An effect of finite reservoir size on pressure gradient generation in a pinched injection sample plug generation in cross design electroosmotic microfluidic device

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Abstract. In performing microfluidic electrophoresis, sample loading must be prioritized since the shape of the initial sample plug injected into the separation channel immensely influences the electrophoretic separation efficiency. Non-zero pressure gradient due to different liquid level in finite size reservoirs is generated as a result of continuous electroosmotic flow (EOF) pumping, resulting in undesired parabolic pressure profile both in the same and opposite direction of sample propagation. This issue could be alleviated by fabricating larger reservoirs which can maintain the liquid level due to gradual volume changing as time elapses. This work presents experimental and numerical study on effect of 3.5 – 8.0 mm reservoir size on Rhodamine B plug flow generation from pinched injection sample loading method in cross design microfluidic device. COMSOL Multiphysics AC/DC module was used in calculating electric field distribution from desired applied voltages. The shapes of the injected sample plugs in the beginning of separation step were studied by varying the time of injection step. The experimental result shows that long-tailed sample plug and pressure profiles were generated when the injection time is 2 minutes or more. For the 8 mm diameter reservoirs, the flow profile illustrates pure EOF plug flow when the injection time does not exceed 1.5 minutes. This implies pressure gradient is virtually disappeared. The result of this study will be later applied for protein transferrin electrophoretic separation.

1. Introduction
Microfluidic electrophoresis is one of the most widely used techniques in separating molecules according to their electrical charge. For an ideal electrophoretic process, electroosmotic flow (EOF) is used in electrokinetically driving an ionic solution along a microchannel for both sample introduction and the separation process [1]. Voltages applied to reservoirs at the ends of microchannels generate electric fields that lead to EOF. Flow characteristic under EOF is called plug flow, in which virtually every molecule of the background solution moves at the same speed under the applied voltages. Since reservoirs have a finite size, EOF induces fluid buildup at reservoirs, creating a non-zero pressure gradient. This results in the destruction of plug flow profile, which in turn reduces the electrophoretic separation efficiency. It has been shown that for a straight microchannel, enlarging the two reservoirs helps alleviate pressure gradient generation [2]. In this work, the investigation was extended to a more practical cross-design microfluidic...
device to inspect the role of pressure buildup in reservoirs during the pinched sample injection step and on the analyte flow profile during the separation step in the devices with reservoirs of various sizes.

2. Theory
In microfluidics, the flow velocity field of fluids with ionic species under the influence of an external electric field can be described as a superposition of steady EOF due to a Coulomb force and time–dependent pressure driven flow due to the change of induced pressure ($\nabla p$) from the liquid level difference in the reservoirs with time as shown in equation (1) as follows [3]:

$$v(y, z, t) = v_{eo} + v_p(y, z, t),$$

$$v(y, z, t) = -\frac{\varepsilon \zeta}{\mu} E - \frac{1}{2\mu} \frac{\partial p(x, t)}{\partial x} \left[ \frac{h^2}{4} - z^2 + \frac{8}{h} \sum_{n=1}^{\infty} \frac{(-1)^n \cosh(my)}{m^3} \cosh(mz) \right] \hat{x}. \quad (2)$$

The first term on the right-hand side of equation (2) is characteristic of the plug–like EOF profile in a straight microchannel where $\varepsilon$ is the fluid’s permittivity, $\zeta$ is the zeta potential, and $E$ is the external applied electric field parallel to the wall [4]. The second term is the quasi–static pressure driven flow profile in a rectangular cross–section microchannel where $\mu$ is the fluid’s dynamic viscosity, $h$ and $b$ are height and width of microchannel, respectively, and $m = (2n-1)\frac{\pi}{h}$ [5].

3. Materials and Methods
The microfluidic device was fabricated from polydimethylsiloxane (PDMS) (Sylgard 184, Dow Corning) bonded with a PDMS coated glass slide. The device consisted of 100 $\mu$m wide microchannels with cross–shaped intersection where the injection and separation channels were 16.1 mm and 50 mm long respectively as shown in figure 1 (a).

Figure 1. (a) Diagram showing the dimensions of the cross-shaped microchannels and (b) the fabricated microchip for the pinched injection plug generation study with (c) a magnified intersection area.

Reservoirs of size 3.5 – 8.0 mm were made by punching 4 mm thickness of PDMS replicas using biopsy punches (Integra Militec, Integra York PA, Inc.) at each end of microchannel. The background electrolyte (BGE) used was 50 mM Tris–HCl buffer at pH 8.5 added with 0.2% w/v sodium dodecyl sulfate (SDS). The initial volume of the buffer solution in the reservoirs were equally filled using a micropipette. The fluid volume in the sample reservoir was replaced with Rhodamine B prior to conducting the experiment. The high DC potentials supplied to each reservoir (SmartPower™ 4000, STRATAGENE) during analyte injection and separation steps are shown in table 1. The pressure gradient corresponding to equation (2) was numerically calculated using the least squares fitting method with the experimental data.

4. Results and Discussion
The fabricated microfluidic chip for the plug generation study is shown in figure 1 (b). The actual dimensions of the fabricated microchannels were 89 $\mu$m in width and 30 $\mu$m in height. The resulting
Table 1. Voltage sequence for a pinched injected sample plug generation.

| Reservoir                              | Voltage (V) |
|----------------------------------------|-------------|
|                                        | Injection step | Separation step |
| Sample reservoir (SR)                  | 133          | 543            |
| Sample waste reservoir (SW)            | 0            | 543            |
| Buffer reservoir (BR)                  | 131          | 700            |
| Buffer waste reservoir (BW)            | 267          | 0              |

corners at the channels’ intersection shown in figure 1 (c) were curved instead of the designed right–angle due to limited resolution in the mask making process.

When performing pinched injection, the shape of the injected sample column is shown in figure 2 (a), confined according to the electric field distribution from SR to SW as shown in figure 2 (b).

Figure 2. (a) The microscopic image of Rhodamine B column shape during pinched injection and the simulated electric field distribution of the (b) injection step and (c) separation step at the intersection area with black, solid electric field streamlines.

Figure 3. The microscopic images of injected Rhodamine B plug propagation along separation channel of microchips with (a – c) 3.5 mm diameter reservoirs, (d – f) 6 mm diameter reservoirs, and (g – i) 8 mm diameter reservoirs using injection times as depicted.

For the separation step, it can be seen from figure 3 that the 2–minute injection time was too long for all reservoir sizes as parabolic flow profiles were clearly visible. The pressure gradient generation for the
3.5 mm reservoir was most severe as liquid built up in SW at a faster rate. The instantaneous velocity field at 1.5 mm away from the intersection was measured by tracking the front profiles on two consecutive frames in order to determine the velocity component due to pressure gradient. It was assumed that at this position the flow is fully developed as pressure gradient is time independent. With $\zeta = 25$ mV and 14 kV/m applied electric field in the separation channel, EOF velocity is assumed to be $280 \mu$m/s. The calculated EOF velocity and pressure gradients are illustrated in figure 4.

![Figure 4](image)

**Figure 4.** (a) The numerically adjusted velocity field corresponding with equation (2) compared to the experimental results. (b) The summarized pressure gradient of different injection times from numerical calculation.

Figure 4 (a) demonstrates how the velocity component due to pressure gradient was obtained for the 3.5 mm reservoir with a 1–minute injection time. The numerical calculation of pressure gradient at different injection times is also shown in figure 4 (b). The magnitude of pressure gradient was lowest in the 8 mm reservoirs with a 1–minute injection time, confirming the previous 2D study [2] that larger reservoirs help reduced pressure gradient generation.

**5. Conclusion**

In this study of finite reservoir size effect on pinched injection sample plug generation, both experimental observations and numerical calculations were performed to determine the pressure gradient induced from increasing of injection time. The experimental results show that the microchip with 8 mm diameter reservoirs were preferable for longer periods of injection step due to less parabolic profile generation on injection plugs corresponding with the minimum magnitude of induced pressure gradient. However, the additional study of backpressure in separation step, which can be also generated due to accumulation of liquid in buffer waste reservoir, is needed to efficiently achieve further electrophoretic applications.

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