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Research Article

Improved hydrostatic pressure sample injection by tilting the microchip towards the disposable miniaturized CE device

A simple method of hydrostatic pressure sample injection towards a disposable microchip CE device was developed. The liquid level in the sample reservoir was higher than that in the sample waste reservoir (SWR) by tilting microchip and hydrostatic pressure was generated, the sample was driven to pass through injection channel into SWR. After sample loading, the microchip was levelled for separation under applied high separation voltage. Effects of tilted angle, initial liquid height and injection duration on electrophoresis were investigated. With enough injection duration, the injection result was little affected by tilted angle and initial liquid heights in the reservoirs. Injection duration for obtaining a stable sample plug was mainly dependent on the tilted angle rather than the initial height of liquid. Experimental results were consistent with theoretical prediction. Fluorescence observation and electrochemical detection of dopamine and catechol were employed to verify the feasibility of tilted microchip hydrostatic pressure injection. Good reproducibility of this injection method was obtained. Because the instrumentation was simplified and no additional hardware was needed in this technology, the proposed method would be potentially useful in disposable devices.

Keywords:
Disposable device / Hydrostatic pressure sample injection / Microchip capillary electrophoresis / Tilting microchip
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1 Introduction

The ability to perform laboratory operations on a small scale using miniaturized devices is very appealing [1, 2]. Small volumes reduce the time taken to synthesize and analyze a product, the unique behavior of liquids at microscale allows greater control of molecular concentrations and interactions, and reagent costs and the amount of chemical waste can be much reduced. Compact devices also allow samples to be analyzed at the point of need rather than a centralized laboratory. Applications of microfluidics-based lab-on-a-chip would include healthcare delivery and monitoring in developing economies, home healthcare and use in doctor's offices in developed economies, uses in homeland security and counterterrorism, use by first responders (police, paramedics and fire departments), applications in veterinary medicine and incorporation into environmental and food safety monitoring.

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Abbreviations: BR, buffer reservoir; **BWR,** buffer waste reservoir; **SR,** sample reservoir; **SWR,** sample waste reservoir

Microchip CE has been widely researched [3–5] since the planar glass chip was introduced [6, 7]. The controlled injection of minute quantities of sample solution in a separation channel is a prerequisite for a successful analysis. Considerable effort has been devoted to the optimization of injection mode. Of all the proposed injection methods, electrokinetic injection based on EOF is the main preference. Two of the main limitations of electrokinetic injection are its strong dependency on surface properties of channel walls and the bias effect towards different species [8, 9]. To avoid biased injection, sample loading by pressure injection methods, which are insensitive to channel surface and sample properties, have been developed [10–19].

Bai *et al.* [10] proposed pressure pinched injection of nanoliter volumes by a multiport injection valve and three syringe pumps. Solignac and Gijs [11] developed a system in which the samples were injected hydrodynamically by applying pressure pulse to a membrane on the reservoir using mechanical actuator. Futterer *et al.* [12] reported an injection system based on dynamic control of the reservoir pressures at the end of each channel. Wu *et al.* [13] proposed a sample introduction method based on push/pull pressure flow using dual-syringe pump with switching valve. Cho *et*

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al. [14] developed a pneumatic injection channel surfaces. Baldock *et al*. [15] designed a novel sample injector, capable of delivering variable volume samples to microdevice with both hydrostatic pumping and syringe pumps, for miniaturized isotachophoretic separations. Kaniansky *et al*. [16] used fixed volume sample loops and four peristaltic micropumps on a column coupling chip for both multicomponent and high salinity samples. Zhang *et al*. [17] developed an approach for injecting well-defined nonbiased picoliter sample plugs into separation channel of microfluidic chip-based CE system, the negative pressure-driven flow was generated by a syringe pump combined with EOF and static pressure created by differential liquid levels in the on-chip reservoirs. Lin and co-workers [18] reported injection by hydrostatic pressure in conjunction with electrokinetic force on a microfluidic chip, the major flaw of this injection method is the introduction of a tail. This tail affects, sometimes even destroys, the separation. In order to overcome the limitation, the same group developed double-cross hydrostatic pressure sample injection to eliminate the tail by the addition of a controlling channel into the crossinjector [19]. The concept of gravi-cell was presented by DiagnoSwiss (http://www.diagnoswiss.com. 2007) where the sample was deposited dropwise on a tilted chip, but it is a pity that the detailed operation was not introduced.

In this report, we describe an improved hydrostatic pressure sample injection method in a classical simple crossform microchip CE. Hydrostatic pressure injection was achieved by tilting the microchip simply (Fig. 1), after sample loading, the microchip was levelled for separation. Towards disposable devices, the technique for sample injection was preferred because no additional hardware was needed and nonbiased sample plug was obtained. Efficiency of the injection mode was discussed theoretically and experimentally. Fluorescence observation and electrochemical detection of dopamine and catechol were employed to verify the feasibility of tilted microchip hydrostatic pressure injection.

2 Materials and methods

2.1 Chemicals

All reagents were of analytical grade. FITC, dopamine and catechol were obtained from Sigma (St. Louis, MO, USA). Sylgard 184 (PDMS) was from Dow Corning (Midland, MI). 20 mM PBS (pH 7.0) was prepared with Na_2HPO_4 and KH_2PO_4 (Nanjing Chemical Reagents Factory, Nanjing, China). All solutions were prepared with doubly distilled water and passed through a 0.22 μm cellulose acetate filter (Shanghai Bandao Factory, Shanghai, China).

2.2 Microfluidic chip system

A laboratory-made microchip with cross-type channel combined with in-channel amperometric detector was used in this study. The amperometric detector was located in the detection reservoir and consisted of an Ag/AgCl wire reference electrode, a Pt wire counter electrode and a laboratory-made carbon fibre working electrode (id 7 μm) [20]. Amperometric detection was performed with a CHI 832b electrochemical workstation (CHI, Shanghai, China).

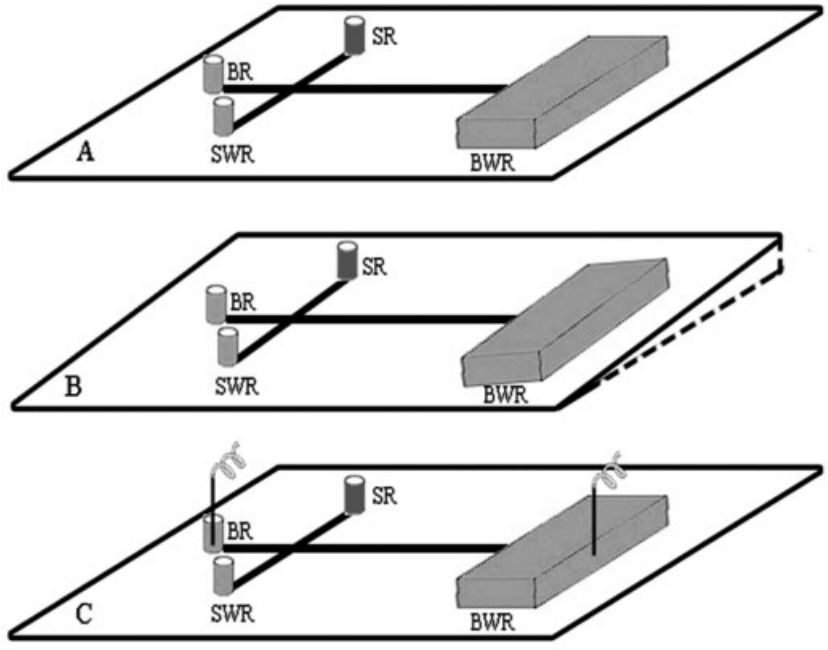

Figure 1. Scheme of microchip status in the process of injection. (A) Before injection, four reservoirs were filled with desired solution; (B) in injection stage, the microchip was tilted and the sample was driven to pass through the injection channel; (C) after sample loading, the microchip was levelled and separation voltage was applied.

The laboratory-made power supply had a voltage ranging from 0 to 5000 V. Current can be monitored graphically in real time. Electrical contact with the solutions was achieved by placing platinum wires into each of the reservoirs.

The PDMS microchip used for this work was fabricated with a positive relief structure of GaAs master [21]. Channel design is shown in Fig. 2A, the channels were fabricated to a depth of 30 μm and a width of 60 μm. Three 0.3 cm id and 0.3 cm deep holes were punched on the chip, serving as reservoirs with volumes of approximately 15 μL each. Buffer waste reservoir (BWR) was designed on the platform on which microchip was set, and it was located at the end of the microchip and served as detection reservoir. The channel CD between sample reservoir (SR) and sample waste reservoir (SWR) was used for sampling and the channel AB between buffer reservoir (BR) and BWR was used for separation. The PDMS pieces were oxidized in a plasma oxidizer (60 s, 18 W) and sealed permanently. AE, CE and DE were 0.5 cm long and BE 3.5 cm long.

2.3 Fluorescent image

Fluorescence images were obtained by means of a microscope (Nikon ECLIPSE TE2000-U, Japan).

2.4 Electrophoresis procedures

In initial state, the microchip was levelled, 13.5 μL of sample was filled in SR (approximately 0.27 cm height liquid level), 15 μL of 20 mM PBS running buffer was filled in SWR and BR (approximately 0.30 cm height liquid level), and BWR was filled with 20 mM PBS running buffer to keep the same liquid level as BR (Fig. 1A). Then microchip was tilted for injection, a wedge with defined height was used to tilt the microchip for desired angle (Fig. 1B). After 40 s of sample loading, the microchip was levelled and electrophoresis was implemented under high separation voltage simultaneously (Fig. 1C).

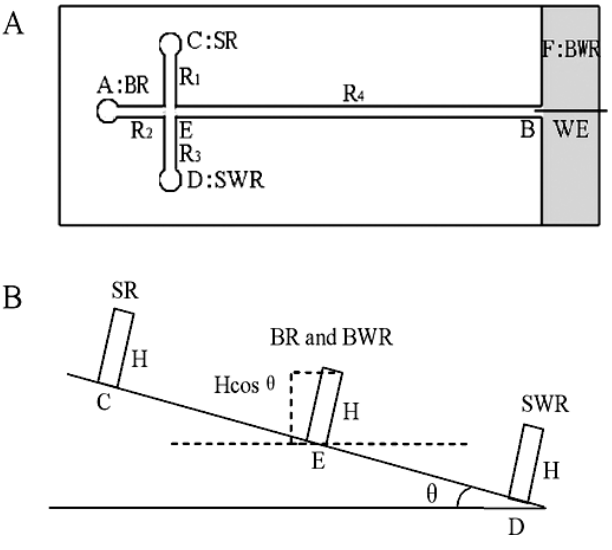

Figure 2. (A) Schematic drawing of the simple crosschannel microchip used in the present work and (B) side view of the tilted microchip.

In a routine CE procedure, the microchip was tilted with 17°, separation voltage was 800 V, detection voltage was +1.4 V (*vs.* Ag/AgCl wire). All experiments were performed at room temperature. CE with electrokinetic injection was performed with the procedure reported previously [21]. Briefly, a laboratory-made programme for the power supply was used to control the voltage switching from sampling to separation. Sampling mode was simple crossing without pinch, while in the separation procedure, both reservoirs were kept floating.

3 Results and discussion

The motivation of the sample injection design is to pursue a simple natural injection method towards disposable microchip CE devices. Hydrostatic pressure is preferred among the number of power for injection due to nonbiased sample loading and additional equipment free. In this study, the effects of tilted angle, initial liquid height and injection duration on electrophoresis were investigated.

3.1 Effect of tilted angle on sample plug

A defined sample plug is a prerequisite for a successful analysis. Microfluidic flow in the chip was determined by liquid level difference between reservoirs in the injection procedure. In order to obtain an optimized stable microfluidic flow, different tilted angles were investigated for injection.

A theoretical result was deduced with equal initial liquid level in four reservoirs. After the microchip is tilted, hydrostatic pressure exists not only between SR and SWR, but also between the reservoirs of different relative liquid levels (SR *vs.* BR and BWR, SWR *vs.* BR and BWR). These pressures drive the solutions into SWR *via* cross-intersection. Therefore, three strands of flow converge at the intersection, forming a pressure-pinching configuration [8]. This pinching profile resembles that of electrokinetic pinching [22].

Using a common analogy between electricity and fluidics [23], the analogy suggests to define flow resistances in the channel R, which is proportional to its length

$$R = kL \tag{1}$$

where k is the coefficient. Accordingly, the flow resistances of channels were defined as R_1, R_2, R_3 and R_4, respectively (shown in Fig. 2A). The pressure corresponds to the electrical voltage P. The pressure-dynamics at the knot E, P_0, is obtained using the corresponding Kirchhoff's law (the sum over all currents at a knot vanishes). The law is expressed in the following equation in this issue:

$$\frac{P_S - P_0}{R_1} + \frac{P_B - P_0}{R_2} + \frac{P_{BW} - P_0}{R_4} = \frac{P_0 - P_{SW}}{R_3} \quad (2)$$

where P_S, P_B, P_{BW} and P_{SW} are hydrostatic pressures in SR, BR, BWR and SWR, respectively. In a microchannel, the hydrostatic pressure difference, ΔP, can be quantified using the following equation:

$$\Delta P = \varrho g \Delta H \quad (3)$$

Then the hydrostatic pressures in different reservoirs (*vs.* the intersection *E*) are quantified as follows:

$$P_S = \varrho g(H \cos\theta + L_{CE} \sin\theta) \quad (4)$$

$$P_B = P_{BW} = \varrho g H \cos\theta \quad (5)$$

$$P_{SW} = \varrho g(H \cos\theta - L_{DE} \sin\theta) \quad (6)$$

Combining Eqs. (1)–(6), the following equation can be readily deduced:

$$P_0 = \varrho g H \cos\theta \quad (7)$$

The pressure-dynamics at the knot *E*, P_0, corresponding to $\rho gH\cos\theta$ hydrostatic pressure, is equal to the hydrostatic pressure in BR and BWR. The sample can be driven into SWR by tilting the microchip but not leak to separation channels, neglecting diffusion at the interface of buffer and sample flow.

The data indicate that the hydrostatic pressure difference between intersection *E* and BR and BWR is not dependent on tilted angles, channels' lengths and initial liquid levels in the reservoirs.

Actually, diffusion exists in the intersection in experiments. The sample plug was broadened with injection duration. In order to overcome the problem, we slightly decreased the sample height in SR to keep the hydrostatic pressure at the point *E* less than that in BR and BWR. Sample (13.5 μL instead of 15 μL) was filled in SR, the diffusion of sample into separation channel was eliminated. According to Eq. (2), P_0 is corresponding to the hydrostatic pressure of 0.29 cm liquid height with 17° tilted angle in the microchip. Fluorescence images (shown in Fig. 3) demonstrated the results.

Model analytes of dopamine and catechol were employed to evaluate the efficiency of the injection mode. Separations of 500 μM of dopamine and catechol were implemented with different tilted angle injection such as 30° (ΔH = 0.50 cm), 17° (ΔH = 0.30 cm) and 9° (ΔH = 0.15 cm), the liquid height of sample was set as 0.45, 0.27 and 0.13 cm, respectively. Electrophoretic results agreed well with theoretical prediction. Electropherograms are shown in Fig. 4. We could observe that the same electropherograms were obtained after enough injection duration (100 s) independently on the angles employed for injection, *i.e.*, the changes in tilted angles had little effects on the sample plug with this injection method.

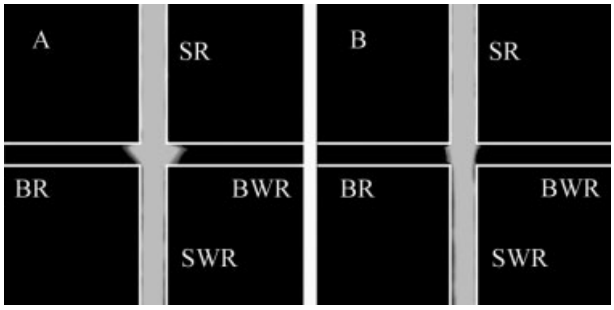

Figure 3. Fluorescence images of sample loading profile with different liquid height in SR with tilted angle of 17° after 100 s injection. (A) Sample liquid level 0.30 cm height and (B) sample liquid level 0.27 cm height.

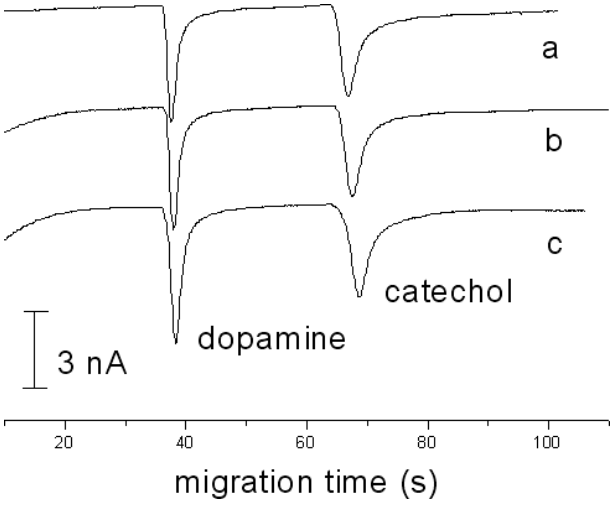

Figure 4. Electropherograms for the separation of 500 μM of dopamine and catechol with different tilted angle injection, sample loading time: 100 s. (a) 30° (ΔH = 0.50 cm); (b) 17° (ΔH = 0.30 cm) and (c) 9° (ΔH = 0.15 cm).

3.2 Effect of tilted angle on injection time

For an injection method, sample loading time is an important parameter. In this tilted microchip hydrostatic pressure injection method, injection duration with different tilted angles was investigated. Dopamine was used as electroactive sample. The electrochemical detector was set at the channel exit of SWR, the microchip was tilted as injection and the signal was recorded. Current–time plots are shown in Fig. 5. We could observe that the signal of dopamine was constant after 30, 60 and 130 s in different tilted angles of 30° (ΔH = 0.50 cm), 17° (ΔH = 0.30 cm) and 9° (ΔH = 0.15 cm), respectively. When the tilted angle was too big, the three-electrode detector would be affected due to the changed liquid state. Conside-

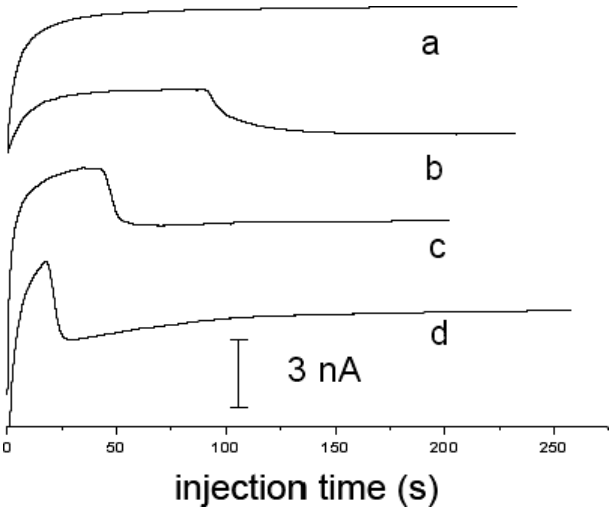

Figure 5. Measured current of 500 μM dopamine at the SWR exit as a function of injection time. The microfluidic flow was generated by differential hydrostatic pressure between SR and SWR with different tilted angles. (a) 17° tilted angle, with blank solution injection; (b) 9° tilted angle; (c) 17° tilted angle; (d) 30° tilted angle. Detection potential: +1.4 V.

ring analysis efficiency and stability of the detector, the 17° tilted angle was selected for injection in this research. According to the electropherogram in Fig. 5, the concentration of analyte was constant at the SWR exit with 60 s injection duration. Here the sample plug was stable at the cross-section *E* after 40 s with 17° tilted angle for injection. Forty seconds was selected as injection time in this research.

Theoretical prediction was also made to demonstrate the experimental results. Hydrostatic pressure drop (ΔP) through the capillary is presented in Eq. (3), when the microchip was tilted, the hydrostatic pressure difference between SR and SWR could be calculated as:

$$\Delta P = \varrho g \Delta H = \varrho g(H_S \cos\theta + L_{CD} \sin\theta - H\cos\theta) \quad (8)$$

where H_S is the liquid height of sample. Therefore, ΔP is mainly determined by L_{CD} sinθ, and the maximum average velocity (v) on the capillary can be given by Eq. (9), known as Hagen–Poiseuille's equation [24, 25].

$$v = \frac{d^2}{32\mu}\frac{\Delta P}{L} \quad (9)$$

Here, L is the length of the channel, μ is the viscosity of the solution, and d is the inner diameter of the channel. For the research microchip, using a 1 cm long, 30 μm id injection channel, according to these equations, ΔH is approximately 0.30 cm; as a result the theoretical flow rate is 84 μm/s, and 119 s is needed for the sample reached SWR. Theoretical prediction was agreed with the experimental results in consideration of the approximate inner channel diameter and measurement errors.

From the electropherograms in Fig. 5, another phenomenon could be observed, namely that the signal of current increased with the increase of liquid flow rate. This result was consistent with the literature [26, 27].

3.3 Effect of initial liquid height on injection time

The effect of initial liquid height in reservoirs on injection time was investigated. A 17° tilted angle was employed for injection, electrophoresis was implemented with different initial liquid height of 0.40, 0.30 and 0.20 cm in the reservoirs except SR, and the sample liquid height was set as 0.36, 0.27 and 0.18 cm, respectively. We observed that the injection time for obtaining a stable sample plug was not affected by the absolute liquid level in the reservoirs. In experiments, the reservoirs with different volumes were achieved by adjusting microchip thickness. The result was also demonstrated by Eqs. (8) and (9). The average velocity was little affected by the initial liquid level with a defined tilted angle.

3.4 CE performance

CE with this injection mode was studied using dopamine and catechol as model analytes. Reproducibility of the hydrostatic pressure sample injection was investigated with a standard solution of 500 μM dopamine and 500 μM catechol. The sample consumption was about 0.012 μL at the loading time of 40 s for each cycle. Further increasing loading time resulted in the same sample plug at the channel intersection. Even after 30 analytical cycles (running buffer in BR need to be refreshed periodically) or 20 min injection duration (evaporation influence sample concentration with long injection duration), a good reproducible electropherogram can also be obtained. In this study, five sequential injections were performed (Fig. 6), the consecutive sample peaks demonstrate excellent reproducibility. Peak height precisions for dopamine and catechol were 3.1 and 4.4% RSD (n = 11), respectively.

In addition, a comparison between the present method and electrokinetic sample injection mode was made. According to the electropherograms shown in Fig. 7, the bias

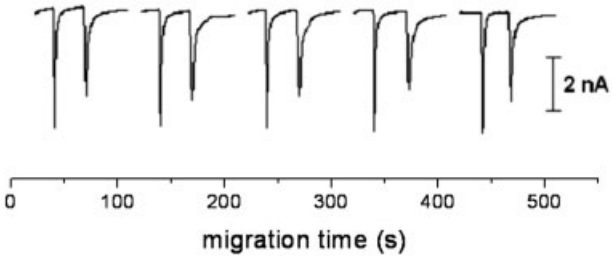

Figure 6. Electropherogram of a sequence separation of 500 μM dopamine and catechol with 17° tilted microchip hydrostatic pressure injection, periodical injection time is 40 s. Detection potential: +1.4 V.

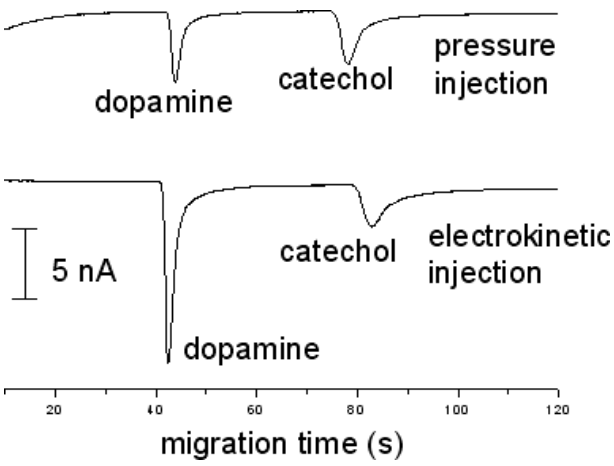

Figure 7. Electropherograms for the separation of 500 μM dopamine and catechol with different injection mode. Separation voltage: 800 V, detection voltage: +1.4 V. (A) Tilted microchip hydrostatic pressure injection with 17° tilted angle, injection duration: 40 s. (B) Electrokinetic injection, injection voltage: 600 V, injection time: 2 s.

effect towards different species existed in electrokinetic sample injection method. The results agreed with literatures reported [8, 9].

4 Concluding remarks

The improved hydrostatic pressure sample injection approach for chip-based CE presented here proved to be a potential method for disposable devices. The technology was not only enabling bias-free analyte transport to the injection point, independently of the properties of sample and buffer solutions, but also producing well-defined sample zones that are little affected by related experimental conditions. Good reproducibility was achieved for analysis of model analytes with electrochemical detector. The operational procedure of this approach was also considerably simplified, with only a single high-voltage power supply used at constant voltage. The technique is particularly useful in commercial microchip towards disposable devices, which will be operated by untrained personnel.

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