Functional Integration of Serial Dilution and Capillary Electrophoresis on a PDMS Microchip

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Abstract For the quantitative analysis of an unknown sample a calibration curve should be obtained, as analytical instruments give relative, rather than absolute measurements. Therefore, researchers should make standard samples with various known concentrations, measure each standard and the unknown sample, and then determine the concentration of the unknown by comparing the measured value to those of the standards. These procedures are tedious and time-consuming. Therefore, we developed a polymer based microfluidic device from polydimethylsiloxane, which integrates serial dilution and capillary electrophoresis functions in a single device. The integrated microchip can provide a one-step analytical tool, and thus replace the complex experimental procedures. Two plastic syringes, one containing a buffer solution and the other a standard solution, were connected to two inlet holes on a microchip, and pushed by a hydrodynamic force. The standard sample is serially diluted to various concentrations through the microfluidic networks. The diluted samples are sequentially introduced through microchannels by electro-osmotic force, and their laser-induced fluorescence signals measured by capillary electrophoresis. We demonstrate the integrated microchip performance by measuring the fluorescence signals of fluorescein at various concentrations. The calibration curve obtained from the electropherograms showed the expected linearity.

Keywords: serial dilution, capillary electrophoresis, microchip, polydimethylsiloxane (PDMS), lab-on-a-chip

INTRODUCTION

The manipulation of liquids in microchannels, with widths of several tens or hundreds μm, is becoming a central technology in chemical, biological and medical applications [1-5]. Many research groups have developed microfluidic systems, or lab-on-a-chip devices [6,7], and have shown the possibility for simple and high throughput analysis of nucleic acids [8-10], enzyme assays [11, 12] and immunoassays [13-15].

The most popular driving forces in the move to fluids in microchannels are the electrokinetic and hydrodynamic forces. The electrokinetic force is generated by applying an electric field at a microchannel or capillary. This is widely used to separate chemical and biological samples in a capillary; the so called, capillary electrophoresis (CE). Especially with CE on a microchip, where a capillary can be replaced with a microchannel or microchannels on plates made of materials such as glass, polydimethylsiloxane (PDMS), and polymethylmethacrylate, has attracted the attention of many researchers [16] due to their speed, simplicity, small reagent consumption and selectivity in handling fluids in order to deliver many chemical and biochemical components through a complex network [17]. Even though electrokinetics as the driving force has advantages, it also has the disadvantages of sample bias effects [18,19], and is dependent on the channel surface, characteristics [20].

Hydrodynamic flow control is also widely used in microfluidics [21,22]. The use of external pumps, or hydrodynamic force from syringes, to directly force liquids through microchannels, make it possible to move fluids at high flow rates, but the accurate control of such a small flow, with an incompressible liquid, is extremely difficult.

One of the reasons for the researcher's interest in microfluidics on microchips is that several functionalities can be integrated into a single microchip, which makes the lab-on-a-chip technology a reality. Burns *et al.* [23] developed a device capable of mixing, amplifying, separating and detecting DNA on a microchip with no other devices, except for a light sources, by integrating microfabricated fluidic channels, heaters, temperature sensors

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and fluorescence detectors into a single chip. Woolley *et al.* [24] have demonstrated microfabricated reactors for polymerase chain reactions (PCR), and CE chips have been successfully integrated to perform fast analysis of DNA. Wilding *et al.* [25] developed microfabricated devices for performing cell isolation and PCR in a single device.

In this paper, we describe microfluidic devices that integrate serial dilution and CE. Standard sample preparations, with various concentrations, are performed daily in biological and chemical laboratories by the serial dilution of standard samples of a known concentration. However, these preparations are ordinarily processed by repetitive manual pipetting, which is very tedious for researchers, and the tolerance of manual pipetting is poor for many quantitative analyses, and is very dependent on the skill of the analyst. As mentioned in our previous report [26], we developed a device that dilutes a sample, with a buffer solution, to several concentrations of equal intervals, and have demonstrated the device to be fast, simple and reliable.

The microfluidic flows in our serial dilution, and CE chips, are generated by hydrodynamic and electrokinetic forces, respectively. Jacobson *et al.* [27] demonstrated microfabricated devices for the parallel and serial mixing of fluids using only electrokinetic flows, but we have used the hydrodynamic flows instead, for fast dilution. By integrating serial dilution and CE into a single three-dimensional chip, and using different driving forces for their operation, we could simplify the time-consuming experimental steps.

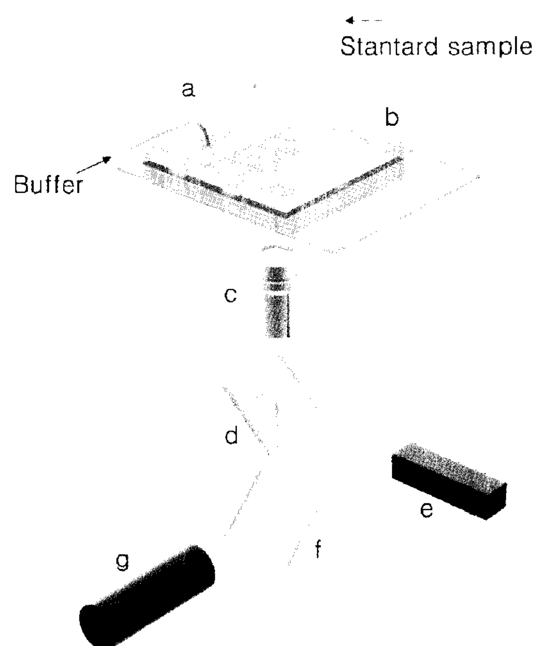


Fig. 1. The schematic design of the fluorescence detection module and integrated microchip. Serial dilution microchip, **a**; CE microchip, **b**; objective lens, **c**; beam splitter, **d**; photomultiplier tube, **e**; mirror, **f**; and laser, **g**.

MATERIALS AND METHODS

Fabrication

We made both devices, for serial dilution and CE, using soft lithography and replica molding [28]. A master was fabricated by spin-coating of negative PR (SU-8, MicroChem, MA, USA). We molded the top layer of a CE chip with PDMS, and a permanent seal was formed between the PDMS layer and a slide glass, making a CE chip with microchannels, after surface treatment with an O_2 plasma. A dilution layer was also molded using the same procedures, and attached to the top layer of the CE chip to form micro fluidic networks. The three-dimensionally bonded chip was then baked in an oven at 80°C for 85 min to intensify the bonding strength. The integrated chip has a serial dilution device on the upper layer, and a CE chip on the bottom.

Apparatus

The electrokinetic flow control and detection were performed by a modified laser-induced fluorescence detection system from Microfluidic Tool Kit instrument (Micralyne Inc., Edmonton, Canada) [29]. An epiluminescent confocal detection module makes use of an objective lens (40×, 0.55 NA) to focus the light beam from a frequency-doubled Nd:YAG diode-pumped solid-state laser (532 nm, 5 mW) onto a channel on a microchip. The fluorescence is deflected, and collected through a dichroic mirror, toward the PMT, as shown in Fig. 1. The whole system was controlled by a LabVIEW program (National Instruments, Austin, TX, USA) and electric fields were applied from ground (GND) to maximum values. The absorbance of the serially diluted samples was measured by a spectrophotometer (Ultrospec1000, Amersham Biosciences, Piscataway, NJ, USA) at 488 nm, to confirm the performance of the serial dilution device.

Reagents

The running buffer solution was prepared with 20 mM boric acid and 20 mM sodium borate (Sigma-Aldrich, MO, USA), adjusted to pH 9.0. A 1 mM solution of fluorescein (Sigma-Aldrich, MO, USA) was prepared in the same buffer as a standard sample. 0.1 M solutions of NaOH and HCl were also prepared for cleaning the microchannels. All the chemicals were of at least reagent-grade, and used as received. Each solution was filtered through a 0.2 μm syringe filter (Minisart, Sartorius, Goettingen, Germany) prior to the experiments.

EOF Measurement

For the electro-osmotic flow (EOF) measurement, we followed the procedure of Huang *et al.* [30]. Briefly, all of the microchannels, and the reservoirs, on a CE chip were at first filled with the 20 mM borate buffer (pH 9.0),

and the EOF then replaced the buffer with a 10 mM borate buffer (pH 9.0) by applying a high voltage across one of the microchannels, with the others left floating. As the 20 mM borate buffer in the microchannel was replaced with the 10 mM borate buffer, the electric current dropped linearly with time. By measuring the time, t, required for the current to stabilize, that is, for the 10 mM borate buffer to fill the entire channel, the EOF velocity, v_{eo}, could be determined as $v_{eo} = L/t$ where L is the channel length. The electro-osmotic mobility, μ_{eo} can also be determined via the equation,

$$v_{eo} = \mu_{eo}\left(V/L\right) \quad (1)$$

where V is the voltage applied to the microchannel. In our devices the electro-osmotic mobility was determined to be 4.3×10^{-4} cm^2/Vs, which was similar to other reported values [31].

Experimental Procedures

Before the experiments, the microchannels for CE in the integrated microchip were thoroughly washed by applying a vacuum to one reservoir, while filling the other with a 0.1 M HCl solution for 5 min, followed by flushing with deionized (DI) water for further 5 min. The microchannels were also rinsed with a 0.1 M NaOH solution for 15 min to activate the PDMS surface. The DI water cleaning was repeated, and the microchannels were finally rinsed and filled with the 20 mM borate buffer and solution. To stabilize the EOF, an electric field was applied to the microchannels for 5 min prior to sample loading.

The volume of each of the sample reservoirs in a serial dilution chip was 45 μL, with a total volume for the five sample reservoirs of 225 μL. The borate buffer and fluorescein solutions, 112.5 μL of each, were separately contained in two syringes. The contents of the syringes were pushed into the microchip, serially diluted and consecutively injected by electrokinetic force, and detected by laser-induced fluorescence.

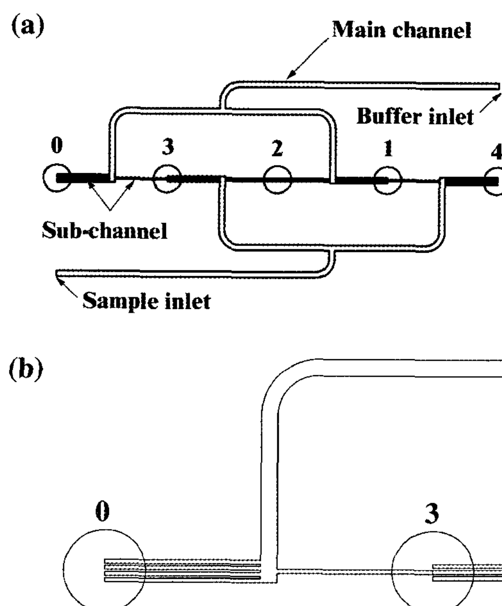


Fig. 2. (a) The schematic design of the serial dilution microchip. The microchip has two main channels, which split into two paths, and finally into five sub-channels. (b) The enlargement at the end of one of the branches. The branch shows four sub-channels on the left side, and one on the right side, respectively.

Table 1. Sample concentrations according to the number of sub-channels of sample and buffer solutions

Port number (nth reservoir from left)	0	3	2	1	4
The number of the sample sub-channels	0	1	2	3	4
The number of the buffer sub-channels	4	3	2	1	0
Nominal sample concentration ($\times 10^{-3}$ M)	0/4	1/4	2/4	3/4	4/4

RESULTS AND DISCUSSION

Serial Dilution Microchip

The serial dilution microchip, as shown in Fig. 2(a), has two inlet ports and five reservoirs (the overall chip size is 42 mm by 20 mm). The width of the two main channels connected to the inlet ports is 400 μm, which successively splits into five sub-channels of 100 μm in width, as shown in Fig. 2(b). The sample solution is injected from one of the two inlet ports into one main channel, and a buffer solution from the other, with simple disposable syringes. Each reservoir was numbered in the order of the sample concentrations. The 0th reservoir had only four sub-channels, directly connected to the main channel, for the buffer solution, the 1st reservoir had three sub-channels connected to the main channel for the buffer solution, and one sub-channel was connected to the main channel for the sample solution, and so on. Thus, the volume ratio of sample to total solutions of the 1st reservoir was 1/4. Similarly each reservoir had a different ratio according to the number of the sub-channels connected to the buffer and sample solutions. Theoretically, all the channels had the same flow resistance, as they consisted of an equal number of sub-channels, with the five serially different concentrations generated having accurate integer-to-integer ratios. The standard sample and buffer solutions were mixed in the reservoirs of the serial dilution chip, according to the ratios shown in Table 1, and the mixed solutions automatically fell downward to the corresponding sample reservoir of the CE chip.

The solutions were more easily mixed in the reservoirs than in the microchannels due to the laminar liquid flow in microchannels. Most micromixers [32,33] can be classified as either active or passive. Passive mixers typically

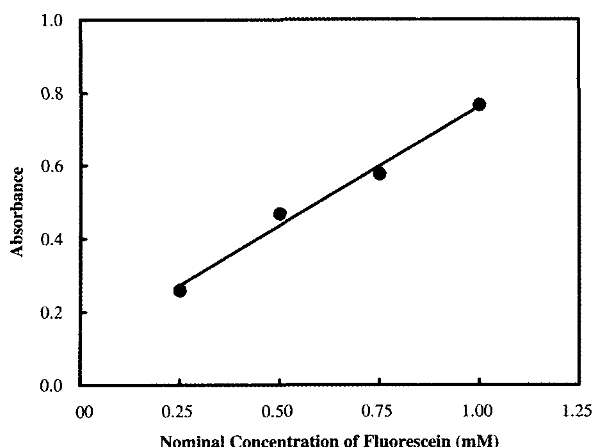


Fig. 3. UV-VIS absorbance data for the solutions taken from the serial dilution microchip of Fig. 2.

use channel geometry in the interfacial area between the liquids to be mixed and accomplish mixing by molecular diffusion. In our system, the mixing occurred in open reservoirs, not the microchannels, so the mixing procedure was simple and accurate [26]

We investigated the proper functionality of the serial dilution device by measuring the UV-VIS absorbance of the serially diluted solutions from the serial dilution chip. A dye solution of 1 mM fluorescein was serially diluted with the borate buffer (20 mM, pH 9.0) to several concentrations, through the microfluidic channels, as mentioned earlier. The samples were taken from the reservoirs of the serial dilution chip in 2 μL aliquots, and diluted 50 times with DI water, to make sufficient volumes to fill the cuvette of the spectrophotometer. The absorbance was linearly dependent upon the nominal concentrations shown in Table 1 and Fig. 3, where with a correlation coefficient, R^2 = 0.987. This indicates that the serial dilution device functioned correctly.

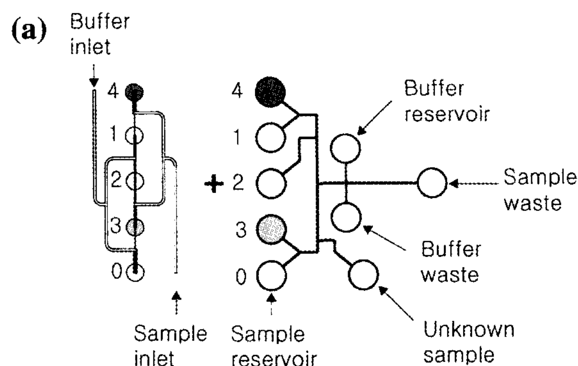
CE Microchip

A CE chip, as shown to the right side in Fig. 4(a), has six sample reservoirs, and three further reservoirs for buffer, buffer waste and sample waste. Five reservoirs of the sample reservoirs precisely coincide with those of the serial dilution chip at relative locations, and are shared by a three-dimensional connection, as shown in Fig. 4(b). The remaining reservoir is for an unknown sample for future study. The cross section of the microchannels of the CE chip is rectangular, with a width and height of 50 μm. The overall chip size was 42 mm by 42 mm. All the channel lengths, between each sample reservoir and the sample waste reservoir, were designed to be the same because the current, that is the flow rate, was inversely proportional to the channel length.

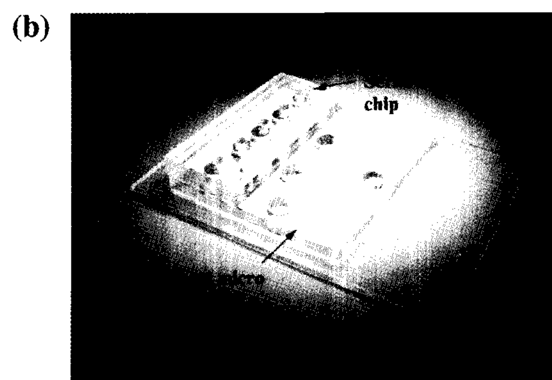
Integration and Data Acquisition

Fluorescein was used as a test sample to investigate the proper working of the integrated microchip. The dye had

(a)



(b)



(c)

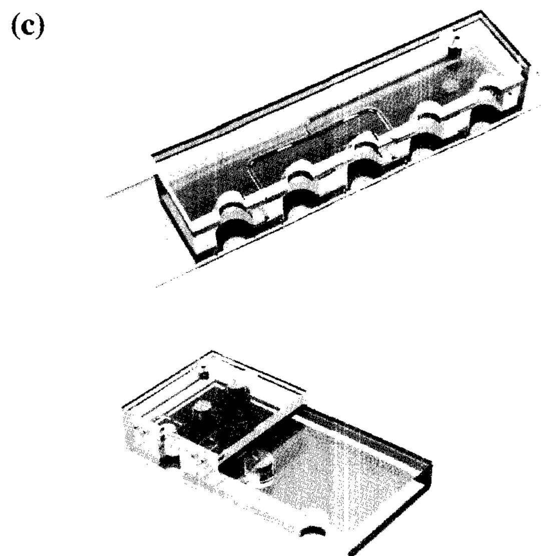


Fig. 4. The schematic design (a), the picture (b) of an integrated microchip and the cross-sectional schematic figure (c). The left side of (a) is a serial dilution microchip and the right of (a) is a CE microchip. The upper and bottom of (c) shows the cross-sectional array of the reservoirs for the dilution microchip and CE microchip at different viewpoints.

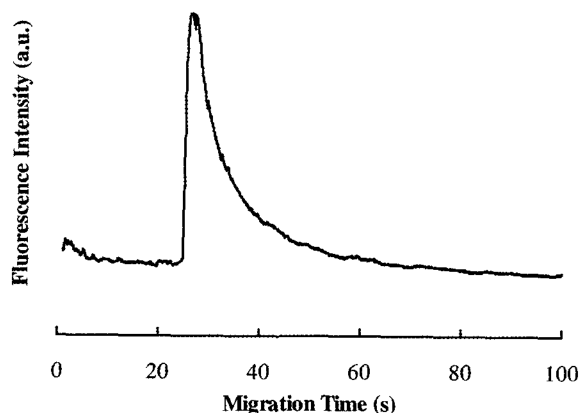


Fig. 5. The electropherogram of 1 mM Rhodamine B on a PDMS microchip. The running buffer was 20 mM borate buffer (pH 9.0).

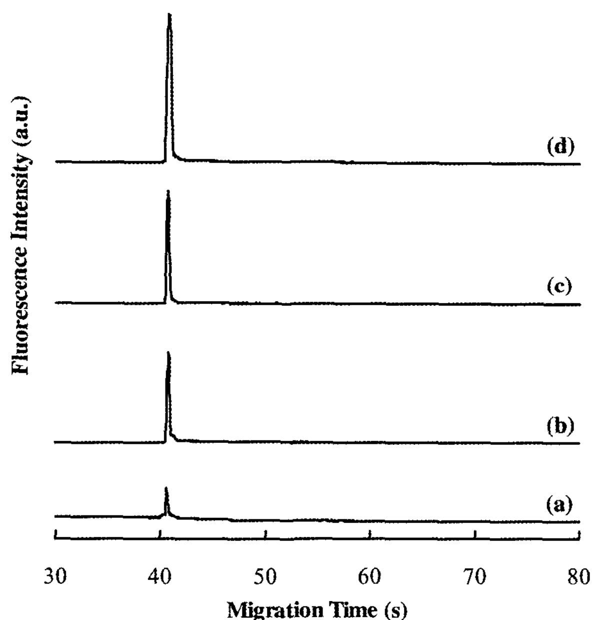


Fig. 6. The electropherograms obtained from the integrated microchip. The nominal concentrations of fluorescein are 0.25 mM (a), 0.50 mM (b), 0.75 mM (c) and 1.0 mM (d).

an excitation maximum at 488 nm. The laser used had a wavelength of 532 nm, and rhodamine was generally used as the fluorophore. Fluorescein, rather than rhodamine, was selected as the fluorophore as rhodamine is adsorbed onto the PDMS surfaces, and the plug generated, according to Slentz *et al.* [34], and from our experimental results (Fig. 5), is unstable. Fluorescein is also more suitable for the comparison with less sensitive UV-VIS absorbance results.

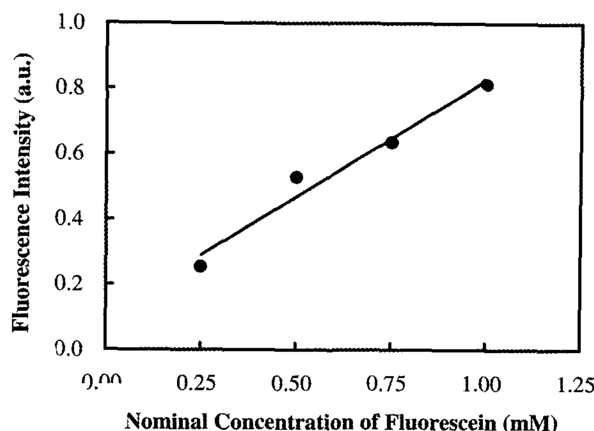


Fig. 7. The calibration curve obtained from the Fig. 6.

The dye solution was serially diluted, to several known concentrations, as previously described. The diluted samples were collected in the sample reservoirs of the CE chip sharing the reservoir of the serial dilution chip, as shown in Fig. 4(b), and sequentially introduced through the microchannels by electro-osmotic force. The fluorescence signals were measured by laser-induced fluorescence detection. Sample injections were accomplished by the scheme shown in Table 2. The procedures were repeated for all the diluted samples. The electropherograms obtained are shown in Fig. 6, and the fluorescence intensities of the electropherograms are plotted in Fig. 7, according to the nominal concentrations in Table 1. The concentrations correlated well with each other, with a correlation coefficient, R^2 = 0.968.

Because our devices have multiple sample reservoirs, anomalies, such as unlike simple and single injection, the peak shape and the background drift [29] occurred. As the experiments continued, the level in the sample waste reservoir gradually increased due to EOF pumping. Thus, leakage from sample waste reservoir, to the other reservoirs, occurred, and the anomalies due to plug generation took place. This was alleviated by standing a glass tip on the PDMS reservoirs.

Table 2. Two-step high voltage program used to form a plug of sample at the channel intersection and push it to the detection window

Step	Reservoir potential (kV)				Duration (s)
	Sample	Buffer	Sample waste	Buffer waste	
Plug formation	3.0	1.2	GND	1.1	40
Detection	0.3	1.6	0.5	GND	40

CONCLUSION

We have demonstrated a three-dimensional microstructure of two components; serial dilution and CE, which were operated by hydrodynamic and electroosmotic forces, respectively. Their functional integration is expected to reduce an analysis time and the tedious manual procedures. We are still attempting to improve the functionalities of our integrated microchip, and hopefully a one-step automatic analysis will be possible from a standard sample preparation, calibration, and separation of an unknown sample. It is believed that this functional integration is a practically useful example of 'lab-on-a-chip' development for analysis.

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