*Anal. Chem.* **2009,** *81,* 3693–3698

# Microfluidic Picoliter-Scale Translational Spontaneous Sample Introduction for High-Speed Capillary Electrophoresis

**Ting Zhang, Qun Fang,* Wen-Bin Du,† and Jing-Lin Fu‡**

*Institute of Microanalytical Systems, Zhejiang University, Hangzhou, 310058, China*

**A novel microfluidic picoliter-scale sample introduction approach was developed by combining the spontaneous injection approach with a capillary electrophoresis (CE) system based on a short capillary and slotted-vial array. A droplet splitting phenomenon at the capillary inlet end during the spontaneous sample introduction process was observed for the first time. On the basis of this phenomenon, a translational spontaneous injection approach was established to reduce sample injection volumes to the sub-100 pL range. A versatile high-speed capillary electrophoresis (HSCE) system was built on the basis of this sample injection approach with separation performance comparable to or even better than those reported in microfluidic chip-based CE systems. The HSCE system was composed of a short fused-silica capillary and an automated sample introduction system with slotted sample and buffer reservoirs and a computer-programmed translational stage. The translational spontaneous sample injection was performed by linearly moving the stage, allowing the capillary inlet first to enter the sample solution and then removing it. A droplet was left at the tip end and spontaneously drawn into the capillary by surface tension effect to achieve sample injection. The stage was continuously moved to allow the capillary inlet to be immersed into the buffer solution, and CE separation was performed by applying a high voltage between the buffer and waste reservoirs. With the use of the novel system, high-speed and efficient capillary zone electrophoresis (CZE) separation of a mixture of five fluorescein isothiocyanate (FITC) labeled amino acids was achieved within 5.4 s in a short capillary with a separation length of 15 mm, reaching separation efficiencies up to 0.40 $\mu$m plate height. Outstanding peak height precisions ranging from 1.2% to 3.7% RSD were achieved in 51 consecutive separations. By extension of the separation length to 50 mm, both high-speed and high-resolution CZE separation of eight FITC-labeled amino acids could be obtained in less than 21 s with theoretical plates ranging from 163 000 to 251 000 (corresponding to 0.31−0.20 $\mu$m plate heights). The present HSCE system also allowed fast chiral separations of FITC-labeled amino acids under micellar electrokinetic chromatography (MEKC) mode within 6.5 s.**

Since the landmark work by Jorgenson and Lukacs in the early 1980s,[1,2] capillary electrophoresis (CE) has become a powerful and versatile analysis technique, combining the advantages of gel electrophoresis with those of high-performance liquid chromatography. Currently, CE has been widely applied in analytical and biological research, such as separations of amino acids, peptides, proteins, DNA and RNA fragments, small molecules, single cell analysis, and chiral separation.[3−6]

In conventional CE systems, separation is usually performed in capillaries with a separation length of 20−100 cm, electric separation field lower than 500 V/cm, and sample volume in the range of 1−10 nL. Excellent separation efficiencies (>100 000 plates) can be obtained in less than 30 min. Since the 1990s, various high speed capillary electrophoresis (HSCE) systems[7−16] featuring the use of short separation lengths (<15 cm) and high separation voltage (>500 V/cm) were developed, aiming to obtain both high speed (<100 s) and high efficiency (<1 $\mu$m plate height) separations. As separation lengths are reduced to only a few centimeters to achieve high speed separation, narrow sample plugs less than 100 $\mu$m (corresponding to picoliter scale plug volume) are usually required to ensure sufficient high separation efficiency. Various picoliter-scale sample injection approaches, including optical-gating injection,[7,8] flow-gating injection,[9,10] and

* To whom correspondence should be addressed. E-mail: fangqun@zju.edu.cn. Phone: +86-571-8820 6771. Fax: +86-571-8827 3572.

† Present address: Department of Chemistry, University of Chicago, Chicago, IL 60637.

‡ Present address: Center for BioOptical Nanotechnology, Biodesign Institute, Arizona State University, Tempe, AZ 85287.

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10.1021/ac900573x CCC: $40.75 
Published on Web 04/07/2009

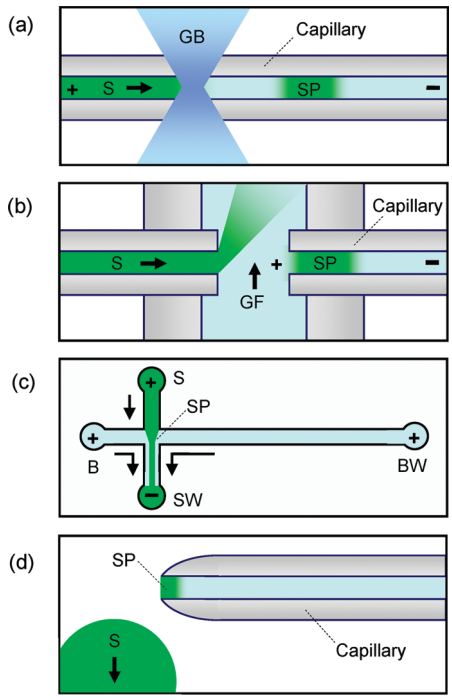


**Figure 1.** Schematic diagrams of four typical sample introduction approaches for HSCE systems: (a) optical-gating injection; (b) flow-gating injection; (c) pinched injection on a microfluidic chip with a cross channel configuration; and (d) microfluidic translational spontaneous injection. S, sample; SW, sample waste; B, buffer; BW, buffer waste; GB, gating beam; SP, sample plug; GF, gating flow; +, anode; −, cathode. The arrows indicate the direction of the flow.

microchip-based injection[13–16] have been developed for HSCE systems.

In 1991, Jorgenson's group developed the optical-gating injection technique for HSCE systems.[7,8] Under this injection mode (Figure 1a), an intense laser was used to create a narrow sample plug (usually <100 $\mu$m) in the capillary by photodegrading the fluorescent analytes. Both high-speed (<100 s) and high-efficiency (<1 $\mu$m plate height) separations were obtained using this approach. However, it could only be applied for photodegradable fluorescent analytes with fluorescence detectors. Later, the same group proposed the flow-gating injection technique,[9,10] which used the switching of sample and buffer flows to obtain a narrow sample plug for HSCE separation (Figure 1b). Compared with optical-gating injection, flow-gating injection has advantages of simple structure, ease of building, and versatility for different samples and detectors. However, both the above-mentioned approaches suffered from the sampling bias effect of electrokinetic injection.[11]

Since the pioneer work reported by Manz et al. in 1992,[13] HSCE separations have been achieved in microfluidic chips. As shown in Figure 1c, micrometer-scale sample plugs could be obtained within the microchannel network under precisely programmed high-voltage control, which enabled a high-speed and high-efficiency CE separation on microfluidic chips by using short separation lengths and high electric field strengths.[14–16] However, the fabrication of microchips usually requires expensive equipments and complicated operation, which may limit their broad application in routine analysis.

In conventional CE systems, nanoliter-scale sample injection approaches, such as electrokinetic injection[1,2] and hydrodynamic injection,[17] are frequently used. However, such injection approaches are difficult to achieve picoliter-scale sample injection as in HSCE systems, due to the existence of spontaneous injection phenomenon during the injection process. This phenomenon was first reported by Zare's group.[18] When a capillary inlet is removed from a sample solution after normal electrokinetic or hydrodynamic injection process, a sample droplet is left at the inlet end of the capillary and drawn into the capillary channel by surface tension effect, which results in an additional and unintentional sample plug of 1 mm long (~4 nL).[18] This phenomenon deteriorated the quantification accuracy and separation efficiency of CE systems using electrokinetic or hydrodynamic injection approaches. Therefore, efforts were made to suppress its influence.[18,19] In 1994, based on this phenomenon, Zare's group developed a novel sample introduction technique for CE systems, i.e., the spontaneous sample injection.[20] The reported smallest sample injection volumes were among 0.35−0.5 nL by using this approach.[18,20] Thereafter, no further research work has been reported on the application of this technique, probably because its operation affected by multiple factors was difficult to control.

In this work, we combined the spontaneous sample injection technique[18,20] with a CE system using a short capillary and an automated sample introduction system based on a slotted-vial array,[21,22] in order to develop a picoliter scale sample injection approach for HSCE.[23] The slotted-vial-array technique was first developed in 2005 in the author's group and has been applied in a miniaturized flow injection analysis system,[21,24] and chip-based[25,26] and capillary-based[27,28] CE systems to perform automated sample injection. However, in these capillary-based CE systems,[27,28] the sample injection volumes were still in the nanoliter range due to the use of the common electrokinetic injection approach, which resulted in their relatively lower separation efficiencies than those obtained in HSCE systems.

In the present system, with the combination of the spontaneous injection and slotted-vial array techniques, a droplet splitting phenomenon during the sample injection process was observed for the first time. On the basis of the investigation and optimization of the droplet splitting effect, a novel microfluidic translational spontaneous sample introduction approach (Figure 1d) in the sub-100 pL range was developed.[23] With this approach, a simple and versatile HSCE system was built, and its performance was demonstrated in the separation of fluorescein isothiocyanate

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(FITC) labeled amino acids[3] under capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) modes.

## EXPERIMENTAL SECTION

**Chemicals and Reagents.** A solution of 15% Acid Red 18 (Sinopharm Chemical Reagent Co., Shanghai, China) was used as a model sample for the observation of the sample introduction process. A 1 $\mu$M sodium fluorescein solution was used for optimization of the HSCE system. A concentration of 1 mM FITC (isomer I, Sigma Chemicals, St. Louis, MO) labeled amino acid (Kangda Amino Acid Works, Shanghai, China) solutions were prepared as previously described elsewhere,[29] except that equimolar amounts of amino acid and FITC were used. The labeled products were fluorescein thiocarbamyl (FTC) derivatives of the amino acids. The sample solution containing a mixture of 1 $\mu$M each amino acid was prepared by mixing the labeled amino acid solutions and diluting with 5 mM borate buffer before analysis. A 5 mM borate buffer (pH 9.2) was used as the working electrolyte for the achiral CE separation. A 5 mM borate buffer (pH 9.2) with 8 mM $\beta$-cyclodextrin ($\beta$-CD) and 12 mM sodium taurocholate (STC) (Sigma Chemicals, St. Louis, MO) was used as the working electrolyte for the chiral CE separation.

**Capillary Preparation.** Fused silica capillaries (50 $\mu$m i.d., 375 $\mu$m o.d., polyimide coating thickness 20 $\mu$m, Refine Chromatography Co., Yongnian, China) with lengths of 3 and 6 cm were used as separation columns. The inlet end of the capillary was carefully cut using a ceramic cleaving stone (Polymicro Technologies, Phoenix, AZ) to get a flat end. For the experiments that used tapered tip capillaries, the inlet end of the capillary was mechanically ground into a cone using sandpaper with a procedure similar to those reported previously[10,30] (see Supporting Information for details). The outer surface of the capillary inlet was silanized using dimethyldichlorosilane to reduce carryover[21,24] (see Supporting Information for details).

**High-Speed Capillary Electrophoresis System.** The setup of the HSCE system based on the translational spontaneous sample introduction is shown in Figure 2. The capillary was horizontally fixed on a glass plate by epoxy glue to facilitate good heat dissipation and easy handling. The automated sample introduction system was composed of slotted sandwich reservoirs for sample and buffer solutions (see Supporting Information for details) and a computer-programmed translational platform. The sample and buffer reservoirs were horizontally fixed on a translational stage controlled by a motion controller (TSA150-AB, Zolix Instruments Co., Beijing, China). The translational stage with the reservoirs could linearly move along the direction at an angle of 90° to the capillary, allowing the capillary inlet to sweep through the slots and immerse in the solutions filled in the reservoirs. The capillary outlet end was immersed in the solution filled in the waste reservoir positioned on the same level as the buffer reservoir.

A home-built high-voltage power supply, variable in the range of 0 to −6000 V, was used for the CE separation. To avoid the discharge phenomenon between the buffer solution in the buffer reservoir and the capillary inlet under high voltages, a high-voltage relay (GRL-1202, Hangzhou Zhonghuo Electronic Co., Hangzhou, China) was used to switch the high voltage during the analysis process.

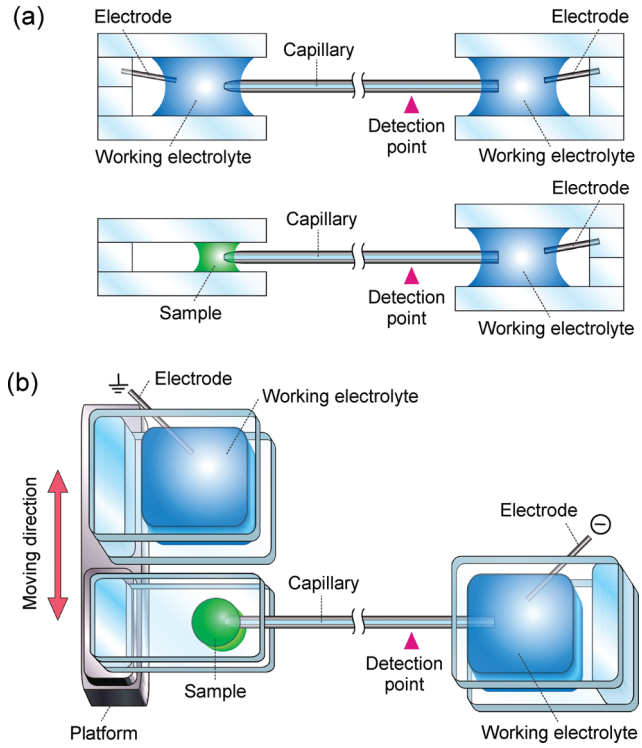


**Figure 2.** Schematic diagram of the HSCE system (not to scale).: (a) side view and (b) top view. The arrows indicate the moving direction of the translational stage.

On-capillary detection was performed using a home-built confocal laser induced fluorescence (LIF) detection system with a 473 nm laser as the light source. A stereomicroscope (XTB-1, Nanjing Jiangnan Novel Optics Co., Nanjing, China) equipped with a CCD video camera (YH-2088, Yonghui Technology Co., Shenzhen, China) was used to directly observe the sample introduction process and quantitate the sample injection plugs.

**Procedures.** Before use, the reservoirs and the capillary were sequentially rinsed with 1 M NaOH followed by water and then working electrolyte solution. The sample reservoir was filled with 20 $\mu$L of sample solution and the buffer and waste reservoirs with 500 $\mu$L of working electrolyte.

The translational spontaneous sample introduction was performed by linearly moving the translational platform, allowing the inlet end first to enter the sample solution without high voltage applied between the sample and waste reservoirs and then removing the sample solution back to its initial position, during which spontaneous sample introduction was achieved at the capillary inlet end. A high voltage was applied between the buffer and waste reservoirs to perform CE separation as soon as the capillary inlet end was immersed into the working electrolyte solution in the buffer reservoir.

**Safety Consideration.** The experiments of silanization of the capillary inlet or using heated concentrated sulfuric acid should be performed in a well-ventilated hood, while wearing protective gloves and goggles.

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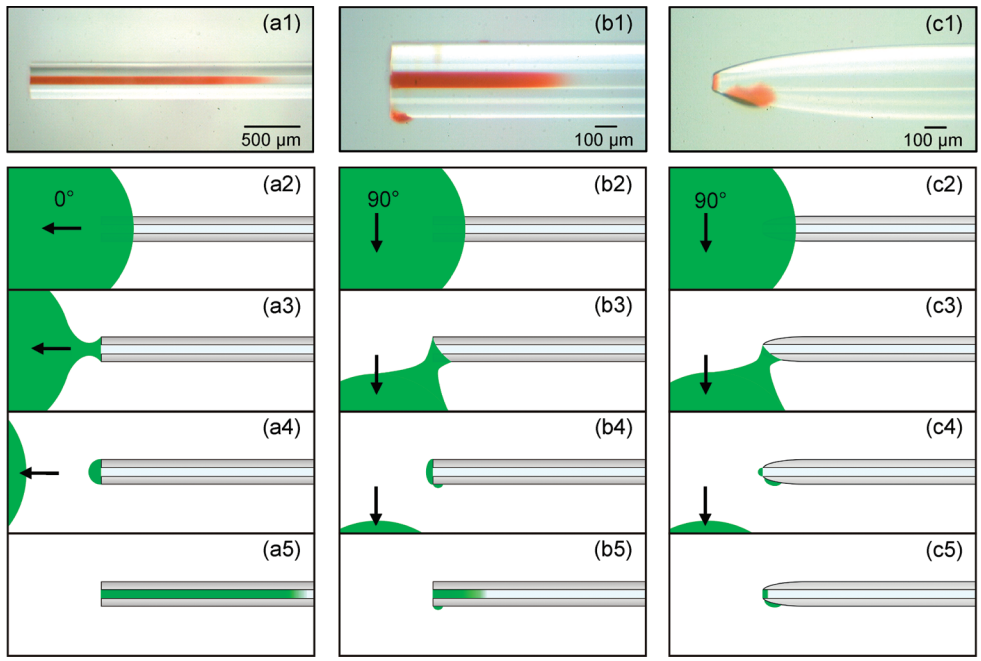


**Figure 3.** Typical images of injected sample plugs (a1, b1, c1) and principle schematic diagrams (a2−a5, b2−b5, c2−c5, not to scale) of the spontaneous sample injection at a removing angle of 0° with a flat-end-capillary (a1−a5), 90° with a flat-end-capillary (b1−b5), and 90° with a tapered-tip capillary (c1−c5). Conditions for images (a1, b1, c1): model sample, 15% Acid Red 18; capillary, 3 cm long, 50 μm i.d., 335 μm o.d.; removing speed of the sample solution, 3.12 mm/s; immersion depth of the capillary inlet in the sample solution, 0.35 mm. The arrows indicate the removing direction of the sample solution.

## RESULTS AND DISCUSSION

**Spontaneous Sample Introduction.** In conventional CE systems, after sample injection, the sample reservoir left the capillary inlet usually with a removing angle of 0° between the capillary and the moving direction of the sample reservoir (Figure 3a2). With the removal of the sample reservoir, a sample liquid bridge first formed between the sample reservoir and the capillary inlet end (Figure 3a3) and then broke up, resulting in a sample droplet remaining on the end-wall of the capillary inlet (Figure 3a4). Subsequently, this sample droplet was sipped into the capillary channel by surface tension forming a sample plug about 2.0 mm long (3.9 nL) (parts a5 and a1 of Figure 3). In this work, the effects of the removing angle of the sample reservoir and the shape of the capillary inlet end on the spontaneous sample introduction process were investigated to develop a simple picoliter-scale liquid introduction approach.

In the experiments on the effects of removing angles, we observed, for the first time, a droplet splitting phenomenon at the capillary inlet during the removing process of the sample solution from the capillary inlet. At a removing angle of 90° (Figure 3b2), the liquid bridge adhered to both the end-wall and side-wall of the capillary inlet when it broke up (Figure 3b3) during the removing process, leading to the splitting of the remained sample droplet at the capillary inlet into two droplets (Figure 3b4). One droplet adhered to the side-wall of the capillary inlet. Another droplet remained on the end-wall and was immediately sipped into the capillary channel, forming a sample plug less than 700 μm long (~1.4 nL) (parts b5 and b1 of Figure 3). It seemed that the splitting ratio of the sample solution left on the side-wall and end-wall after the liquid bridge broke up was related to the surface area ratio ($A_{side}/A_{end}$) of both walls to which the sample liquid bridge adhered. A large $A_{side}/A_{end}$ value would result in a large splitting ratio, i.e., more sample solution left on the side-wall and less sample on the end-wall, which led to a relatively small amount of sample introduced into the capillary.

Therefore, instead of the flat end-capillary, a tapered tip capillary with a relatively small end-wall area ($A_{end}$) was used to perform the spontaneous sample introduction. The spontaneously injected sample volume was further reduced to less than 40 pL (Figure 3c1) using a capillary with a tapered tip diameter of ~50 μm and a removing angle of 90°. Figure 3c2−c5 schematically shows the principle of the sample introduction process using the sharp tip capillary. The use of the sharp tip capillary significantly reduced the $A_{end}$ value and thereby increased the $A_{side}/A_{end}$ value, which resulted in the evident increase of the splitting ratio by ~100 times (estimated from the CCD images) and the subsequent decrease of the injected sample volume to the tens of picoliter scale.

Fishman et al.[18] also reported that the spontaneous injection volume could be reduced from 4 to 0.35 nL by etching the outer diameter of the capillary to form a tapered tip end. However, maybe because a removing angle of 0° was used in those systems without the use of droplet splitting effect, the injected sample volumes were still in the nanoliter range. In this work, with the translational spontaneous sample introduction approach utilizing the droplet splitting effect, picoliter-scale sample introduction with sample plug lengths less than 20 μm was achieved, which is particularly suitable for HSCE analysis.

**Building of the HSCE System.** On the basis of the above results, a versatile HSCE system (Figure 2) based on a short capillary was built. A novel type of slotted vial, sandwich liquid reservoir, was used to obtain stable liquid levels of the solutions filled in the reservoirs during a prolonged working process. A sample reservoir with thinner space than those of buffer and waste reservoirs was used to generate a tiny counterflow when the

capillary inlet was immersed in the sample solution (Figure 2a), in order to eliminate the interference of molecule diffusion of the analytes[31] to the spontaneous sample injection. The buffer and waste reservoirs had the same space to ensure equal liquid levels between the two reservoirs during the CE separation process.

A comparison CE experiment adopting the three different injection modes as shown in parts a1, b1, and c1 of Figure 3 was performed, using 1 $\mu$M sodium fluorescein as the sample with a 15 mm separation length and 900 V/cm separation electric field. Plate heights of 25, 2.6, and 0.44 $\mu$m were obtained, respectively. Therefore, the translational spontaneous injection mode using a tapered-tip capillary and a removing angle of 90° for the reservoirs as shown in Figure 3c1 was adopted in the HSCE system to obtain the highest separation efficiency.

The effects of the removing speed of the sample reservoir on the spontaneous sample injection were tested. In the range of 1.25−3.12 mm/s, no evident influence of removing speed on the separation efficiency and peak height of fluorescein was observed. Thus, a removing speed of 3.12 mm/s, which was the highest possible one for the stage used in this work, was employed to increase the analysis throughput.

The effects of the electric field strength for CE separation on the separation efficiency and migration time were studied with an effective separation length of 15 mm. The current vs electric field strength curve of the capillary channel showed a linear relationship between 100 and 900 V/cm and deviated from the linear behavior above 1000 V/cm. A significant decrease of the migration time was observed with the field strength from 100 to 1300 V/cm. The plate height decreased with the field strength from 100 to 900 V/cm, reached a minimum value of 0.44 $\mu$m at 900 V/cm, and increased at further higher field strengths due to the zone dispersion caused by the Joule heating effect.

**Performance of the HSCE System.** Under optimized conditions, the present system was applied in the separation of a mixture of FITC-labeled amino acids. High separation efficiencies ranging from 0.40 to 0.46 $\mu$m plate height (Figure 4b) were obtained with a separation length of 15 mm, which were comparable to most of the microfluidic chip-based HSCE systems. The limit of detection for the HSCE system under translational spontaneous injection mode was 4.4 nM sodium fluorescein, corresponding to a mass detection limit of 0.18 amol within the 40 pL injection volume. Outstanding peak height precisions ranging from 3.7% RSD for FTC-aspartic acid to 1.2% RSD for FTC-glycine were achieved in 51 consecutive separations with a high throughput of 137 $h^{-1}$ (Figure 5). No obvious carryover was observed during this process, which demonstrated that the carryover effect on the sample analysis produced by the sample droplet (usually less than 2 nL) remaining on the side-wall of the capillary tip could be omitted.

A much faster separation could be achieved using a very short separation length of only 5 mm. Most of the amino acids were separated within 1.7 s (Figure 4a) with a highest plate number of 7 180 for FTC-glycine.

By extending the separation length to 50 mm, both high-speed and high-resolution CE separations were obtained (Figure 4c) in less than 21 s with separation efficiencies ranging from 163 000 to 251 000 theoretical plates (corresponding to 0.31−0.20 $\mu$m plate height) for the mixture of eight amino acids. These results were better than those obtained in most chip-based CE systems and implied the possibility of the present system coupling the high-speed advantage of chip-based CE with the high-resolution advantage of conventional CE.[32]

We also applied this HSCE system to chiral separation of amino acid enantiomers. As shown in Figure 4d, amino acid enantiomers were resolved in 6.5 s over a separation length of 15 mm, using $\beta$-CD and STC as chiral selectors.[33]

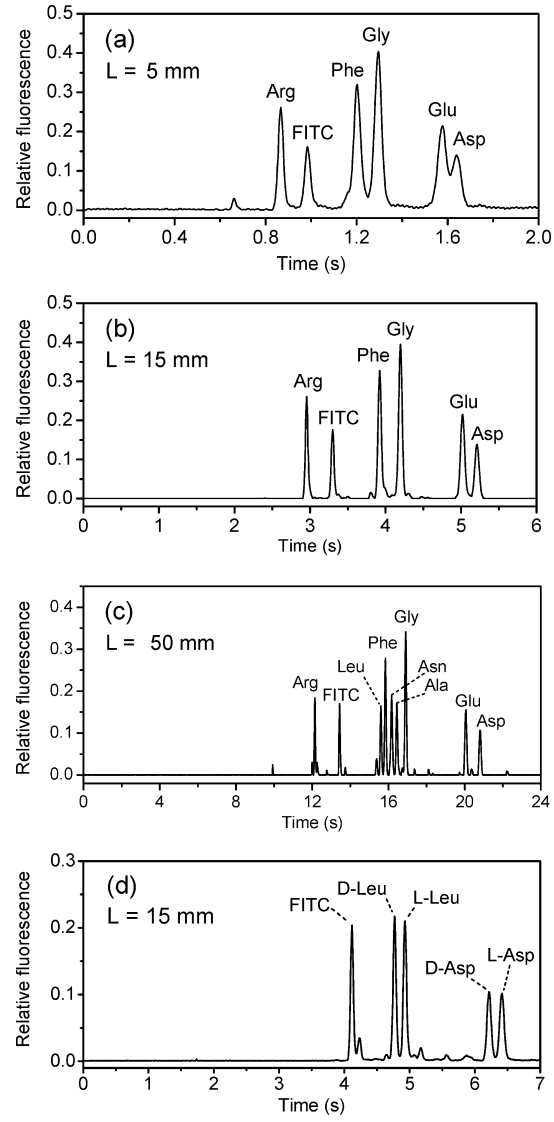


**Figure 4.** Electropherograms of achiral and chiral separations of a mixture of 1 $\mu$M FITC-labeled amino acids with effective separation lengths of (a) 5, (b) 15, (c) 50, and (d) 15 mm under optimized conditions: tapered-tip capillary; removing angle, 90°; removing speed, 3.12 mm/s; immersion depth of the capillary tip in the sample solution, 0.35 mm; electric field strength, 900 V/cm; working electrolyte for achiral separations (a−c), 5 mM borate buffer (pH 9.2); working electrolyte for chiral separation (d), 5 mM borate buffer (pH 9.2) with 8 mM $\beta$-CD and 12 mM STC.

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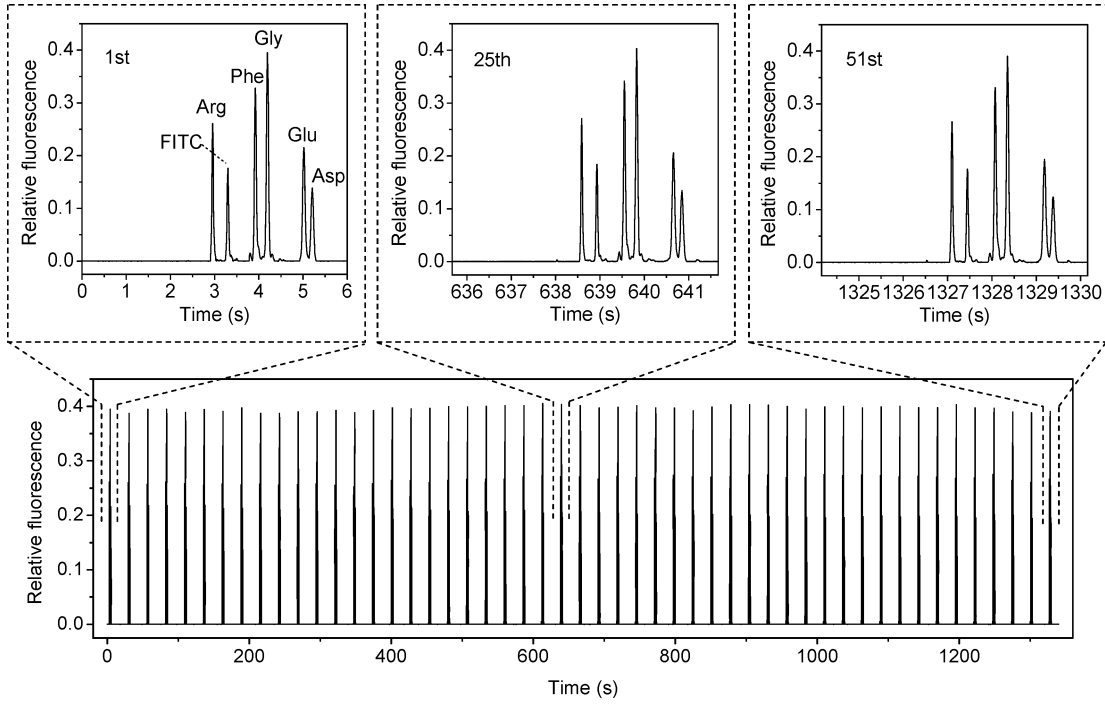


**Figure 5.** Electropherograms of 51 consecutive separations of a mixture of 1 $\mu$M FITC-labeled amino acids to show the reproducibility of the present HSCE system. Conditions as in Figure 4b.

## CONCLUSION

In this work, a microfluidic spontaneous sample injection approach in the picoliter range was developed for a HSCE system. The use of the droplet splitting effect and the translational sample introduction system significantly reduced the sample introduction volumes to the picoliter-scale. The percentage contributions from the injected sample plug length to total plate height in the present HSCE system were lower than 1% (see Supporting Information for details), which demonstrated the applicability of the translational spontaneous sample injection approach in the capillary-based HSCE system. The advantages of the present injection approach include simple structure, ease of operation, low cost, and versatility in application. Besides the successful application under CZE and MEKC modes, some preliminary studies also revealed the feasibility of this approach under other separation modes such as capillary gel electrophoresis (CGE) and capillary electrokinetic chromatography (CEC), which implied that the application of the present HSCE system could be extended to other types of samples such as peptide, nucleic acid, and protein. Furthermore, because of its simplicity in system structure and operation, the present HSCE system also has potential to be further developed into a miniaturized portable HSCE instrument, which could be applied in in situ analysis or point-of-care testing.

Finally, in addition to the application in HSCE systems, the translational spontaneous injection approach also provided a novel microfluidic technique to perform picoliter-scale liquid introduction and manipulation for capillary-based microfluidic systems without the requirement of complicated microfabricated devices.

## ACKNOWLEDGMENT

Financial support from the National Natural Science Foundation of China (Grants 20775071, 20825517, and 20890020) and the Ministry of Science and Technology of China (Grants 2007CB714503 and 2007CB914100) is gratefully acknowledged. The authors also thank Mr. Tao Zhang and Jianzhang Pan for inspiring discussions.

## SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text and a movie of translational spontaneous sample introduction and the CE separation process. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review March 19, 2009. Accepted March 26, 2009.

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