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Study of injection bias in a simple hydrodynamic injection in microchip capillary electrophoresis

Maojun Gong¹, Kenneth R. Wehmeyer², Apryll M. Stalcup¹, Patrick A. Limbach¹, and William R. Heineman¹

¹Department of Chemistry, University of Cincinnati, P.O. Box 210172, Cincinnati, OH 45221-0172, USA

²Procter and Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason-Montgomery Rd, Mason, OH 45040, USA

Abstract

The electrokinetically pinched method is the most commonly used mode for sample injection in microchip capillary electrophoresis (μ CE) due to its simplicity and well-defined sample volume. However, the limited injection volume and the electrophoretic bias of the pinched injection may limit its universal usage to specific applications. Several hydrodynamic injection methods in μ CE have been reported; however, almost all claimed that their methods are bias-free without considering the dispensing bias. To investigate the dispensing bias, a simple hydrodynamic injection was developed in single-T and double-T glass microchips. The sample flow was produced by hydrostatic pressure generated by the liquid level difference between the sample reservoir and the other reservoirs. The reproducibility of peak area and peak area ratio was improved to a significant extent by using large-surface reservoirs for the buffer reservoir and the sample waste reservoir to reduce the Laplace pressure effect. Without a voltage applied on the sample solution, the voltage-related sample bias was eliminated. The dispensing bias was analyzed theoretically and studied experimentally. It was demonstrated that the dispensing bias existed and could be reduced significantly by appropriately setting up the voltage configuration and by controlling the appropriate liquid level difference.

Keywords

Capillary electrophoresis; Hydrodynamic injection; Injection bias; Microchip; Laplace pressure

1 Introduction

Many techniques developed for traditional capillary electrophoresis (CE) have been transferred onto microchip CE (μ CE) [1-5], which is one of the most powerful and promising analytical miniaturization modes for micro total analysis systems. Advantages of μ CE over traditional CE include rapid analysis, reduced reagent consumption and easy automation [6]. However, some limitations, such as difficulties in doing hydrodynamic sample injection, for μ CE relative to traditional CE exist due to the complications caused by the channel networks at the cross region of the microchip [7].

Injections in μ CE routinely rely on some variant of electrokinetic injection. The common modes are pinched [8], floating [8], gated [9], and dynamic[10] injections. Pinched injection provides an accurate, precise and well-defined volume of sample solution with high separation efficiency, but the injection volume is limited by the dimensions of the microchip intersection,

and the tight focus of the sample may be detrimental to detection sensitivity due to dilution and dispersion effects [11]. Floating injection supplies a little more sample than pinched injection depending on the loading time, but the sample volume is still limited. Gated injection introduces varied volumes of sample solution by varying the injection voltage and time duration and maintains continuous sample solution flow, but it also produces significant injection bias with respect to the electrophoretic mobilities of analytes and transradial electrokinetic selection [12]. Dynamic injection, like gated injection, introduces variable sample plugs, but the injection bias is significant during the dynamic step. All of these electrokinetic injection schemes have advantages and disadvantages, and all have more or less injection bias [12-14]. For qualitative analysis or migration time measurements, sample injection bias is not a significant problem. However, the injection bias can be problematic for quantitative applications of μ CE.

The ideal injection method should satisfy the following conditions: *a) No or minimum bias.* Sample injection bias is detrimental to quantitative analysis. *b) No voltage applied on the sample reservoir.* This voltage may change the sample pH due to electrolysis, possibly produces sample bias [15] and may perturb the analyte interactions, such as complexation. *c) Variable injection volume.* The separation efficiency or the detection sensitivity can be improved by decreasing or by increasing the sample volume, respectively.

To solve the sample injection problems associated with the electrokinetic injection modes, researchers have focused on hydrodynamic injection approaches widely applied in traditional CE, and several schemes have been developed for μ CE [6,15-18]. Among these, the injection driving force includes external pressure or hydrostatic force, and the flow control in microchip networks is carried out by valveless gated flow or a valve integrated on the microchip. Lin et al. [6] used a syringe pump to push the sample flow into the intersection of a single-T microchip and used the voltage-gated scheme to prevent the sample solution from entering the separation channel except during the sample injection step. Backofen et al. [16] used hydrostatic pressure by adding a little more sample solution into the sample reservoir (SR) relative to the buffer reservoir (BR) and the sample waste reservoir (SW) on a double-T microchip. They also used gated flow to push back the sample solution and prevent its entry into the separation channel. A voltage was still applied on the SR to drive the sample flow. Solignac and Gijs [15] used a pressure pulse produced from a flexible poly(dimethylsiloxane) (PDMS) membrane covered on the SR to push sample solution to the cross of a single-T microchip. The three schemes mentioned above only reduce sample loading bias although the authors claimed their injection methods are bias-free. The dispensing bias may still exist and is overlooked [19]. The most promising hydrodynamic injection scheme would be a pressure injection as in traditional CE but achieved by integrating valves on microchips [17,18]. By opening and closing the other channels during the sample loading or the dispensing step, truly non-biased sample solution may be introduced into the separation channel. However, the opening and closing of valves on microchips requires sophisticated controls which are not commonly available at present.

In this paper, we describe a simple hydrodynamic injection scheme in commercially available single-T and double-T microchips using large cuboid BR and SW. The driving force for the sample solution flow is the hydrostatic pressure produced by a higher level of the sample solution in the SR. The performance was evaluated by comparing with gated injection and diffusion injection in a gated valve. The dispensing bias was studied by changing electric field strength in the BR channel.

2 Experimental

2.1 Instrumentation

The custom-built μ CE system has been described [2,20]. Briefly, the light source was a 150-watt xenon lamp. The excitation wavelength was filtered to 480 ± 20 nm, and the fluorescence

emission was collected with a 20x objective and was filtered spatially through a 1.0-mm pinhole and optically through a bandpass filter (535 ± 25 nm). The voltage configuration was applied and controlled by the Labview program (National Instrument Corporation, Austin, TX, USA), and the data were recorded and processed by Turbochrome software (PE Nelson, San Jose, CA, USA). A CCD-100 camera system (Dage-MTI, Inc., Michigan City, IN) was coupled to the bottom of the microscope and was used together with a RCA video recorder and monitor (Model BWMC, Javelin Systems, Torrance, CA) to qualitatively observe dye plug movement and to capture the flows in the microchip channels. The video captured on the video tape was transferred to a computer by Pinnacle Studio software (Version 7.15), and selected pictures of the flow progress were obtained from the video.

The standard borofloat glass microchips were purchased from Micralyne Inc. (Edmonton, AB, Canada). Both the single-T and double-T microchips have two channels with lengths of 85.0 and 8.0 mm, respectively. The short channel is bisected by the long channel at 5.0 mm. The two side channels of the double-T microchip are 100 μ m apart. Polypropylene reservoirs cut from 200 μ L pipette tips were attached at the SR, the SW, the BR and the buffer waste (BW) using epoxy as shown in Figure 1a. Polypropylene cuboid reservoirs of 16 mm in length and 4.0 mm in width were attached on the BR and SW for reducing the Laplace pressure effect (Figure 1b).

2.2 Chemicals and reagents

BODIPY 505/515 (4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene) and fluorescein-5-isothiocyanate (FITC) were obtained from Molecular Probes (Eugene, OR, USA). Fluorescein disodium salt (FL) was from ICN Biomedicals (Aurora, OH, USA). 5-carboxyfluorescein (5-FAM) was from Aldrich Chemical Company (Milwaukee, WI, USA). Sodium tetraborate and sodium hydroxide were from Fisher Scientific (Fair Lawn, NJ, USA). All chemicals were used without further purification.

All the water used was deionized through a NANOpure system (Sybron Barnstead Corp., Boston, MA, USA). Tetraborate stock solution (100 mM) was prepared in deionized water, and the tetraborate separation buffer (20 mM) was prepared by diluting the stock buffer with water as needed. BODIPY 505/515 stock solution was prepared at a concentration of 10 mM in methanol. FITC, FL and 5-FAM stock solutions were prepared in water/methanol (70:30; v-v) at concentrations of 0.25, 1.0 and 0.44 mM, respectively. The working dye sample solutions were finally prepared by diluting the stock solutions with water and tetraborate buffer. All solutions put into the microchip reservoirs were filtered through 0.22- μ m syringe filters purchased from Gelman Laboratory, Pall Corp. (Ann Arbor, MI, USA); the buffer and sample solutions were degassed under a vacuum.

2.3 Procedure

The channels of the microchip were conditioned by filling the SR, BR and SW reservoirs sequentially with 1.0 M NaOH, water, and separation buffer for 5 min each by applying a vacuum to the BW. After NaOH rinse, all reservoirs were flushed several times with water. For the microchip with all circular reservoirs, separation buffer was pipetted into the BR (30 μ L), BW (30 μ L) and SW (28 μ L). After a short application of a vacuum on the SR, the microchip was put on the microscope platform and the corresponding electrodes were placed into the reservoirs except the SR in which no electrode was needed. After a volume of 60 μ L sample solution was pipetted into the SR, the detection point was rapidly focused and the voltage program and the Turbochrome software were switched on simultaneously. For the microchip with cuboid reservoirs, the SR, SW, BR and BW were filled with 80- μ L sample and 80-, 80- and 40- μ L separation buffer, respectively.

The injection and dispensing process was monitored and recorded using FITC at 50 μM in separation buffer (20 mM tetraborate). A 10x microscope objective was focused on the microchip intersection. The light beam was adjusted to be larger to illuminate the intersection. Electropherograms were obtained with BODIPY 505/515 at 100 μM , FITC and FL at 1.0 μM each and 5-FAM at 2.0 μM . Various voltage programs used for the hydrodynamic and gated injections are detailed in the appropriate sections of the Results and discussion.

3 Results and discussion

3.1 Process of hydrodynamic injection

Hydrodynamic injection was carried out by keeping a higher liquid level in the SR than in the other reservoirs either by adding a larger volume of sample solution to the SR or by using larger reservoirs for the SW and BR. The resulting hydrostatic pressure pushed the sample solution through the cross region. The injection and dispensing process in the single-cross microchip was monitored using 50- μM FITC solution in the SR as shown in Figure 2. The sample flow from the SR under the force of the hydrostatic pressure continued during both the injection and dispensing processes, and the sample flow was gated by the separation buffer flowing from the BR (Figure 2a). The sample injection was performed by simply floating all the electrodes for a period of time (such as 0.5-30 s) that was adjusted as needed (Figure 2b). Because the flow velocity was proportional to the channel length under the same pressure drop, more sample solution entered the two branch channels (4.0 mm) than the separation channel (80.0 mm). When the dispensing process (Figure 2c) was immediately switched on after the injection step, the sample solution in the BR channel was divided into the separation channel and the SW channel.

3.2 Comparison with gated injection

Gated injection was first described by Jacobson et al. [21]. The sample flow was electrokinetically pumped through the cross region, and was gated by the buffer flow at the cross. During injection, the BR and/or SW electrodes were kept floated for a short time. The injection volume depends on the apparent velocity of the analyte and the injection duration. Injected analytes were electrophoretically biased, to some extent, depending on the relative electrophoretic velocities of analytes. In bare silica microchips, for instance, less anionic analytes are injected than neutrals. In addition, the sample solution is also biased by the continuous electrokinetic flows, so that the anionic analytes are slightly concentrated in the SR as the process proceeds over time. To reduce the injection bias, the diffusion injection combined with the gated loading can be used by simply keeping all the electrodes floated for a short time during the injection process [12].

The performance of the hydrodynamic injection (All used circular reservoirs as shown in Figure 1a) in the single-T microchip (Figure 2) was compared with the gated electrokinetic and the gated diffusion injections in the same microchip using the voltage program shown in Figure 3. The results are summarized in Table 1. As can be seen, both the electrokinetic and the diffusion injections produced poor reproducibilities of the peak area and peak area ratio, presumably due to the nonuniform distribution of analytes in the electrokinetically loaded sample solution. The difference in peak size comes from the variable injection volumes. On the other hand, the hydrodynamic injection produced good reproducibility of the peak area with a relative standard deviation (RSD) below 4%, and a peak area ratio with a RSD of 1.7%. In addition, the electrokinetic injection produced a peak area ratio of 1.7 (5-FAM/FITC), which is much lower than that produced by diffusion (4.1) or the hydrodynamic (4.0) injection. This smaller area ratio reflects the large electrophoretic velocity difference between FITC (-2) and 5-FAM (-3) under the same electric field strength during the injection process.

3.3 Injection volume control

The injection volume affects the resolution and detection sensitivity. Larger injection plugs produce higher signals allowing the detection of lower analyte levels. Small sample plugs produce sharp peaks with better resolution at the cost of lower detection sensitivity. In practical applications, the injected sample plug should be controlled according to the experimental requirements. However, the commonly used pinched injection just introduces a relatively fixed sample volume, which might be inadequate, depending on sample requirement. One of the advantages of the gated loading scheme is the variable injection volume achieved by changing the injection time and/or the electric field strength. Injection time can be easily adjusted by setting up the required time duration in the voltage program. The hydrostatic pressure used as the sample flow force is managed by controlling the liquid level difference which can be easily changed by adding more or less sample solution in the SR. However, a high liquid level difference between the SR and the other reservoirs provides a high pressure effect that may lead to peak broadening during separation. When a 160- μ L sample solution was added into the SR, no obvious peak anomalies were observed. This may be due to a small pressure effect in the much longer separation channel (80.0 mm) relative to the branches (4.0 mm). For the microchip used here, the injection volume is limited by the BR channel (4.0 mm) into which the sample solution mainly flows instead of the separation channel under the hydrostatic force.

3.4 Laplace pressure effect

The microchip has access holes with a diameter of 2.0 mm. Plastic reservoirs with a diameter of 5.0 mm cut from 200- μ L pipet tips are usually attached with epoxy to contain large volumes of sample/buffer solution as shown in Figure 1a. BODIPY 505/515, FL and 5-FAM were selected as the model dyes with 0, -2 and -3 charges, respectively. As can be seen in Figure 4, the peak height (Figure 4a) and the peak area ratio (Figure 4b) increased with the successive injections (46 injections, the first group is the leaking analytes before operation and is not counted) for each analyte. Peak size depends on the amount of analyte injected into the BR channel and the dispensed amount of this sample plug into the separation channel. With successive injections and separations, the liquid level in the BR should slightly decrease while the level in the SW should increase if there is no surface tension between solutions and reservoirs. With solution pumping into or out of the reservoirs from the bottom, a positive Laplace pressure in the SW and a negative Laplace pressure in the BR are produced. The total effect pushes more sample solution into the BR during the same injection period. Moreover, the positive Laplace pressure in the SW also increases the dispensed amount of analyte into the separation channel. In addition, the Laplace pressure may play a more important role in this process than liquid level difference as discussed by Crabtree [22] and by Heo [23]. As shown in Figure 4b, the peak area ratio of 5-FAM/BODIPY changed more than the other two pairs, FL/BODIPY and 5-FAM/FL, with successive injections. This may be due to the large electrophoretic velocity difference between 5-FAM (-3 charge) and BODIPY (neutral) while the difference for FL (-2 charge)/BODIPY and the 5-FAM/FL pair is smaller. Therefore, when an internal standard (IS) is selected, the IS and the target analyte should have as similar electrophoretic mobilities as possible for practical applications.

To reduce the Laplace pressure effect produced by the liquid in the SW and BR, the reservoirs attached to the SW and BR were changed by using cuboids as shown in Figure 1b. The length and width of the cuboid are 16.0 mm and 4.0 mm, respectively. The same experiment as in Figure 4 was carried out and the results are shown in Figure 5. The reproducibility of both peak height (Figure 5a) and the peak area ratios (Figure 5b) was significantly improved. This improvement may be attributed to the reduction in the Laplace pressure produced by the liquid meniscus [22] in the cuboid reservoirs relative to the circular ones.

3.5 Dispensing bias

The injection bias can be effectively reduced by using hydrodynamic sample flow. However, the bias during the dispensing process may still exist. As discussed previously [13,19], the dispensing process produces bias for analytes with different electrophoretic mobilities. The analyte distribution between the separation channel and SW channel was analyzed theoretically in the following part.

The analyte injected into the separation channel is mainly from the BR channel in which the sample solution is pushed by the hydrostatic pressure. This analyte plug will be split into two parts during the dispensing process: one part is injected into the separation channel for the subsequent separation, and the other is pushed into the SW channel (Figure 2c). The apparent velocities of analytes in the BR channel (V_{BR}) and the separation channel (V_{BW}) are determined by Equations 1 and 2.

$$V_{BR} = E_{BR}\mu_a - V_{PBR} \quad (1)$$

$$V_{BW} = E_{BW}\mu_a + V_{PBW} \quad (2)$$

where E_{BR} and E_{BW} are the electric field strength, V_{PBR} and V_{PBW} are the pressure-induced velocities in the BR and separation channels, respectively; μ_a is the apparent mobility of an analyte. If the sectional channel dimensions are uniform except the intersection and if the analyte is evenly distributed in the sample plug, the percentage (r_{inj}) of an analyte injected into the separation channel is determined by Equation 3.

$$r_{inj} = \frac{V_{BW}}{V_{BR}} \quad (3)$$

According to Equations 1-3, the bias of dispensed analytes with different electrophoretic mobilities can be determined with Equation 4.

$$\frac{r_{inj}^m}{r_{inj}^n} = \frac{(\mu_a^m + V_{PBW}/E_{BW})(\mu_a^n - V_{PBR}/E_{BR})}{(\mu_a^n + V_{PBW}/E_{BW})(\mu_a^m - V_{PBR}/E_{BR})} = r_{mn}r_{nm} \quad (4)$$

where

$$r_{mn} = \frac{(\mu_a^m + V_{PBW}/E_{BW})}{(\mu_a^n + V_{PBW}/E_{BW})} \quad (5)$$

$$r_{nm} = \frac{(\mu_a^n - V_{PBR}/E_{BR})}{(\mu_a^m - V_{PBR}/E_{BR})} \quad (6)$$

where m and n mark two different analytes. Suppose $\mu_a^n > \mu_a^m$, then $r_{mn} \geq \mu_a^m/\mu_a^n$ and $r_{nm} \geq \mu_a^n/\mu_a^m$. Therefore, only when there is no pressure-induced flow, $r_{mn}r_{nm} = 1$, which means there is no dispensing bias for analytes with different electrophoretic mobilities. However, the higher level of solution in SR produces pressure-induced flows. In addition, the Laplace pressure produced in SW plays a more significant role than the level difference [22]. According to Equations 4-6, this bias can be reduced by decreasing the ratio of V_{PBW}/E_{BW} and/or V_{PBR}/E_{BR} .

One way to affect the V_{PBW}/E_{BW} ratio is to vary the pressure-induced flow which is related to the liquid level difference, as well as the Laplace pressure produced in the SW. According to equations 4-6, the injection bias would be expected to increase with an increase in the pressure-induced velocity. The liquid level difference was varied by adding various volumes (40 to 120 μ L) of sample solution in SR and averaging the results from replicate ($n = 5$) injections at each volume. The 5-FAM/FL peak area ratio increased from 2.02 to 2.16 (RSD < 1%) in

agreement with the prediction of Equation 4. Note that the Laplace pressure effect was reduced by using large reservoirs but it was not completely eliminated.

An alternative approach is to affect the V_{PBR}/E_{BR} ratio by varying E_{BR} while maintaining liquid level differences between SR and the other reservoirs by adding a fixed volume of solution to each reservoir. The voltage setup is shown in Table 2. The SR and the BW were floated and grounded, respectively. The voltage setup produced a fixed electric field strength of 600 V/cm in the separation channel. The increase of the field in the BR channel virtually caused the field increase in the SW channel. Therefore, the EOF in the SW channel varied while it was constant in the separation channel. The first series of experiments was performed from condition #1 to #6 (Table 2) as a series of six continuous injections for each condition. A second series was performed from #6 to #1, again with six continuous injections for each condition. The results for the six continuous injections under each condition were averaged (Table 2) in order to reduce artifacts caused by the change in pressure with cumulative injections. The applied voltage was calculated with Equations 7-9.

$$U_{SW} = E_{BW}l_{BW} - E_{SW}l_{SW} \quad (7)$$

$$U_{BR} = E_{BW}l_{BW} - E_{BR}l_{BR} \quad (8)$$

$$E_{BR} = E_{BW} + E_{SW} \quad (9)$$

where U , E and l are applied voltage, electric field strength and the channel length, respectively; the subscripts indicate the related reservoirs. For the single-T microchip, $l_{BW} = 8.00$, $l_{SW} = 0.50$, and $l_{BR} = 0.40$ cm. As can be seen in Table 2, the peak area ratio in each group is decreasing with the increase of the electric field strength (E_{BR}) in the BR channel. This trend is consistent with Equations 4. That is to say, the injection bias due to different electrophoretic mobilities was reduced by increasing E_{BR} . We can also see that the decreasing slope is largest for 5-FAM/BODIPY pair and smallest for 5-FAM/FL pair. This situation can be attributed to the magnitude of the electrophoretic mobility differences between the analytes making up each ratio pair.

3.6 Double-T microchip

The situation of the injection and dispensing process in a double-T microchip in many aspects is similar to that in the single-T microchip. The process was also monitored using 50- μ M FITC in separation buffer in the SR as shown in Figure 6. SR and SW can essentially be exchanged [16]. Considering the convenience of the gated process, the reservoir assignment is as shown Figure 6a. The sample flow from SR split into two flows in the opposite directions in the injection step (Figure 6b). When the dispensing process (Figure 6c) was immediately switched on after the injection step, the sample solution in the BR channel was divided perpendicularly into the separation channel and the SW channel.

As in the single-T microchip, the cuboid reservoirs greatly improved the reproducibilities of the peak heights and the peak area ratios for replicate injections from an initial filling of the SR with the sample solution. Similar experiments for the bias studies were also carried out on the double-T microchip with cuboid reservoirs attached to the BR and SW. As in the single-T microchip experiments, the peak area ratio for the 5-FAM/FL pair was found to decrease with increased electric field strength in the BR channel. The peak area ratio of 5-FAM/FL decreased from 2.71 to 2.54 (RSD < 1%) when the electric field strength in the BR channel was increased from 1034 to 1789 V/cm. Therefore, Equations 4-6 are also applicable to the double-T microchip.

4 Concluding remarks

With the miniaturization and integration of CE instrumentation, the usual pressure injection becomes complicated due to the presence of branch channels with open communication to the main separation channel. However, by properly controlling the flows in the channels, hydrodynamic injection can be realized in μ CE.

This simple hydrodynamic injection developed in single-T and double-T microchips produces nearly bias-free sample injection and bias-reduced sample dispensing by appropriately setting up the voltage program and adding an appropriate volume of sample solution. The liquid level differences in the reservoirs must be tightly controlled and should not be too small or too large. A small liquid level difference requires a long time for the sample flow to enter the BR channel, so diffusion will play a role in resolution. While a large liquid level difference produces a large electrophoretic analyte dispensing bias. For the reservoir size used here, 80 μ L of solution produces a 2-3 mm level difference. The sample flow from the SR continues as soon as sample solution is pipetted into the SR. To reduce this flow effect, the sample solution should be added in the SR after the microchip already sits on the microscope; and the voltage and data recording start as soon as possible after the sample loading.

This hydrodynamic injection scheme described here will be used in protein-DNA binding studies which requires bias-free injection of the sample solution because the peak area ratio of the free and bound DNAs [24] needs to be determined by μ CE.

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Abbreviations

CE, capillary electrophoresis; μ CE, microchip capillary electrophoresis; FITC, fluorescein-5-isothiocyanate; FL, Fluorescein disodium salt; 5-FAM, 5-carboxyfluorescein; RSD, relative standard deviation; BR, buffer reservoir; SR, sample reservoir; BW, buffer waste reservoir; SW, sample waste reservoir.

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Figure 1.

Schematics of single-T microchips with attached plastic reservoirs: (a) four attached reservoirs were cut from 200- μ l pipette tips with a diameter of 5.0 mm and (b) two reservoirs are 200 μ l pipette tips, and the other two reservoirs are polypropylene cuboids with the dimensions 16 mm $\times$ 4 mm.

Figure 2. Injection and dispensing process in a single-T microchip. (a) gated flows; (b) hydrodynamic injection under a hydrostatic pressure; and (c) analyte dispensing. The right side shows voltage (kV) configuration and bulk flow directions in each step.

Figure 3.

Voltage program for gated electrokinetic or diffusion injection: (a) injection and (b) dispensing. The voltages (kV) used are shown in each step along with the bulk flow directions.

Figure 4.

Variations of the peak height and peak area ratio for successive injections in a single-T microchip with four circular reservoirs. (a) Successive injections ($n = 46$) detected at 4.0 mm from the intersection. Three peaks in each injection are BODIPY, FL and 5-FAM respectively from left to right. (b) Peak area ratio in each run.

Figure 5.

Variations of the peak height and peak area ratio for successive injections in a single-T microchip with two circular and two cuboid reservoirs. (a) Successive injections ($n = 46$) detected at 4.0 mm from the intersection. Three peaks in each injection are BODIPY, FL and 5-FAM, respectively from left to right. (b) Peak area ratio in each run.

Figure 6.

Injection and dispensing process in a double-T microchip: (a) gated flows; (b) hydrodynamic injection under a hydrostatic pressure and (c) analyte dispensing. The right side shows voltage configuration and bulk flow directions in each step.

Table 1
Reproducibility comparison of peaks produced with different injection

	Electrokinetic ^a		Diffusion ^b		Hydrodynamic ^c	
	FTTC	5-FAM	FTTC	5-FAM	FTTC	5-FAM
Peak Area	AVE %RSD	182.7 21.8	307.8 3.0	119.1 20.6	475.6 11.7	156.8 3.7
Area Ratio	AVE %RSD	1.7 17.0	4.1 16.2		4.0 1.7	

^aElectrokinetically gated sample loading followed by 1 s electrokinetic injection (Figure 3).

^bElectrokinetically gated sample loading followed by 10 s diffusion injection (Figure 3).

^cGated hydrodynamic sample loading followed by 5 s hydrodynamic injection (Figure 2).

Table 2

Electric field strength effect on bias

#	E _{BR} V/cm	V _{BR} kV	V _{SW} kV	FAM/B ^a	FL/B ^a	FAM/FL ^a
1	1050	5.22	4.62	1.00	1.00	1.00
2	1200	5.28	4.56	0.98	0.99	1.00
3	1350	5.34	4.50	0.95	0.96	0.99
4	1500	5.40	4.44	0.92	0.94	0.99
5	1650	5.46	4.38	0.88	0.91	0.98
6	1800	5.52	4.32	0.88	0.90	0.97

^aRelative peak area ratio, suppose #1 is 1.00. Obtained with two loadings; one was run from #1 to #6, the other was from #6 to #1. Final is theaverage of two sets, 6 runs each. VBW was grounded and VSR floated. B=BODIPY 503/505.