Chapter 9

Micro-Total-Analysis Systems (μTAS)

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9.1 Lab-on-a-Chip

9.1.1 Introduction

Micro-total-analysis systems (μTAS), and the subset of devices referred to as
lab-on-a-chip (LOC), derive from application of “hard” and “soft” fabrication
techniques for the manufacture of miniaturized devices that perform all or part
of a biochemical analysis. μTAS may be hybrids of multiple chips, integrated elec-
tronics, and external supports; while LOC refers more specifically to a microfluidic
chip or other device that performs a well-defined analytical task [Guber et al.,
2004]. Biochips more generically include the LOC devices and microarray
devices. The definition of these terms varies with different authors and may be
used interchangeably. Commonly used materials, fabrication techniques, and the
history of these devices have been discussed in previous chapters.

Microanalysis devices can be broadly classified as either microfluidic-based or
microarray-based microdevices [Krishnan et al., 2001]. DNA chips (for the study
of DNA and RNA) include both DNA LOC devices and DNA microarrays,
while protein chips include both LOC devices and protein microarrays. These
are discussed in Chapters 11 and 12.

The goal of μTAS and LOC devices is to achieve increased efficiency through
smaller scales and to undertake analysis that cannot be done conveniently by other
means. Advantages of smaller scales include the following:

1. improved transport through use of electrokinetic effects and miniaturized
   pumps;
2. efficient cells, molecular and particle separation, and immobilization;
3. smaller sample requirements and carrier volumes;
4. reduced reagent consumption; and
5. integration of channels, mixers, separators, reactions chambers, electrodes,
   and detectors into single devices.

Improved throughput of analytes occurs as a consequence of miniaturization and
integration. LOC devices may incorporate microfluidic components, microsensors,
microactuators, and customized surfaces created by chemical modification or
coatings with inorganic and organic materials. Microspheres and beads are also
integral to numerous LOC devices.

Most of the techniques employed for μTAS and LOC devices are investiga-
tional; hence, much of the discussion here derives from current research efforts.
While certain representative works have been selected to introduce basic concepts,
not all techniques are reported, and much can be gained by going directly to the
literature and searching a topic of interest.

9.1.2 Chemical analysis

Although electrophoretic methods, especially for DNA analysis, have been the
catalyst for LOC devices, new technologies have emerged for continuous and
sequential chemical processing. These devices are typically microfluidic in design, and incorporate either novel electrokinetic effects or pressure-driven systems for movement of fluids and solids.

Recall from the discussion on diffusion in Chapter 5 that the mean-square displacement of a particle from its origin is proportional to time, and that surface area to volume is proportional to the scale of the microchannel. As the scale decreases, surface area to volume increases, while time for diffusion decreases. Figure 9.1 shows the scale dependence of the molecular transportation time and the specific interface area. In LOC devices, the channel size of 150–250 μm means diffusion across the channel may occur in seconds.

Pressure-driven continuous-flow chemical processing chips with stabilized laminar flow, suitable for a variety of inorganic, organic, and physical chemistry reactions, are described by Sato et al. (2003). Applications include chemical analysis, multi-ion sensing, immunoassays, and cell analysis. An immunoassay chip, for example, is shown in Fig. 9.2. The following summarizes the process steps required to fabricate this chip:

Mechanically polished 0.7-mm thick Pyrex glass plates were used (top and bottom plates). Inlet and outlet holes were drilled by ultrasonic sandblasting on the top plate. For good contact between the substrates and the photoresist, and protection of the substrates during glass etching, 20-nm thick Cr and 100-nm thick Au layers were deposited on the substrates by physical vapor deposition. 2-mm thick positive photoresist was spin-coated on the Au metal layer and baked at 90°C for 30 minutes. UV light was exposed through a photomask by using a mask aligner to transfer the microchannel pattern onto the photoresist. The photoresist was developed and a pattern with 10-mm wide lines was obtained. The Au and Cr layers were etched with I₂/NH₄I and Ce(NH₄)₂(NO₃)₆ solutions. The bare glass surface with the microchannel pattern was etched with a 50% HF solution at an etching rate of 13 mm/min. After glass etching, the remaining photoresist was removed in acetone and metals were removed in I₂/NH₄I and Ce(NH₄)₂(NO₃)₆ solutions.

Figure 9.1  Scale dependence of the molecular transportation time and the specific interface area. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]
Microchannels were designed with guide structures (Fig. 9.3), forming a stable liquid-liquid interface. These required three photomasks and three independent etching cycles. Surface-chemical modification with octadecylsilane stabilized liquid flow in organic solvent carrying channels.

Figure 9.2 Immunoassay chip. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]

Figure 9.3 Microchannels with guide structures forming a stable liquid-liquid interface. [Reprinted with permission from Sato et al. (2003), copyright Elsevier.]
As an alternative to traditional ELISA, this immunoassay chip is able to measure ultra-trace amounts of human carcinoembryonic antigen (CEA) (a serum colon cancer marker) and interferon. The methodology employs antibody-coated polystyrene beads packed in the microchannels and thermal-lens microscopy for detection.

9.2 Capillary Electrophoresis Arrays (CEA)

9.2.1 Capillary electrophoresis (CE)

A microfluidic system for capillary electrophoresis (CE) is shown in Fig. 9.4, where sample introduction and electrophoretic separation are accomplished in each of two crossing channels. The sample is driven through the short sample channel across the separation channel by application of a potential, and the introduced sample “plug” is then electrophoretically separated by application...
of another potential. Optical or electrochemical detection occurs at the end of the separation channel, and provisions for buffer flow, discarding the sample, and separation wastes are included [Guber et al., 2004]. Microreactor chips for capillary electrophoresis and mixing by diffusion are shown in Fig. 9.5.

9.2.2 Linear and radial arrays

Multiplexing, or running multiple samples in parallel, increases speed and the ability to run control and test samples simultaneously under the same conditions, as well as the ability to analyze multiple time-delayed samples from various reactions at the same time [Felton, 2003].

The evolution from linear to radial capillary array electrophoresis (CAE) devices at U.C. Berkeley is shown in Fig. 9.6.

Rectilinear format μCAE devices appeared in 1994. These chip-based devices involved etching 15 independent channels in a glass microscope slide, then bonding a cover slide with drilled ports for sample, waste, cathode, and anode. The walls of the channels were coated with covalently bonded linear polyacrylamide to prevent electro-osmotic flow and adsorption of DNA fragments. “Direct” and “plug” injection methods were investigated, revealing better separation with a cross-sectional methodology [Oosterbroek and van den Berg, 2003].

Radial-format μCAE devices, which appeared in 1999, maximize use of substrate surface, simplify channel routing, eliminate linear scanning, and are reproducible. The first devices were on 100-mm wafers with 96 capillaries and 3.3-cm tubes, using two-color allele specific PCR (ET-ASPCR analysis). Later, a 150-mm wafer with 5.5-cm tubes was fabricated, and then a 200-mm wafer with 8-cm-long separation channels (24 quartets, allowing for 96 samples). Detection schemes are reviewed below [Oosterbroek and van den Berg, 2003].