameters, inappropriate injections or large detection volumes. At least two publications have been presented in the literature on the use of photolithographically fabricated microstructures for gas [7] and liquid chromatography [8]. Recently, we proposed a 15-nl detector cell for absorption measurements (optical pathlength 1 mm [4–6]). This

☆ Permanent address: University of Alberta, Edmonton, Canada.

☆☆ Present address: Ciba-Corning Diagnostic Corp., Medfield, MA, USA.

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paper presents a technique for the manufacture of entire microchannel systems with very high precision. Such systems allow injections in the pl or nl range, dilutions, pre- or post-column reactions and sophisticated small-volume detections to be combined with, for example, capillary electrophoresis (CE).

THEORY AND MINIATURIZATION

Two approaches provide information on the behaviour of a simple flow system when it is miniaturized: (1) a set of numerical values can be calculated, using standard formulae to give the order of magnitude for a specific paramater; and (2) consideration of the proportionalities, *i.e.* the parameter of interest as a function of the variables to be miniaturized (space and time), shows the major trends of a parameter during its down-scale. In the case of capillary separation systems, the two approaches are equally interesting.

Table I depicts the results of an analysis carried out according to approach 1. Choice of the desired number of theoretical plates at a given retention time, as well as the heating power per length (in the case of CE), allows comparison of the resulting capillary dimensions and operation conditions for CE, liquid chromatographic (LC) and supercritical fluid chromatographic (SFC) separation experiments. The microchannels must be a few micrometers in diameter (2.8–24 μm), a few centimeters in length (6.5–20 cm) and need small-volume detectors (3.3–94 pl). Although these values cannot replace experimental results, they give an indication of values forbidden by theory. Approach 1 is very meaningful if the values of the given parameters are clear and if the optimum performance is well defined, as is the case with the Golay equation for capillary LC and SFC.

In the case of CE, the optimum performance is basically determined by the maximum voltage applied to the system. The higher the voltage, the better the separation performance and, at the same time, the faster the analysis. The limitation is usually given by the heat produced in the capillary. Three parameters may be relevant: the power per

TABLE I

CALCULATED PARAMETER SETS FOR A GIVEN SEPARATION PERFORMANCE OBTAINED WITH CE, LC AND SFC

Assumed constants are: diffusion coefficients of the sample in the mobile phase, $1.6 \cdot 10^{-9}$ m^2/s (CE, LC) and 10^{-8} m^2/s (SFC); viscosities of the mobile phase, 10^{-3} Ns/m^2 (CE, LC) and $5 \cdot 10^{-5}$ Ns/m^2 (SFC); electrical conductivity of the mobile phase, 0.3 S/m (CE); electrical permittivity × zeta potential 5.6×10^{-11} N/V (CE)

Parameter	Symbol (unit)	CE (micellar)	Capillary LC	Capillary SFC
Number of theoretical plates	N	100 000	100 000	100 000
Analysis time	$t(k'=5)$ (min)	1	1	1
Heating power	P/L (W/m)	1.1	–	–
Capillary inner diater	d (μm)	24	2.8	6.9
Capillary length	L (cm)	6.5	8.1	20
Pressure drop	Δp (atm)	–	26	1.4[a]
Voltage	ΔU (kV)	5.8	–	–
Signal bandwidth	σ_x (mm)	0.21	0.56	1.4
Signal bandwidth	σ_t (ms)	42	70	70
Signal bandwidth	σ_v (pl)	94	3.3	52
Ratio length/diameter of an eluting peak	σ_x/d	*ca.* 10	*ca.* 200	*ca.* 200
Detection volume requirements	$\sigma_v/2$ (pl)	<47	<1.6	<26
Optical pathlength parallel to flow	$\sigma_x/2$ (μm)	<105	<280	<700
Optical pathlength perpendicular to flow	d (μm)	<24	<2.8	<6.9
Response time requirements	$\sigma_t/2$ (ms)	<21	<35	<35

[a] The pressure needed to maintain the mobile phase in the supercritical state may exceed this value, *e.g.* for carbon dioxide the inlet and outlet pressure could be 75.4 and 74 bar, respectively.

TABLE II

EXAMPLE OF A PROPORTIONALITY ANALYSIS FOR CE

The given miniaturization factors are d and L. Three arbitrarily chosen time dependencies are shown here. The remaining parameters are then calculated using the basic definition of d, L and time

Parameter	Symbol	L system	$d \cdot L$ system	$d^2 \cdot L$ system
Diameter of capillary	d	d	d	d
Length of capillary	L	L	L	L
Time	t	L	$d \cdot L$	$d^2 \cdot L$
Linear flow-rate	$u = L/t$	Constant	$1/d$	$1/d^2$
Péclet number	$v \propto u \cdot d$	d	Constant	$1/d$
Reduced plate height	$h = 2/v$	$1/d$	Constant	d
Number of theoretical plates	$N = L/(d \cdot h)$	L	L/d	L/d^2
Electric field	$E \propto u$	Constant	$1/d$	$1/d^2$
Applied voltage	$U = E \cdot L$	L	L/d	L/d^2
Electric current	$I \propto U \cdot d^2/L$	d^2	d	Constant
Power per volume	$\propto U \cdot I/(d^2 \cdot L)$	Constant	$1/d^2$	$1/d^4$
Power per length	$= U \cdot I/L$	d^2	Constant	$1/d^2$
Temperature difference	$\Delta T \propto I^2$	d^4	d^2	Constant

unit volume, the power per unit length, and the temperature difference generated in a steady-state thermal diffusion system. An example of a proportionality analysis according to approach 2 is shown in Table II. The miniaturization of a CE system is characterized by the inner diameter d of the capillary, by its length L and by the time t. Time cannot be set into a well defined relation with d or L, which means that we have one degree of freedom. Out of the numerous possible dependencies, a set of three have been chosen: time proportional to L (length), to $d \cdot L$ (area) and to $d^2 \cdot L$ (volume), with power per unit volume, power per unit length and temperature difference as a constant, respectively. All of the remaining parameters of interest are then strictly based on d and L.

The L system is characterized by a time scale forced into proportionality with L. For example, a ten-fold shorter capillary implies a ten-fold shorter retention time if the electric field strength (and the linear flow-rate) are kept constant. The number of theoretical plates must decrease. The influence of d is restricted to the current flow and the power generated in the capillary.

In the $d \cdot L$ system, the time scale depends on d multiplied by L. This implies that all the reduced variables used in capillary LC, *e.g.* Péclet number or reduced plate height [9], are kept constant. In the same way the power per unit length, which is often used as a measure of thermal effects [10], remains constant. In this case, an improvement of both separation performance and analysis time can be achieved if d is miniaturized more drastically than L. For example, a ten-fold decrease in d and a five-fold decrease in L would double the number of theoretical plates in 1/50th of the retention time. Starting from a known CE experiment ($d = 70$ μm, $L = 1$ m, $n = 10^6$ and $t = 30$ min), we would obtain $2 \cdot 10^6$ theoretical plates within 36 s using a capillary of 20 cm × 7 μm I.D. The increase in plate number can be understood as arising from the increased electric field and from the decreased migration times, since longitudinal diffusion plays a major role. This system has been experimentally proven to be true by Monnig and Jorgenson [10].

The $d^2 \cdot L$ system is based on a constant temperature difference in the capillary when steady temperature diffusion is taken into account [11]. It is far more optimistic than the $d \cdot L$ system. Experimentally, it has not yet been possible to prove this system to be valid at all.

MICROMACHINING

Originated by the microelectronics industry, the photolithographic patterning of layer structures on the surface of silicon wafers has become a well known and high-tech standard procedure. In addition to its semiconductor qualities, monocrystalline silicon is abundant and inexpensive, can be produced and processed controllably to extremely high standards of purity and perfection, has excellent mechanical and chemical properties (yield strength better than steel, Young's modulus identical to steel, Knoop hardness comparable to quartz, chemical inertness comparable to glass) and is highly amenable to miniaturization (down into the micrometer range). The surface treatment to obtain mechanical structures is called micromachining [12] and includes fabrication steps such as film deposition, photolithography, etching and bonding. A simple process for obtaining a channel in silicon is shown in Fig. 1. It is obvious that the two-dimensional shape of the channel layout is given by the photomask, but the particular pattern does not affect the complexity of the process at all. As soon as a variation in depth (third dimension) or material (*e.g.* a metal layer) is needed, additional processes must be added to the sequence.

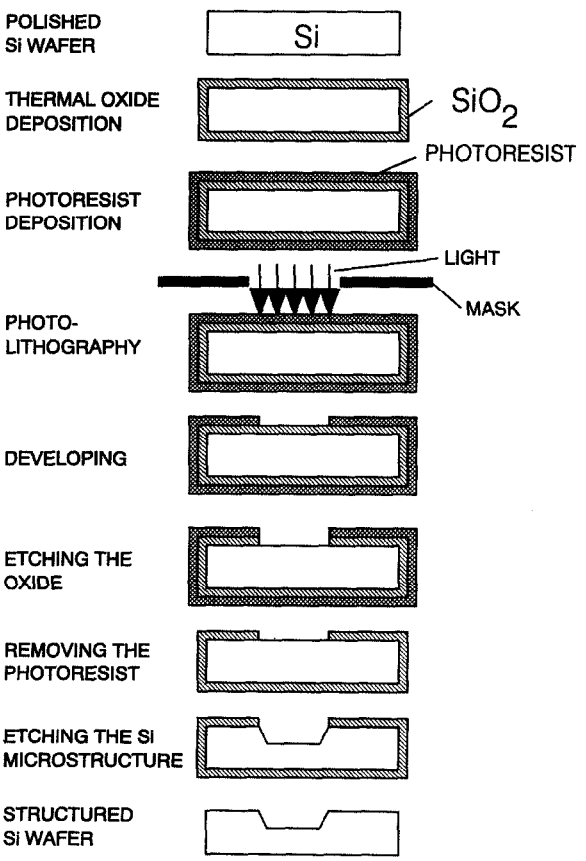

Fig. 1. Process steps of a standard one-mask micromachining procedure to etch a channel structure into silicon.

There are mainly four different groups of processes:

(1) Film deposition includes spin coating, thermal oxidation, physical vapour deposition (PVD) and chemical vapour deposition (CVD), low-pressure CVD, plasma-enhanced CVD, sputtering, etc. A large variety of metals, inorganic oxides, polymers and other materials can be deposited using these techniques.

(2) Photolithography, a technique used to transfer a layout pattern from a mask onto a photosensitive film, can be done using visible light for structures larger than 1 μm. For special applications such as submicron patterning, UV, X-ray or electron beam lithography is used.

(3) Etching is performed either as a wet chemical process or as a plasma process. Isotropic as well as anisotripic processes are known.

(4) Bonding means the assembly of pieces of silicon onto silicon, glass or other substrates. The subject of micromachining has been dealt with in great detail in many sources in the literature. For more information, see for instance ref. 13, which gives a good review of this huge field.

Silicon-, quartz- and glass-based physical and chemical sensors and actuators are currently a focus of interest [14]. Compared with conventional machining, photolithographic processes allow cheap mass fabrication of complicated microstructures. Hundreds to thousands of structures may be fabricated in the same batch. The precision and reproducibility of the structure elements are excellent. Silicon allows monolithic integration of electronics, sensors and actuators, but micromachining has to be done under clean-room conditions and needs high-tech instrumentation. However, in the last few years, the number of companies offering custom-made silicon, quartz and glass microstructures has significantly increased.

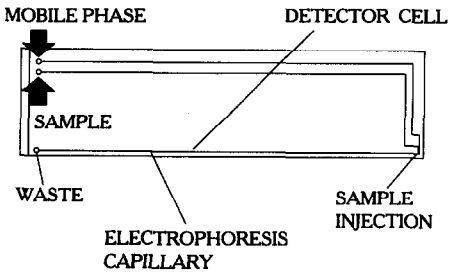

Fig. 2. Glass microstructure for injection and CE. Size 15 × 4 cm. Electrophoresis channel 30 × 10 μm. The external laser fluorescence detector was positioned 6.5 cm from the point of injection.

CAPILLARY ELECTROPHORESIS ON A CHIP

We have made six different structures in silicon (covered by Pyrex glass) [15] and one in amorphous glass [16]. The silicon structures, even with state-of-the-art insulating films (SiO_2 and Si_3N_4), exhibited poor voltage breakdown characteristics. In the best case, 950 V could be applied on a single device for a few minutes. Better results have been obtained with amorphous glass or quartz.

The photolithographically fabricated glass device shown in Fig. 2 was used to perform a first CE experiment "on a chip". The overall size of the structure is 150 × 40 × 10 mm. It consists of two glass plates, one of them containing the etched channels and the other serving as a cover. The three channels, two of them being 10 μm deep and 30 μm wide, the other 10 μm by 1 mm, meet at one point. The intersection has a volume of 9 pl, which means that no extra dead volume exists. The cross-section of the channels is not exactly rectangular, but rounded at the corners (compare with refs. 17–19). Detection was done using a laser fluorescence set-up similar to the one described previously [20,21] located somewhere downstream from the electrophoresis capillary (0–135 mm after the point of injection). The buffer reservoirs containing the platinum electrodes were pipette tips mounted directly into the drilled holes at the ends of the channels.

To set the experiment up, the background electrolyte and the fluorescent sample mixture were both driven past the injection and detection points by externally applied voltages, allowing positioning of the detector. The carrier electrolyte was then driven through the electrophoresis channel to flush it out. A 30-s pulse of 500 V applied to the sample channel provided the injection. To run the electropherogram, 3000 V were applied to the carrier electrolyte and the separation capillary (200 V/cm). The resulting separation of two fluorescent dyes is shown in Fig. 3. For calcein, a performance of 18 000 theoretical plates has been obtained. The height equivalent to a theoretical plate is 3.6 μm, which is comparable to a non-optimized standard CE experiment. The standard deviation of the peak in terms of time, length and volume was 1.4 s, 0.49 mm and 145 pl, respectively.

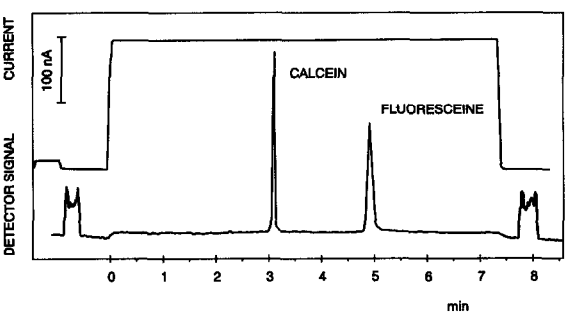

Fig. 3. CE separation of two fluorescent dyes. Sample: 20 μM calcein, 20 μM fluorescein. Background electrolyte: 50 mM borate, 50 mM Tris, pH 8.5, 3000 V on 13 cm. Detection at 6.5 cm, fluorescence, excitation 490 nm, collection 520 nm, injection through side channel, 500 V for 30 s.

CONCLUSIONS

Consideration of hydrodynamics and diffusion processes indicates faster and more efficient chromatographic separations, faster electrophoretic separations and shorter transport times for miniaturized TAS. The consumption of carrier, reagent or electrophoresis buffer is dramatically reduced. Micromachining, especially photolithographic processes, offers access to novel analytical microstructures such as branched-channel systems having no dead volume.

The access to electrophoretic separations within a planar glass structure shown here is a first step towards an integrated microflow system using CE together with injection, sample pretreatment and post-column reactions, etc. We anticipate that higher voltages can be applied to the structure to speed

up the separation and to increase its performance. Silicon structures would show a problem at high applied voltages. The reasonable range might be up to 200 V. This, of course, implies a poor efficiency (small number of theoretical plates), but relatively short retention times.

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