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Journal of Chromatography A, 1063 (2005) 253–256
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Bias-free pneumatic sample injection in microchip electrophoresis

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Received 18 September 2004; received in revised form 8 November 2004; accepted 23 November 2004
Available online 13 December 2004

Abstract

We have developed a new microfluidic chip capable of accurate metering, pneumatic sample injection, and subsequent electrophoretic separation. The pneumatic injection scheme, enabling us to introduce a solution without sampling bias unlike electrokinetic injection, is based upon the hydrophobicity and wettability of channel surfaces. An accurately metered solution of 10 nL could be injected by pneumatic pressure into a hydrophilic separation channel through Y-shaped hydrophobic valves, which consist of polydimethylsiloxane (PDMS) and fluorocarbon (FC) film layers. We demonstrated the successful pneumatic injection of a red ink solution into the separation channel as a proof of the concept. A mixture of fluorescein and dichlorofluorescein (DCF) could be baseline-separated using a single power source in microchip electrophoresis.

Keywords: Microchip electrophoresis; Pneumatic sample injection; Hydrophobic valve; Sampling bias

1. Introduction

Microchip electrophoresis, one of the examples of a successful lab-on-a-chip, is a promising analytical technique due to its high performance and ability to incorporate various channels. A variety of designs for microchip electrophoresis have been fabricated to mix, react, concentrate, and separate analytes [1,2]. These have facilitated chemical and biological analyses including enzyme assay, chiral separation, DNA sequencing, immunoassay, etc. [1–8].

Usually, sample solutions are introduced by electrokinetic methods, such as pinched injection [9,10] or gated injection [11,12] in microchip electrophoresis. Even though these methods are easy to use, there are problems such as sampling bias and difficulty in measuring the injection volume precisely [13]. Thus, many attempts have been made to overcome these problems in electrokinetic injection [14–17]. For bias-free sample injection, Slentz et al. introduced samples into the separation channel using the diffusion of molecules after turning off the applied potential [14]. Bai et al. investigated pressure pinched injection of nanoliter volumes by a multiport injection valve and syringe pumps [15]. Solignac and Gijs injected a sample hydrodynamically by applying a pressure pulse to a membrane on a reservoir using a mechanical actuator [16]. Tabuchi et al. also developed a pressurization technique to separate protein mixtures in a 12-microchannel array [17]. Such injection methods can solve the problem of sampling bias. However, the absolute amount injected is still ambiguous. Yamada and Seki developed a microdispenser system, which can measure and inject a fixed nanoliter-sized droplet pneumatically [18].

Recently, we have developed a novel nanoliter-fluidic chip, capable of nanoliter metering, reaction, and mixing, by controlling the capillary pressure and wettability of channel surfaces [19]. Precise quantitative sample handling and analysis for an enzymatic reaction were demonstrated with the chip. Applying this sample handling system to microchip electrophoresis is a promising technique since a desired vol-

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doi:10.1016/j.chroma.2004.11.088

ume can be accurately measured and the complicated manipulation of high voltages avoided since only a single power source is required. The biggest problem to overcome, however, is to design an interface between the sample handling system and the electrophoretic separation channel. In this study, we introduce a new microchip electrophoresis system enabling precise sample metering, hydrodynamic injection, and electrophoretic separation. The sample manipulation in the microfluidic chip was performed by controlling the capillary pressure and the wettability of channel surface as before [19]. The injection and electrophoretic separation were demonstrated using a mixture of fluorescein and dichlorofluorescein (DCF).

2. Experimental

2.1. Materials

Sodium fluorescein was from Junsei (Tokyo, Japan). DCF and sodium borate were from Sigma (St. Louis, MO, USA). A run buffer for electrophoretic separation was prepared by adjusting the pH of 20 mM sodium borate with 0.1 M NaOH to pH 9.2. All reagents were of analytical grade and were used without further purification. De-ionized water was obtained from a NANOpure purification system (Barnstead, Dubuque, IA, USA).

2.2. Microchip electrophoresis

The schematic of microfluidic chip is shown in Fig. 1a. The microfluidic chip was fabricated using isotropic wet etching, lift-off patterning of the fluorocarbon (FC) film, and PDMS replica molding techniques, as described elsewhere [19]. The main structure of the microfluidic chip was composed of two layers; one fabricated on a glass wafer for electrophoretic separation and the other for microfluidic sample handling fabricated on polydimethylsiloxane (PDMS). On a glass surface, a separation microchannel (13 cm long, 150 μm wide, and 50 μm deep) was fabricated and then a hydrophobic FC film was patterned in order to adjust the surface interfacial energy of the hydrophilic glass wafer. The PDMS layer contains sample loading reservoirs, two metering microchannel networks, a serpentine mixing channel, a sample-injection hydrophobic valve, and air venting channels. The dimension of the assembled microfluidic chip was 46 mm × 25 mm.

A handler for liquid sample injection and pneumatic solution control was constructed as before [19]. This was composed of plastic syringes, springs, a micrometer, and acrylic plastic housing (Fig. 1b). The rotational motion of the micrometer was converted into the linear motion of the piston of a syringe, letting a sample solution to be either pushed into or withdrawn from the microchannels. The motions of the sample solution were monitored using a CCD-camera-equipped optical microscope (Fig. 1c). After solution manipulations, the microchip was released from the handler and immediately

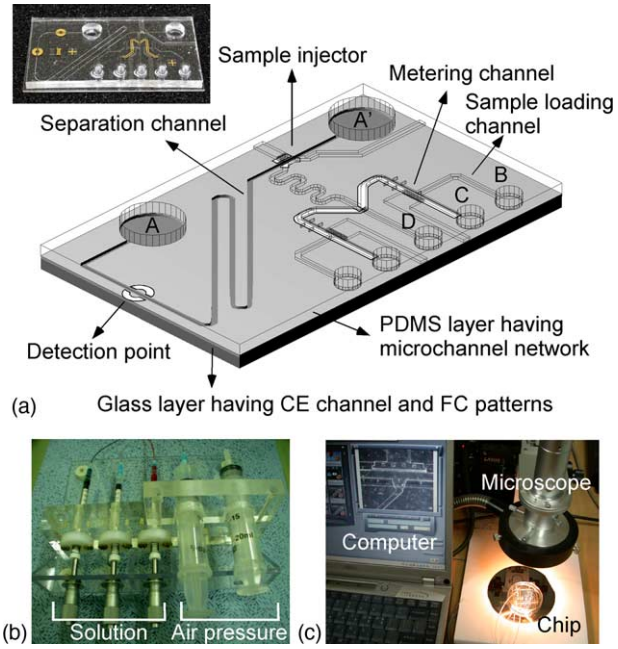

Fig. 1. Schematic of a microfluidic chip and a sample handling system. (a) Reservoirs are labeled as (A, A′) run buffer, (B) sample loading port, (C) metering port, and (D) pneumatic control port. The separation channel depth was 50 μm and width was 150 μm. The inset is an image of the fabricated microfluidic chip (size: 46 mm × 25 mm). Half of the sample loading and metering channel was designed for later use. (b) A handler for liquid sample injection and pneumatic solution control. (c) Setup to monitor sample manipulations using a CCD-camera-equipped optical microscope.

mounted on a stage for microchip electrophoresis. The electrophoretic separation was performed using a single power source, as described previously [5,6]. A laser-induced fluorescence detection system was used to detect the analytes with an excitation/detection at 488 nm/520 nm, respectively.

3. Results and discussion

For a quantitative analysis, it is important to meter precisely the amount of solution injected into a microchannel. The scheme developed by Lee et al. [19] was used. Due to the coating of a hydrophobic FC film on the glass surface (white area in Fig. 2a), an aqueous solution from reservoir B stopped in front of the metering channel. The solution could

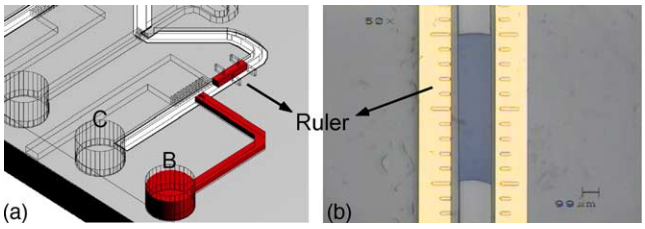

Fig. 2. Metering of a sample solution. (a) Simplified schematic of a metering channel. The injected amount was measured using a ruler. The illustration was not drawn to scale. (b) Microscope image of a 10-nL metered solution. The volume between two scale bars corresponds to 1 nL.

be introduced further into the metering channel when an additional pressure was applied through reservoir B using a sample handler. After introducing a desired amount of the solution, a pressure through reservoir C split the solution as shown in Fig. 2a. Then the solution was metered using a ruler fabricated inside a microchannel. One scale bar corresponded to 1 nL. Fig. 2b shows a 10-nL metered solution.

To inject samples of nanoliter size into a separation microchannel without any loss, we modified the previous nanoliter-handling device. The simple design coupling two microchannels in the same plane, as shown Fig. 5 in ref. [19], would have led to the introduction of air bubbles into a separation channel. Thus, we devised a cross-shaped sample injector, which is composed of an upper PDMS layer and a lower glass layer; a sample-metering channel carved on the PDMS surface overlapped crosswise with the separation channel on the glass as shown in Fig. 3a [20]. At the crossing of the two channels, a square window was formed. Since the glass surface around the injection window was coated with a hydrophobic FC film and the separation channel surface was hydrophilic, the injected aqueous solution did not overflow out of the injection window. However, there was a small amount of solution loss due to the undesired adhesion of the solution to the edge of the upper channel in the previous design [20]. Hence a Y-shaped injector has been devised to prevent such a solution loss, as shown in Fig. 3b. The injection window was widened by overlapping the two channels at a slanted angle. A venting channel was added to reduce the disturbance of a solution in the separation channel during sample injection. Due to the hydrophobic nature of the upper channel and the FC patterning on the glass surface around the injection window, there was no overflow into the upper hydrophobic channel unless extremely high pressure was applied to the separation channel.

The injection of a red ink solution into the separation channel was monitored using an optical microscope to demonstrate the injection scheme (Fig. 3). First, the separation channel was filled with de-ionized water by the capillary force. A 10-nL metered ink solution was pushed slowly towards the solution in the separation channel by pressure. The ink droplet was pulled into the separation channel by the cohesion force as soon as the two liquid phases met. Small PDMS pillars on the other side prevented the ink solution from crossing the separation channel. Air could be easily vented through two microchannels on the PDMS layer. As shown in Fig. 3c, the ink solution could be injected into the separation channel without any loss.

In order to test the performance of the current microchip, a mixture of 0.1 μM DCF and fluorescein was separated in a run buffer of 20 mM borate (pH 9.2). The electrophoretic separation scheme is as follows. First, the separation microchannel was filled with a run buffer by the capillary force. Second, a sample solution was placed in reservoir B and pushed into a microchannel by applying pressure. A desired amount of the sample solution was precisely measured and split by pneumatic controls through reservoir C. The metered sample droplet was transported to the sample injection window and injected into the separation channel by pressure into reservoir D. Finally, electrophoretic separation was carried out by applying a potential between reservoirs A and A′. In the previous report, DCF and fluorescein could not be baseline-separated (resolution of 0.7) [20]. It was reported that a turn of small curvature in a separation channel could give rise to undesired band broadening [21]. Thus, the number of turns was reduced to as few as possible in the current design. As shown in Fig. 4, DCF and fluorescein could be successfully baseline-separated with the resolution of 2.4 ± 0.1. DCF was detected at 6.4 ± 0.1 min with the efficiency of 5700 ± 10

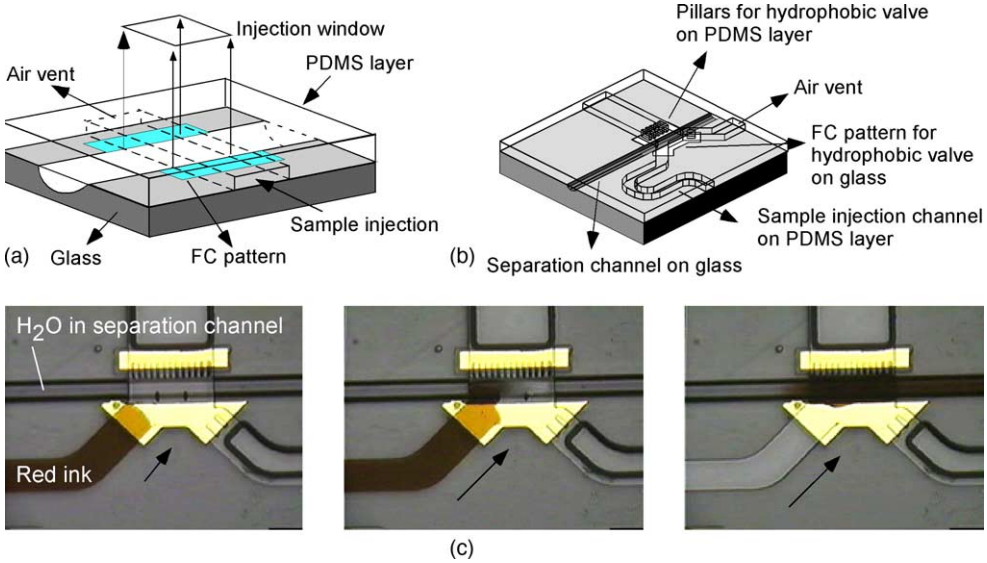

Fig. 3. Pneumatic sample injection. (a) Simplified schematic of the previous pneumatic sample injector. (b) Detailed schematic of the pneumatic sample injector. (c) Microscope images for injecting a diluted red ink solution into de-ionized water filled in the separation channel (sequence from left to right).

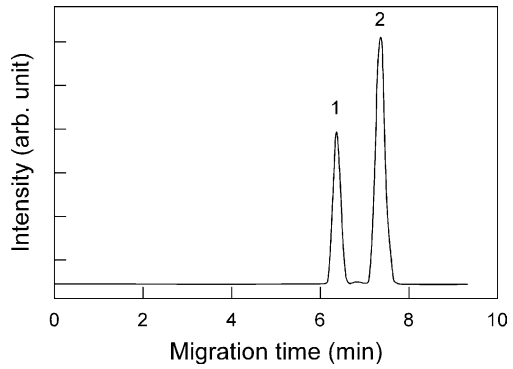

Fig. 4. Electropherogram for the separation of 0.1 μM DCF (1) and 0.1 μM fluorescein (2) in a 20 mM sodium borate buffer of pH 9.2. Conditions: Separation channel length, 13.0 cm (length to detection point: 12.2 cm); applied voltage, 3 kV; injection amount, 10 nL; and fluorescence excitation and detection at 488 and 520 nm, respectively.

and fluorescein detected at 7.4 ± 0.1 min with the efficiency of 5320 ± 10. When an analyte in a highly saline solution is injected electrokinetically, the sampling bias becomes serious and the injected amount of the analyte and thus peak heights are significantly reduced [13,14]. However, such a decrease of peak height was not observed in spite of the presence of high concentration salt (100 mM NaCl) in the current study since sample injection was performed pneumatically.

4. Conclusions

We have successfully performed the pneumatic sample injection, and electrophoretic separation on a newly developed microfluidic chip by controlling the capillary pressure, the wettability of the channel surface, and external air pressure, instead of relying upon a complicated electric field control. Nanoliter-sized sample solution of 10 nL could be precisely measured using the integrated metering system. By modifying the shape of an injection window and increasing the air-ventilation area, a sample solution was injected into the separation channel without any loss. A mixture of fluorescein and DCF, injected without sampling bias, were successfully baseline-separated by decreasing the number of channel turns. The developed microfluidic chip will be very useful, especially when precise measurement and biasless sample injection are required in microchip electrophoresis.

Acknowledgement

This work was supported by the International Mobile Telecommunications 2000 R&D Project (Ministry of Information and Communication).

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