Micro Capillary Electrophoresis Chips with Sample Pre-concentration Devices Utilizing Alternating Current (AC) Electroosmosis Effect

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Abstract

The present paper reports a new micro capillary electrophoresis (CE) chip integrated with a sample pre-concentration device utilizing alternating current (AC) electro-osmosis effect. Micro capillary electrophoresis chip has proven to be a powerful tool for sample separation. Not only does it miniaturize the size of the analytical apparatus, but it also executes sample separation in a faster, more efficient way using less samples and reagents. However, the minimum detectable concentration of the samples could still remain an issue. To increase the detectable limits, pre-concentration micro-devices prior to sample separation/detection are of crucial needs. In this study, we utilize a pair of electrodes to generate AC electroosmosis forces such that DNA samples could be focused in a concentration zone, and thus increasing the fluorescence signals. A combination of high-voltage power supplies, high-voltage relays and function generators is used to apply the AC electric signals on the pre-concentration electrodes. Sample plugs in the microchannel are thus concentrated in the pre-concentration zone. Concentrated samples are then injection into the subsequent separation channels. At last, a PMT (Photo-multiplier tube) module is used to detect the fluorescence signals enhanced by the AC electroosmosis effect. Three DNA samples, including λ-DNA (12 bps), φX-174 DNA marker (11 segments) and detection gene for Group A streptococcus (777 bps), have been tested. Successful concentration and separation of these samples have been experimentally verified. The developed micro CE devices with the pre-concentration devices could have significant potential for the analysis of the dilute and low concentration DNA samples.

Key Words: AC Electroosmosis, DNA Analysis, Micro Capillary Electrophoresis, Pre-concentration

1. Introduction

In the last decade, microfabrication of micro capillary electrophoresis (CE) chips for chemical and biomedical applications has been widely investigated [1–4]. Successful separation of DNA and protein samples has been demonstrated. Compared to their large-scale counterparts, these micro CE devices provide several significant advantages, including higher separation efficiency, faster analysis, less sample/reagent consumption. More importantly, these devices could be integrated with other microfluidic devices such that their functionality and reliability could be greatly enhanced. Several integrated microfluidic systems, including CE/mass spectrometry [5,6], CE/optics [7,8], and sample pretreatment/CE [9,10], have been successfully reported in published literature.

For micro CE devices, it provides a fast analysis of complicated samples. However, a detectable concentration limit still exists (~ 10^-9 M) for laser induced fluorescence (LIF) detection, which is one of the most popular schemes for detection of separated samples [11]. Otherwise, the signal-to-noise level will not be acceptable for...
optical detection after separation process. To concentrate the samples, several traditional methods such as filtration, centrifugation, liquid phase extraction, and solid phase extraction were commonly used. However, even though quite useful, these methods may suffer from several drawbacks, including long processing time, intensive human labor, and complicated fabrication process to miniaturize these devices. More importantly, it still remains a challenge to integrate functional miniaturized sample pre-concentration devices with other microfluidic devices. General speaking, concentration of biological molecules is of great needs for most of the microfluidic systems, especially for micro CE devices. Several approaches using microfabrication techniques to concentrate DNA samples have been reported in literature. For example, a DC electric field was used to attract DNA molecules on the electrodes since they are negatively-charged [12,13]. However, these DNA molecules may adhere onto the surface of the electrodes and requires other forces to detach. Therefore, in this study, we propose to use a pair of electrodes to generate AC electroosmosis forces to concentrate bio-samples, especially for DNA molecules. Due to the suitability of the microfluidic systems and relatively simple fabrication process of the micro electrode structures, using electrokinetic forces to manipulate the sub-micron scale bio-molecules was proven to be a promising approach [14–19]. To the best of the current authors’ knowledge, the proposed design represents the first attempt to utilize AC electroosmosis forces to realize the concentration of DNA samples within a micro capillary electrophoresis chip.

DC electroosmosis forces using a constant electric field were generally used to pump fluids inside microchannels and became a popular approach for micro capillary electrophoresis application [20]. Alternatively, AC electroosmosis forces, implying that fluid flow could be induced by an AC electric field, have been reported in previous literature and proven to be a promising approach for manipulation of bio-molecules. In order to detect samples with a low concentration, the pre-concentration chip was used to partially condense samples by using AC electroosmosis forces [21]. The fluid flow could be induced by the AC electric field, and such fluid flows have been reported in many electrokinetic applications. For example, the techniques have been demonstrated for enhancing mixing and improving detection efficiency in microfluidic systems [22–24]. Hence, it is beneficial to develop a microfluidic device, which is capable to integrate these electrokinetic device and other microfluidic modules, to concentrate and detect diversification of biological samples.

In this study, we integrate a pair of AC electroosmosis electrodes and micro CE channels to form a microfluidic chip capable of sample pre-concentration and DNA separation and detection. These electrodes are used to concentrate the dilute concentrations of DNA samples for the subsequent fluorescence detection. By applying an AC potential with an appropriate driving frequency, concentration of the biological particles such as DNA molecules could be achieved. CCD (charge-coupled device) images of the concentrated samples and laser induced fluorescence signals showed that significant concentration of the samples has been achieved. The pre-concentration device was integrated with a micro capillary electrophoresis chip such that on-line sample injection and separation could be performed.

2. Nomenclature

AC: alternating current
ADC: analog to digital converter
BOE: buffered oxide etchant
bp: base pair
CCD: charge-coupled device
CE: capillary electrophoresis
DC: direct current
DNA: deoxyribonucleic acid
EDL: electric double layer
EOF: electroosmosis flow
HPMC: hydroxyl-propyl-methyl-cellulose
LIF: laser induced fluorescence
PMT: photo-multiplier tube
SOG: spin-on-glass

3. Design and Fabrication

AC Electroosmosis is one type of electrokinetics occurring at frequencies below 1 MHz [22–24]. When an electric potential is applied to the electrode, the charge density near the electrode surface is induced and an electric double layer (EDL) is then formed, which is usually referred as electro-polarization process [25]. Figure 1 shows a schematic illustration of fluid flow induced by
the AC electroosmosis forces. Note that the force is induced on the surface of the electrodes and may cause the motion of fluid towards one of the electrodes.

Velocity of the fluid flow around the surface of the electrodes could be represented as follows [26]:

\[
< v > = \frac{1}{8} \frac{\varepsilon V_0^2 \Omega^2}{\mu r (1 + \Omega^2)}
\]

(1)

where \( \varepsilon \) is the permittivity of the electrolyte, \( V_0 \) is the potential applied to the electrodes, \( \mu \) is the viscosity of the electrolyte, and \( r \) is the distance from the center to the electrode gap. The non-dimensional frequency \( (\Omega) \) is given by

\[
\Omega = \omega r \frac{\varepsilon}{\sigma} \frac{\pi}{2} \kappa
\]

(2)

where \( \omega \) is the frequency applied to the electrodes, \( \sigma \) is the conductivity of the electrolyte, and \( \kappa \) is the reciprocal Debye length of the electrical double layer. Previous works also showed that the motion of the fluid induced by the AC electro-osmosis effect depended on the geometry of the electrodes [22]. In order to concentrate DNA samples around the intersection of a cross-shape CE channels (see Figure 2a), we designed a pair of electrodes right at the intersection (Figure 2). After the samples have been concentrated, then a sample plug will be injected into the separation channel for subsequent sample separation and detection. Figure 2 represents the schematic of the proposed micro CE systems with the pre-concentration devices. The micro CE device was constructed by two substrates. The upper plate (Figure

Figure 1. Electro-polarization and formation of AC electro-osmosis flow. The dotted lines represent the flow trace.

Figure 2. Schematic illustrations of micro CE chips with sample pre-concentration device. There are two major steps of the process, namely microfluidic channel fabrication and AC electroosmosis electrode fabrication.
2a) comprises the microchannel structures by using wet glass etching technique. The dimension of the sample flow channels is 80 µm in width and 30 µm in depth, and total length of the separation channel is 6 cm. A close-up view of the electrodes for generating the AC electroosmosis forces was also shown in Figure 2(a). The dimension of the electrodes is 25 µm in width, and the distance between two electrodes is 20 µm. The bottom substrate with the AC electroosmosis driving electrodes to control the motion of fluid was formed by using thin-film deposition techniques. Figure 2(b) illustrates the complete microfluidic chips. After two steps of fabrication process were completed, drilling through the upper glass substrate was performed to create the sample injection/waste chambers and was followed by the alignment of the upper and bottom substrates under an optical microscope. These two substrates were then fusion-bonded in a vacuum oven at 660 °C for 30 minutes. Finally, Spin-on-glass (SOG) was filled into the micro-channels to avoid hydrolysis when the electrode was applied with a high voltage [27,28]. Besides, the SOG coating also enhances the separation efficiency of the samples by reducing the zeta potential on the glass surface [29,30].

A simplified fabrication process for this micro CE chip is shown in Figure 3. There are two major steps of the process, namely microfluidic channel fabrication and AC electroosmosis electrode fabrication. A rapid and reliable method to fabricate microfluidic channels on 1.1 mm thick soda-lime glass substrates (G-Tech Optoelectronics, Shenchu, Taiwan) was used [31]. Briefly, a thin layer of AZ4620 (Clariant, Charlotte, NC, USA) photoresist was spun on the surface of the plate (Figure 3a). After a simple process of lithography, a thin film of the photoresist was used to pattern the microchannel structures on the upper substrate as an etching mask (Figure 3b). To form the microchannel, a commercially available solution (buffered oxide etch, BOE; NH4:HF = 6:1; J. T. Baker, Phillipsburg, NJ, USA) was then used to etch the soda-lime glass for 30 minutes (Figure 3c). After wet etching process was completed, KOH was used to remove the photoresist film (Figure 3d). To form the micro-electrode structures, an electron-beam evaporator was used to deposit a thin layer of chromium onto the glass surface and then a thin layer of gold onto the chromium layer (Figure 3e). The thickness of the chromium and gold thin layer was 100 Å and 3000 Å, respectively. After patterning the thin film of AZ4620 and metal etching as shown in Figures 3(f) and 3(g), the AC electroosmosis electrodes were finally formed (Figure 3h).

Figure 4a shows a photograph of the developed microchip CE device after assembly. The size of the chip is 4 cm in width and 6.35 cm in length. In order to generate the electroosmotic and electrophoretic flows to drive the sample plugs in the channels, five DC electrodes were also integrated onto the chip and placed on each reservoir. For
the purpose of the sample pre-concentration, two AC electrodes were placed around the intersection of the microchannels (Figure 4b). To ensure that the AC electrodes could be enclosed in the microchannels structures, the width of the AC electrodes was 25 µm, and the distance between two electrodes was 20 µm.

4. Experimental Setup

Figure 5 shows the experimental setup for the micro CE system with sample pre-concentration device. To inject and separate the DNA samples, high-voltage power supplies were used to provide DC voltages to generate the bulk flow of the samples by generating electroosmotic and electrophoretic flows. Before the operation process of the proposed micro CE chip device, the DNA sample was loaded into the reservoir 1 (Figure 2a). For sample injection process, a DC potential was applied between reservoirs 1 and 2 by using a high-voltage power supply (FP-3500, Major Science Inc., Taiwan), the sample was driven to the reservoir 2 from the reservoir 1 by DC electroosmotic flow. After the injection process was completed, another DC potential was applied between reservoirs 3 and 5 to drive the sample plug to the pre-concentration zone. For the purpose of sample pre-concentration, AC potentials were applied to the AC electroosmosis electrodes for 30 seconds by using a function generator (8648B, HP, USA) and a power amplifier. After the pre-concentration process was completed, a DC potential was applied between reservoirs 4 and 5 to separate and drive the sample plug to the detection region. Please note the voltages between reservoirs 3 and 5 and reservoirs 4 and 5 were the same. The excitation light source for fluorescence detection was Hg lamp which was filtered using a band pass filter and focusing lens. The excitation light source with 488 nm wavelength, which was located 1.5 cm behind the sample pre-concentration zone, was used for the detection of induced fluorescence signals. The emitted fluorescence signals were then collected using a PMT module (C3830, R928, Hamamatsu, Japan) via a 10X objective focus lens. The amplified optical signals were converted into corresponding analog signals and acquired by a commercially available dual-channel 24-bit ADC module (Model 0224-2, SISC, Taipei, Taiwan) using a personal computer.

5. Results and Discussion

Figure 6 represents the close-up view of the sample pre-concentration zone of the micro chip device. The sample used in the experiment was 12-base λ-DNA. The buffer used was HPMC (hydroxyl-propyl-methyl-cellulose, 1.2%) with a conductivity of 1.2 × 10^{-3} S m^{-1}. In order to observe the DNA under a fluorescent microscope (E400, Nikon, Japan), a fluorescent dye, YORPRO-1(Y3603, Molecular Probes, Inc., Eugene, USA) was mixed with the buffer and injected into the microchannels prior to the injection of the samples. Figure 6(a) shows the CCD (charge-coupled device) images of the DNA samples around the sample pre-concentration zone while no AC

![Figure 5. Schematic of the experimental setup. The system consists of an excitation light source, a fluorescence signal detector, DC high voltage power supplies, and an AC function generator.](image)

![Figure 6. The close-up view of the sample pre-concentration zone (a) without and (b) with AC electroosmosis effect.](image)
potential was applied on the electrodes. To concentrate the \( \lambda \)-DNA molecules, an optimum operating condition with an AC potential of 5 Volts (peak-to-peak) at a frequency of 1 kHz was applied [32]. Note that voltages higher than 5 Volts may cause electrolysis and generate air bubbles. By applying the AC potential of 5 Volts (peak-to-peak) for duration of 30 seconds, a significant increase of the fluorescence signals was observed (Figure 6b). The experimental data clearly represents the effect of the AC electro-osmosis, which induces the motion of the flow and thus concentrates the sample. After converting the intensity of the CCD images, the concentration of the fluorescence signals was enhanced by 50%.

The pre-concentrated samples were then injected into the subsequent microchannel for sample separation and detection using the procedure described in the previous section. Figure 7 indicates the fluorescence signals of the \( \Phi X \)-174 DNA marker (concentration = 50 ppm) labeled by YOPRO-1 dyes. The fluorescence signals for both cases, with and without the AC electroosmosis effect, were shown in the same figure for comparison. Note that the signals were collected by the PMT modules, and the running buffer was HPMC (1.2%). In both cases, the injection of the sample was achieved at 0.6 kV for a 1 minute of loading time, while the separation process was set at 1.2 kV for 2 minutes. Again, 5-Volt (peak-to-peak) AC potential was applied for duration of 30 seconds at a frequency of 1 kHz. It is clearly seen that all eleven segments of the DNA marker could be successfully separated and detected in both cases within two minutes. Experimental data also show the signal amplitude could be enhanced 1.4-folds while the AC potential was applied on the micro electrode.

Another DNA samples related to detection of Group A streptococcus (777 base-pairs) were also used to characterize the performance of the developed CE chips. Figure 8 represents the fluorescence signals of the DNA samples. For fluorescence detection, the fluorescent dye, YOPRO-1, was mixed with the HPMC buffer (1.2%) and then injected into the microchannel. The loading of the sample was performed at 0.5 kV for 1 minute and the injection process was conducted at 1.2 kV for 2 minute. Again, an AC potential (5 Volts, peak-to-peak) at 1 kHz was applied for a duration of 30 seconds to generate AC electroosmosis forces. The experimental data clearly show that the signal amplitude could be increased by approximately 1.4 folds. The results of Figures 7 and 8 confirm that the proposed pre-concentration device is capable of concentrating bio-samples and enhance the fluorescence signals.

6. Conclusion

The present study demonstrated a micro CE chip integrated a pre-concentration device using micro-fabrication technology to successfully concentrate DNA molecules for subsequent analysis. With this approach, sample concentration could be enhanced and fluorescence signals in capillary electrophoresis chips could be improved. Three DNA samples, including \( \lambda \)-DNA, \( \Phi X \)-174 DNA markers and a
detection gene for Group A streptococcus, were used to verify the performance of the proposed device. Using a pair of electrodes to generate AC electroosmosis forces, the signal amplitude could be enhanced about 1.4-folds. Further attempt to increase the effect of pre-concentration is undergoing. For example, cascaded electrode structures may be used to generate multiplier of sample concentrators. The developed micro CE device could be crucial for DNA analysis.

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