Miniaturization of Chemical Analysis Systems – A Look into Next Century's Technology or Just a Fashionable Craze?

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Abstract. Miniaturization of already existing techniques in on-line analytical chemistry is an alternative to compound-selective chemical sensors. Theory points in the direction of higher efficiency, faster analysis time, and lower reagent consumption. Micromachining, a well known photolithographic technique for structures in the micrometer range, is introduced and documented with structures as examples for flow injection analysis, electrophoresis, and a detector cell.

Fig. 2. *Comparison of response time, analysis time, and cycle time for an ideal chemical sensor, a flow injection analysis based and a chromatography based TAS*

1. Introduction

The continuous monitoring of a chemical parameter, usually the concentration of a chemical species, is gaining increasing attention in biotechnology, process control [1], and the environmental and medical sciences. The chemical compound of interest is usually accompanied by interfering species. Due to the severe selectivity requirements, and the fact that developments were made by researchers of different fields starting at different points, many possibilities resulted in the approach to this topic. Recently, we presented a general concept for a miniaturized total chemical analysis system [2].

Selective chemical sensors, flow injection analysis (FIA), and separation techniques (chromatography, electrophoresis) can be used as 'stand alone techniques', *i.e.* an apparatus, standing in a lab, operated by a qualified technician. For monitoring purposes, the instrument needs to be fully automatic, from sampling through information evaluation. The state-of-the-art strategy is the so called 'Total Chemical Analysis System' (TAS), which periodically transforms chemical information into electronic information. Sampling, sample transport, any necessary chemical reactions, chromatographic or electrophoretic separations, and detection are automatically performed. Most of these methods are precise and reproducible, but also time consuming. Because the sample pretreatment serves to eliminate most of the interfering chemical compounds, the detector or sensor in a TAS needs not be highly selective. Furthermore, calibration can be incorporated into the system. Examples of TAS were presented earlier (gas

chromatography monitor [3], on-line glucose analyzer [4]).

2. Concept for Miniaturization

A miniaturized TAS must be defined both in relation to a chemical sensor and to a TAS (*Fig. 1*) [2]. If a TAS performs all sample handling steps extremely close to the place of measurement, then we propose that it be called a 'Miniaturized Total Chemical Analysis System' (μ-TAS).

The interface to the control and measurement electronics could include, for instance, tubing for mass flow and optical fibers. If the analysis time of a μ-TAS is comparable to the response time of a selective chemical sensor, then both become very similar in
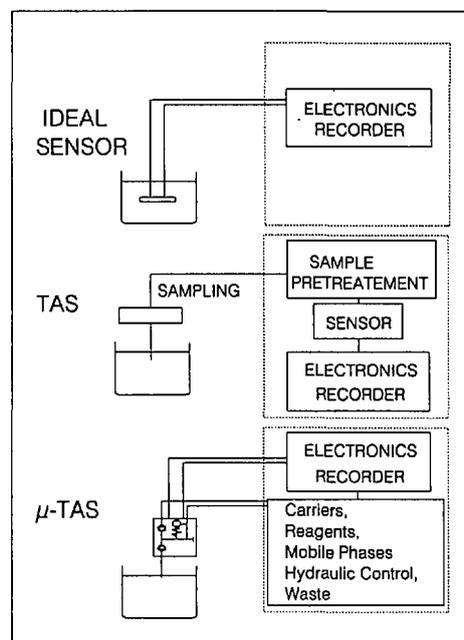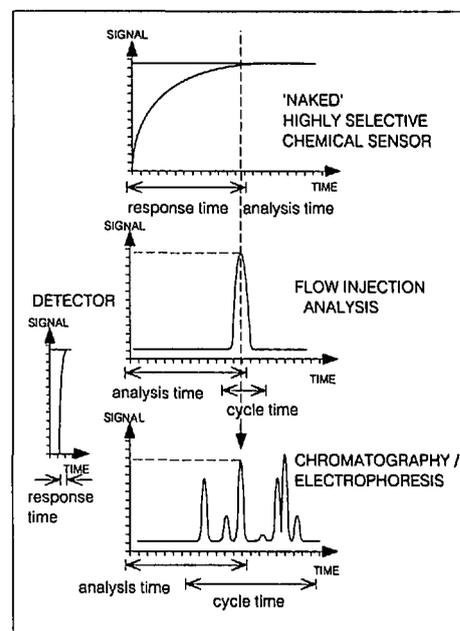

Fig. 1. *Schematic diagram of an ideal chemical sensor, a total chemical analysis system (TAS), and a miniaturized TAS (μ-TAS)*

appearance and use as visualized in *Fig. 2*. Several research groups have done basic developmental work on micro pumps and valves [5–7], small flow-injection analysis systems [8], and open-tubular column chromatography, *e.g.* [9][10]. The most advanced micro-technology definitely is capillary electrophoresis [11]. Recently, *Monnig* and *Jorgenson* presented a high-speed separation using a 10-mm capillary with elution taking 0.5–2 s [12]. Our main reason for the miniaturization of the TAS is related to an enhancement of its analytical performance (*Table*), rather than a reduction of its size.

3. Theory of Miniaturization

General

Let d be a typical length in a given system (for example, the diameter of a tube). By multiplying each variable by d^n and the appropriate constants, it can be reduced to a dimensionless parameter which is independent of the spatial scale of the given system (for example, the flow rate becomes the *Péclet* number). Similar systems of different sizes can then be easily compared. If we assume that a miniaturization is a simple 3-dimensional down-scale (extensively discussed in [2]), we can easily demonstrate the behaviour of the relevant physical variables. There remains then one degree of freedom for the mechanical parameters: time.

Time Constant System

In this case, the time scale is the same for the large and for the small system. Consequently, all relevant time variables (analysis time, transport time, response time) do not change. But a linear flow rate in a tube would decrease by d, a volume flow rate by d^3, the *Reynolds* number by d^2 and a pressure drop needed would be a constant. This system

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Table. *Calculated Parameter Sets for a Given Separation Performance Obtained with Capillary Electrophoresis (CE), Liquid (LC), and Supercritical Fluid Chromatography (SFC).* Assumed constants are: diffusion coefficients of the sample in the mobile phase 1.6×10^{-9} m²/s (CE, LC) and 10^{-8} m²/s (SFC), viscosities of the mobile phase 10^{-3} Ns/m² (CE, LC) and 5×10^{-5} Ns/m² (SFC), electrical conductivity of the mobile phase 0.3 S/m (CE), electrical permittivity × zeta potential 5.6×10^{-11} N/V (CE).

Parameter		Capillary electrophoresis [micellar]	Capillary liquid chromatography	Capillary supercritical fluid chromatography
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 diameter	d [μm]	24	2.8	6.9
Capillary length	l [cm]	6.5	8.1	20
Pressure drop	Δp [atm]	–	26	1.4
Voltage	ΔU [kV]	5.8	–	–
Peak capacity	n	180	220	220
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

behaviour is important for simple transportation and flow injection analysis systems. Diffusion would certainly be predominant. The main advantage is saving carriers or reagents. A 10-fold decrease in size, for example, would cause a 1000-fold decrease in carrier or reagent consumption.

The *diffusion-controlled system* becomes important, when molecular diffusion, heat diffusion, or flow characteristics control the separation efficiency in the given system. In this system, the time scale is treated as a surface, *i.e.*, time is proportional to d^2. This system is in perfect agreement with standard chromatographic and electrophoretic band-broadening theory, *e.g. van Deemter* or *Golay* equations. All reduced parameters, including *Reynolds* number, *Péclet* number (flow rate), *Fourier* number (elution time), and *Bodenstein* number (pressure drop), remain constant regardless of the size of the system [13]. Hydrodynamic, heat, and diffusion effects are compensated.

This indicates that a down-scale to 1/10 of the original size (diameter of a tube) reduces the related time variables (analysis time, required response time of a detector) to 1/100. The pressure requirements increase by a factor 100, but the voltage requirements (for electrophoresis/electroosmosis) remain a constant. The main advantage is a considerably higher speed of separation with a comparable efficiency. Some theoretical values comparing capillary electrophoresis (micellar solutions), capillary liquid chromatography, and capillary supercritical fluid chromatography are given in the *Table*. The resulting channel structures are a few μm in diameter (2.8–24), a few cm long (6.5–20) 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.

4. 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. Besides its semiconductor qualities, monocrystalline silicon is abundant and inexpensive, can be produced and processed controllably to unparalleled standards of purity and perfection, has excellent mechanical and chemical properties (yield strength better than steel, *Young's* modulus about identical, *Knoop* hardness
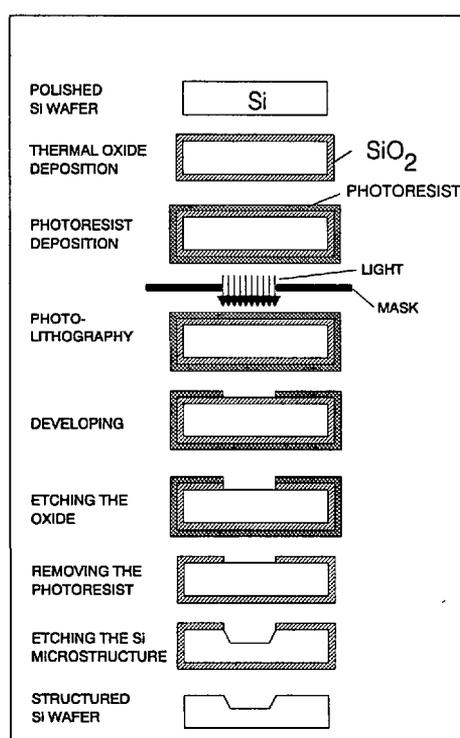

Fig. 3. *Process steps of a standard one-mask micromachining procedure*

comparable to quartz, chemical inertness comparable to glass) and is highly amenable to miniaturization (down into the μm range). The surface treatment used to obtain mechanical structures is called micromachining [14]. It includes fabrication steps such as film deposition, photolithography, etching, and bonding. A simple process for obtaining a channel in silicon is shown in *Fig. 3*. It is obvious that the two-dimensional shape of the channel layout is given by the photomask, but does not affect the complexity of the process at all. As soon as a variation in depth (3rd dimension) or material (*e.g.* a metal layer) is needed, additional processes have to be added to the sequence.

Film-deposition processes used include spin coating, thermal oxidation, physical-(PVD) and chemical-vapour deposition (CVD), low-pressure CVD, plasma-enhanced CVD, sputtering, *etc.* A large variety of metals, inorganic oxides, polymers, and others can be deposited.

Photolithography can be done using visible light for structures larger than 1 μm. For special applications such as submicron patterning, UV, X-ray or e-beam photolithography is used.

Etching is performed either as a wet chemical process or as a plasma process. Isotropic as well as anisotropic processes are known.

Bonding means the assembly of a piece of silicon onto silicon, glass or other substrates.

Silicon-based physical and chemical sensors and actuators are in the focus of interest nowadays [15]. Compared to conventional machining, photolithographical processes allow cheap mass fabrication of complicated microstructures. Hundreds to thousands of structures are fabricated in the same batch. Precision and reproducibility of the structure elements is excellent (see *Fig. 4*). Although silicon allows monolithic integration of electronics, sensors and actuators, micromachining has to be done under clean room conditions and needs high-tech instrumentation, both of which represent a large financial investment.

5. Examples of Structures

Two examples of photolithographically fabricated structures are shown. The chemical analysis system shown in *Fig. 5* combines a flow injection analysis technique with a capillary electrophoretic separation. The device consists of two glass plates, 40×155 mm. The upper plate contains the etched channel system (30 μm wide, 10 μm deep) and the lower plate the platinum electrode pairs (20×30 μm each).

The carrier liquids are fed through the holes into the system using electroosmotic/electrophoretic flow. The flow in the system can be controlled by applying appropriate voltages to the different external electrolyte containers and to the electrodes at the end of
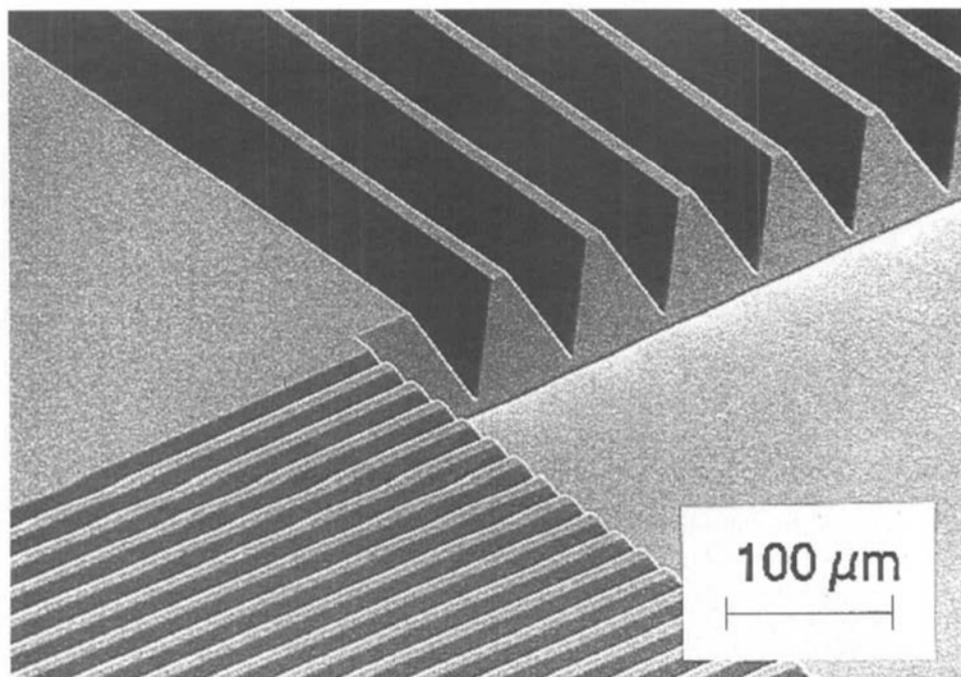
Fig. 4. *Scanning electron micrograph of a micromachined silicon structure, demonstrating the precision and reproducibility of the process*

some of the channels. The sample is injected, automatically diluted, derivatized, and then injected into the electrophoresis capillary without the use of valves. Conductivity, amperometric, or fluorescence (external fluorescence microscope) measurements are used for detection [16].

Fig. 6 shows an optical detector cell for use in chemical analysis. The absorption follows an optical path of 1-mm length at a total volume of 1 nl only. The structure is fabricated in two pieces of silicon. The upper chip provides the inlet and outlet holes as well as the optical windows, whereas the lower chip includes the channels and the

anisotropically etched optical mirrors in the cell (defined by silicon crystal planes) [17].

6. Conclusion

A basic theory of hydrodynamics and diffusion indicates faster and more efficient chromatographic separations, faster electrophoretic separations, and shorter transport times for miniaturized TAS. The consumption of carrier, reagent, or mobile phase is dramatically reduced. Micromachining, especially photolithographic processes, offer a wide variety of analytical microstructu-

res which were absolutely inaccessible until now.

Because there has been little direct experimental evidence to date supporting the above theory, one could argue that miniaturized chemical analysis systems are just a fashionable craze. However, it is difficult to foresee the impact a new technological concept will have, when it is in its early stages of development. After all, the early experiments with microelectronics did not predict the overwhelming success of this technology, either. The history of silicon-chip technology teaches that a well documented and theoretically positive idea can be accepted within a few decades. An inherent problem is that a heavy investment in $R \& D$ has to be made, if positive results are to be obtained at all. Even easy fabrication and satisfactory analytical performance will not suffice to make miniaturized total chemical analysis systems part of the next century's technology. A swing of political opinion in favour of this research, a strong financial support for $R \& D$, a healthy competition among research labs worldwide, and a stronger interest in the competitive marketing of these new products in a > 5 billion US$ p.a. market would certainly trigger an increased rate of development in this field [18].

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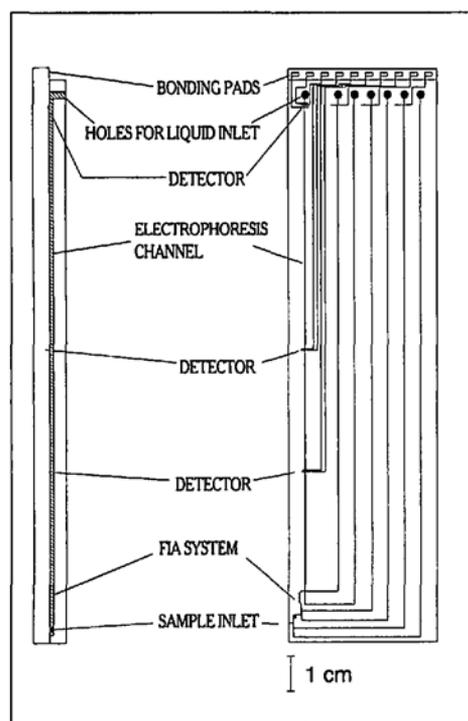[18] You see, Prof. Simon, I have learned a lot from you! (A. Manz)

Fig. 5. *Layout of an electroosmotically driven flow injection analysis device used for automated injection, dilution and capillary electrophoresis of a sample*
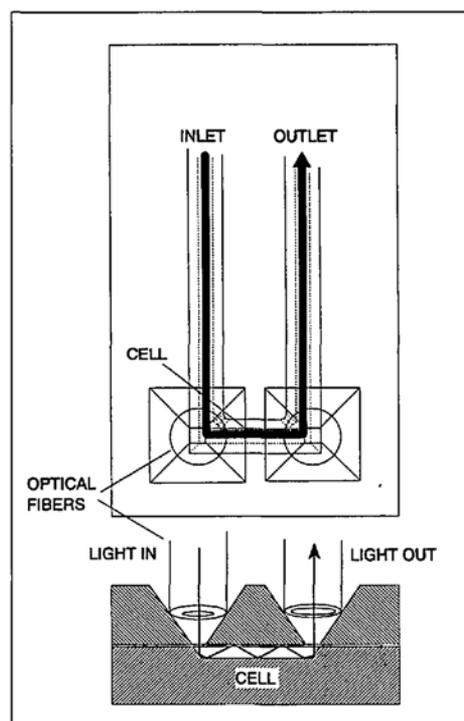

Fig. 6. *Layout and cross-sectional view of an optical, small volume flow cell.* The optical pathlength is considerably larger than the cross-section of the channel.