Electrokinetic Microsystems for Biochemical Applications

Chuan-Hua Chen

Abstract Micro-total-analysis systems (µTAS) integrates various functions of conventional biochemical lab onto a small chip. Electrokinetic (EK) microsystems is a popular choice to realize µTAS. This paper introduces the underlying principle for electrokinetics, describes the design and fabrication of microsystems, reviews the major functionality of EK microsystems, and comments on the applications of EK microsystems in biochemical analysis.

1. Introduction

Microsystems technology, which integrates multiple functions on a small device, is a technology originally extended from microelectronics industry. The field of microsystems dates back to 1970s, when a group at Stanford University demonstrated the first micromachined gas chromatography system on a 5cm-diameter Silicon wafer. Microsystems was commonly called micro-electro-mechanical systems (MEMS), due to the dominance of electrical and mechanical engineers in the field [2]. As the technology develops, it becomes apparent that microsystems devices can extend far beyond electrical and mechanical engineering. In particular, the biochemical functionality has drawn vast attention because of the huge biomedical market. Micro-total-analysis systems (µTAS) was coined in the late 80s to accommodate this new technological trend [3]. µTAS offers integrated functionality with faster speed, smaller sample consumption, and cheaper price.

A truly integrated µTAS device, sometimes called “lab-on-a-chip”, needs to fulfill various functions on a single chip, including sampling, sample pre-treatment (filtration, concentration, mixing, reaction, separation, etc.) and detection [4]. Although it is unlikely that any single chip at current research stage can replace a conventional biochemical lab, electrokinetic (EK) microsystems seems to be a promising choice to accomplish most of the functions of conventional biochemical labs. The advantages of electrokinetically driven microsystems over other (e.g., pressure-driven) microsystems include: (1) Positive scaling effects for microscale devices, for example, faster separation speed and smaller sample consumption; (2) Accurate control of transportation and manipulation of liquid sample by electrical field, and no solid moving parts required; (3) Directly adaptable knowledge from mature electrokinetic research on conventional macro-scale devices.

This paper will focus on the electrokinetic microsystems for applications in biochemical analysis. Part 2 introduces the principle of electrokinetics, Part 3 describes the design issues, Part 4 introduces fabrication techniques, Part 5 reviews the functions of EK microsystems, and Part 6 comments on the practical applications.

2. Electrokinetics

Electrokinetics includes electroosmosis and electrophoresis. Electroosmosis is utilized to transport liquid sample, while electrophoresis is utilized to separate different species.

As shown in Figure 1, when a dielectric (say, glass) solid surface comes into contact with a typical (e.g., pH > 3) solution, the glass surface becomes charged due to the spontaneous deprotonation of surface silanol groups. The charged surface attracts counterions and repels coions, forming a charge double layer, the outer layer of ions (on the solution side) of this double layer are mobile. If an electrical field is applied parallel to the wall, Coulombic forces are exerted on these mobile ions and the electromigration of these ions will drag the bulk fluid through viscous interaction, resulting in electroosmosis [5]. In the thin-double-layer limit, the velocity is plug-like

\[ V_{\text{electroosmotic}} = -\frac{\varepsilon \zeta E}{\mu} \]

where \( \varepsilon \) and \( \mu \) are the permittivity and viscosity of the working fluid respectively, \( E \) is the electric field, \( \zeta \) is the Zeta potential of the wall with respect to the bulk fluid. The Zeta potential characterizes the influence of the surface condition on electroosmotic flow.

**Figure 1. Electroosmosis and electrophoresis**

Electroosmosis: mobile ions in the charge double layer are driven by the applied electrical field, the moving ions will drag the bulk fluid through viscous forces, resulting in the electroosmotic flow.

Electrophoresis: different species have different charge-to-mass ratio, the balance between electrical body force (proportional to charge) and viscous drag (related to body size and shape) will result in different electrophoretic velocities.

Electrophoresis can be explained by the double layer theory too. We shall adopt a simplified view here to show the basic principle. When an ion (or particle) in solution is under an external electrical field, there will be electrical body force exerted on the ion,
where $q$ is the charge on the ion. On the other hand, the moving ions will experience viscous drag by the solution, if the ion is assumed to be a perfect sphere,

$$F_{\text{friction}} = 6\pi r \eta \nu$$

where $r$ is the radius of the sphere. The balance between the electric body force and the viscous drag will give the electrophoretic mobility of the ion (assuming a shape of perfect sphere)

$$V_{\text{electrophoretic}} = \frac{qE}{6\pi \eta \nu}$$

Generally, different species have different electrophoretic velocities, which is the basis for electrophoretic separation [6].

The double layer is usually thin compared to the channel dimension. In the thin-double-layer-limit, the electroosmotic velocity profile will be plug-like (Equation 1), unlike the parabolic velocity profile in pressure driven flow. The plug-like velocity profile will minimize dispersion and increase the electrophoretic separation efficiency [7]. Since the velocity profile is linearly proportional to electrical field, the electroosmotic flow can be precisely controlled through simple electronics.

Zeta potential is a strong function of surface conditions, therefore, the electroosmotic velocity can be drastically altered by techniques such as surface coating. As a result, electroosmosis can either be exploited as a transporting mechanism or be suppressed if not desired (e.g., in isoelectric focusing) [5].

3. Design

The functions of a biochemical analysis system can be roughly divided into three categories: sampling, sample pre-treatment and sample detection [4].

Sampling involves the interface between micro-world to macro-world. The interface is extremely hard for µTAS because of its fluidic and biochemical nature. The fluidic nature requires special sealing and transportation, and the biochemical nature raises concern for compatibility and reusability. Taking blood analyzer for example, there is no straightforward way to directly transport blood from human body into microsystems, so sampling is still not directly incorporated into commercially available microsystems [9].

As to detection, most µTAS devices require quantitative information (other than threshold detection). Furthermore, biochemical samples usually have low concentrations, given the low sample volume in microsystems, sensitive detection is usually a must. Popular detection schemes for µTAS systems include fluorescent detection, UV detection, and electrochemical detection. Therefore, it is unlikely that the whole detection unit will be integrated onto the microsystems which might be single-use devices.
In the current and subsequent sections, we will focus on the design and fabrication microchips involved in sample-pretreatment (such as mixing and separation). A typical microchip of this kind is shown in Figure 2 [9].

![Microchip Image]

The glass chip has a total dimension of 16mm x 95mm x 2.2mm, and the channel cross section is approximately semicircular. The sample reservoir is 2 mm in diameter.

The first design decision when making an electrokinetic microchip is the cross-sectional dimensions. Due to the low Reynolds number, mixing is accomplished only through diffusion, which sets the upper limit of the channel dimension. For a typical small molecule, its diffusivity $D$ is on the order of $10^{-10} \text{ m}^2/\text{s}$, in order to achieve mixing time scale (t) on the order of 10 second, the channel dimension based on diffusion length argument ($x = \sqrt{2Dt}$) is about 50 $\mu$m. On the other hand, a typical sensitivity requirement for biological assays is about 1 nM. Generally $\geq 1,000$ molecules are needed for accurate detection, that means a volume of $(12 \mu m)^3$ is needed. These two requirements stipulate the dimension of channel cross section to be somewhat between 10 $\mu m$ and 100 $\mu m$ [10].

Secondly, the length of the channel needs to be decided. For a diffusion-limited separation, the separation resolution is defined as [11]:

$$SR = \frac{L}{\sigma} = \frac{\Delta \mu}{\mu_{av}} \frac{L}{\sqrt{2D}} \left( \frac{E\mu_{av}}{L} \right)$$

where $L$ is the separation length, $\Delta \mu$ is the mobility difference, $\mu_{av}$ is the average mobility, $D$ is the diffusion coefficient, and $E$ is the electrical field. The separation resolution is therefore proportional to $(EL)^{1/2}$ for a diffusion-limited separation. The upper limit of electrical field is set by the cross-sectional dimension which affects the heat dissipation capacity. Hence, long separation length is desired. The microchip format limits the length of a straight channel to be several inches at most, so serpentine turns are usually necessary to achieve long separation length. However, the turns will increase dispersion, which requires optimization of turn geometry [11].
Lastly, the design of sample reservoirs needs to be considered. As commented at the beginning of this section, interface between micro-world to macro-world is still under research. Currently, simple through-holes (Figure 2) usually serve as the sample reservoirs. In addition, platinum electrodes are dipped into the reservoirs to provide high voltage sources.

4. Fabrication

In choosing the materials for microchip, one has to take into account the economic issues such as cost and disposability and technical issues such as surface properties and manufacturability. Among the top choices of materials are glass, polymer and silicon [10]. The microfabrication processes will mainly be determined by substrate materials and desired resolutions. This paper will comment on general fabrication processes, the reader can find the details in the references [12, 13].

For glass chips, several types of materials have been used, such as soda lime glass, borosilicate glass and quartz. The fabrication involves conventional drilling process to produce through holes, micro-lithography and wet-etching to produce microchannel, and thermal bonding to enclose the microchannel. Glass chips are relative expensive, but they can be flushed with sodium hydroxide solution and reused. Glass chips are commercially available from Micralyne [9].

Polymer chips are best suited for single-use devices, they are also popular in applications where electroosmosis needs to be suppressed. Polymer materials for microchips include polydimethylsiloxane, polystyrene, and acrylic. Microfabrication processes vary from case to case, and conventional machining techniques like laser ablation and hot embossing are applied when applicable. Acrylic chips are commercially available from Aclara [14].

Some of the earlier work in electrokinetic microsystems used Silicon as the substrate [15]. However, Silicon itself does not support electroosmosis, and requires surface coating of materials like Silicon Nitride [16] and Silicon Dioxide[17]. Furthermore, the electrical breakdown issues limit the electrical field. Since the resolution of microchannels is usually 10 µm or above, which can be easily achieved by state-of-art microfarbication techniques, Silicon is only used when special functions are needed [16, 17].

5. Functionality

Pumping and valving The fluidic transport is accomplished by electrokinetic pumping and valving, both of which are based on electroosmotic flow. Electroosmosis can pump working fluids of a wide range of conductivity, ranging from organic solvent to buffered solution [18]. The manipulation of cells through electrokinetics has been
reported as shown in Figure 3 [19]. Electrokinetic valving has been thoroughly researched. Both pinched valve and gated valve are developed [20]. Using these two these two kinds of valving operation, desired volume of sample plugs can be obtained by injection at either a simple cross or a twin-T cross (the two kinds of crosses are shown in Figure 2).

**Diluting and concentrating** Sample pre-diluting is based on diffusive mixing of the sample with buffer solution, which can be easily achieved at a microfluidic T-junction. Sample pre-concentrating has been realized by porous membrane structure, as shown in Figure 4 [21]. Dilution and concentration of greater than two orders of magnitude have been reported.

**Mixing** The Reynolds number in electrokinetic microsystems is usually small (Re < 1), so the mixing commonly relies on molecular diffusion. Fortunately, the channel dimension is generally small (~ 10 µm), so diffusive mixing in tens of seconds is possible. Many designs are simply aimed at decreasing the length that the diffusion has to cover to achieve mixing, and Figure 5 is an example of such efforts [22]. The microfluidics group at Stanford University has discovered instability phenomena associate with low Reynolds number electrokinetic flow, which is a potential solution to efficient mixing in microsystems [23].

**Separation** Separation is the major functionality of most electrokinetic microsystems. Microsystems has large surface-to-volume ration and is very efficient in heat dissipation, this property permits high electric fields on the order of 1 kV/cm. As shown by Equation 5, high electrical field leads to high separation resolution. Interesting development in on-chip separation include sub-millisecond electrophoresis [24] and 2D electrophoresis [24, 25]. As shown in Figure 6, sub-millisecond electrophoresis exploits the high resistance of the thin channel in the center to produce locally high electrical field. Figure 7 shows a schematic of the realization of 2D-separation on-chip, this is an important step toward 2D electrophoresis.
Figure 5. Photomicrograph of the micro-mixer
The small image in the lower left corner is the SEM of the mixer. This mixer is about 100 by 200 µm wide and 10 µm in depth.

Figure 6. High-speed microchip
The microchip is used for high-speed electrophoretic separations. The inset is the enlargement of the injection valve and separation channel.

Figure 7. 2D-separation microchip
The first-dimension separation channel extends 69 mm from the first cross intersection to the second. The second separation channel extends 10 mm from the second cross to the point of detection.
6. Applications

Electrokinetic microsystems has broad applications in biochemical analysis. Representative applications include DNA and protein separations, DNA sequencing, immunoassay and enzyme assay [4, 10]. However, most of these applications are still at research stage, EK microsystems-based companies like Aclara and Caliper are still looking for the right market. The road toward micro total analysis systems seems to be much longer than some people have initially thought.

One of the major obstacles is the reluctance toward novel methods in biochemical community. Biochemical research is very tedious and researchers would rather rely on the well-established analytical methods, unless the new method is proven to have robust and accurate performance with a higher performance-to-price ratio. During the past decade, the EK microsystems research community has generally concentrated its effort on discovering new functionality and looking for possible applications. Up to now, various functionalities of EK microsystems in sample pre-treatment have been shown, and potential applications in genomics and proteomics have been explored, it is time to evaluate the reliability and accuracy of EK microsystems [27].

The goal of miniaturization is to look for better and novel functionality. As mentioned before, the scaling down of electrokinetic analytical systems offers faster speed in a compact design at lower price. What’s more, the miniaturization enables parallel processing and system-level integration. Parallel processing is vital for application with a huge set of samples, such as drug screening. The system-level integration of reaction and separation functions, essential for combinatorial chemistry, will beat microarray in terms of functionality. Future research needs to treat microsystems as an “enabling” technology rather than a micro-version of conventional technology [12, 13].
References


