Fabrication of universal serial bus flash disk type microfluidic chip electrophoresis and application for protein analysis under ultra low voltage

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A simple and effective universal serial bus (USB) flash disk type microfluidic chip electrophoresis (MCE) was developed by using poly(dimethylsiloxane) based soft lithography and dry film based printed circuit board etching techniques in this paper. The MCE had a microchannel diameter of 375 μm and an effective length of 25 mm. Equipped with a conventional online electrochemical detector, the device enabled effectively separation of bovine serum albumin, lysozyme, and cytochrome c in 80 s under the ultra low voltage from a computer USB interface. Compared with traditional capillary electrophoresis, the USB flash disk type MCE is not only portable and inexpensive but also fast with high separation efficiency. © 2016 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4943915>]

I. INTRODUCTION

The advances in device miniaturization have led to the development of integrated microfluidic devices, the so called lab-on-a-chip system.¹ Microfluidic chip electrophoresis (MCE), an important branch of the miniaturized total analysis system, has been keeping on developing in many fields. The ultimate goal of this technology is to detect and separate samples with a chip of small size, fast analysis, low cost, high integration, and automatic level.² A mass of experiments prove that the MCE has wide development potential and feasibility in biology, drug screening, DNA sequencing, protein detection, and environmental monitoring.^{3–12} Besides, the MCE has great potential because of its miniaturization, integration, and automation.^{13–18}

In terms of the current research for MCE and related technology, there still exist a lot of challenges. For example, the separation voltage of conventional microchip electrophoresis is generally a few hundred volts to several kilovolts. The high electric voltage may cause overheating and low resolution; at the meantime, it is too difficult to meet the portable requirements of the separation instrument because carrying an extra high voltage power supply is unavoidable. Therefore, the idea of low voltage separation instead of high voltage separation was first put forward by Lin and co-workers.¹⁹ At present, the research in low voltage MCE has been gradually mature. For instance, Urresti *et al.* developed a novel lateral punch-through transient voltage suppressor (TVS) structure which is based on NPN or PNP open-base bipolar transistors attached to MCE for low voltage applications.²⁰ The lateral TVS structures could provide the leakage current on the main geometrical and technological parameters. Xu and co-workers reported a new approach for the separation of amino acids on the low-voltage-driven MCE.²¹ The results showed that the phenylalanine and lysine mixture were effectively separated in less

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than 7 min. Hauser *et al.* developed a compact portable microchip equipped with contactless conductivity detection.²² The detection limits for Br^- and NH_4^+ ions are $3\ \mu\text{M}$ and $5\ \mu\text{M}$, respectively. Recently, Li *et al.* presented a technology to fabricate a microchip in which microchannels and microelectrodes of sensors were integrated directly into the copper sheet on a printed circuit board (PCB).²³ They demonstrated the generation of oil-in-water and water-in-oil emulsion droplets on this microchip driven by a universal serial bus (USB) interface, and the size of droplets was detected by the microelectrodes on the downstream microchannel.

The online electrochemical detection method depending on analyzing the electrical signals which converted from the chemical signals is simple, easy, and low cost, although the electrode life and selectivity are limited and the impurities in analytes can greatly influence the detection results. For example, Dossi *et al.* developed a method based on MCE with electrochemical detection for separation of 2,4-dinitrophenylhydrazine (DNPH) derivatives.²⁴ The detection limits of 4.5, 6.6, 6.8, and $13.1\ \mu\text{M}$ were obtained for acetaldehyde-DNPH, propionaldehyde-DNPH, butyraldehyde-DNPH, and hexylaldehyde-DNPH derivatives, respectively. Li and co-workers applied in-channel indirect amperometric detection mode in MCE for separation of heavy metal ions.²⁵ The results showed that Pb^{2+} , Cd^{2+} , and Cu^{2+} were efficiently separated within 80 s in a short poly(dimethylsiloxane) (PDMS) channel. By using the same detection mode, they also separated the chloride, chlorate, and perchlorate with a PDMS MCE.²⁶ The detection limits of Cl^- , ClO_3^- , and ClO_4^- were 1.9, 3.6, and $2.8\ \mu\text{M}$, respectively. Medina-Sánchez *et al.* fabricated a flexible hybrid PDMS-polycarbonate microchip with integrated electrodes and applied it for electrochemical quantum dots detection.²⁷ The detection of cadmium sulfide quantum dots in a range between 50 and $8000\ \text{ng ml}^{-1}$ with a sensitivity of $0.0009\ \mu\text{A}/(\text{ng ml}^{-1})$ was achieved.

In this paper, an ultra-low-voltage-driven USB flash disk type MCE equipped with PDMS microchannels and electrochemical detectors was designed and fabricated. Compared with traditional capillary electrophoresis (CE), the device is not only portable and inexpensive but also fast with high separation efficiency. Taking electricity with an ultra low voltage ($\sim +5\ \text{V}$) directly from a computer USB interface, it can completely separate three basic proteins of bovine serum albumin (BSA), Lysozyme (Lys), and cytochrome c (Cyt-c) in 80 s.

II. EXPERIMENTAL

A. Materials

BSA, Lys, and Cyt-C were purchased from Aldrich (St. Louis, USA). PDMS (Sylgard 184) were purchased as a two-component kit that contained the vinyl-terminated base and curing agent from Dow Corning (Midland, USA). Monosodium orthophosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 99.0%) and dibasic sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 99.0%) were bought from Shunqiang Chemical Reagent Company (Tianjin, China). Sodium hydroxide (NaOH, 96.0%) and hydrogen nitrate (HNO_3 , 65.0%) were purchased from Hongyan Reagent Company (Tianjin, China). Ethanol ($\text{C}_2\text{H}_5\text{OH}$, 99.7%) was bought from Sanhe Chemical Reagent Company (Tianjin, China). The dry film was obtained from Longfeiyu Science and Technology Company (Shenzhen, China). The single-sided copper-clad laminate (CCL) was purchased from Songgang Pengyu Electronic Company (Shenzhen, China). Phosphate buffer was used as separation medium, and the pH value was adjusted by NaOH (100 mM) and NaH_2PO_4 (40 mM). The 30 wt. % HNO_3 solution was used as etchant. The NaOH solution with concentration of 1 wt. % and 5 wt. % was used as the developer and etchant, respectively. The concentrations of BSA, Lys, and Cyt-c in the testing samples were all $0.5\ \text{mg ml}^{-1}$. All solutions were filtered through a $0.45\ \mu\text{m}$ membrane before usage.

B. Fabrication of USB flash disk type MCE

As shown in Fig. 1(a), the PCB ($6\ \text{cm} \times 2.7\ \text{cm} \times 0.15\ \text{cm}$) with desired circuits was fabricated by dry film photolithography and copper etching on the CCL. A layer of dry film was laminated on the copper-clad first. A printed photomask that contained the desired patterns on transparent polymer films (Mylar, DuPont) was then positioned above the dry film in a contact mode. Next, the dry film underneath was exposed to UV light of 365 nm for about 90 s at 2.32

mW cm^{-2} . During exposure, the exposed region is photopolymerized, while the unexposed region remains uncured. Subsequently, the unexposed dry film was developed in 1 wt. % NaOH solution for about 2 min. After etching away the uncovered copper by 30 wt. % HNO_3 solution for about 30 s and then the exposed dry film by 5 wt. % NaOH solution for about 60 s on the copper-clad, the obtained PCB with desired copper electrodes and circuits was rinsed with water and blow dried with nitrogen gas. Finally, a USB interface was welded at the top of the PCB.

As shown in Fig. 1(b), the PDMS microfluidic chip ($5\text{ cm} \times 2.5\text{ cm} \times 0.2\text{ cm}$) with rounded channels was fabricated by soft lithography using rounded fused-silica capillary ($375\text{ }\mu\text{m}$, O. D.) as template. Water soluble polyvinyl alcohol (PVA) elastic adhesive was used to glue the fused-silica capillary into a master framework. Then, the 10:1 mixture of the PDMS prepolymer and curing agent was degassed and poured onto a Teflon mold containing the fused-silica capillary framework. After cured at 80°C for 2 h, rounded microfluidic channels in PDMS with a diameter of $375\text{ }\mu\text{m}$ were formed by withdrawing the silica capillary templates. The remaining tiny amount of PVA glue in the channel was removed by flushing the channel with 70°C hot water for 1 h. After extending the liquid storage pools by another piece of templated PDMS ($5\text{ cm} \times 2.5\text{ cm} \times 0.2\text{ cm}$), the two pieces were covalently bonded together through free radical reactions generated in oxygen plasma (for 30 s at 30 W). A stereomicroscope (SMZ-T1, China) equipped with a digital camera was used to characterize the morphologies of microchannels.

As shown in Fig. 1, the PDMS microchip and the PCB were packaged together with liquid storage pools aligned and connected to the copper electrodes. After sealed the seam between the PDMS microchip and the PCB around the liquid storage pool with PDMS prepolymer, the obtained MCE was cured at 80°C for 2 h before testing.

C. Separation of proteins by USB flash disk type MCE

As shown in Fig. 2, the electrochemical measurements were adopted to detect the samples using a CHI-832C electrochemical analyzer (CH Instruments, China) at room temperature. An integrated microelectrode system was put into the detection reservoir with a Pt wire (CHI-115) as counter electrode, a bare gold electrode (CHI-105) as working electrode, and a Ag/AgCl electrode (CHI-111) as reference electrode. Before electrophoresis, the PDMS microchannel was flushed with 100 mM NaOH solution and deionized water, and then filled with 40 mM phosphate buffer solution ($\text{pH} = 6.0$) from the reservoirs. Meanwhile, the mixed sample of

FIG. 1. Illustration of the fabrication process of USB flash disk type MCE.

FIG. 2. Illustration of the experimental setup for USB flash disk type MCE. Inset (a) shows a microscope section image of the rounded microfluidic channels. Inset (b) shows an illustration of the integrated microelectrode system.

BSA, Lys, and Cyt-c (0.5 mg ml^{-1} for each protein) was injected via an electrokinetic injection system from the injection channel for 2 s. Finally, an ultra low direct current voltage of +5, +6, or +12 V was applied by a Hawk HPH703 high speed USB hub equipped with independent switch and transformer (E-sense Technology Company, China) to the two buffer reservoirs from the USB interface to start the MCE separation, and the detection signal was recorded by the online electrochemical analyzer in a current-time mode.

III. RESULTS AND DISCUSSION

Fig. 3 shows the separation of a mixture of three proteins (BSA, Lys, and Cyt-c) using the USB flash disk type MCE equipped with electrochemical detector at ultra low voltages of +6 V and +12 V, respectively. The three proteins are completely separated in less than 70 s, indicating the high separation efficiency of the USB flash disk type MCE under the ultra low voltage. With a baseline separation time less than 30 s, the separation efficiency of the USB flash disk type MCE at +12 V is better than that of +6 V.

FIG. 3. Separation of three proteins under +6 V (a) and +12 V (b) using the USB flash disk type MCE. Separation conditions: buffer, 40 mM phosphate (pH = 6.0); injection voltage, +6 V for (a) and +12 V for (b); sample, 0.5 mg ml^{-1} for each protein; capillary, $375 \mu\text{m} \times 45 \text{ mm}$ (25 mm effective); and temperature, 25°C . Peak identification: 1, Cyt-c; 2, Lys; and 3, BSA.

FIG. 4. Comparison of the USB flash disk type MCE system (a) with traditional CE apparatus system (b) in dimensions.

The comparison of the USB flash disk type MCE system with traditional CE apparatus system in dimensions is shown in Fig. 4. The USB flash disk type MCE has a dimension of $7.5\text{ cm} \times 2.7\text{ cm} \times 0.6\text{ cm}$ and a weight of about 10 g, while the traditional CE apparatus (CL-1020, Huayang Limin Instruments, China) has a dimension of $44\text{ cm} \times 36.3\text{ cm} \times 40.4\text{ cm}$ and a weight of 30 kg. Obviously, the USB flash disk type MCE is much smaller, lighter, and more portable than the traditional CE apparatus.

The comparison of the USB flash disk type MCE with traditional CE apparatus in separation performance is shown in Fig. 5. The USB flash disk type MCE enabled baseline separation of BSA, Lys, and Cyt-c in 80 s under the ultra low voltage of +5 V from a convenient computer USB interface with an effective microchannel length of 25 mm, while the traditional CE apparatus separated only 2 proteins in more than 12 min under the high voltage of +12 kV from an extra cumbersome high voltage power supply with an effective bare capillary length of 41 cm. The reason for the bad separation of three proteins in the traditional CE apparatus lies in the adsorption of biomacromolecules, particularly proteins onto fused silica capillaries, which severely degrades the separation performance of CE, leading to sample loss, peak broadening, and long migration times.^{28–32} The USB flash disk type MCE can avoid the disadvantage of traditional CE by using a more anti-protein-fouling microchannel of PDMS.

Table I shows that the run-to-run ($n=5$) relative standard deviation (RSD) of migration time for the proteins in the USB flash disk type MCE is less than 1%, day-to-day ($n=5$) RSD

FIG. 5. Comparison of the USB flash disk type MCE (a) with traditional CE apparatus (b) in separation performance. (a) Separation conditions: buffer, 40 mM phosphate (pH = 6.0); injection voltage, +5 V; applied voltage, +5 V; sample, 0.5 mg ml^{-1} for each protein; capillary, $375\text{ }\mu\text{m} \times 45\text{ mm}$ (25 mm effective); and temperature, $25\text{ }^{\circ}\text{C}$. Peak identification: 1, Cyt-c; 2, Lys; and 3, BSA. (b) Separation conditions: buffer, 40 mM phosphate (pH = 6.0); injection, 20 s with a height difference of 20 cm; applied voltage, +12 kV; UV detection, 214 nm; sample, 0.5 mg ml^{-1} for each protein; capillary, $75\text{ }\mu\text{m} \times 50\text{ cm}$ (41 cm effective); and temperature, $25\text{ }^{\circ}\text{C}$. Peak identification: 1, Cyt-c; 2, Lys; and 3, BSA.

TABLE I. RSDs of migration time and peak height of the USB flash disk type MCE for protein separation.

Proteins ^a	Run to run (n = 5)		Day to day (n = 5)		Chip to chip (n = 5)	
	migration time	peak height	migration time	peak height	migration time	peak height
Cyt-c	0.68	2.45	1.37	3.11	2.27	4.17
Lys	0.89	2.64	1.74	3.47	2.43	4.55
BSA	0.74	2.56	1.63	3.35	2.32	4.41

^aSeparation conditions: the same as Fig. 5(a).

is less than 2%, and chip-to-chip (n = 5) RSD is less than 3%. At the meantime, the run-to-run (n = 5) RSD of peak height for the proteins in the USB flash disk type MCE is less than 3%, day-to-day (n = 5) RSD is less than 4%, and chip-to-chip (n = 5) RSD is less than 5%. The RSD results were in accordance with the reported RSD value of similar protein separation by MCE.^{33,34} The detection limits can be further improved if more sensitive detection technique is applied.^{35–39} Additionally, the USB flash disk type MCE makes full use of the computer USB interface, and there is no need to carry an extra cumbersome high voltage power supply. Therefore, the USB flash disk type MCE is quiet energy saving and convenient. It has the great potential to be adopted as a rapid and portable onsite detection device for different CE applications in the future.

IV. CONCLUSIONS

In this work, a simple and effective USB flash disk type MCE is developed successfully by using PDMS based soft lithography and dry film based PCB etching techniques. After equipped with a conventional online electrochemical detector, the device enabled baseline separation of BSA, Lys, and Cyt-c in 80 s under the ultra low voltage of +5 V from a computer USB interface. At voltage of +12 V, the baseline separation of three proteins was achieved in 30 s. Compared with traditional CE, the USB flash disk type MCE is not only portable and inexpensive but also fast with high separation efficiency. Because of making full use of the computer USB interface, the USB flash disk type MCE is quiet energy saving and convenient. It has the great potential to be adopted as a rapid and portable onsite detection device for different CE applications in the future.

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