Qi Li
Ting Zhang*
Ying Zhu
Yong-Qiang Cheng*
Qing-Hu Lin*
Qun Fang

Department of Chemistry, Institute of Microanalytical Systems, Zhejiang University, Hangzhou, P. R. China

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Short Communication

Automated high-speed CE system for multiple samples

A high-speed CE system for multiple samples was developed based on a short capillary and an automated sample introduction device consisting of a commercial multi-well plate and an *x*-*y*-*z* translation stage. The spontaneous injection method was used to achieve picoliter-scale sample injection from different sample wells. Under the optimized conditions, a 40 μm-long sample plug (corresponding to 78-pL plug volume) was obtained in a 50 μm id capillary, which ensured both the high separation speed and high separation efficiency. The performance of the system was demonstrated in the separation of FITC-labeled amino acids with LIF detection. Five FITC-labeled amino acids including arginine, phenylalanine, glycine, glutamic acid, and asparagine were separated within 15 s with an effective separation length of 1.5 cm. The separation efficiency ranged from 7.96×10^5/m to 1.12×10^6 /m (corresponding to 1.26–0.89 μm plate heights). The repeatability of the peak heights calibrated with an inner standard for different sample wells was 2.4 and 2.7% ($n = 20$) for arginine and phenylalanine, respectively. The present system was also applied in consecutive separations of 20 different samples of FITC-labeled amino acids with a whole separation time of less than 6 min.

Keywords:
High-speed CE / Multi-well plate / Multiple sample analysis / Short capillary / Spontaneous injection
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CE is a useful separation technique for various samples including amino acid [1], protein [2], DNA [3], cell [4], enzyme [5], and carbohydrate [6] samples owing to its advantages of rapid separation, high efficiency, and low consumption. In 1990s, high-speed CE (HSCE) systems [7, 8] were developed, which usually use short separation lengths (<15 cm) to obtain both high separation speed (<1 min analysis time) and high separation efficiency (>10^6/m plate numbers) over conventional CE systems [9–11].

Currently, most HSCE systems are built on the basis of microfluidic chips [8] or short capillaries [7]. Although microchip-based HSCE systems can obtain relatively high separation speed and efficiency, they usually require expensive microfabrication equipments and complicated operation, which may limit their broad application in routine analysis. In contrast to microchips, capillary-based HSCE systems have several practical benefits such as easy to build, inexpensive, and convenient to use.

Fang et al. [12] developed a capillary-based HSCE system coupled with a sequential injection system to achieve automated sample introduction and fast separation of amino acids. This technique has been applied in fast separation with amperometric [13], UV [14], and fluorometric [15] detection. In 2005, the authors' group [16] developed a slotted-vial-array (SVA) system to perform automated and continuous sample introduction in microfluidic systems. This automated sample presenting system was coupled to CE systems to achieve fast separation of multiple samples [17, 18]. The bottom-slotted vials in the SVA array were filled alternately with samples and buffers, and horizontally positioned on a programmable

Correspondence: Professor Qun Fang, Department of Chemistry, Institute of Microanalytical Systems, Chemistry Experiment Building, Room 101, Zhejiang University (Zijingang Campus), Hangzhou 310058, P. R. China
E-mail: fangqun@zju.edu.cn
Fax: +86-571-88273572

Abbreviations: HSCE, high-speed CE; **SVA**, slotted-vial-array

*Current address: Dr. Ting Zhang, Department of Chemistry and Department of Pharmacology, University of Michigan, Ann Arbor 48109-1055, USA.

Dr. Yong-Qiang Cheng, Institute of Oceanographic Instrumentation, Shandong Academy of Sciences, Qingdao 266001, P. R. China.

Qing-Hu Lin, CNNC Hainan Nuclear Power Co., Haikou 570125, P. R. China

Colour Online: See the article online to view Fig. 1 in colour.

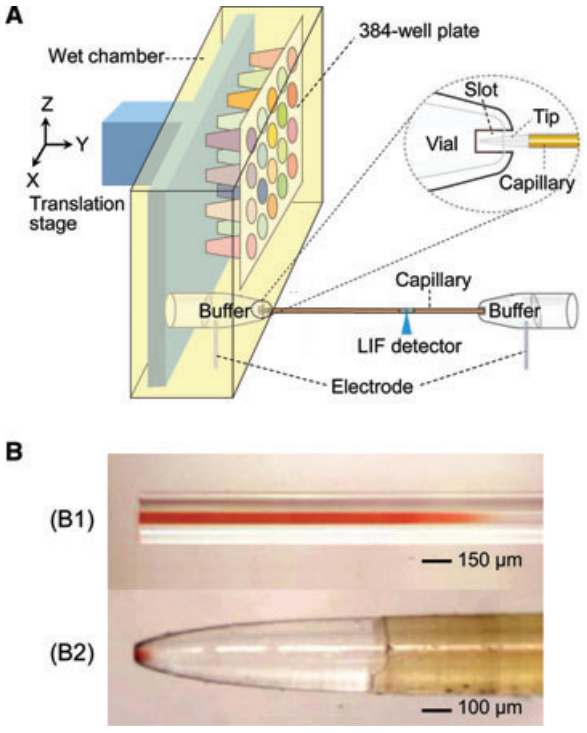

Figure 1. (A) Schematic diagram of the HSCE system (not to scale). (B) Typical images of injected sample plugs obtained at a removing angle of 0° with a flat-end capillary (B1) and a tapered-tip capillary (B2). Conditions: model sample, 15% Acid Red 18 solution; capillary, 50 μm id, 375-μm od; removing speed of sample vial, 1000 mm/min.

translational platform. By linearly moving the SVA to sequentially switch the sample vials to the sampling probe, multiple samples were introduced electrokinetically in turn into the probe for separation. Fan et al. [19] and Xu et al. [20] developed different types of SVA coupling with capillary-based CE systems for reducing the volume of vials and increasing the number of vials to perform CE separation for multiple samples. Although automated fast separation for multiple samples has been achieved in the above-mentioned systems, their separation speeds and efficiencies are not comparable to HSCE systems, due to the relatively longer separation lengths and larger sample injection volume in the nanoliter range.

The sample injection volume is an important factor governing the column efficiency of CE separations. In an HSCE system, separation length is usually reduced to several centimeters to achieve high-speed separation, which requires narrow sample plug with <100 μm lengths (picoliter range) to ensure high separation efficiency. In 2009, the authors' group has developed a picoliter-scale translational spontaneous sample injection technique for short capillary-based HSCE separations [11]. This technique has the advantages of convenient operation, ease of changing samples, rapid separation, and high efficiency, which was successfully applied in the high-speed separation of amino acids, DNA fragments [21], and protein mixtures [22] with separation performances comparable to microchip-based HSCE systems. However, in these systems, slotted sandwich reservoirs [11] or linear SVA

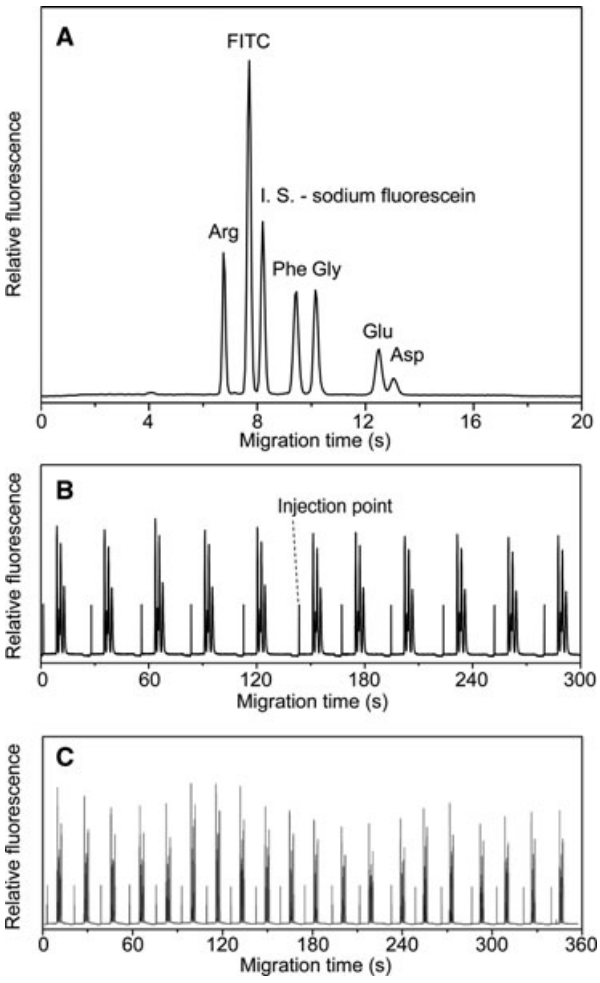

Figure 2. (A) A typical electropherogram of a mixture of five FITC-labeled amino acids. (B) Electropherograms of continuous separation of a mixture of FITC-labeled amino acids from the same sample well in repeatability experiment. (C) Electropherograms of continuous separation of a mixture of FITC-labeled amino acids from 20 sample wells in repeatability experiment. Conditions: tapered-tip coated capillary, 1.5 cm effective length, 50 μm id; removing angle of sample well, 0°; removing speed, 1000 mm/min; separation field strength, 400 V/cm; working electrolyte, 5 mM borate buffer (pH 9.2).

[21,22] were used for containing sample and buffer solutions, in which the number of sample vials are limited (usually <10 samples). Typically, commercial CE instruments employ multi-well plates to contain sample and buffer solutions for improving sample capacity.

In this work, on the basis of the spontaneous injection method, we developed a short capillary-based HSCE system capable of coupling to a commercial multi-well plate to perform automated high-speed CE separation for multiple different samples. The HSCE system consisted of a short tapered capillary and an automated sample introduction system based on a 384-well plate (Fig. 1A). The 384-well plate was fixed vertically on an *x*-*y*-*z* translation stage to match the horizontally placed tapered capillary. The movement of the translation stage was under control of a computer program to present

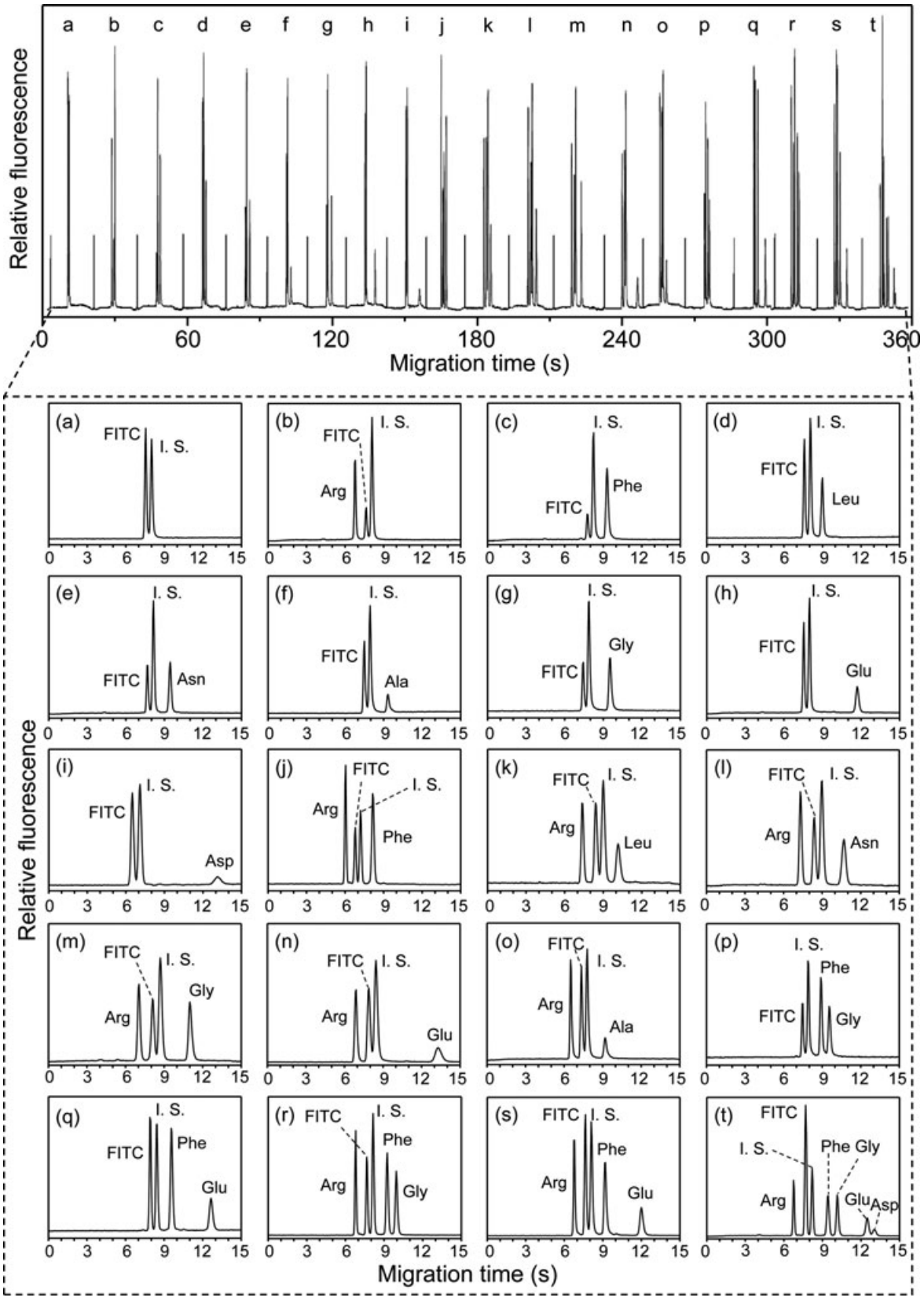

Figure 3. Electropherograms of consecutive separations of 20 different FITC-labeled amino acid mixtures from 20 sample wells. Conditions as in Fig. 2.

different sample wells to the capillary inlet end. This design allowed the present system to be easily coupled with commercial multi-well plate and thus significantly increased its sample capacity. We tested if the movement of the translation stage would lead to the leakage of sample solutions from the vertically placed 384-well plate. The results show that the sample solutions could be held in wells without leakage even at the highest moving speed (1000 mm/min) of the translation stage due to the surface tension of sample solutions in wells and the relatively small diameter (3.5 mm) of the wells.

In the present system, the spontaneous injection method was used to achieve picoliter-scale sample injection from different sample wells. Sample injection and separation were performed by moving the *x*-*y*-*z* translation stage, allowing the capillary inlet end first to enter the sample well without high voltage applied between the sample well and waste vial, and then quickly remove from it and enter the buffer vial. A high voltage was applied between the buffer and waste vials to carry out CE separation as soon as the capillary inlet end was immersed into the buffer vial.

During the spontaneous injection process, the 384-well plate was removed from the capillary tip with a removing angle of 0° to the capillary because there was no slot at each well, differing from the 90° angle used in previously reported HSCE systems with slotted reservoirs or vials. Therefore, there was no droplet-splitting phenomenon occurred during the spontaneous injection process [11]. When the 384-well plate was removed from the capillary with the removing angle of 0°, only one droplet remained at the end-wall of the capillary tip, and subsequently this droplet was entirely sucked into the capillary channel by surface tension, forming a sample plug with a length of ~40 μm (corresponding to ~78 pL plug volume, Fig. 1B). Although this sample plug volume was relatively higher than that (40 pL) in the previous HSCE system [11], it is still in the picoliter range, which is sufficient for obtaining high separation efficiency. Furthermore, the cross-contamination produced by the sample droplet remaining on the side wall of the capillary tip as in previous HSCE systems with 90° removing angle could be avoided in the present system.

To optimize the system, we studied the effects of the removing speed of the sample wells on the spontaneous sample injection and the effects of the electric field strength on separation efficiency (see Supporting Information for details). Under the optimized conditions, i.e. 1000 mm/min removing speed of the sample wells and 400 V/cm electric field strength in CE separation, the present system was applied in the separation of mixtures of FITC-labeled amino acids. As shown in Fig. 2A, five FITC-labeled amino acids including arginine (Arg), phenylalanine (Phe), glycine (Gly), glutamic (Glu), and asparagine (Asp) were separated in 15 s with 1.5-cm-long effective separation length. High separation efficiencies ranging from 7.96×10^5 /m to 1.12×10^6 /m (corresponding to 1.26 μm to 0.89 μm plate heights) were obtained for the amino acids, which are comparable to most of the microchip-based HSCE systems. The LOD of the system for sodium fluorescein was 8.6×10^{-10} mol/L.

Figure 2B shows the electropherograms of a mixture of two FITC-labeled amino acids from one sample well in repeatability experiment. The repeatabilities for the peak heights of Arg and Phe were 4.5 and 4.0% RSD ($n = 11$), which were improved to 1.3 and 2.1% after calibrated with an inner standard (I.S.) of sodium fluorescein, respectively. Both the repeatabilities for the migration times of Arg and Phe were 0.4% RSD ($n = 11$). We also tested the repeatability of the system for different sample wells by sequentially separating the same mixture of FITC-labeled Arg and Phe filled in 20 wells (Fig. 2C). The RSDs of migration time of Arg and Phe were between 1.5 and 1.8% ($n = 20$), while the RSDs of the peak height were 14.0 and 14.4% ($n = 20$) for Arg and Phe, respectively. The relatively larger RSD values of the peak height may be caused by the fluctuation of sample injection volumes between different sample wells produced by the effect of the different air–liquid interface shapes in these wells on the spontaneous injection process. Since the moving distances from different wells to the buffer vial were also different, this would also result in the fluctuation of the peak height due to the inconsistent diffusion times of the injected sample plugs from the different wells before the separation operation was started. Therefore, an internal standard, sodium fluorescein, was added to the sample solution to calibrate the variation of the peak height. The RSDs of the peak height for Arg and Phe were significantly improved to 2.4 and 2.7% ($n = 20$), respectively.

The present system was also applied in the consecutive separations of 20 different samples of FITC-labeled amino acids from 20 sample wells (Fig. 3). All the analytes in the 20 different samples were baseline resolved, and the whole separation was achieved in less than 6 min.

In this study, we have developed a capillary-based HSCE system suitable for the analysis of multiple samples. The present system has attractive features such as simple structure, ease of building without the requirement of microfabricated devices, convenient operation, low cost, and high speed. It offers a new platform to analyze multiple samples and has potential applications in drug discovery, inhibitor screening, and binding constant determination.

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