An automated electrokinetic continuous sample introduction system for microfluidic chip-based capillary electrophoresis

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An automated and continuous sample introduction system for microfluidic chip-based capillary electrophoresis (CE) was developed in this work. An efficient world-to-chip interface for chip-based CE separation was produced by horizontally connecting a Z-shaped fused silica capillary sampling probe to the sample loading channel of a crossed-channel chip. The sample presentation system was composed of an array of bottom-slotted sample vials filled alternately with samples and working electrolyte, horizontally positioned on a programmable linearly moving platform. On moving the array from one vial to the next, and scanning the probe, which was fixed with a platinum electrode on its tip, through the slots of the vials, a series of samples, each followed by a flow of working electrolyte was continuously introduced electrokinetically from the off-chip vials into the sample loading channel of the chip. The performance of the system was demonstrated in the separation and determination of FITC-labeled arginine and phenylalanine with LIF detection, by continuously introducing a train of different samples. Employing 4.5 kV sampling voltage (1000 V cm^{-1} field strength) for 30 s and 1.8 kV separation voltage (400 V cm^{-1} field strength) for 70 s, throughputs of 36 h^{-1} were achieved with $<1.0\%$ carryover and 4.6, 3.2 and 4.0% RSD for arginine, FITC and phenylalanine, respectively ($n = 11$). Net sample consumption was only 240 nL for each sample.

Introduction

Since the first demonstrations of rapid capillary electrophoresis (CE) separations on microfabricated chips in the early nineties, microfluidic chip-based analysis has made great development in recent years.[1–3] Various microfluidic systems have been applied for the analysis of enzymes,[4,5] antibodies,[6,7] peptides[8,9] DNA,[10–12] and other biological samples.[13–15] The miniaturized version of CE significantly improves the analysis speed and sample/reagent consumption and potentially allows the automation and integration of the complete analytical system, including sample pretreatment.

Hitherto, in most chip-based capillary electrophoresis systems, much research interest has been focused on the innovation of efficient on-chip fluid manipulation and detection techniques, while batchwise and often manual approaches were adopted for achieving sample loading and sample change. Currently, on-chip fabricated reservoirs are used most often for containing sample and reagent solutions. With this broadly practised approach, a typical sample change requires that the sample reservoir be emptied, rinsed, and refilled with the new sample solution before further on-chip treatment, implying power interruption as well as temporal termination of the analytical process. Such time-consuming operations drastically lower the overall throughput and denude an important advantage of microfluidic systems in achieving high analytical speed.

Various approaches were developed to perform continuous and automated sample introduction and changing on chips. One strategy to achieve such goals is to dispense a series of samples to multiple sample reservoirs fabricated on a chip before analysis, and sequentially delivering the sample solutions in different reservoirs into a sample loading channel on the chip for further injection and separation.[16–19] With this approach, the sample dispensing is often operated manually, while the electrokinetic sample loading is achieved with a separate electrode and power terminal for each sample reservoir. Due to the complications in chip-design, as well as the sophistication of peripheral equipment increasing proportionally with the number of reservoirs, usually the number of on-chip sample reservoirs is limited to a maximum of 10–20. Although hundreds of reservoirs have been fabricated on a single chip with highly integrated channel arrays, these are reserved for different separation channels in highly efficient parallel analysis, and not for sample change using the same separation channel.[20] Another approach employed for continuous sample introduction is to sequentially introduce different sample solutions through a split-flow interface fabricated on the chip, where a small fraction of the sample was electrokinetically introduced into the sample loading channel of the chip for subsequent injection and CE separation.[21] Various interfacing designs have been developed based on this approach, mainly for achieving continuous sample introduction on chip-based CE systems. We reported a miniaturized CE system with an H-channel design to achieve continuous sample introduction by coupling a wide-bored sampling channel to a CE separation capillary,[22] in which pressure-driven flows in the capillary were insignificant during pumped sample loading and CE separations. Harrison's group reported a microfabricated chip-based CE system for achieving

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continuous sample introduction by fabricating a split-flow sample introduction interface on the chip.[23] A similar principle was adopted by exploiting the differences in volume flow resistance of the interfacing sampling channel and that of the sample loading channel of the CE chip for achieving on-chip continuous sample introduction. Pumped sample flow rates of up to 1 mL min^{-1} were used without perturbing the solutions within the separation channels. Later, Ausserer *et al.* adopted a similar approach for continuous sample introduction on a chip-based CE system for DNA separation, using vacuum to introduce samples into the sampling channel.[24] In addition to the large dimensional difference of the sampling channel and sample loading/separation channels, a viscous medium was filled into the separation channel that further increased the impedance against pressure-induced flows. Lin *et al.* employed a similar chip design to couple CE microchips with flow-through analyzers. A syringe pump was used to load the external sample into a large cross-sectional sampling channel, and electrokinetic gated injection was used to inject the sample in the sample loading channel into the separation channel.[25] Later, Lin *et al.* improved the chip configuration and demonstrated automated sample introduction for chip-based CE by connecting an autosampler to the inlet of the sample loading channel.[26]

Recently, we improved the performance of the split-flow interface using a flow-through sampling reservoir featuring a guided overflow design to substitute the sampling channel.[27] The system was designed to allow continuous flow-through of sample solutions without changing the liquid head above the separation channel, which may deteriorate the separation efficiency and stability of the CE system. Pressure driven flows in the sample loading channels were almost completely avoided. The performance of the system in achieving sample change with high frequency and low carryover was demonstrated in the separation and determination of FITC-labeled amino acids with LIF detection during continuous monitoring of the FITC derivatizing process over a period of 4 h, involving 166 analytical cycles. More recently, the flow-through reservoir design was modified by using a falling-drop approach to further reduce the dead volume of the split-flow sampling interface and to isolate the high-voltage system for CE separation from the external sample introduction system.[28]

In most of the above cited systems involving a split flow interfacing approach, the volumes of sample introduced through the sampling channel of the chips were in the 5–100 μL range, whereas the sample volumes introduced into sample loading channel of the chips were often only a few hundred nanoliters. Therefore sample utilization rates were usually very low. If automation of sample introduction were pursued, a further reduction of the utilization rate could occur owing to increase of the dead volume in transporting lines used for interrogating various designs of autosamplers, particularly when low carryover between neighboring samples is required.

In this work, a simple and robust microfluidic chip-based CE system capable of achieving automated sample introduction and change with extremely low sample consumption and high sample utilization rate was developed. Electrokinetic sample introduction from fixed sample reservoirs or sampling channels on CE chips, was substituted by sampling directly from an array of off-chip sample vials, and the on-chip sample reservoir was substituted by a short capillary sampling probe.

Experimental

Chemicals and reagents

All reagents were of analytical reagent grade and deionized water was used throughout. Fluorescein isothiocyanate (FITC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Arginine and D-, L-β-phenylalanine were obtained from Kangda Amino Acid Works (Shanghai, China). Stock solutions of each amino acid (10 mM) were prepared in water. The FITC-labeled amino acid stock solutions each with concentrations of 2.5 mM were prepared by mixing 250 μL of each stock solution with 100 μL FITC solution (40 mM in acetone) and 650 μL 10 mM sodium tetraborate buffer (pH 9.2). The reaction was carried out for 12 h in the dark. Labeled amino acids were stored in a refrigerator at 4 °C. Sodium tetraborate buffer (5 mM, pH 9.2) was used as working electrolyte for CE separation. Working sample solutions were prepared daily by diluting FITC-amino acid stock solutions with 5 mM borate buffer.

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 laser induced fluorescence (LIF) system, modified from an optical microscope (XD-101-2B, Jiangnan Optoelectrical Co., Nanjing, China), equipped with an ocular and a 473 nm diode laser (10 mW, Optoelectronic Technologies, Changchun, China) was used for observation and detection. The laser beam was reflected and focused to a 20-μm point on the channel recess from below the chip. The emitted light was collected by the same focusing system, and detected by a PMT (Hamamatsu, Beijing, China). The signal output from the detector was recorded using a model XWTD-164 chart recorder (Dahua Instruments. Shanghai, China).

Fabrication of the microchip

Photolithographic and wet chemical etching techniques were used for fabricating channels onto a 1.7-mm thick 2 × 6 cm glass plate with chromium and photoresist coating (AZ1805, Shaoguang Microelectronics Corp., Changsha, China). The chip design (as shown in Fig. 1) was transferred onto the glass plate following an UV exposure using a model JKG-2A photolithographic equipment (Photomechanical Co. Shanghai, China). The channels were etched into the plate in

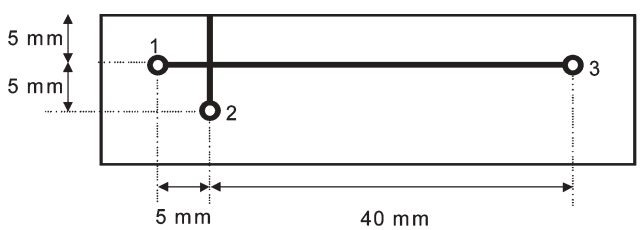

Fig. 1 Schematic figure of the microchip design.

a well-stirred, dilute HF/NH_4F bath with an etching time of 15 min. Three 1.2-mm-diameter access holes were drilled on the etched plate at channel terminals (as shown in Fig. 1) using a 1.2-mm-diameter diamond-tipped drill bit.

Microchips with a crossed-channel design were used for CE separations. Channel dimensions were 20 μm deep and 100 μm wide, with a 10-mm long sample loading channel, and 45-mm separation channel. An identical sized blank glass plate (SG2506, Shaoguang Microelectronics Corp., Changsha, China) was used as cover plate. Room temperature bonding of the etched and cover plates was achieved using a procedure detailed elsewhere.[29] In some studies, the bonded chip was subjected to a thermal treatment at 550 °C to achieve permanent bonding. The buffer and waste reservoirs were produced from a 9-mm-id, 10-mm-section plastic tube, cut from a 1.5 mL centrifuge tube, perpendicularly affixed with epoxy on the etched plate, surrounding the access hole.

A 35-mm long polymer coated fused-silica capillary (50-μm id, 375-μm od Reafine Chromatography Ltd., Yongnian, China), functioning as the sampling probe, was connected to the inlet of the sample introduction channel on the glass chip. Connection between the capillary and the chip channel was achieved mainly following a procedure described by Bings *et al.*[30] with some modifications. Briefly, the terminal of the channel on the edge of the glass chip was drilled 2 mm deep with a 400 μm diameter flat-tipped diamond-tip drill bit, the inlet terminal of the capillary was inserted into the hole, and the joint was sealed with epoxy. In order to minimize the dead volume at the interface of the capillary and chip channel, and also to avoid blocking of the channels by epoxy, an epoxy-freezing approach was employed in the sealing operation.[31] The epoxy components A and B were mixed and filled into the small gap between the hole and the capillary probe by capillary action until the epoxy nearly reached a point about 10 μm from the end of the capillary, observed under a microscope. The chip was immediately put into a refrigerator and maintained at −18 °C for 5 h to freeze the epoxy flow at this point. The epoxy was partially cured during this operation. The chip was then removed from the refrigerator and the epoxy was fully cured at room temperature for 6 h. The capillary sampling probe was bent into a Z-shape using pins to fix the capillary on a piece of plastic foam. The form of the bent capillary was then fixed with epoxy and the foam was removed (see Fig. 2). A platinum electrode (0.2 mm od, 10 mm long) was tightly bound to the probe by a thin plastic cannula.

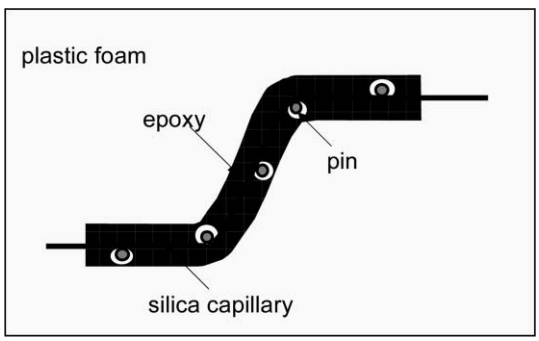

Fig. 2 Schematic illustration on fabrication of the Z-shaped capillary probe.

Sample introduction system

The automated sample introduction system was composed of a microchip with a capillary sampling probe and an array of micro-sample vials fixed on a computer controlled auto-sampler platform (as shown in Fig. 3). The array of micro-sample vials was produced mainly following a procedure detailed elsewhere.[32] Briefly, the sample vials were produced from 0.2 mL Microtubes (Porex, Petaluma) by fabricating 1.5-mm wide, 2-mm deep slots on the conical bottom of the tubes. The slotted sample vials were horizontally fixed on the platform in an array, with the slot of each vial positioned horizontally. The autosampler platform was modified from a chart recorder (LM14-164, Dahua Instruments, Shanghai, China), with a moving speed of 10 cm s^{-1} and spatial precision of 0.25 mm. The movement of the recorder was programmed by a computer.

Procedures

Before use, the conduits in the chip were sequentially flushed with 0.1 M NaOH, water, and 5 mM borate buffer, each for 15 min. The three reservoirs on the chip were filled with 500 μL 5 mM sodium tetraborate buffer, and platinum electrodes were inserted into the reservoirs. The microchip position was adjusted on the X–Y–Z stage of the microscope relative to the focus point of the laser beam to illuminate the detection point on the separation channel, and this position was kept unchanged during the analysis process of a series of samples.

The vials of the sample vial array were filled alternately with 10–20 μL samples and 0.2 mL buffer solution. Sample loading was performed by linearly moving the array of vials to immerse the probe and electrode in the sample solution of the vial under investigation. Voltages were then applied to the electrodes, with 4.5 kV at the sample vial, 2.5 kV at reservoirs 1 and 3, and zero voltage at reservoir 2 to achieve a pinched mode loading. Pinched sample injection and separation were then carried out by applying 1.8 kV to reservoir 1 and 0.8 kV to reservoir 2, while reservoirs 3 and sample vial were maintained at ground. The sample loading and separation times were 30 and 70 s, respectively. After sample injection, the vial array was shifted one vial position allowing the probe and electrode to transfer into the buffer solution in the next vial. After one analysis cycle was completed, sample changing was performed by moving 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.

Results and discussion

Design of the automated sample introduction system

The automated sample introduction system for microfluidic CE chips developed in this work was designed both to significantly reduce sample consumption and to achieve high sample throughput with low carryover for chip-based CE. These issues are as yet weaker links in world-to-chip interfacing, and are also challenges to be faced in serial treatment of large numbers of few microliter volume samples in real life. The design used in this work was adapted from the

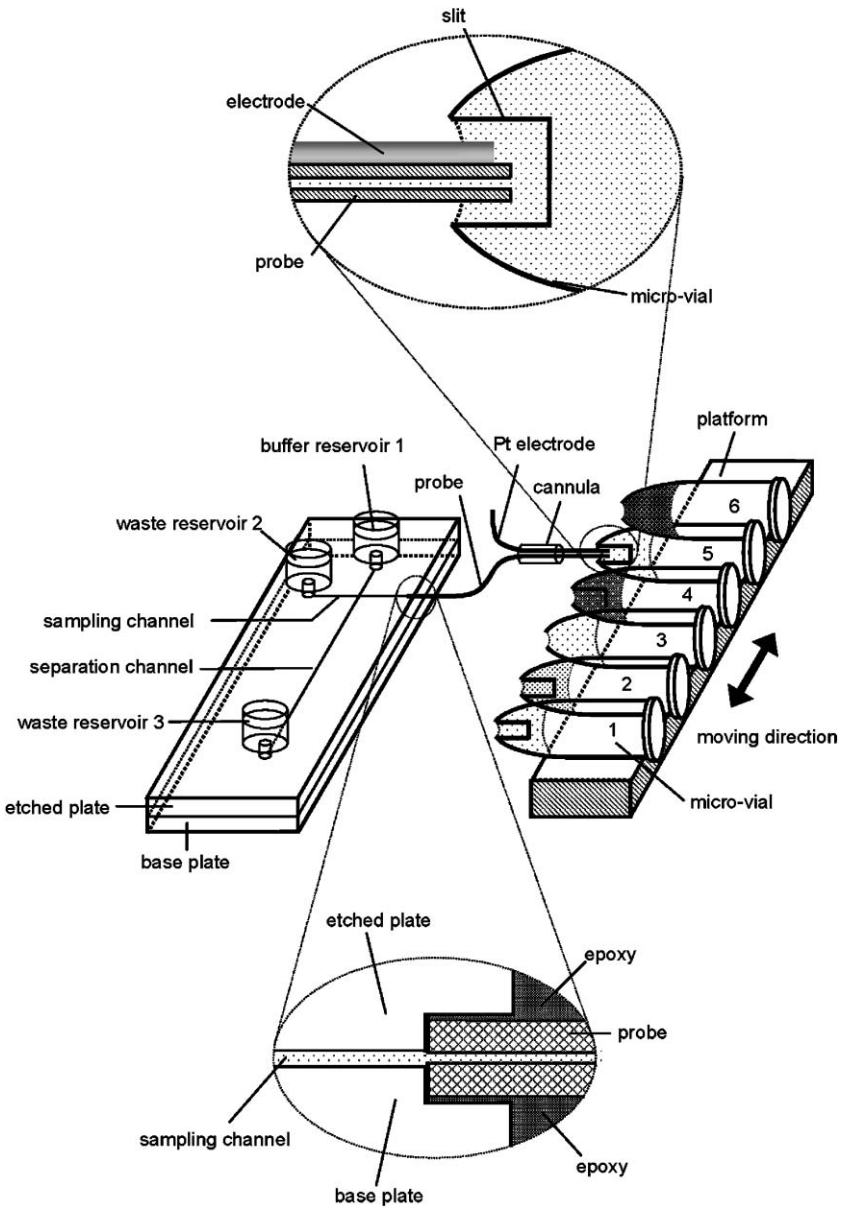

Fig. 3 Automated electrokinetic continuous sample introduction system for microfluidic chip-based capillary electrophoresis. (not to scale). Vials 1, 3 and 5: buffer solution, vials 2, 4 and 6: samples. See text for further details.

one we applied successfully for performing high throughput nanoliter flow injection analysis on a microfabricated chip with pressure driven flows,[32] mainly characterized by an on-chip integrated capillary sampling probe and an off-chip sample vial array, with horizontal linear movement under computer control. In this work the sampling probe-sample vial array combination was demonstrated to be equally effective under electrokinetically driven flows for chip-based CE systems. The design demonstrates significant advantages over split-flow sample introduction interfacing designs reported previously for achieving continuous sample introduction and sample changing in microfluidic CE systems, in being simpler, more robust and much more sample-economic.

Sampling probe. A 35 mm-long silica capillary sampling probe, connected to the sample loading channel of the chip, as described in the Experimental section, reached each sample vial, transferring samples directly from the vial into the sample loading channel. Sample loss through flow-splitting was thus avoided. In this work, even using a relatively large sample-loading channel width of 100 μm, sample consumption was reduced more than 95% (see Analytical performance section). The 4-terminal high-voltage power supply used for CE separation was used for sample introduction without additional driving device for sample introduction as in most other continuous sample introduction systems. Ausserer *et al.*[24] reported a sample introduction system with a capillary probe attached to a microfluidic chip for sampling from a 96-well plate. In their system, however, the sampling probe was attached perpendicularly to the plane of the chip for sampling from the well plate placed beneath the chip. Such a design produced a hydrostatic pressure induced by more than 2.5 cm

liquid level difference between the chip reservoirs and sampling probe inlet. Although this pressure had no evident effects on the CE separation of DNA samples in their work, owing to the use of a viscous sieving gel as separation matrix, it could pose problems in CE separation systems where working solutions of relatively low viscosities are used. Differences of liquid levels in the reservoirs of the chip could induce Poiseuille flows that deteriorate CE separations. To avoid such effects, in this work, the sampling probe was attached to the microchannel outlet at the side-wall of the chip, and shaped into a Z-configuration with a vertical distance of 8 mm between the probe inlet and the plane of the chip. The fluid levels in the reservoirs level were adjusted equal to that of the probe inlet, and were maintained almost constant during extended operations by using large-diameter (9 mm) reservoirs.

Using a fused silica capillary with polymer coating as the sampling probe, it was not possible to produce a permanent Z-shape without a suitable support. Initially, we burnt off the polymer coating, and bent the silica capillary by heating the bending points in a burner flame. This required considerable expertise, even then, the yield was low, and the probe was extremely fragile owing to removal of the polymer coating. To overcome these limitations, the capillary was pre-configured into a Z-shape on a board of plastic foam with the aid of pins, and subsequently fixed with epoxy as shown in Fig. 2. With this approach, the probes were easily produced with high yield and sufficiently robust to avoid accidental breakage and easy binding to a platinum electrode. The latter was required for electrokinetic delivery of samples from the vials into the chip channels. This mode of sample delivery is advantageous in that no extra driving devices were required apart from those normally used for chip-based CE separation, comparing favorably with the system described by Ausserer *et al.*,[24] where a vacuum source was required for sample aspiration.

Automated sample presentation system. In most reported chip-based CE systems, including those employing continuous sample introduction interfacing, the sample presentation issue has rarely been addressed, the operations usually being performed manually by moving the inlet tip of the sample inlet tube from one sample vessel to the next with intermediate rinses to minimize carryover. Such operations restrict further increase of the sampling throughput and application of the system in routine analysis, especially when dealing with large numbers of samples. Ausserer *et al.* reported the use of a commercial auto-sampler in connection with a chip-based CE system for achieving automated sample presentation.[24] Although effective, the approach also implies significant additional cost as well as a departure from the general aim for miniaturization of equipment.

In this work, the sample presentation system was composed of an array of bottom-slotted sample vials, horizontally positioned on a linearly moving platform. This approach, which has been applied successfully for sample presentation in a chip-based flow injection system under pressure driven flows,[32] was adapted to work with chip-based CE in this work. Automated sample change was performed by linearly moving the platform carrying the sample vial array in one direction, with the sampling probe sequentially entering into different sample vials through the slot fabricated on the bottom of each sample vial (see Fig. 3).

The sample vials in the array were fixed horizontally on the platform to maintain constant hydrostatic pressure in the chip channels during the entire sample loading/injection/separation process. Fluids were kept within the horizontal vials by surface tension, and the liquid levels remained unchanged while sample or blank buffer solutions in the vials were electrokinetically delivered into the chip channel during the sample loading or separation stages, respectively.

In the vial array, vials filled with blank solution placed between neighboring sample vials were used for reducing carryover. With the present system, the liquid volume carried over to neighboring vials was about 0.05 μL. This was mainly produced by retention of the solution from the previous vial within the small gap between the sampling probe and the electrode through surface tension. The retained solution was subsequently carried into the next vial by the probe, and created a carryover of about 0.3%. In this work, the carryover between neighboring sample vials was reduced to $<0.1\%$ by inserting a vial containing blank working electrolyte solution between samples (*i.e.* with the array arranged in a sequence of sample-blank-sample). Dilution of the sample solution by working electrolyte back flow following pinched sample injection could also be avoided with such a sample vial array, by moving the probe from the sample vial into the blank immediately after sample injection. The integrity of the used sample is thus preserved for other purposes.

Effects of sample loading voltage and time

In the present system, samples were introduced electrokinetically from their respective vials into the sample loading channel of a crossed-channel chip. Both the applied loading voltages and loading time are important for obtaining a non-biased sample composition at the injection crossing of the channels. Firstly, the effects of sample loading voltage applied between the sample vial and reservoir 2 (with reservoirs 1 and 3 kept floating), was studied by alternately introducing 2 μM FITC solution and 5 mM borate buffer into the loading channel with loading voltages in the range 1.5–5 kV, and fixing the LIF detection point on the crossing of the separation and sample loading channel to monitor the sample loading process. The experimental results showed that sample loading time decreased with the increase of sample loading voltage, however, gas bubbles tend to form at the junction between the loading channel and the probe capillary when the voltage exceeded 5.0 kV, owing to Joule heating effects. In order to increase sampling throughput as far as possible, a relatively high sample loading voltage of 4.5 kV (1 kV cm^{-1} field strength) was adopted in this work.

Further studies on the effects of sampling time on carryover were conducted with this sample loading voltage applied to the sample vials, 2.5 kV pinch voltages applied to reservoirs 1 and 3, and reservoir 2 grounded. A sample series containing 2.4, 1.2, and 0 μM FITC was introduced, and subjected to CE separation. The results in Fig. 4 show that a sampling time of 25 s was sufficient to ensure $<3.0\%$ carryover for the FITC dye. Since the mobility of phenylalanine-FITC, the slowest

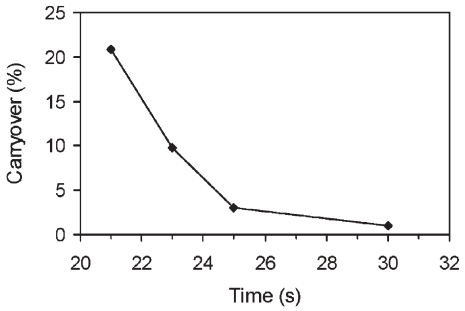

Fig. 4 Effects of sampling time (21, 23, 25, and 30 s) on carryover in chip-based CE separation, obtained by continuously introducing 2.4, 1.2 and 0 μM FITC with working electrolyte in-between. Working electrolyte, 5 mM borate buffer; applied field strengths for pinched mode sample loading and CE separation, 1000 and 400 V cm^{-1}, respectively; effective separation length, 2.5 cm.

moving analyte in this study, was not much lower than FITC, this sampling time of 25 s was found to be also sufficient for all other analytes studied, and 30 s sampling time was employed for most studies in this work.

Analytical performance of the system

The performance of the system was demonstrated in the separation and LIF determination of FITC-labeled amino acids, arginine and phenylalanine. The reproducibility of the system was tested by repeatedly injecting a mixture of 2 μM FITC-labeled arginine and phenylalanine (as shown in Fig. 5), achieving peak height RSD's of 4.6, 3.2 and 4.0% for arginine, FITC and phenylalanine, respectively (n = 11).

An important feature of the present system is its capability of continuously performing automated sample changing with negligible carryover. Fig. 6a shows an electropherogram of a series of samples with different compositions introduced sequentially at a throughput of 36 h^{-1}. No observable cross-talk occurred between sequentially introduced neighboring samples. Fig. 6b shows an electropherogram of two cycles of a sample series containing 2.0, 1.0 μM FITC-labeled amino acids, followed by a blank, introduced at a throughput of 36 h^{-1}. The estimated carryover was less than 1.0%. The sample consumed during sample change was 240 nL, which is lower than most previously reported chip-based CE systems.

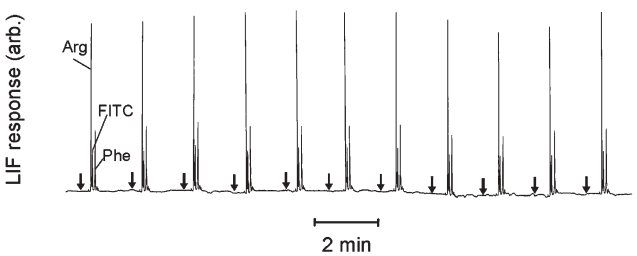

Fig. 5 Electropherograms recorded by continuous introduction of 2 μM FITC-labeled amino acid mixtures, with working electrolyte in-between, for 11 cycles to show reproducibility. Sampling frequency, 36 h^{-1}. Other conditions as in Fig. 4.

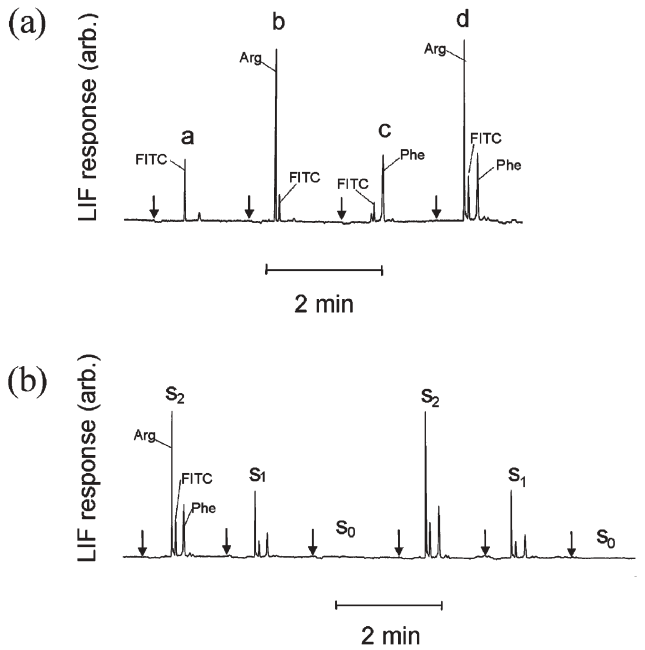

Fig. 6 Electropherograms recorded by sequentially introducing (a) 1 μM FITC, 2 μM arginine-FITC, 2 μM phenylalanine-FITC, and mixture of 2 μM arginine-FITC and phenylalanine-FITC (a, b, c, and d, respectively) to show cross-talk effects; (b) 2 μM, 1 μM FITC-labeled amino acid mixtures and a blank sample buffer solution for two cycles (S_2, S_1 and S_0, respectively) to show carryover effects. Employing 1000 V cm^{-1} sampling field strength 30 s and 400 V cm^{-1} separation field strength. Other conditions as in Fig. 4.

Conclusions

The present system proved to be an efficient, sample-economic and robust means for achieving automated sample changing and introduction for chip-based CE systems. Sub-microliter sample consumption was achieved without requirement of additional fluid drives for sample introduction. This system should prove to be particularly useful for CE separation dealing with large numbers of valuable samples. The sample composition is not changed during the analysis, and virtually all of the sample may be recovered for further use as in nondestructive measurements. The sample consumption of the system could be reduced further by decreasing the sampling probe length and inner diameter, and sample consumption of a few tens of nanoliters is foreseeable.

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