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Miniaturization and chip technology. What can we expect?*

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Abstract: Miniaturization and chip technology play an important role for analytical chemistry instrumentation in the future. A brief theory of the relevant microfluidics with reasons for miniaturization is given.

INTRODUCTION

The immediate and accurate determination of chemical or biological parameters is a key issue in environmental analysis, medical diagnostics, chemical and biotechnological production, and many other fields. Since in most cases, the compound of interest is a minor part of a complex chemical mixture, the analyte has to be distinguished against its background.

Basically, two different concepts have been followed to satisfy the demands of these fields:

- The chemical or biological sensor was proposed as an extension of the well-established pH-electrode. The sensor offers short response time and *in situ* application, but, therefore, needs high selectivity, high sensitivity, and reasonable stability under these conditions.
- A total analysis system (TAS) transforms periodically chemical information into electronic information. Sampling, sample transport, sample pretreatment, separation, and detection are automatically carried out. The initial TAS concept used conventional laboratory instrumentation and was, therefore, a bulky but reliable system.

Modifying the TAS approach by downsizing and integrating multiple steps (injection, reaction, separation, detection) onto a single device, yields a sensor-like system with fast response time, low sample consumption, on-site operation, and high stability; this concept is termed μ-TAS [1–3]. The technology developed for microfabrication of electronics opened the way to producing complex three-dimensional structures in silicon and other materials.

MICROMACHINING

Today's μ-TAS devices are usually fabricated by standard photolithographic methods. The processes developed for microelectronics can be applied to silicon and glass substrates producing channel networks in two dimensions for sample transport, mixing, separation, and detection systems on a monolithic chip.

The mask is made as a negative image of the desired channel layout. A UV-light source transfers the layout from the mask to the photoresist (analogous to photographic film), which has been previously

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deposited on the substrate by spin-coating. The photoresist is then developed, exposing the substrate beneath the areas that were exposed to light. The open areas are chemically etched into the substrate. The depth of the channels is controlled by etching time, while the shape of the groove is dependent upon etching conditions and the crystalline orientation of the substrate. For example, dry plasma etching produces nearly vertical channel walls, anisotropic wet etching yields V-shaped grooves in <100> oriented silicon, and isotropic wet etching in silicon or glass yields U-shaped grooves. Once the substrate has been patterned, the photoresist is removed and a coverplate is thermally or anodically bonded to the substrate to close the channel system.

More complex systems can be produced in a relief-like fashion by repeating photolithography on the same substrate. Three-dimensional microfluid handling systems have been made by stacking interconnected silicon chips yielding a complex microflow system. For polymer-based micromachining, hot embossing, injection moulding, and laser ablation have been used. For applications in chemistry, these materials seem far more likely to be of commercial value, because they are more compatible and cheaper.

THEORY OF MINIATURIZATION

The use of micromachining techniques enables cheap mass production of µ-TAS. In this respect, the present state of microprocessor chip technology may hint at an exciting future for µ-TAS. This and other effects of miniaturization can be demonstrated through the use of dimensional analysis and a few simple formulae [2]. Table 1 shows a number of device characteristics as a function of a typical length d. An important criterion is the maximum number of devices that can be arranged on a surface (1 cm^2). This number increases with $1/d^2$ and at a typical length of 10 µm, 250 000 devices can be made per cm^2. Such a host of devices could be used in discrete applications, but also together for parallel processing.

Table 1 A number of device characteristics at three values of the typical length d.

Typical length	1 mm	100 µm	10 µm
Volume	10^{-6} L	10^{-9} L	10^{-12} L
Number of molecules at 1 µM	$6{\cdot}10^{11}$	$6{\cdot}10^{8}$	$6{\cdot}10^{5}$
Diffusion time	15 min	10 s	100 ms
Arrangement	25 volumes/cm^2	2500 volumes/cm^2	$2.5{\cdot}10^{5}$ volumes/cm^2
Maximum information density	1.5 values per min and cm^2	250 values per s and cm^2	$2.5{\cdot}10^{6}$ values per s and cm^2

Downscaling also affects the key processes that take place in a µ-TAS device, i.e., mixing, separation, and detection. In conventional chemistry, mixing is usually performed by convection. However, the same mechanism cannot be used in microstructures since Reynold's numbers in small structures are so low that it is very difficult to generate turbulence. Fortunately, mixing by diffusion is greatly enhanced in small structures. The reason being, that the time a molecule needs to travel a distance d decreases as $1/d^2$. This effect is illustrated in Table 1. It shows that a molecule with a diffusion coefficient of 10^{-9} m^2 s^{-1} needs 15 min to travel a distance of 1 mm, but only 10 s to travel 100 µm and 100 ms for 10 µm. Consequently, efficient mixers can be made when two solutions are brought into contact on a micron scale. Such mixers function as microreactors if they are used to mix the analyte with a reactant.

Shorter diffusion times increase exchange of molecular information on the device and therefore the rate of information generation. When this rate is multiplied by the number of volumes present, a maximum information density number can be defined per time and per surface area. Table 1 shows that it increases with the fourth power of the distance *d*. It should be noted, however, that this limit cannot be reached in all cases. Slow kinetics, fluid handling constraints, and detection requirements will all restrict the information generation. High information densities will be very useful in the evaluation of the millions of compounds produced by combinatorial chemistries, or in the speeding up of clinical DNA diagnostics.

For analytical chemistry applications, there are two categories of approaches: (1) the separation type systems, which need to physically separate volumes taken by different compounds, and (2) the nonseparation type systems, which need selective chemical reactions to perform the analysis. The most common approach for this is flow-injection analysis (FIA).

The main motivation to downsize **FIA-type systems** is the conservation of carrier and reagent solution and, thus, a reduction in maintenance cost. To this end, a time-constant scaling system can successfully be used. In this system, three spatial coordinates are scaled by division through a scaling factor δ. Subsequently, all other system parameters are scaled, applying appropriate factors to give a constant analysis time *t*. Table 2 shows the resulting scaling factors. It should be realized that any choice of scaling system, of course, is entirely arbitrary and that there are many more possible data sets than the ones presented here. The ones chosen provide simple rules for miniaturization.

Table 2 Scaling factors for μ-TAS parameters.

	Time-constant scaling (FIA-type systems)	Diffusion-related scaling (separation-based systems)
Space, x	δ	δ
Time, t	constant	δ^2
Linear flow rate, u	δ	$1/\delta$
Volume flow rate, F	δ^3	δ
Pressure drop (laminar flow), Δp	constant	$1/\delta^2$
Voltage (electroosmotic flow), ΔU	δ^2	constant
Plate number, N	–	constant

As can be seen in Table 2, the volume flow rate decreases with the third power of the scaling factor δ, which gives the desired conservation of carrier and reagent solutions. The time-constant scaling system is also suitable for pressure-driven transport. The required pressure drop, Δp [N m^{-2}], in a cylindrical channel of length L [m] and diameter d [m] filled with a solution of viscosity η [N s m^{-2}] is

$$\Delta p = 32\eta \frac{L^2}{d^2 t}, \tag{1}$$

where t [s] is the (constant) analysis time. Since both L and d are scaled with the same factor δ, the Δp required for fluid transport is not affected by miniaturization in the time-constant system. Therefore, downscaling in this system does not result in high back-pressures, and integrated micropumps can be used. Electroosmotic pumping is an alternative.

The flow of solution in **separation-type systems** can be driven either by a pressure gradient as in HPLC or by an electrical potential gradient as in CE. For on-chip separations, the latter driving force

has almost exclusively been used and therefore its characteristics are considered in detail here. Some scaling figures are given in Table 2. The conditions that will be derived for optimal separation clearly show the advantages of miniaturization.

Under normal conditions, the walls of glass capillaries carry a negative charge. This charge is balanced by cations in the solution directly adjacent to the glass. The resulting sandwich of adjacent layers with negative and positive charges is called the electrical double layer. Its thickness is typically a few nanometers. When a potential difference is applied along the length axis of the capillary, the electric field drives the positively charged solution part of the double layer toward the cathode. The momentum is transferred to the solution in the capillary bore by viscous drag until after about 100 μs a stable flat flow profile is reached. This phenomenon is termed electroosmotic flow. The generated flow profile is essentially flat, in contrast to the parabolic profile generated in pressure-driven flow. The flat profile has been observed experimentally in rectangular capillaries. This feature, in particular, gives electrically driven separation methods a significant advantage over pressure-driven methods, since peak dispersion is strongly reduced. The solution moves with the electroosmotic flow velocity u_{eo} (typical value of 5 mm s^{-1}),

$$u_{eo} = \mu_{eo} E, \tag{2}$$

which is proportional to the electrical field, E [V m^{-1}], with the electroosmotic mobility, μ_{eo} [m^2 s^{-1} V^{-1}], as the proportionality factor:

$$\mu_{eo} = -\frac{\varepsilon_0 \varepsilon_r \zeta}{\eta}. \tag{3}$$

Here, ε_0 [C V^{-1} m^{-1}] is the permittivity of free space, ε_r the dielectric constant of the solution and η [N s m^{-2}] the solution viscosity. The electroosmotic mobility increases with the zeta potential, ζ [V], which is the potential difference between the so-called plane of shear and the solution bulk. The shear plane forms the boundary between the immobile glass wall (including the first layer of immobile adsorbed water molecules and cations) and the mobile solution. The zeta potential increases with the solution pH and ionic strength. The typical range is between –50 and –100 mV at pH = 7–10. The electroosmotic flow decreases when the solution viscosity, η, rises. A very important feature of electroosmotic flow is that its velocity is independent of capillary bore size. However, there is a lower limit to the capillary diameter. The electroosmotic flow decreases markedly when the ratio of the capillary diameter and the double layer thickness is smaller than 20.

Generally, all dissolved species in the capillary are transported with the solution toward the cathode by the electroosmotic flow. During the time of transport to the detector, different ionic species are separated because they move with different electrophoretic velocities. The electrophoretic velocity, u_{ep} [m s^{-1}], is defined as

$$u_{ep} = \mu_{ep} E. \tag{4}$$

It is proportional to the applied field, E, with the factor called the electrophoretic mobility, μ_{ep} [m^2 s^{-1} V^{-1}],

$$\mu_{ep} = \frac{\varepsilon_0 \varepsilon_r \zeta' a}{\eta}. \tag{5}$$

In this equation, the zeta potential of the transported species, ζ', occurs (*c.f.* ζ in eq. 3). Its value increases with the ionic charge and decreases with its size, the latter including its hydrating ions. For example, typical values are ±120 mV for small inorganic ions (z = ±1,2,3) and about –50 mV for fluorescein isothiocyanate-(FITC-) labeled amino acids (z = –2.5). The geometry factor a depends on the ionic size relative to the thickness of the particle double layer. It is 2/3 for small inorganic ions and unity for very large ions. The total migration velocity, u, of a given species is

$$u = u_{eo} + u_{ep} = E\left(\mu_{eo} + \mu_{ep}\right). \tag{6}$$

The migration velocity does not depend on the capillary diameter, but only on the applied field and the sum of the mobilities. Neutral species have no electrophoretic velocity and are transported by electroosmosis alone.

The ability of the system to separate different species is reduced by peak broadening. Assuming a detector signal in the form of a Gaussian distribution curve, the peak width is characterized by the standard deviation σ [m]. Its square is the peak variance σ^2 [m^2]. In CE, extra-column variance is introduced by injection (the finite volume of the injection plug, σ^2_{inj}) and detection (the detection window, σ^2_{det}). On-column variance is introduced by species adsorption onto the capillary wall (σ^2_{ads}), the capillary temperature increase (σ^2_T), and longitudinal diffusion during separation (σ^2_{dif}). Especially relevant for chip-based capillary electrophoresis (CE) with short separation lengths is the variance introduced by pressure drops in the system giving rise to a parabolic flow profile (σ^2_P), and the variance introduced by corners, curves, and any change in diameter or direction of the separation channel (σ^2_G). Therefore,

$$\sigma^2 = \sigma^2_{inj} + \sigma^2_{det} + \sigma^2_{ads} + \sigma^2_T + \sigma^2_G + \sigma^2_P + \sigma^2_{dif}. \tag{7}$$

Of these different contributions, only σ^2_{dif}, σ^2_{inj}, and σ^2_{det} are truly unavoidable. All others can, in principle be eliminated. Of these three, the injection and detection variance generally can be made smaller than a quarter of the diffusional variance, with the exception of CE separations of DNA and large proteins. Here, we will therefore consider an ideal case in which only diffusional variance occurs. Diffusional variance results from species diffusion along the capillary axis during the migration time toward the detector, giving rise to a Gaussian concentration profile. Peaks are sharper, and hence peak resolution is better if diffusional dispersion is small relative to the migration velocity. The average diffusion distance of a particle within the analysis time t equals $(2Dt)^{1/2}$ (where D [m^2 s^{-1}] is the diffusion coefficient) and corresponds to the standard deviation σ of the Gaussian curve. Noting that the analysis time $t = L/u$ (with L the separation length), the resulting band variance of concentration at the detector, σ^2_{dif}, is given by

$$\sigma^2_{dif} = \frac{2DL}{u}. \tag{8}$$

The height equivalent to a theoretical plate, H [m], equals σ^2/L, so that

$$H = \frac{2D}{E\left(\mu_{eo} + \mu_{ep}\right)}. \tag{9}$$

At a field strength of 2 kV cm^{-1} and typical values for D and μ, H is only 58 nm! This demonstrates that in CE the plate height can theoretically be made vanishingly small by increasing the electric field E.

The efficiency of a separation is expressed in terms of the plate number, $N = L/H$. Noting that the field E equals the ratio of applied potential U [V] and capillary length L, we derive

$$N = U\frac{\left(\mu_{eo} + \mu_{ep}\right)}{2D}. \tag{10}$$

Thus, separation efficiency increases with the applied potential. It does not depend on capillary dimensions and, therefore, is not influenced by miniaturization. Theoretically, N can be indefinitely increased by increasing the applied potential, but practical limits are imposed due to Joule heating in the capillary. In addition, corona formation and arcing can occur at electrodes above 35 kV. At this voltage and

for typical values of μ and D, 3 million plates are theoretically achievable. The number of plates needed to separate different species will depend on their differences in mobility and diffusion coefficients. As a rule of thumb, about 500 plates are needed for separation of small ions differing by one charge unit, 500 000 plates for the separation of larger ions of similar charge (e.g., fluorescently labeled amino acids) and more than 10^7 plates are desirable for DNA separations.

The analysis time t is equal to L/u. Substituting eq. 6 for the migration velocity it is found that

$$t = \frac{L^2}{U\left(\mu_{eo} + \mu_{ep}\right)}. \tag{11}$$

The analysis time, therefore, can be considerably reduced by shortening the separation channel and also by increasing the applied potential.

A reduction of the capillary cross-sectional area is favorable in reducing the Joule heating of the solution. Joule heating is caused by the solution resistance to current transport. The temperature rise can be considerable, and results in a reduced efficiency at high applied fields. Several explanations for the observed drop in efficiency have been cited in the literature. They are based on the induced radial temperature gradients in the solution that increase radial diffusion and cause convection. Generally, a heat generation of 1 W m^{-1} is considered to be the maximum for circular capillaries when passive cooling is used. Due to the larger surface area of the rectangular channels in planar μ-TAS devices, heat dissipation is superior and heat generation can be larger. It can be derived from equations for the temperature gradient inside circular and rectangular capillaries, that the maximum heat generation in rectangular channels is $2a/\pi$ W m^{-1}. The heat Q [W m^{-1}] generated per unit length in a rectangular channel of height h and width ha (with a the aspect ratio) is

$$Q = \frac{U^2 h^2 a}{L^2} \lambda c, \tag{12}$$

where λ [m^2 mol^{-1} Ω^{-1}] is the (pH-dependent) molar conductivity of the buffer and c [mol m^{-3}] its concentration. The equation shows that, unfortunately, heat generation increases when we increase U (to obtain a high N) or when we decrease L (to obtain a short t). The maximum field strength, $E = U/L$, that can be applied without overheating follows from substituting Q = $2a/\pi$ W m^{-1} into eq. 12:

$$\left(\frac{U}{L}\right)_{max} = \frac{1}{h}\sqrt{\frac{2}{\pi\lambda c}}. \tag{13}$$

The equation shows that high potentials or small channel lengths (and hence large field strengths) can be used if λ or c, and especially if the channel height h are minimized. An expression for the plate generation rate can be derived from eqs. 10 and 11, and shows a dependence on the square of the electric field strength.

DETECTION

It has been long recognized that the final limits to miniaturization of FIA and separation-based microsystems are set by the system detector. The problem is illustrated in Table 1. This table shows the number of molecules that are present in different (detector) volumes at a typical analyte concentration of 1 μM. When the detector is a cube with sides of 1 mm, its volume is 1 μL and 600 billion molecules are present. A diversity of conventional detection methods can be used at this level. When the detector measures $10 \times 10 \times 10$ μm, 600 000 molecules are present in the detector cube at any time. Such a low-molecular population can only be measured using laboratory methods like laser-induced fluorescence. Further volume reduction will lead to a situation where, for a significant amount of time, no analyte

molecules may be present in the detector volume. Detection issues thus define a lower limit to the ultimate size of μ-TAS.

EXAMPLES

In my oral presentation at the IUPAC conference, Tokyo, I gave a few examples of miniaturized chip-based analytical systems we use in our lab, for example, capillary electrophoresis, electro-chemiluminescence detection [4,5], particle speed measurements [6], continuous-flow chemical reactors [7–10], batch bioreactors [11], plasma emission spectroscopy [12–14], and using plasma devices for analog computing [15].

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