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Research Article

Fabrication of a monolithic sampling probe system for automated and continuous sample introduction in microchip-based CE

A fabrication process for producing monolithic sampling probes on glass chips, with tip diameters of a few hundred micrometers was developed, using simple tools including a glass cutter and a bench drill. Microfluidic chips with probes fabricated by this approach were coupled to a linearly moving slotted-vial array sample presentation system for performing continuous sample introduction in the chip-based CE system. On-chip horizontal tubular reservoirs containing working electrolyte and waste were used to maintain a stable hydrostatic pressure in the chip channels during prolonged working periods. The performance of the system was demonstrated in the separation of FITC-labeled amino acids with LIF detection, by continuously introducing a train of different samples without interruption. Throughputs of 30–60/h were achieved with <1.0% carry-over and reproducibilities in peak height of 3.6, 3.3, and 3.5% RSD for arginine, FITC, and phenylalanine, respectively ($n = 11$). Continuous analysis of a mixture of FITC-labeled amino acids for 2 h, involving 60 analytical cycles, yielded an RSD of 7.5 and 6.8% for arginine and FITC ($n = 60$), respectively. An extremely low sample consumption of 30 nL for each analysis was obtained. Separation efficiencies in plate numbers were in the range of $0.8–2 \times 10^5$/m. In addition to the application in sample introduction, the sample/reagent introduction system was also used to produce working electrolyte gradients during a CE separation to improve the separation efficiency. Comparing with isocratic electrophoresis separation, gradient CE demonstrated better separation efficiencies for a mixture of FITC-labeled amino acids.

Keywords:
CE / Microfluidic chip / Sample introduction

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1 Introduction

Chip-based microfluidic systems have attracted broad interest in recent years as a major form for realizing the lab-on-a-chip, or micrototal analysis system (μTAS) concept to achieve the integration of sample introduction, pretreatment, reaction, separation, and detection in a miniaturized analysis system [1–9]. For sample introduction, hitherto, in most of the reported chip-based systems, including CE systems, on-chip fabricated reservoirs connected with microchannels are often employed to contain the sample solution and perform sample introduction [10]. However, in these systems, the operation for sample change in reservoir was often carried out by manual approach, which is time-consuming and difficult to be automated. The efficient interface between the chip and the outside world is considered as a major challenge. Various approaches have been developed to achieve continuous and automated sample introduction. One strategy is to fabricate multiple sample reservoirs or analytical cells on a single chip to achieve different sample introduction among a series of samples [11–14]. However, the increase in the number of on-chip sample reservoirs is limited by the chip size and the sophistication of the peripheral equipment increasing proportionally with the number of reservoirs. Another strategy is characterized by employing a split-flow interface fabricated on the chip to achieve continuous sample introduction [15–21]. By the use of this type of interface, continuous and automated sample introduction with high sampling throughput could be achieved, while the sample consumption usually falls in the range of 10–100 μL.

Recently, a novel strategy employing an on-chip sampling probe that substituted the on-chip sample reservoir was proposed to achieve a continuous sample introduction [22–24]. The characteristic of this system was that the sam-

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pling probe was directly connected to the microchannel of the chip, sample introduction was realized simply by inserting the inlet of the sampling probe into off-chip sample vials. Zhang *et al.* [22] reported an approach for fabricating a sampling probe on a microchip that was coupled to MS detection. The procedure included, first fabricating a guiding channel on the chip for sample-loading capillary, then inserting a 1.5-cm long capillary probe into the guiding channel, and finally fixing with silicone sealant. Automated and continuous sample introduction was achieved by sequentially inserting the capillary probe into each microwell of the 96 well sample plates fixed on an *x-y-z* translation stage. More recently, we reported an efficient world-to-chip interface for chip-based CE separation by horizontally connecting a Z-shaped fused-silica capillary sampling probe to the sample-loading channel of a crossed-channel CE chip [23]. Electrokinetic sample introduction was performed by sampling from an off-chip programmed moving sample vial array with an extremely low sample consumption of 240 nL. However, the fabrication process for the sampling probe involved the production of a guide hole for the capillary in the outlet terminal of the microchannel, sealing of the interface between the capillary and the chip channel using epoxy with minimized dead volume, and bending the capillary probe into a "Z" shape to facilitate sampling operation. The procedure is relatively complicated, and the operator needs considerable training before satisfactory fabrication can be achieved. Although the epoxy-freezing approach was employed in the sealing operation [25], a dead volume larger than 1 nL still existed at the interface of the capillary and the microchannel. When electroosmosis flow was used as the driving force for flows in sample introduction channel, gas bubbles may be formed and trapped at the interface of the capillary and the microchannel, expanding with the increase in analysis time, which resulted in the decrease or interruption of the electroosmosis flow. Chen and Wang [24] reported a simple sample introduction approach for CE chips with single channel design. The inlet of the separation channel was fabricated with a diamond saw into a tip shape. Electrokinetic sample introduction and separation were carried out by manually inserting the channel inlet tip sequentially into the sample and the working electrolyte for 3 and 36 s, respectively. However, the probe fabricated using such an approach was sharp only from the top view, but could be as thick as 2 mm from the side (judging from the fabrication method used), which is difficult to be coupled with the slotted sample vial array presenting system that usually has a vial slot thickness lower than 2 mm [23].

In this work, a simple fabrication technique for monolithic sampling probes on chips with tip size in the range of several hundred microns was developed. On-chip monolithic sampling probes fabricated by this approach were easily coupled with the slotted-vial array presenting system to perform a continuous sample introduction. This fabrication technique also completely avoided introducing any dead volumes associated with the connection of the chip with external probes, and was advantageous for reducing carryover between neighboring samples to a minimum. In addition to the application in sample introduction, for the first time, the monolithic probe-based solution introduction system was used for achieving gradient CE separation. The performance of the systems was demonstrated in the separation and determination of FITC-labeled amino acids with LIF detection.

2 Materials and methods

2.1 Chemicals and reagents

All reagents used were of analytical reagent grade and demineralized water was used throughout. FITC was purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Arginine, L-lysine, L-glutamic acid, L-glycine, DL-alanine, and DL-β-phenylalanine were obtained from Kangda Amino Acid Works (Shanghai, China), and L-asparagine from Shanghai Third Reagent Works (Shanghai, China).

The stock solutions for each FITC-labeled amino acid (2.5 mM) were prepared as described elsewhere [19], and stored in a refrigerator at 4°C. Working sample solutions were prepared daily by diluting FITC-labeled amino acid stock solutions with working electrolyte.

5 mM sodium tertraborate buffer (pH 9.2) was used as working electrolyte in the testing experiments for sample introduction system. Buffer solution A containing 10 mM sodium tertraborate and 10 mM Tris (pH 10.3) and buffer solution B containing 10 mM sodium tertraborate, 10 mM Tris, and 10% ethanol (pH 10.3) were used as working electrolytes in the separation of the mixture solution of FITC-labeled amino acids, which contained 2 μM L-arginine, L-lysine, DL-β-phenylalanine, L-glycine, DL-alanine, L-glutamic acid, and L-asparagine.

2.2 Apparatus

A home-built programmable high-voltage power supply, with four electrode terminals, variable in the 0–6000 V range, was used for on-chip sample loading and CE separation. A home-built confocal microscope LIF system, modified from an optical microscope (XD-101-2B, Jiangnan Novel Optics, Nanjing, China), equipped with an ocular and a 473 nm diode laser (10 mW, New Industries Optoelectronics Technologies, Changchun, China) was used for observation and detection. The laser beam was reflected and focused to a 20-μm point on the channel from below the chip. The fluorescence intensity was detected by a photomultiplier tube (CR114, Hamamatsu, Beijing, China) and recorded using a model XWTD-164 chart recorder (Dahua Instruments, Shanghai, China). A bench drill (Xihu Bench Drilling Machine Manufactory, Hangzhou, China) was used to fabricate the probes on the chip.

2.3 Fabrication of the microchip

Standard photolithographic and wet chemical etching techniques were used for fabricating microchannels on glass microchip with a crossed-channel configuration, as described elsewhere [19]. Figure 1a shows a schematic presentation of the channel design implemented for the experiments. The separation channel (2–4) was 50 mm long, intersecting the 20 mm long sample-loading channel (1–3) at a distance of 10 mm from the channel inlet 2. The microchip dimension was 20 mm × 50 mm × 3.4 mm with channel depth of 15 μm and width of 70 μm. The room-temperature prebonding and high-temperature (550°C) thermal bonding procedures were employed to achieve the bonding of etched plate and cover plate [26].

2.4 Fabrication of on-chip sampling probe

Before fabrication, the inlet of the sampling channel (1–3) on the chip was preblocked using epoxy glue so as to prevent the blockage by glass particles produced during the fabrication process. The unsolidified epoxy glue was first drawn into the sampling channel for a distance of 0.5–1 mm based on capillary effect by contacting the inlet of sampling channel with the epoxy for 1 s, and then allowing it to solidify under 60°C for 30 min.

The fabrication process for on-chip sampling probe is as described in Fig. 1. A 2 mm × 5 mm × 3.4 mm rectangular probe was first produced at the terminal of the sampling channel by cutting the chip using a glass cutter with a diamond tip (as shown in Figs. 1b and b′). A 2-mm-diameter flat-tipped emery drill installed in the bench drill with a rotating speed of 2900 rpm was used in further fabrication of the probe (as shown in Fig. 2). The square probe was ground by the rotating sidewall of the drill first into a wedge shape (Fig. 1c′), then tetrahedron shape (Fig. 1d′), and finally conical shape with a tip diameter of *ca.* 300 μm (Figs. 1f and e′), keeping the sampling channel in the center of the probe. During the fabrication process, water was continuously added to the interface of the glass probe and emery drill not only to lubricate and cool the drill but also to make the channel in the sampling probe easily observable. The chips with two probes for sampling and working buffer were used in gradient CE separation of the mixture of FITC-labeled amino acids, and the chips with four tips were used for continuously changing each solution including samples, buffer solutions, and waste solutions in CE separation. All probes were fabricated using the same procedure as described above (Figs. 1g and h).

After the fabrication of probes was completed, the chip was immersed into concentrated sulfuric acid and heated to 70°C for 5 min, to remove the epoxy in the sampling channel.

The outer surface of each probe tip, except its 1-mm tip section (as shown in Fig. 1f′), was coated with a thin epoxy layer to form a relatively hydrophobic surface to avoid wetting of the solution on the probe surface, which may result in serious crosstalk between neighboring vials.

2.5 Fabrication of on-chip reservoirs and slotted vials

Figure 3 shows the microfluidic chip systems for continuous sample introduction and gradient CE separation. The on-chip buffer and waste reservoirs were produced from three 5.0 mm id, 15-mm long plastic tubes, which were horizontally affixed with epoxy on the sidewall of the chip, surrounding individual channel inlets. Two holes (*ca.* 1 mm diameter) were made on the wall of the reservoir, one used as an entry for Pt electrode and another as a vent hole during CE separation process respectively while the reservoir was covered at the outlet to prevent the inside solution from evaporating.

Slotted vials with a vial volume of 0.2 mL and slot dimension of 1.5-mm wide and 2-mm deep were produced as described elsewhere [27].

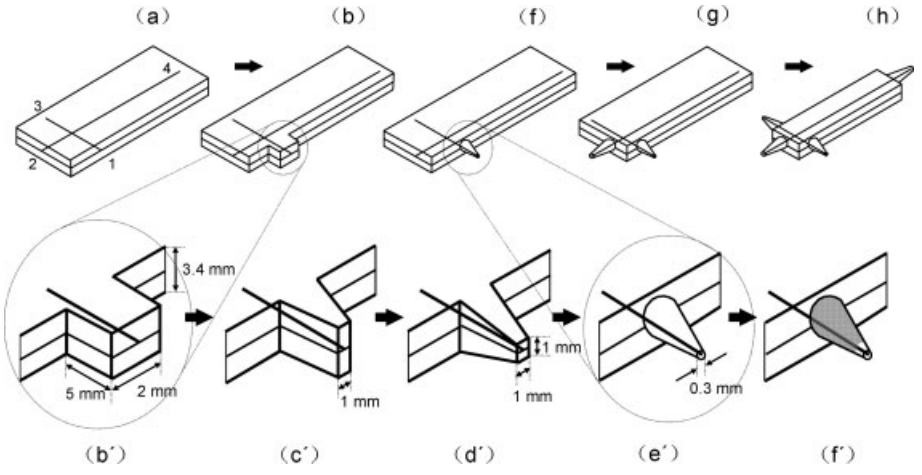

Figure 1. Schematic diagram of probe tips fabrication process (not to scale).

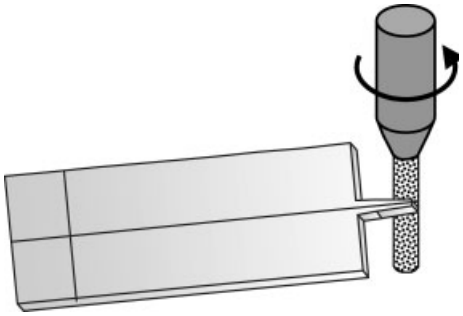

Figure 2. Schematic illustration on fabrication of the sharp probe tip.

2.6 Procedures

2.6.1 Continuous sample introduction

As shown in Fig. 3a, the automated continuous sample introduction system for microfluidic chip-based CE separation was composed of an on-chip sampling probe and a sample presentation system. The vials of the sample vial array were filled alternately with 20 μL of samples and 0.2 mL of 5 mM borate buffer solution, and fixed horizontally on the platform. Sample changing was performed by linearly moving the platform carrying the sample vial array in one direction, with the sampling probe and electrode sequentially immersing into different sample vials through the slot fabricated on the bottom of each sample vial.

Before use, the microchannels in the chip were sequentially flushed with 1 M NaOH, water, and 5 mM borate buffer, each for 15 min, and then filled with 5 mM borate buffer. The three reservoirs on the chip were filled with 0.25 mL of 5 mM borate buffer. Pt electrodes were inserted, respectively, through the holes of each of the three reservoirs into the buffer soultions filled in the reservoirs. The sampling probe attached with a Pt electrode was immersed through the vial slot, 1 mm into the buffer solution. Pinched injection mode was employed in chip-based CE separation. Sample loading was performed by linearly moving the array of vials to immerse the sampling probe and Pt electrode in the sample solution filled in the sample vial, and applying 3.0, 1.8, 1.8, and 0 kV voltages at sample vial, reservoirs 1, 3, and 2, respectively. Sample separation was then carried out by applying 2.0, 1.0, and 1.0 kV to reservoirs 1, 2, and sample vial, while reservoir 3 was maintained at ground. The vial array was moved 2 s after the initiation of sample separation, allowing the sampling probe and electrode to immerse into the 5 mM borate buffer solution filled in the next vial. The sample loading and separation times were 10 and 50–110 s, respectively. After one analysis cycle was completed, sample changing was performed by moving the vial-array to transfer a new sample vial to the sampling probe position. Detection signals were recorded by a chart recorder, and peak heights and half-peak widths were evaluated manually.

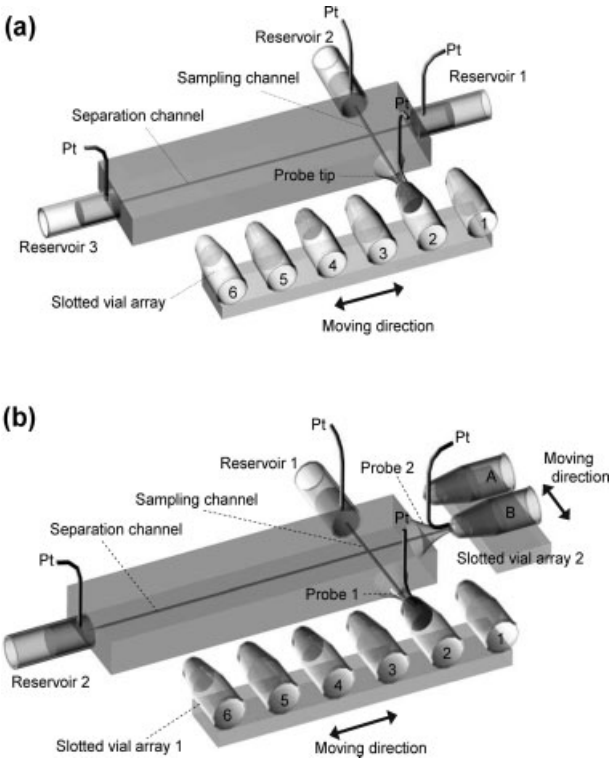

Figure 3. Schematic diagrams of the microfluidic chip systems for CE with (a) electrokinetic continuous sample introduction system and (b) gradient chip-based CE separation system (not to scale). (a) Slotted-vial array, vials 1, 3, and 5 were filled with the same buffer solution (working electrolyte), and vials 2, 4, and 6 with samples 1, 2, and 3, respectively; (b) slotted-vial array 1, vials 1, 3, and 5 were filled with the same buffer solution (working electrolyte), and vials 2, 4, and 6 with samples 1, 2, and 3, respectively; slotted-vial array 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.

2.6.2 Separation of the mixture of FITC-labeled amino acids with gradient working electrolyte

In the experiments, besides the on-chip sampling probe, another on-chip probe was fabricated at the position of reservoir 1 to achieve a change of composition of the working electrolyte during CE separation process with the aim of improving the separation efficiency. Two different working electrolytes, 0.2 mL of buffers A and B were filled, respectively, into individual slotted vials fixed horizontally on a platform (as shown in Fig. 3b). The microchannels in the chip were treated as the procedure described for continuous sample introduction experiments except that buffer A was employed instead of 5 mM borate buffer. Pinched injection approach was also used in CE separation for the mixture of FITC-labeled amino acids with the same high-voltage setting program as in above experiments. The probe for the introduction of working electrolyte was immersed in buffer A

during 40 s sample loading stage. Sample separation was subsequently carried out by sequentially immersing the buffer probe attached with electrode into buffer B for 90 s and buffer A for 150 s by linearly moving the vial array.

3 Results and discussion

3.1 Fabrication of on-chip monolithic sampling probe

In this work, the sampling probes were fabricated using a rotating emery-tipped drill to grind the chip walls surrounding the sample-loading microchannel to form a monolithic probe structure. Special care was taken during the fabrication of the monolithic probe to avoid blocking of the chip channel by glass powder produced in the grinding process. In this work, this was effectively achieved by first filling the channel terminal with epoxy, and subsequently removing it with hot concentrated sulfuric acid after producing the probe tip.

Another important factor in the fabrication of the monolithic probe is to avoid overgrinding the tip and longitudinally exposing the chip channel, while ensuring a sharp tip of a few hundred micrometers diameter, and simultaneously maintaining the channel outlet at the center of the tip. This required a good transparency of the glass substrate to facilitate observation during the fabrication process. However, the surface of the glass probe ground by the rotating drill gradually became opaque owing to light scattering from the coarse surface, posing difficulties for the direct observation of channel position within the probe. Under such conditions, the lubricating water flow described in Section 2 helped to significantly reduce light scatter, and the substrate became sufficiently transparent for direct monitoring of the channel position within the probe tip. The water flow also helped to cool the chip during grinding and avoided cracking.

Using the present fabrication approach, a probe with an outer tip diameter of *ca*. 300 μm was readily produced within 20 min. Such an outer diameter is close to that of common fused-silica capillaries (375 μm), and sufficiently thin to serve as a sampling probe capable of sweeping through the slots of microvials in the present system. Figure 4 shows the CCD image of the tips of a monolithic probe tip and a polymer-coated fused-silica capillary (75 μm id, 375 μm od). Actually, by using this fabrication process, a probe tip with an outer-diameter of 50 μm and a channel width of 10 μm had been successfully produced. Such thin monolithic probes were not necessary in the present work, but could be used to advantage for other applications, such as serving as a nanospray nozzle for chip-MS systems, or as a sampling probe for cells.

3.2 Horizontal tubular reservoirs

The objective of producing a microfluidic chip CE system capable of continuous sample introduction implies that the system should be able to deal with a large number of samples without the interruption of operations. However, for chip-based CE systems using stationary reservoirs vertically fixed on the chip, variations of liquid levels in the reservoirs are bound to occur during prolonged operations (*e.g.*, 30 min), leading to the generation of Poiseuille flows in the separation channel, which interferes in the electrophoresis process [28]. In this work, horizontal tubular reservoirs containing working electrolyte and waste, with a length of 15 mm and inner diameter of 5.0 mm, were used to maintain a stable hydrostatic pressure in the chip channels during prolonged working periods. A similar strategy was employed in our previously reported work [29, 30] to obtain stable flowrates for gravity-driven fluids during prolonged working periods. In the present system, the horizontal tubular reservoirs were fixed on the sidewalls of the chip at the same liquid level as that of the vial array containing sample/buffer or working electrolytes. Fluids were kept within the horizontal reservoirs by surface tension, the liquid levels maintained constant while gradually being consumed in the working electrolyte reservoir, or accumulated in the waste reservoirs by electroosmosis flows. With the electrophoresis conditions and reservoir capacity used in this work, uninterrupted working for several hours was feasible. Samples were continuously introduced and separated for 60 cycles during a period of 2 h, with a peak height RSD of less than 8%, without adjusting the liquid volume in the reservoirs.

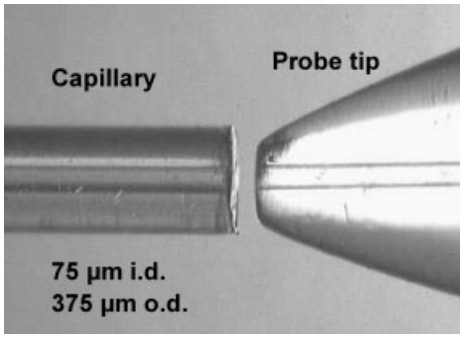

Figure 4. CCD image of the tip section of a monolithic probe and a polymer coated fused-silica capillary.

3.3 Performance in continuous sample introduction

The performance of the described system was demonstrated by separation of various mixtures of FITC-labeled amino acid samples by moving the microvial array to achieve a sample change. The throughput of the system was in the range of 30–60/h, with a sample loading time of 10 s and CE separation time of 50–110 s. Figure 5a shows an electropherogram of a series of samples with different compositions introduced sequentially at a throughput of 45/h. Compared to most of the chip-based CE systems employing reservoirs or flow-through sample introduction systems with sample consumption in the 10–100 μL range and sample changing time in the 10–100 s range, the sample consumption of the

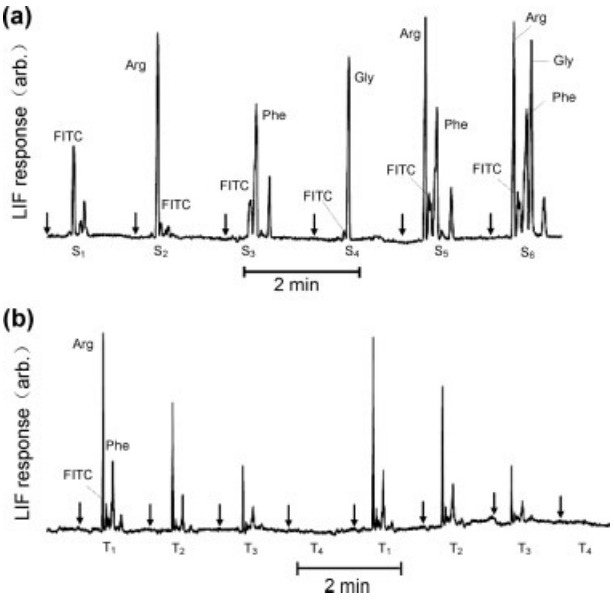

Figure 5. Electropherograms recorded by sequentially introducing (a) S_1, 1 μM FITC; S_2, 2 μM arginine-FITC; S_3, 4 μM phenylalanine-FITC; S_4, 2 μM glycine-FITC; S_5, mixture of 2 μM arginine-FITC and 4 μM phenylalanine-FITC; S_6, mixture of 2 μM arginine-FITC, 4 μM phenylalanine-FITC and 2 μM glycine-FITC; (b) T_1, mixture of 6 μM arginine-FITC and 6 μM phenylalanine-FITC; T_2, mixture of 4 μM arginine-FITC and 4 μM phenylalanine-FITC; T_3, mixture of 2 μM arginine-FITC and 2 μM phenylalanine-FITC; T_4, blank sample buffer solution for two cycles to show carry-over effects. 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.

present system was extremely low, *ca.* 30 nL, and the sample changing time was shortened to less than 0.5 s. To the best of our knowledge, this is the lowest level for sample consumption in the hitherto reported chip-based CE systems employing crossed-channel configuration and pinched injection. Another important feature of the present system is its capability of performing high-throughput sample changing under continuous operation with negligible cross-talk and carry-over. This was achieved by placing vials filled with blank buffer solution between neighboring sample vials in the vial array as described elsewhere [23, 27]. No evidence of cross-talk between the neighboring samples is observed in Fig. 5a. Figure 5b shows an electropherogram of two cycles of a sample series containing a mixture of 6.0, 4.0, and 2.0 μM of each FITC-labeled amino acids, followed by a blank at a throughput of 45/h. The carryover, estimated by measuring the peak height of the tallest peak, was less than 1.0%.

The reproducibility of the system was demonstrated by repeatedly injecting a mixture of 2 μM FITC-labeled arginine and phenylalanine 11 times, achieving peak height RSD values of 3.6, 3.3, and 3.5% for arginine, FITC, and phenylalanine, respectively. Samples were continuously introduced and separated for 60 cycles during a period of 2 h, with RSD for peak heights of 7.5 and 6.8% for arginine and FITC, respectively, without adjusting the liquid level in the reservoirs. Optimum separation efficiencies expressed in plate number were in the range of $0.8–2 \times 10^5$/m.

3.4 Separation of FITC-labeled amino acids with gradient working electrolyte

In most of the chip-based CEs, the type and concentration of working electrolytes were maintained unchanged during the separation process. However, when dealing with samples with complicated composition, it may be difficult to obtain a complete separation for all of the components in the sample by using a single working electrolyte. Kutter *et al.* [31] reported a solvent-programmed microchip with a mixing tee at one of the side arms of a crossed-channel, in which two different solvents were mixed. The solvent gradients were produced by adjusting the flow rate of the two EOF-driven streams controlled by a programmed high-voltage supply. The advantages of the approach are its integratable structure and simple operation. However, the mixing ratio of the two solvent streams could be affected by variations in EOF during CE separation process.

In this work, we conducted some preliminary studies on using the monolithic sampling probe combined with microvial array sample/reagent presentation to produce a working-electrolyte gradient in a chip-based CE system. This was readily achieved by sequentially introducing different working electrolytes contained in individual slotted vials into the separation channel on the run. The performance of this working-electrolyte gradient system was demonstrated in the separation of mixture of FITC-labeled amino acids, with results shown in Fig. 6 and Table 1. With an isocratic working electrolyte of buffer A in the absence of ethanol, the arginine and lysine were not baseline-separated. With an isocratic working electrolyte of buffer B containing ethanol, the resolution for arginine and lysine was significantly improved owing to the addition of ethanol, which reduced EOF in the separation channel, while the separation time was increased to 4 min and separation efficiencies deteriorated. With a gradient working electrolyte of buffer A and B, good resolution and separation efficiencies were simultaneously achieved (see Fig. 6b and Table 1).

4 Concluding remarks

The process for fabricating monolithic sampling probes on glass chips described in this work, albeit seemingly tedious and labor-intensive, proved to be mastered easily by relatively untrained personnel while achieving high yields. The use of monolithic probes combined with the slotted-vial array sample/reagent presentation system significantly simplified and improved the efficiency of solution introduction and changing with very low solution consumption and carry-over.

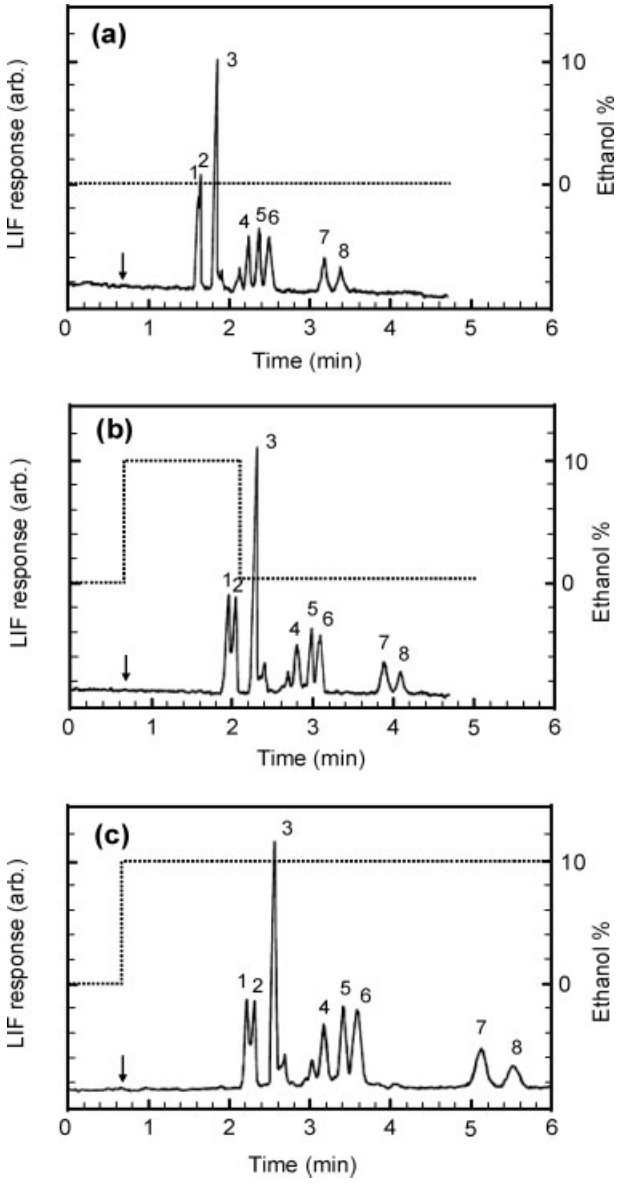

Figure 6. Electropherograms of isocratic and gradient CE separation for seven FITC-labeled amino acids. (a) Isocratic CE separation using buffer A (10 mM sodium tertraborate–10 mM Tris, pH 10.3); (b) gradient CE separation, using buffer B (10 mM sodium tertraborate–10 mM Tris–10% ethanol solution, pH 10.3) for 90 s, and buffer A for 150 s; (c) isocratic CE separation using buffer solution B. 1, L-arginine; 2, L-lysine; 3, FITC; 4, DL-β-phenylalanine; 5, L-glycine; 6, DL-alanine; 7, L-glutamic acid; 8, L-asparagine. Sample loading time, 40 s; other conditions as in Fig. 5.

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 much easier, such as cutting a thin chip into probe shapes using a razor blade or a pair of scissors.

Table 1. Separation performance under different working electrolyte conditions

	(a)[b]	(b)[b]	(c)[b]
R_S[a]	—[c]	1.3	1.3
N_{Arg} (m^{-1})[a]	—[c]	2.7×10^5	2.8×10^5
N_{Lys} (m^{-1})[a]	—[c]	2.7×10^5	2.9×10^5
N_{FITC} (m^{-1})[a]	4.9×10^5	3.0×10^5	3.0×10^5
N_{Glu} (m^{-1})[a]	4.0×10^5	3.7×10^5	1.8×10^5
N_{Asp} (m^{-1})[a]	4.1×10^5	3.8×10^5	1.7×10^5

a) R_S, resolution for L-arginine and L-lysine; N_{Arg}, N_{Lys}, N_{FITC}, N_{Glu}, and N_{Asp} are plate numbers *per* meter for L-arginine, L-lysine, FITC, L-glutamic acid, and L-asparagine, respectively.

b) Conditions for experiments (a), (b), and (c) are as shown in Figs. 6a, b, and c, respectively.

c) Under experimental condition (a), resolution and plate number for L-arginine and L-lysine could not be calculated because of peak overlapping.

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