High-throughput microfluidic sample-introduction systems

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We give an overview on recent developments in high-throughput microfluidic sample-introduction techniques based on a capillary sampling probe and a slotted-vial array (SVA). We discuss the advantages and the potential of SVA-based sample-introduction systems as well as their applications in miniaturized flow-injection analysis, sequential-injection analysis, capillary electrophoresis and liquid-liquid extraction. We illustrate the advantages and the potential of SVA-based sample-introduction systems using results obtained recently.

Keywords: Capillary electrophoresis; Flow-injection analysis; High throughput; Liquid-liquid extraction; Microfluidics; Sample introduction; Sampling probe; Sequential-injection analysis; Slotted-vial array

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1. Introduction

In recent years, microfluidic chip-based analysis has made great progress, due to its outstanding advantages including high analysis speed and low sample and reagent consumption, as well as its great potential to allow automation and integration of the complete analytical system on a single chip [1,2].

Currently, particular interest is focused on achieving automated continuous introduction of a series of samples to a microfluidic chip with high throughput and low sample consumption. Introduction of samples to a microfluidic chip requires an interfacing system between samples with volumes in the μL–mL range from the macro-world and microfluidic systems handling fluid volumes in the nL–pL range. World-to-chip interfacing is considered a major challenge, particularly in high-throughput applications requiring frequent sample change (e.g., drug screening or on-line continuous process monitoring).

Currently, various world-to-chip interfacing schemes are being developed to suit different requirements of external sample and microfluidic chip systems (e.g., sampling rate, sample consumption, precision, stability, and degree of automation). Fig. 1 shows possible modes of sample introduction for microfluidic chips.

In mode A, sample and reagent solutions are loaded in the on-chip reservoirs connected with microchannels. Such an approach has been employed frequently in most reported chip-based analytical systems. It has the advantages of a high degree of integration and convenient operation in transferring the solution in the reservoir to the microchannels [3–5]. However, the sample-change operation in the on-chip reservoir in multiple sample measurements, which usually requires the sample reservoir to be manually emptied, rinsed, and refilled with the new sample solution, is time consuming and difficult to automate. Such operations dramatically lower the overall throughput and thwart the important advantage of microfluidic systems in achieving high analytical speed. Although multiple-sample reservoirs could be fabricated on a single chip to improve sample introduction [6], the number of on-chip sample reservoirs is limited by the chip size and the capabilities of peripheral equipment.

Mode B is characterized by employing a split-flow interface fabricated on the chip to achieve continuous sample introduction. Different sample solutions are sequentially delivered to the chip via a connecting tubing through the split-flow interface, where a split sample flow is introduced into the microchannel of the chip for subsequent analysis. Various interfacing designs, based on mode B (e.g., flow-through [7–9], guided overflow [10] and falling drop [11]) have been developed mainly to achieve high-throughput, continuous, automated sample introduction in chip-based capillary

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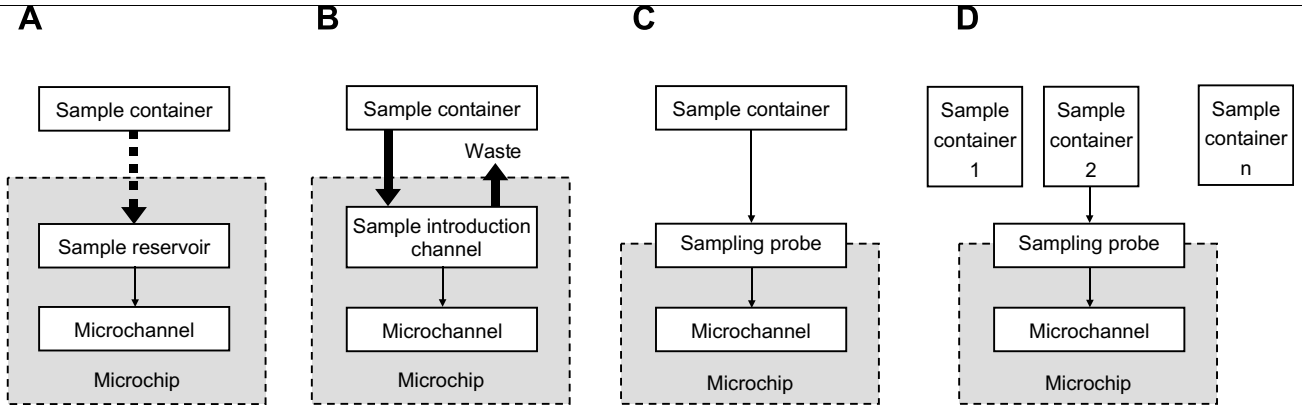

Figure 1. Designs for microfluidic world-to-chip interfacing.

electrophoresis (CE) systems. In most of these systems, a wide-bored sample-introduction channel was usually coupled to a thin-bored microchannel for sample loading in the split-flow interface to avoid the formation of Poiseuille flow in the microchannel during sample introduction. Such a design results in relatively large sample consumptions in the 5–100 μL range with low rates of sample use (<1%).

Mode C involves the use of an on-chip sampling probe for continuous sample introduction, which substitutes the on-chip sample reservoir in mode A and the sample-introduction channel in mode B. In mode C systems, a sampling probe is directly connected to the chip microchannel, and sample introduction is performed simply by inserting the inlet of sampling probe into an off-chip sample vial [8,12,13]. With such a design, the sample consumption in the sample-introduction process could be reduced significantly to the sub-mL level.

However, in most reported chip-based systems performing continuous sample introduction, such as those mentioned above, sample change has rarely been addressed. Usually, sample-change operations were performed manually by moving the inlet tip of the sample inlet tubing from one sample vessel to the next. Such operations further restrict sampling throughput, especially when dealing with large numbers of samples.

Auto-samplers (mode D) based on x-y-z stages were developed to automate sample change for chip-based systems [8,14]. Although these approaches were effective, the cost and the size of the systems were increased significantly.

Recently, we reported a simple sample-introduction system based on an array of microsample vials with a slot on the bottom of each vial [15], which was coupled with a capillary sampling probe to perform high-throughput nL-sample introduction and change in microfluidic analysis systems [16].

This article gives an overview of some of the trends and the potential of this type of sample-introduction technique in flow-injection analysis (FIA), sequential-injection analysis (SIA), CE and miniaturized liquid-liquid extraction (LLE) systems, mainly based on our work.

2. Microfluidic FIA systems

FIA is a well-established technique for automated solution analysis with solution capacity in the 10–10^3-μL range. Since 1995, Haswell's group [17–19] and Ramsey's group [20] have reported chip-based FIA systems employing electroosmotic flow (EOF) for both mobilization of reagents and sample injection. Recently, Leach et al. [21] reported a microfluidic FIA system built on a poly(dimethylsiloxane) (PDMS) chip using multiple pneumatically-driven microvalves and micropumps to achieve sample injection and change. In 2004, we developed a high-throughput sample-introduction system for microfluidic analysis systems based on a capillary sampling probe (SP) and a slotted-vial array (SVA) comprising horizontally-positioned microsample vials each with a slot fabricated on the bottom of it [15]. The SP-SVA sample-introduction system was employed to build a chip-based FIA system capable of achieving high-throughput, automated, continuous sample change and sample injection in the nL range [16].

The μFIA system (as shown schematically in Fig. 2(a)) comprised a microchip with a capillary sampling probe and reagent reservoir, and an SVA sample-introduction system. The slotted vials were produced from 0.2-mL Microtubes (Porex, Petaluma, USA) by fabricating 1.5-mm wide, 2-mm deep slots on the conical bottoms of the tubes for the sampling probe to pass through. The SVA was fixed on a home-built platform modified from a chart recorder, capable of moving linearly under computer control with a maximum moving distance of 30 cm and maximum moving speed of 10 cm/s. In the system, the fluids in the chip channels were driven by hydrostatic pressure produced from differences in liquid levels

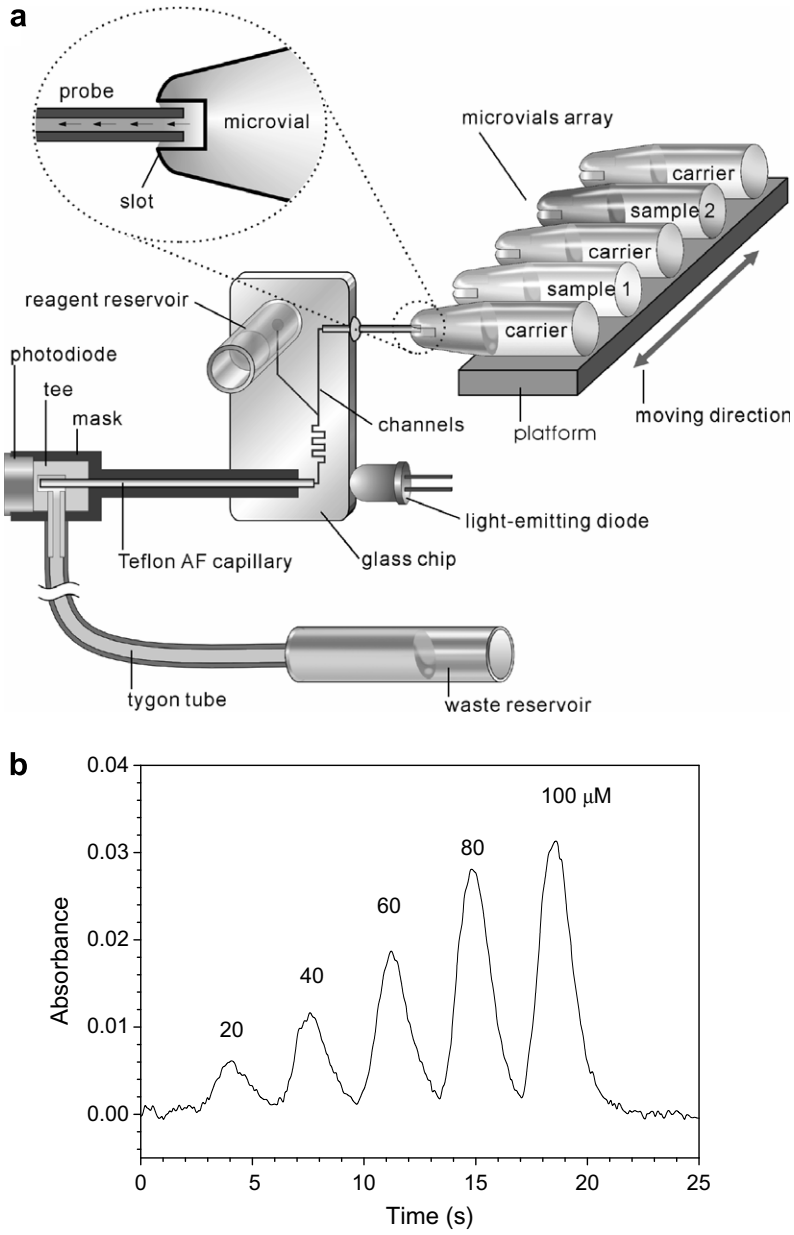

Figure 2. (a) μFIA microchip system; (b) Typical recording of sequentially injected 20, 40, 60, 80, 100 μM Fe(II) standards to show the highest sampling throughput of 1000/h. Sampling time, 0.08 s; injection time for carrier, 3.4 s; sampling flow-rate, 0.5 μL/min (Reprinted with permission from [16]).

between the sample vial or reagent reservoir and the waste reservoir, which has the advantages of not requiring an external power source or driving equipment. FI sample loading and injection were performed by linearly moving the SVA filled alternately with 50-μL samples and carriers, allowing the probe inlet to enter the solutions in the vials through the slots sequentially in the order of carrier, sample 1, carrier, sample 2, carrier …etc; and, the sample and carrier solution were introduced into the chip driven by gravity. The amount of solution injected was determined by the solution flow-rate within the chip channel and the residence time of the probe within the sample vial, which was controlled by the computer. The injected samples mixed with the reagent downstream in the mixing channel, and the reaction product was detected in the liquid-core waveguide (LCW) flow-cell, fabricated from a 20-mm long Teflon AF 2400 capillary (50-μm i.d., 375-μm o.d.) with a cell volume of 40 nL and an effective path-length of 1.7 cm.

The performance of the μFIA system was demonstrated in the determination of Fe(II) with *o*-phenanthroline as reagent. A good reproducibility – 0.6% RSD (n = 18) for peak height of 80 μM Fe(II) standard – was

achieved with a sampling throughput of 300/h. The highest sampling throughput of 1000/h was obtained using an injection time of 0.08 s with a sample-injection volume of only 0.6 nL for each cycle (as shown in Fig. 2(b)). The limit of detection (LOD) for Fe(II) was 0.1 μM (3σ). The sensitivity was comparable with that obtained using conventional FIA systems, which typically consume 10,000-fold more sample.

In this work, we proposed a new concept for chip-based FI sample injection characterized by sweeping an on-chip SP through an off-chip SVA holding samples and carrier. Such an injection mode for chip-based FIA allowed convenient, rapid sample injection in the nL range and sample change with low carry-over and high frequency without requiring mechanical valves and pumps.

In 2006, a similar FI injection strategy was adopted by Guan et al. [22] to study the performance of the micropump based on capillary and evaporation effects in a microfluidic chip-based FI chemiluminescence (CL) detection system. The experimental set-up of the μFI-CL system is shown in Fig. 3. In the sample-injection system, a 15-mm-long silica capillary (75-μm i.d., 375-μm o.d), serving as SP, was connected to the microchannel by inserting it into appropriately bored PDMS tubing fixed in the access hole and sealed with epoxy. The vials in the SVA system for FI injection were made from plastic PCR vials, which were fixed in an array on a holding platform, and filled with sample and carrier solution in the sequence: carrier, sample A, carrier, sample B, carrier, etc.

The performance of the micro FI-CL system driven by the micropump based on capillary and evaporation effects was studied using a model system involving the well-known luminol/hexacyanoferrate/H_2O_2 CL reaction, with luminol as sample. Valveless sample injection was performed by linearly moving the SVA to allow the SP sweep through the slots of vials in the sequence: carrier, sample A, carrier, sample B, carrier, etc., with staying times for the probe immersed in each sample (luminol) and carrier solution of 10 s and 100 s, respectively. The injected sample merged with the reagent flow driven by the micropump, and generated CL in the reaction channel. Sampling frequency was 30/h in continuous analysis of a series of samples. A precision of 1.4% RSD (n = 11) was obtained in the system using a 10-mM luminol sample.

3. Microfluidic SIA systems

Ruzicka and Marshall [23] introduced SIA to improve the performance of FIA systems. Compared to conventional FI systems, the advantages of SIA systems include simple hardware, automated operation, better reliability, and low sample and reagent consumption. However, so far, efforts to miniaturize SIA are rarely reported. In most of the reported miniaturized SIA systems, macroscale multi-way selection valves have still been required to perform sequential injection of sample and reagents, which has resulted in sample and reagent consumption in the μL range.

More recently, we combined the SVA sample-introduction system with a short fused-silica capillary to build an efficient microfluidic SIA system without requirement for any conventional valves and pumps or microfabricated chips [24]. The system set-up is as described in Fig. 4(a). A 6-cm-long fused-silica capillary was used as an SP reactor, as well as a flow-through detection cell. The outlet of the capillary was connected to a horizontally-oriented waste reservoir, which provided liquid-level differences for inducing gravity-driven flows in the capillary. The SVA sample-introduction system comprised a pair of gears driven by a programmed stepping

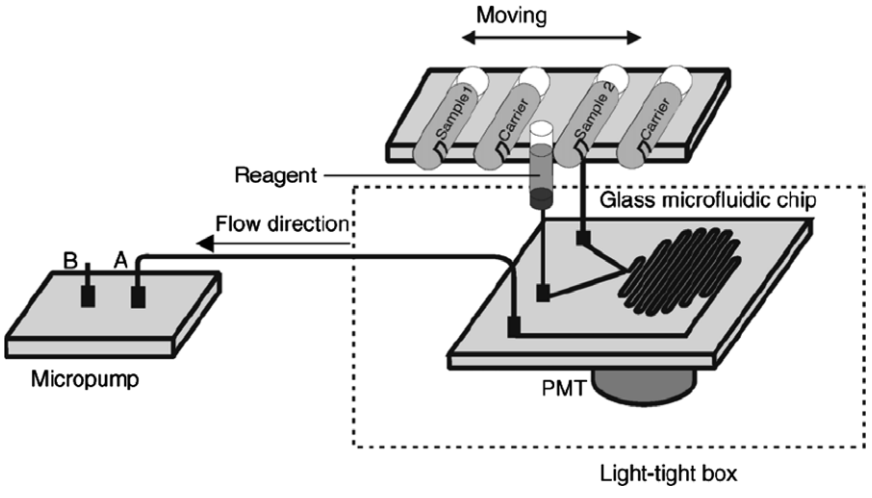

Figure 3. Microfluidic flow-injection chemiluminescence system (Reprinted with permission from [22]).

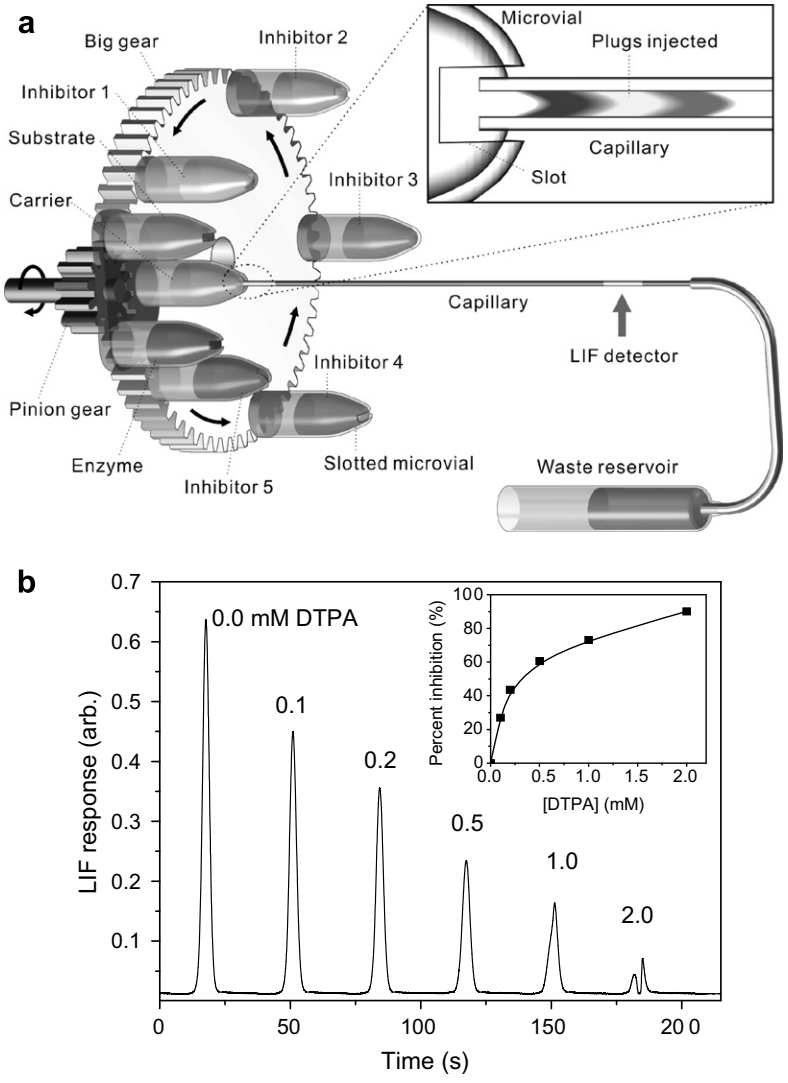

Figure 4. (a) μSIA system (not to scale); (b) typical recordings of enzyme-inhibition assays obtained by varying the inhibitor (DTPA) concentration while holding other conditions constant using a 6-cm long, 50-μm i.d. capillary with 5-cm reactor length, obtained by sequentially injecting 1 s of *β*-Gal (0.1 mg/mL with 83 μM $MgCl_2$), 1 s of DTPA (0–2.0 mM) and 1 s of FDG (100 μM); average flow rate, 4.2 nL/s (Reprinted with permission from [24]).

motor and two horizontally-fixed SVAs for samples and reagents. On-column detection was achieved using a laser-induced fluorescence detector with an excitation wavelength of 473 nm.

Sample and reagent zones were sequentially introduced by sweeping the capillary tip through the vial slots, while vials containing sample, reagent and carrier were sequentially rotated to the capillary by programmed movement of the vial holders. Sequentially-injected serial zones were rapidly mixed within the carrier flow in the capillary, transported through the capillary to the detection point and then to waste by the gravity drive. The mutual mixing between serial nL zones in a thin, straight capillary was based on mutual zone-penetration effects by diffusion and convection in pressure-driven flow, which supplied a different microfluidic mixing strategy from those employed in most microfluidic systems featuring the merging of laminar flows. Such serial-zone mixing behavior proved to conform well to the Taylor dispersion model, and the μSIA system was successfully characterized and optimized under Taylor's dispersion-theory guidelines, achieving good agreement between experimental results and theoretical calculations. Fluorescein was used as a model analyte to study the performance of the system. Both experimental results and theoretical calculations showed that, even with six serial zones, effective mutual mixing between zones could still be achieved in a 5-cm-long 75-μm-i.d. capillary provided the volume of each zone was reduced to less than 25 nL.

The potential of using the μSIA system in high-throughput drug discovery was demonstrated by

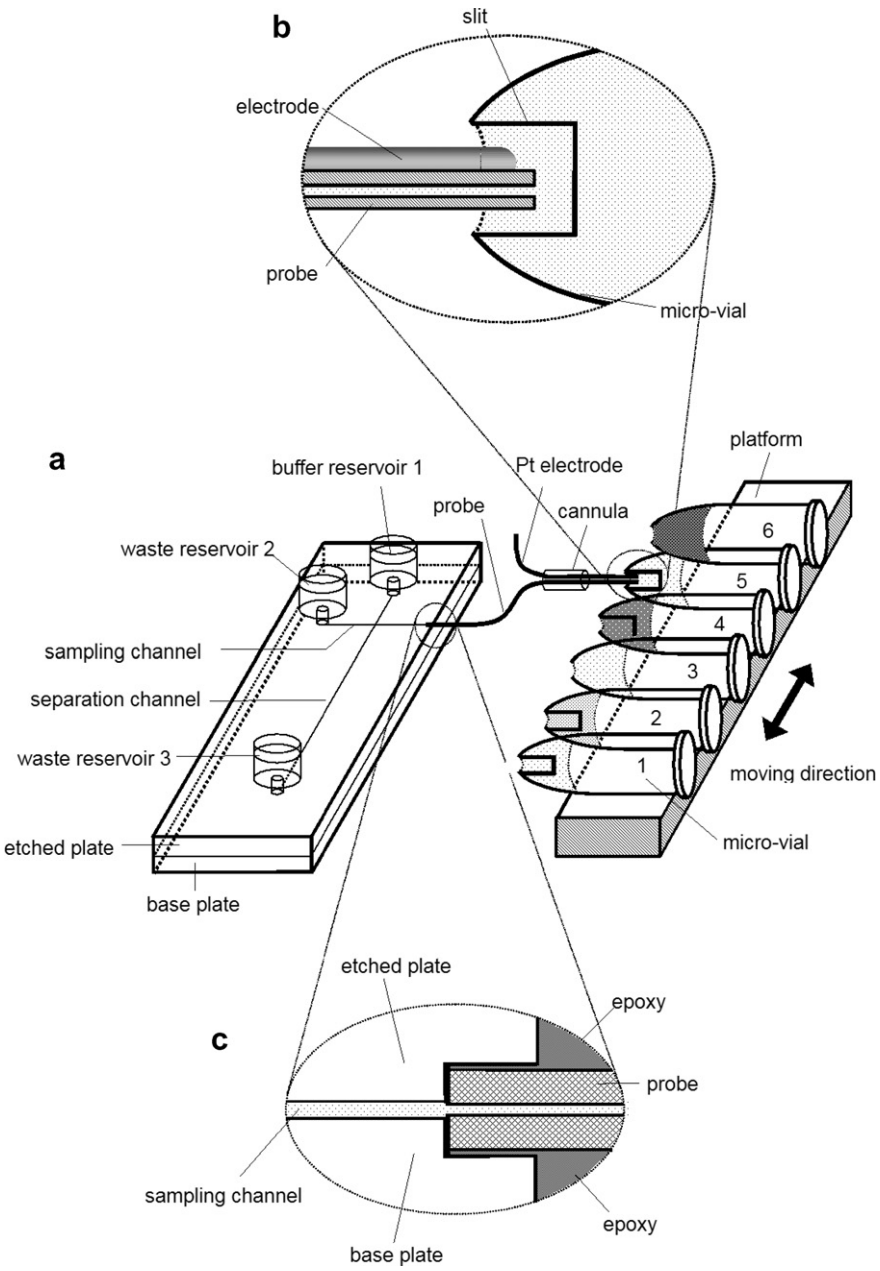

Figure 5. (a) Automated electrokinetic continuous sample-introduction system for microfluidic chip-based capillary electrophoresis (not to scale). Vials 1, 3 and 5 filled with buffer solution; vials 2, 4 and 6 filled with different samples; (b) SP and Pt electrode immersed in solution in the vial through the slot; and, (c) interface connecting SP and microchannel (Reprinted with permission from [13]).

employing the β-galactosidase enzyme-inhibition system as a model. The enzyme-inhibition assay was based on converting fluorescein digalactoside (substrate) to fluorescent hydrolysate via β-galactosidase (enzyme) and the inhibition of β-galactosidase by diethylenetriamine-pentaacetic acid (inhibitor). The SI enzyme-inhibition assay was performed by sequentially introducing sample and reagent solutions into the capillary in the order: carrier, enzyme, inhibitor sample 1, substrate, carrier, enzyme, inhibitor sample 2, substrate, etc. Fig. 4(b) displays the results of an enzyme-inhibition assay. An extremely low sample/reagent consumption of 4.2 nL was obtained with a maximum possible throughput of 300/h.

In this work, an approach for sequentially-introducing multiple solutions in the nL range into a microchannel was developed based on the SP-SVA strategy without resorting to expensive, complicated microfabrication techniques. Such an approach has great potential in bioanalysis involving precious samples and reagents, or for sequential introduction of multiple solutions.

4. Microfluidic CE

Microfluidic chip-based CE, as a main area of development of microfluidic chips, has undergone rapid development in recent years. Chip-based CE systems provide outstanding advantages, including extremely fast separations, low sample and reagent consumption, and potential for achieving high-throughput separations in multi-channel arrays. However, hitherto, in most chip-based CE systems, a discrete, manual approach to changing samples is still adopted, using on-chip reservoirs or a split-flow interface. This has lowered the overall throughput and counteracted the advantage of fast separations.

In 2005, we applied the SP-SVA strategy in chip-based CE systems to perform automated, continuous sample loading and changing [13]. The on-chip sample reservoir or split-flow interface on the CE chip was substituted by an on-chip sampling capillary probe and off-chip SVA system. Electrokinetic sample introduction was performed by using the on-chip probe to sample directly from the off-chip SVA system.

The on-chip SP was produced by horizontally connecting a Z-shaped fused-silica capillary (35-mm long, 50-μm i.d., 375-μm o.d.) to the inlet of the sample-loading channel on a glass CE chip with a crossed-channel design. The sample vials in the SVA system were filled alternately with samples and working electrolyte, horizontally positioned on a programmable linearly moving platform. The set-up is as shown in Fig. 5(a). Sample loading was performed by linearly moving the SVA system to immerse the SP and Pt electrode (Fig. 5(b)) in sample solution filling the sample vial, and applying voltages for pinched sample loading at the sample vial and three reservoirs. Sample injection and separation were then carried out under pinched injection mode. After sample injection, the SVA was moved, allowing the sampling probe and electrode to immerse into the buffer solution in the next vial to avoid cross-talk between neighboring samples. After one separation cycle was completed, a new sample vial was transferred to the SP position by moving the SVA system to achieve sample changing. In the system, a four-terminal, high-voltage power supply used for CE separation was enough to perform sample loading (i.e. introduction) and separation without an additional driving device for sample introduction, as in the continuous sample-introduction systems using a split-flow interface.

The performance of the system was demonstrated in separation and determination of FITC-labeled arginine and phenylalanine with LIF detection, by continuously introducing a train of different samples. An analytical throughput of 36/h was achieved with a sample-changing time of less than 1 s. Net sample consumption was 240 nL for each sample with <1.0% carry-over. Compared with chip-based systems using on-chip sample reservoirs, the sample solution in the vial is easy to recover for further use, as in non-destructive measurements.

However, in the above-mentioned work, the fabrication process for SP involved production of a guide-hole for connecting the capillary to the microchannel, sealing the interface between the capillary and the chip channel (Fig. 5(c)), and bending the capillary probe into a ''Z'' shape, which is relatively complicated, and there was usually a dead volume larger than 1 nL at the interface of capillary and microchannel. When EOF is used as driving force for flows in sample-introduction channel, gas bubbles may be formed and trapped at the interface of the capillary and microchannel, which may result in decrease or interruption of the EOF.

More recently, we developed a simple fabrication approach for producing monolithic SPs on glass chips [25]. A microfluidic chip with the monolithic probes was employed to substitute the chip with a connected capillary SP used in our previous work, and coupled to an SVA sample-introduction system for performing continuous sample-introduction in the chip-based CE system. The system set-up is as shown in Fig. 6(a). The sampling probes were fabricated using a rotating emery-tipped drill to grind the chip walls surrounding the sample-loading microchannel, forming a monolithic conical probe structure. This completely avoided introducing any dead volumes associated with connecting the chip with external probes, and was advantageous for minimizing carry-over between neighboring samples. Using the present fabrication approach, approximately 300-μm outer diameter for the probe tip was readily achieved, which is close to that of capillary SP (375 μm), and sufficiently thin to sweep through the slots of vials in SVAs.

In the system, on-chip horizontal tubular reservoirs containing working electrolyte and waste, instead of on-chip vertically fixed reservoirs, were used to maintain stable hydrostatic pressure in the chip channels during prolonged working periods. The horizontal tubular reservoirs were fixed on the side-walls of the chip at the same liquid level as that of the SVA containing sample or buffer to avoid hydrokinetic flows generated in the chip channels. Fluids were kept within the horizontal reservoirs and vials in the SVA by surface tension, the liquid levels being maintained constant while gradually being consumed or accumulated in the reservoirs or vials by EOF. With a horizontal reservoir capacity of 300 μL for working electrolyte and waste, non-interrupted working for several hours was feasible.

In the system, sample change was carried out with similar SVA system and procedure as those employed in the above-mentioned work [13]. The performance of the

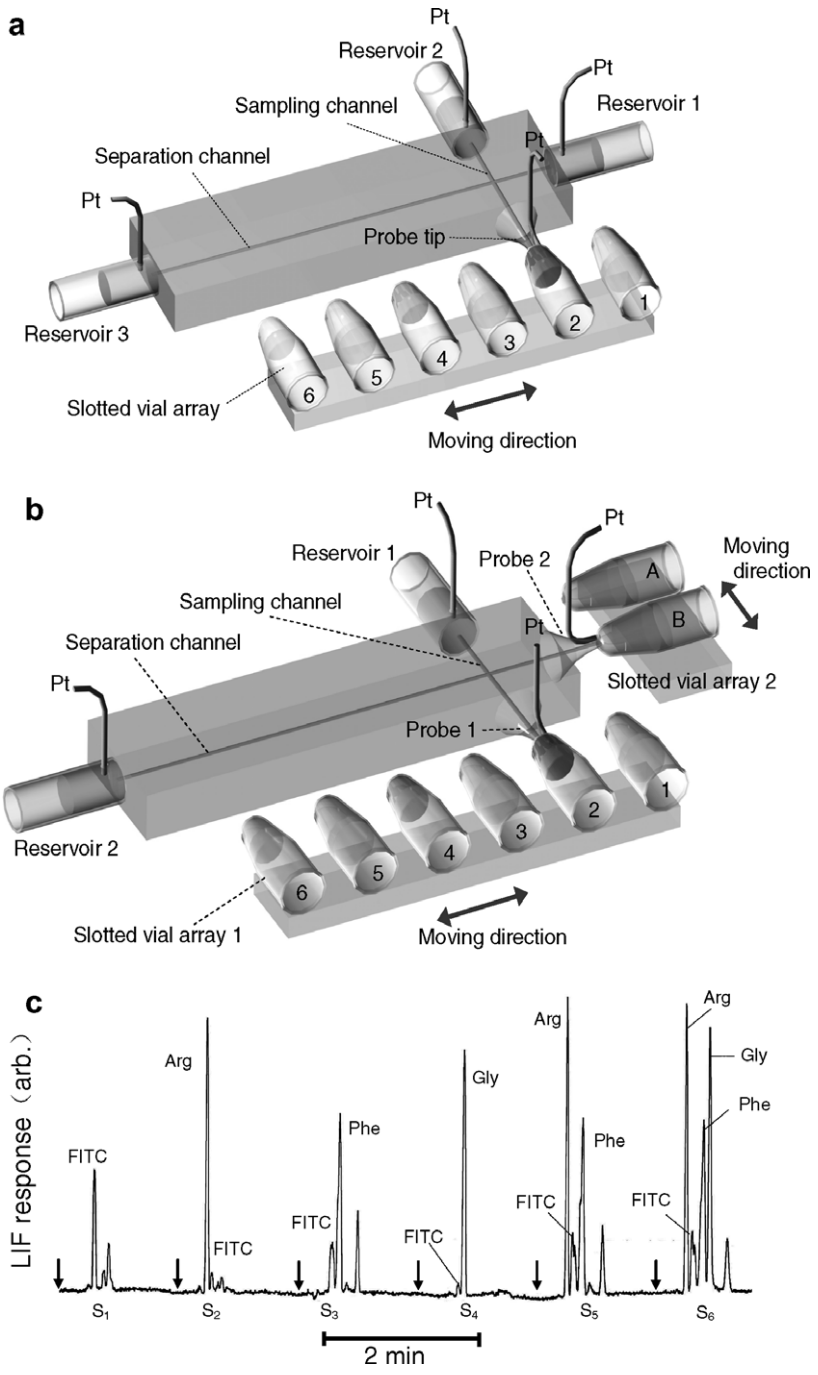

Figure 6. Microfluidic chip systems for CE with (a) electrokinetic continuous sample-introduction system, and (b) gradient chip-based CE separation system (not to scale). (a): slotted vial array (SVA) – vials 1, 3 and 5 were filled with the same buffer solution (working electrolyte), and vials 2, 4 and 6 with sample 1, 2 and 3, respectively; (b): SVA 1 – vials 1, 3 and 5 were filled with the same buffer solution (working electrolyte), and vials 2, 4 and 6 with sample 1, 2 and 3, respectively; SVA 2, vial A was filled with buffer A (10 mM sodium tertraborate-10 mM Tris, pH = 10.3) and vial B with buffer B (10 mM sodium tertraborate-10 mM Tris-10% ethanol solution, pH = 10.3), respectively; and, (c) electropherograms recorded by sequentially introducing S_1,1 μM FITC; S_2, 2 μM arginine-FITC; S_3,4 μM phenylalanine-FITC; S_4, 2 μM glycine-FITC; S_5, mixture of 2 μM arginine-FITC and 4 μM phenylalanine-FITC; S_6, mixture of 2 μM arginine-FITC, 4 μM phenylalanine-FITC and 2 μM glycine-FITC. Working electrolyte, 5 mM borate buffer; Employing 1.5 kV/cm sample loading field strength 10 s and 0.4 kV/cm separation field strength with pinched mode for 80 s; Effective separation length, 2.5 cm (Reprinted with permission from [25]).

system was demonstrated in separating FITC-labeled amino acids by continuously introducing a train of different samples without interruption. Throughputs of $30 \sim 60$/h were achieved with <1.0% carry-over (as shown in Fig. 6(c)) and reproducibilities in peak height of 3.6%, 3.3% and 3.5% RSD for FITC-arginine, FITC

and FITC-phenylalanine, respectively (n = 11). Extremely low sample consumption of 30 nL was obtained for each analysis.

In addition to the application in sample introduction, we also conducted some preliminary studies on using the monolithic sampling probe combined with SVA sample-introduction system to produce a working-electrolyte gradient in a chip-based CE system (as shown in Fig. 6(b)) to improve separation efficiency. In this system, beside the on-chip sampling probe, another on-chip probe was fabricated at the inlet tip of the separation channel, achieving composition change of the working electrolyte during the CE separation process. Step-gradient CE separation was readily achieved by sequentially introducing different working electrolytes contained in individual vials in the SVA into the separation channel on the run. Compared with isocratic electrophoresis separation, gradient CE demonstrated better separation efficiencies for a mixture of FITC-labeled amino acids.

In this work, the use of monolithic probes combined with the SVA sample/reagent-introduction system significantly simplified and improved the efficiency of solution introduction and changing with very low solution consumption and carry-over. Although the work reported here has been restricted to monolithic probes fabricated on glass chips, the solution-introduction strategy can be applied readily to polymer chips, on which monolithic probes could be fabricated more easily, such as cutting a thin chip into probe shapes using a razor blade or a pair of scissors.

As well as being applied in chip-based CE systems, the SVA system was also applied in a conventional, high-speed CE system with UV detection to perform automated sample injection and sample change [26]. In most of the reported automated, high-speed CE systems, a split-injection mode was employed, with the limitations of a complicated sample-introduction system and large sample consumption in the μL range [27,28]. The use of the SVA system significantly simplified the whole CE system (as shown in Fig. 7) and reduced sample consumption to the nL range. Similar to the above-mentioned systems [13,25], the SVA system used in this work comprised an SVA horizontally positioned on a programmable, linearly moving platform with samples and working electrolyte alternately filled in the vials. A silica-fused capillary (9-cm long, 50-μm i.d., 375-μm o.d.) horizontally positioned on a platform was coupled to the SVA system with the capillary inlet inserted into the working electrolyte solution filling the vial of the SVA system. The outlet of the capillary was inserted into a waste reservoir filled with the working electrolyte, which was made from a slotted vial horizontally positioned on the same level as the vials in the SVA system. Sample injection was performed by linearly moving the SVA system to immerse the capillary inlet and Pt electrode in the sample solution filling the sample vial, and applying voltage between sample and waste vials for a definite time for sample injection. Sample separation was then carried out under separation voltage by moving the SVA system, allowing the capillary inlet and electrode to immerse in the working electrolyte solution in the next vial. After one separation cycle, a new sample vial was moved to the capillary inlet to change sample. The system was applied in fast separation of sulphamethoxazole (SMZ) and trimethoprim (TMP) in sulphatrim tablets with an effective separation length of 6.5 cm, achieving a high throughput of 72/h. The sample-injection volume was 1.8 nL with 1.0 s injection time and 200 V/cm injection voltage. Separation efficiency of 11-μm plate height for SMZ was obtained. The LODs (3σ) for SMZ and TMP were 9.8 mg/L and 12.2mg/L, respectively.

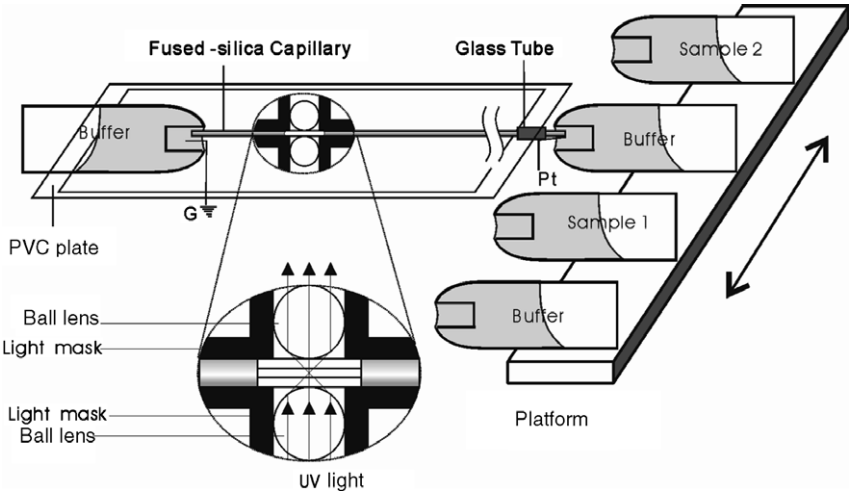

Figure 7. Miniaturized capillary electrophoresis system with UV detection [26].

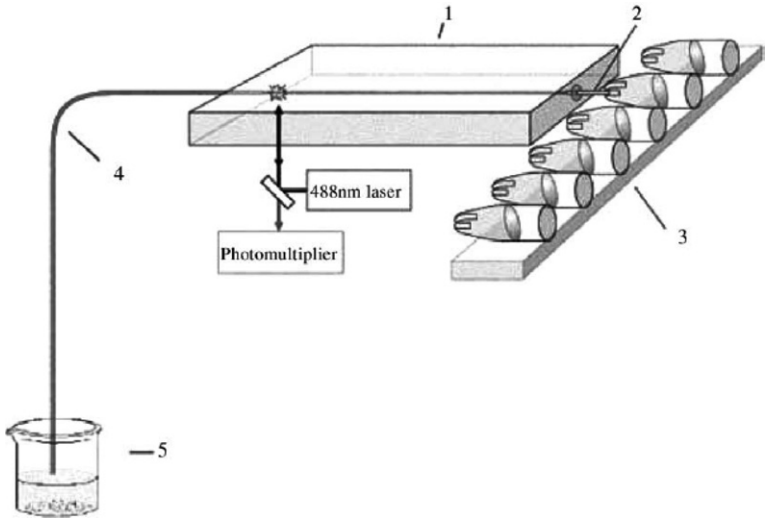

Figure 8. Set-up for chip-based micro flow-injection wetting film extraction (not to scale): (1) microfluidic chip made of polycarbonate; (2) fused-silica capillary sampling probe; (3) slotted vial array for organic solvent, blank and sample solutions; (4) fused-silica capillary for inducing hydrostatic pressure; and, (5) beaker for wastes (Reprinted with permission from [32]).

5. Microfluidic LLE systems

LLE is one of the most widely used sample-pretreatment techniques in analytical chemistry. However, the conventional LLE procedure is time-consuming and labor-intensive, and requires large amounts of toxic organic solvent. Although various novel LLE techniques [29–31] have been developed in recent years, automation of LLE still remains a challenge, especially in miniaturized LLE systems.

In 2005, Chen et al. [32] reported a microchip-based wetting film LLE system (as shown in Fig. 8) using an SVA system coupled with an on-chip SP for sequential introduction of aqueous sample solution and organic solvent into the extraction microchannel. A hydrophobic extraction channel was fabricated on a polycarbonate chip to support the wetting film of the organic solvent. A 1-cm-long fused-silica capillary (200-μm i.d. and 375-μm o.d.) was connected to the inlet of the channel serving as an SP. Hydrostatic pressure generated by the difference in liquid levels was employed to drive the fluids. In the extraction process, the organic solvent for coating, aqueous sample solution, washing water and organic solvent for elution were sequentially introduced into the extraction channel by linearly moving the SVA platform with introduction times of 120 s, 60 s, 2 s and 120 s, respectively. An organic solvent wetting film was first formed at the surface of the extraction channel, then analyte in the aqueous sample was extracted into the film and eluted by the eluting solvent. A LIF detector was employed to measure the concentration of analyte in the eluting solvent. Butyl rhodamine B was used as a model analyte and butanol as both coating and eluting solvent. Under optimized conditions, a 24-fold enrichment factor was obtained with a sample consumption of 3 μL. A good repeatability of 1.5% (RSD, n = 11) for the detected fluorescence signals was obtained.

More recently, we reported a chip-based, trapped-droplet-array LLE system (as shown in Fig. 9) with an SP-SVA sequential-introduction system and CL detection [33]. The microfabricated glass chip had a 35-mm-long extraction channel, with a 134-recess (L100 × W50 × D25 μm) array on both sides of the middle section of the channel. A 20-mm-long silica capillary was connected to the inlet of the channel serving as an SP. The slotted vials in the SVA system for holding sample and reagents were made from micropipettes with a slot fabricated at the tip end of each micropipette. The hydrostatic pressure generated by the difference of liquid levels between the sampling probe and the waste outlet was used for sequential delivery of sample and reagents. Ketonic peroxyoxalate ester solution was first introduced into the extraction channel, filling in the recesses and forming organic droplets. LLE and preconcentration were performed by continuously introducing the aqueous sample into the extraction channel, and analytes were transferred from the aqueous phase into the droplets through molecular diffusion. After that, catalyst and hydrogen peroxide solutions were introduced into the channel, and mixed with analytes and peroxyoxalate ester in the recess array to emit CL light. The performance of the system was tested using butyl rhodamine B as model sample, yielding precision of 4% RSD (n = 5) and an LOD of 10^{-9} M. Within a 17 min analytical cycle, the consumptions of sample and peroxyoxalate solutions were 2.7 μL and 160 nL, respectively.

In this system, the capability of the SP-SVA strategy in dealing with relatively complicated fluid manipulations

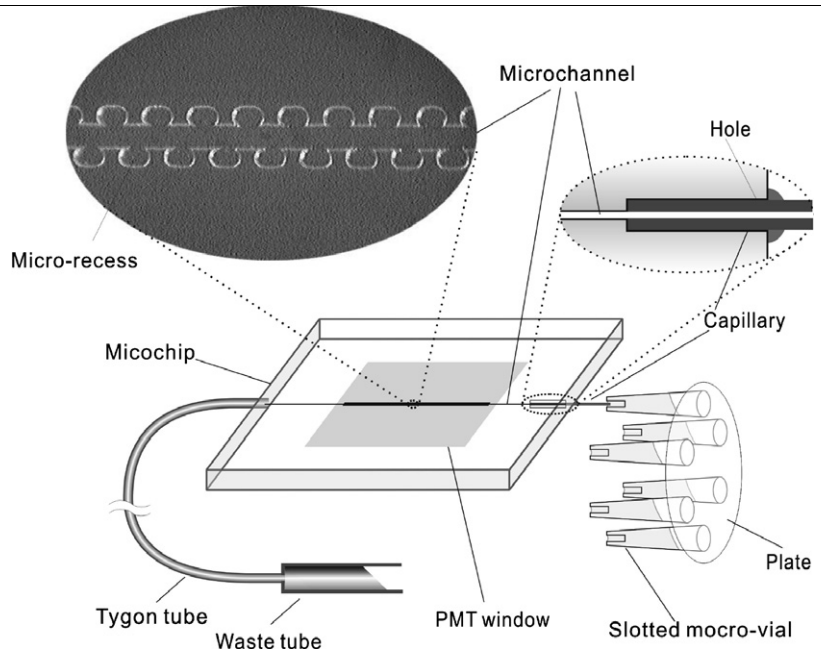

Figure 9. Microfluidic chip-based sequential-injection system with trapped-droplet liquid-liquid extraction (LLE) preconcentration and chemiluminescence (CL) detection (Reprinted with permission from [33]).

was further demonstrated through the enrichment process and subsequent CL detection. Such a mode of operation could readily be extended to other sample-pretreatment operations (e.g., solid-phase extraction).

6. Conclusions

We have shown SP-SVA to be a powerful approach for achieving automated, continuous sample introduction in microfluidic analysis systems, including FIA, SIA, CE and LLE systems. In conventional auto-samplers based on x-y-z stage, sample introduction is performed by moving the sampling probe or sample plate at least in two dimensions. In SP-SVA systems, a similar operation could be achieved by moving the sampling probe or SVA in one dimension. The use of slotted vials significantly facilitates moving the sampling probe among different vials, so sample introduction and change can be performed quickly and conveniently. Based on this mode, various high-throughput sample-introduction techniques in the nL–pL range could be developed.

A limitation of SP-SVA systems is that the number of vials is relatively fewer than that in auto-sampler systems coupled with 96-well or 384-well plates. This could be overcome by reducing the vial size and increasing the length of the SVA system to install more vials in the array, or using multiple SVAs in the sample-introduction system. In the above-mentioned works, no significant evaporation of the solutions filling the slotted vials was observed, since most of the analysis operation could be achieved in less than 30 min due to the high sampling throughput of the SVA systems. However, in a prolonged analysis process with large numbers of samples, the influence of solution evaporation should be considered, and effective means (e.g., installing the SVA system in a closed box with high humidity) should be adopted to suppress evaporation of the solutions filling the slotted vials.

As well as being useful in chip-based microfluidic systems, SP-SVA methodology has also proved to be quite useful in developing a novel type of microfluidic system (i.e. the capillary-based microfluidic analysis system, CBMAS). As a valuable complement to chip-based analysis systems, such a microfluidic system could be built based on a capillary without resorting to microfabrication techniques and equipment, while the analytical performances in sample and reagent consumption and analysis throughput are comparable to chip-based systems. CBMAS may contribute to extending the application of microfluidic analysis in real-world analysis, especially in bioanalysis involving precious samples and reagents, as well as in high-throughput screening.

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References

[1] T. Vilkner, D. Janasek, A. Manz, Anal. Chem. 76 (2004) 3373.
[2] P.S. Dittrich, K. Tachikawa, A. Manz, Anal. Chem. 78 (2006) 3887.
[3] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science (Washington, D.C.) 261 (1993) 895.
[4] S.C. Jacobson, R. Hergenroeder, L.B. Koutny, R.J. Warmack, J.M. Ramsey, Anal. Chem. 66 (1994) 1107.
[5] Y. Shi, P.C. Simpson, J.R. Scherer, D. Wexler, C. Skibola, M.T. Smith, R.A. Mathies, Anal. Chem. 71 (1999) 5354.
[6] http://www.chem.agilent.com/Scripts/PDS.asp?lPage51.
[7] S. Attiya, A.B. Jemere, T. Tang, G. Fitzpatrick, K. Seiler, N. Chiem, D.J. Harrison, Electrophoresis 22 (2001) 318.
[8] W.A. Ausserer, L. Bousse, S.J. Gallagher, C.B. Kennedy, H.L. Phan, JALA 6 (2001) 69.
[9] Y.-H. Lin, G.-B. Lee, C.-W. Li, G.-R. Huang, S.-H. Chen, J. Chromatogr., A 937 (2001) 115.
[10] Q. Fang, G-M. Xu, Z-L. Fang, Anal. Chem. 74 (2002) 1223.
[11] Q. Fang, Z-J. Jia, Z-L. Fang, in: Y. Baba, S. Shoji, A. van den Berg (Editors), Micro Total Analysis Systems 2002, Vol. 1, Kluwer, Dordrecht, The Netherlands, 2002, p. 685.
[12] http://www.caliperls.com/technology_partners/microfluidic/.
[13] Q.-H. He, Q. Fang, W.-B. Du, Z-L. Fang, Analyst (Cambridge, U.K.) 130 (2005) 1052.
[14] S. Ekstrom, P. Onnerfjord, J. Nilsson, M. Bengtsson, T. Laurell, G. Marko-Varga, Anal. Chem. 72 (2000) 286.
[15] Q. Fang, W.-B. Du, Q.-H. He, Z.-L. Fang, Chem. J. Chin. Univ. 25 (2004) 1442.
[16] W.-B. Du, Q. Fang, Q.-H. He, Z.-L. Fang, Anal. Chem. 77 (2005) 1330.
[17] R.N.C. Daykin, S.J. Haswell, Anal. Chim. Acta 313 (1995) 155.
[18] G.N. Doku, S.J. Haswell, Anal. Chim. Acta 382 (1999) 1.
[19] P.H. Petsul, G.M. Greenway, S.J. Haswell, Anal. Chim. Acta 428 (2001) 155.
[20] A.G. Hadd, S.C. Jacobson, J.M. Ramsey, Anal. Chem. 71 (1999) 5206.
[21] A.M. Leach, A.R. Wheeler, R.N. Zare, Anal. Chem. 75 (2003) 967.
[22] Y.-X. Guan, Z.-R. Xu, J. Dai, Z.-L. Fang, Talanta 68 (2006) 1384.
[23] J. Ruzicka, G.D. Marshall, Anal. Chim. Acta 237 (1990) 329.
[24] W.-B. Du, Q. Fang, Z.-L. Fang, Anal. Chem. 78 (2006) 6404.
[25] Q.-H. He, Q. Fang, W.-B. Du, Z.-L. Fang, Electrophoresis. 28 (2007) 2912.
[26] J. Liu, Q. Fang, W.-B. Du, Chin. J. Anal. Chem. 33 (2005) 1799.
[27] C.A. Monning, J.W. Jorgenson, Anal. Chem. 63 (1991) 802.
[28] Q. Fang, F.-R. Wang, S.-L. Wang, S.-S. Liu, S.-K. Xu, Z.-L. Fang, Anal. Chim. Acta 390 (1999) 27.
[29] H. Liu, P.K. Dasgupta, Anal. Chem. 68 (1996) 1817.
[30] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[31] M. Tokeshi, T. Minagawa, T. Kitamori, Anal. Chem. 72 (2000) 1711.
[32] Z.-X. Cai, H.-W. Chen, B. Chen, C.-B. Huang, Talanta 68 (2006) 895.
[33] H. Shen, Q. Fang, Z.-L. Fang, Lab Chip 6 (2006) 1387.