15 Miniaturized Analytical Systems

Learning objectives

- To provide an overview on the principles and developments in miniaturization of chemical analysis systems
- To highlight the advantages of miniaturization in comparison to conventional systems
- To introduce the fabrication method
- To give examples for successful chip designs

15.1 Principles

Chemical analysis nowadays is mostly performed with bench-top systems, roughly the size of a large television set. As we have seen in the previous chapters, there exist a variety of analytical chemistry methods for laboratory analysis of a given sample with respect to component identification, quantification, and possible structural elucidation. The analysis consists of sample collection, pretreatment, sample injection, separation, and detection. Originally, all these steps had to be performed manually with different instruments.

However, for on-line analysis a more automated approach is necessary. In most modern on-line systems, sample handling, separation, and detection stages are integrated into a single instrument, with most stages automated and computer controlled. Examples of these so-called *total analysis systems* (TAS, Fig. 15.1-1b) are flow injection analysis (FIA), electrophoresis, chromatography (Chap. 5) and mass spectrometry (Sec. 9.4). These methods operate ex-situ, i.e., a sample has to be taken and transferred to the laboratory, usually requiring some more or less complicated sample pretreatment prior to analysis. These systems offer the advantages of a high degree of automation, the possibility of inbuilt calibration and, owing to the sample pretreatment, the need for only moderatly sensitive detector systems. The main disadvantages are the reagent consumption, slow transport of samples in solution, and the slow separation speed of liquid chromatographic methods.

As with electronics some decades ago, there is a strong move toward the miniaturization of chemical analysis systems. The potential applications for small, portable analyzers are numerous, for example:

- Environmental measurements
- Pollution control, directly at the sources of contamination
- Pharmaceutical and agrochemical research
- Biomedical screening and other high-throughput medical applications
- Industrial process control, e.g., in the food industry
- Forensic studies
- Artificial senses for "smart machines", e.g., the "artificial nose"
- Domestic applications in the medical and hygiene field

Size reduction in analytical systems has several distinct advantages:

- Faster analysis, due to the reduced transport lengths, optimized mass transport for chemical reactions and separations
- Reduced consumption of reagents
- Less waste production
- Smaller sample volumes
- Portability

Many of the analytical systems described in previous chapters, such as chromatography, electrophoresis, or flow injection analysis, have been subject to this miniaturization, leading to the concept of *miniaturized total analysis systems* (μ-TAS, Fig. 15.1-1c).

Total analysis systems are analytical systems where sample handling, separation and detection are integrated into a single instrument.

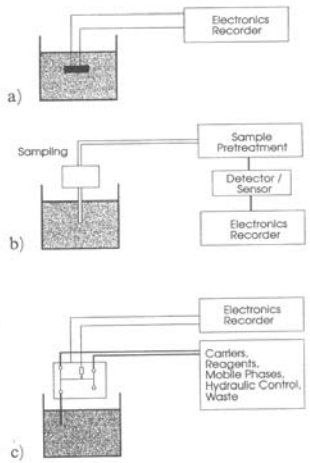

Fig. 15.1-1. Concept comparison between (a) a chemical sensor; (b) a total analysis system and (c) a miniaturized total analysis system

In order to define μ-TAS, one has to take a look at competing concepts in analytical chemistry. On one hand, there is the chemical sensor (Fig. 15.1-1a, see Sec. 7.8 and 7.9). An ideal sensor shows a high sensitivity toward the substance to be detected while at the same time suppressing response to any other substance. It has a large dynamic (e.g., concentration) range, and displays reproducible signals and low noise over an extended period of time. Ideally, it can be used in situ, c.g., the sensor should be small enough to be operated at the place of interest, possibly immersed in the liquid or the gases under consideration. In on-line applications, signal acquisition must be fast and continous. So far, problems remain in obtaining selectivity (partly overcome by the use of sensor arrays) and with the life time of the devices. Furthermore, the development of a market-ready sensor is very time- and labor-consuming.

Miniaturized total analysis systems are TAS, where the analytical functions take place at the location of the measurement itself.

If a TAS can be reduced in size, leading to the advantages listed above, we can define it as a miniaturized total analysis system (μ-TAS), and it should be able to perform all the necessary steps of sampling, handling, and pretreatment at the same location as the measurement itself. This approach, illustrated in Fig. 15.1-1, combines the advantages of chemical sensors with the resolving power of modern bench-top analytical systems. These properties make μ-TAS an ideal concept for high-throughput applications, such as clinical analysis or process control of simple mixtures.

15.2 Microfabrication

The fabrication process for μ-TAS makes use of integrated circuit technology, developed originally for the microelectronics industry. Chip fabrication relies on well-established processes, including photolithography, wet etching, or advanced gas-phase technologies such as reactive ion etching (RIE). Figure 15.2-1 shows a typical fabrication process. A substrate, usually silicon, glass, or quartz, but potentially also polymers, is covered with a metal film (usually chromium or gold with a thin chromium layer to promote adhesion) and a photoresist layer. With a photomask, which contains the structural information for the device, produced by standard lithography processes, the resist film is exposed and then developed, removing the photoresist from the exposed areas. The substrate is then placed in an etching bath, which etches through the metal film in the areas not covered by photoresist. In a second etch step, the substrate itself is etched, typically in HF/HNO_3 or KOH. Depending on the etchant and the substrate, the microchannels have different profiles. In glass or other amorphous materials, one usually has

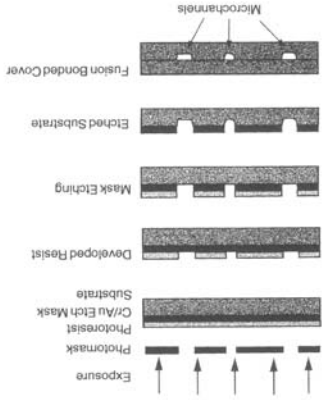

Fig. 15.2-1. Microfabrication process for the production of microchannels

Fig. 15.3-3. Example of a separation of two fluorescent dyes obtained with the HPLC-chip

15.3.2 Free-flow electrophoresis

Free-flow electrophoresis (FFE) is a very useful technique for sample pretreatment. The principle can be explained by Fig. 15.3-4. The sample is fed into a carrier stream. An electric field is applied perpendicular to the flow of the carrier. Ions are then deflected from the flow direction according to their electric charge and electrophoretic mobility. The angle of deflection increases with the applied electric field, the ion charge and mobility, and decreases with the flow speed of the carrier. After the separation process, the different species can be observed as they exit the outlet channel.

Figure 15.3-5 shows the layout of a FFE device fabricated on silicon. The sample is fed into the system at the location marked 'sample inlet' and is transported by the carrier, accessing the chip through the carrier buffer inlet channels. The voltage is applied by two platinum arrays in the side beds, separated by a spacer, consisting of 2500 V-shaped grooves. A device of this type can be used for continuous sample fractioning where specific species are selected for a subsequent analysis step.

Fig. 15.3-4. The principle of free-flow electrophoresis (FFE)

Electrophoretic separation is the separation of individual molecules under the influence of an electric field applied along the capillary.

Electroosmotic flow is the movement of the complete liquid column in a capillary under the influence of an electric field applied along the capillary.

15.3.3 Capillary electrophoresis (CE)

This electrophoretic technique has also been used in μ-TAS. The sample and the buffer liquid are fed into small capillaries. If a voltage is applied at either end of the capillary, two phenomena can be observed. The first effect, the electrophoretic separation, is simply the motion of individual ions, positive or negative, in the fluid under the influence of the applied field. The second effect is called electroosmotic flow and pumps the fluid through the capillary. It arises from the existence of an electric double layer (Helmholtz layer) close to the capillary walls, consisting of a negative immobile charge at the walls (ionized silanol groups) and a layer of positive ions from the liquid which are attracted by this negative charge. If an electric field is applied along the capillary, the mobile positive ions in the fluid start to move, owing to the electrostatic force. Because of the viscosity of the liquid, the complete fluid column is dragged along by the moving ions.

An important parameter for the performance of a separation system is its efficiency. It can be shown theoretically that the separation efficiency, the number of theoretical plates N, is given by:

$$N \propto \frac{L}{d},$$

where L is the capillary length and d its diameter. The analysis time t is given by:

$$t \propto L \cdot d,$$

so a decrease in the capillary diameter leads to a reduction in analysis time as well as an increase in performance. This property makes CE particularly suitable for miniaturization. The limiting factor for the performance of CE chips is the Joule heat because of the current flowing through the capillary. Good thermal coupling of the fluid and the substrate and a high heat dissipation in the substrate material are therefore necessary. In order to achieve sufficiently high electric filed strength, these devices have to be made on glass rather than on a silicon substrate, as silicon is a semiconductor.

Figure 15.3-6 shows a layout of a CE chip. After feeding in the sample between ports 1 and 4, the separation voltage is applied between ports 2 and 5 and a sample plug with a typical volume of less than 100 pL is driven through the separation channel. Figure 15.3-7 shows an electropherogram of six fluorescein-labeled amino acids. The separation length is 24 mm, the field strength 1060 V/cm. A separation efficiency with plate numbers up to 200 000 has been achieved within seconds, rather than minutes as in conventional CE. Several chips have been designed with a pre-column mixing chamber to monitor fast chemical reactions or with post-column reactors to add the fluorescence labeling after the separation.

Fig. 15.3-5. Design of an FFE system on a silicon chip

isotropic etching conditions, yielding the same etch rate in any direction, and therefore forming rounded channel edges. On a monocrystalline silicon or quartz substrate, anisotropic etching is possible with suitable etchants, leading to a channel profile which depends on the direction of the exposed crystal planes. In a final step, a cover lid is fusion-bonded on top of the substrate, closing the channels. Another possibility is to grow a top layer of SiO_2 onto the substrate. Further process steps, such as the metallization of certain areas to act as electrical contacts, or the formation of oxide or nitride layers for insulation, etc., can easily be included.

15.3 Examples and Experimental Results

15.3.1 Chromatography

The credit for the very first fully integrated gas chromatography system on a silicon chip, including valves and a detector system, goes to Terry and co-workers in 1979. But only in recent years have new designs, particularly for liquid chromatography, emerged. A design of an open-capillary liquid chromatography system is shown in Fig. 15.3-2. Typical dimensions of the column are: width 5–50 µm, depth 1–10 µm, length 5–15 cm, yielding a total column volume between 1.5 and 10 nL. The detection is achieved by amperometric or conductometric methods, measuring the current and resistance across the column outlet. The detector volume can be as little as 1.2 pL.

Another design with an optical detection unit is shown in Fig. 15.3-1. It basically consists of a split injector, controlling the portion of the sample entering the separation column, the column itself, a frit at the end of the column (which holds back packing material from the column), and the detector cell. The optical detection, based on the excitation of fluorescence in the sample, demands an optical path-length of the order of 1 mm to achieve satisfactory sensitivity. The light is therefore guided along the whole length of the detector groove, where it is reflected in and out of the groove by the aluminum covered side walls, and transferred to a photomultiplier via an optical fiber. The detector volume is 2.3 nL, with a column volume of 490 nL. Figure 15.3-3 shows a separation of the two fluorescent dyes fluorescein and acridine orange, obtained within less than 1 min.

The main obstacle to these HPLC chips becoming a complete μ-TAS is the current lack of integratable high-pressure pumps and valves suitable for the range between 10 and 400 bar. Although recent years have seen commercially available micromachined silicon valves and pumps, they can operate only at pressures up to a few bar, producing a pulsating flow. New approaches, such as piezoelectric pumps used in ink-jet printers, may be able to overcome this problem. So far, the available HPLC chips give far from optimal performance and require further improvement.

Fig. 15.3-1. Design for a packed column HPLC-chip with optical detection cell

Fig. 15.3-2. Design for an open tubular liquid chromatography system on a chip

path-length with respect to the detection volume, either by using multiple reflections from the channel walls or by probing the capillary in a longitudinal direction. In HPLC chip designs, platinum electrodes have been included in the chip for electrochemical detection. The requirement of floating the detector electronics at a high voltage makes this approach more difficult to use in CE.

General reading

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Questions and problems

1. Why is a miniature analytical system attractive?
   Answer:
   - Performance is better: larger number of theoretical plates and better resolution achievable
   - Time scale is shorter
   - Less reagent consumption and less waste production
   - Equipment is small and possibly cheap
2. An injected sample broadens during a capillary electrophoresis separation by longitudinal diffusion (broadening proportional to square root of time). Miniaturization by a factor of 10 (linear) at maintained voltage would result in 1000 times less volume, 100 times less cross-section, 10 times shorter capillary, and 100 times faster linear flow rate. How is the peak maximum of an injected component affected? Which of the following three detector schemes seem most favorable for this case?
   Laser-induced fluorescence is proportional to the amount of material, sensitivity at 1 µL, 10^{-12} mol/L; amperometric detection is proportional to surface area, sensitivity at 1 µL, 10^{-9} mol/L; potentiometric detection is constant, sensitivity 10^{-6} mol/L injected material.
   Answer:
   Compare the known system at 1 µL with the new system at 1 nL:
   - Detection volume 1000 times smaller
   - Concentration in detection volume = peak maximum, becomes 10 times larger (square root of time)
   - Amount of compound in detection volume 100 times smaller
   - Fluorescence: 100 times smaller, 10^{-19} mol/nL
   - Amperometric: 10 times smaller, 10^{-17} mol
   - Potentiometric 10 times larger, 10^{-16} mol

   Conclusion: fluorescence is still a lot more sensitive at the 1 nL level.
   Note: at the 1 pL level, all three detection methods show about the same sensitivity. (If you have answered this question correctly, or if these kinds of question interest you, consider doing a PhD thesis in this area: e-mail a.manz@ic.ac.uk.)

Fig. 15.3-6. Layout of a CE chip. The numbers denote fluid reservoirs. *1* sample inlet; *2* carrier electrolyte inlet; *3* modifier electrolyte inlet; *4* sample outlet; *5*, *6* outlets

Fig. 15.3-7. Electropherogram of six fluorescein labeled amino acids recorded with a system shown in Fig. 15.3-6

Fig. 15.3-8. Experimental setup for a CE experiment on a chip

15.3.4 Experimental setup

An example of a typical µ-TAS experiment, a capillary electrophoresis device, is shown in Fig. 15.3-8. Fluid reservoirs, usually plastic vials or pipet tips, are glued onto the chips. Through holes drilled into the cover plate they are connected to the microchannels of the CE chip. Platinum electrode wires are placed into these reservoirs and connected to a high-voltage power supply via a relay. For the sake of clarity, the electrodes connected to the lower reservoirs are omitted from the diagram. Detection is usually carried out with laser-induced fluorescence, where light from an Argon ion, He–Ne, or He–Cd laser is coupled into the detection zone via an optical fiber or other optical arrangement. Recently, blue LEDs have also been used as a light source for fluorescence excitation, indicating the possibility of integrating the detection system onto the chip. The excited fluorescence is sampled, either directly with a microscope or indirectly by an optical fiber, filtered to suppress the light of the laser, and detected in a photomultiplier tube or a CCD camera. Another detection method is optical absorbance, used frequently in conventional HPLC systems. As the sample volumes in CE devices are of the order of 1 nL or less, care has to be taken to optimize the coupling of the light in and out of the sample volume. Special flow-cells have been designed to maximize the optical

Laser induced fluorescence is the most sensitive and widely used method for on-chip detection. It enables single molecule detection.