Preconcentration of diluted mixed-species samples following separation and collection in a micro–nanofluidic device

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A microfluidic device consisting of a nanoscale Nafion membrane and a polydimethylsiloxane microchannel is proposed for the preconcentration of diluted multi-mixed species samples then following separation and collection. When an electric field is applied across the microchip, an accumulation of the mixed-species sample occurs at the junction between the microchannel and the membrane by means of ion concentration polarization effect. A separation of the sample then takes place due to the difference in the electrophoretic mobilities of the sample components. Finally, the component of interest is guided to a collection reservoir by manipulating the external potential configuration and is trapped in place by means of a magnetically actuated valve. The preconcentration performance of the proposed device is evaluated in both straight and convergent microchannels using a fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) sample. It is shown that a preconcentration factor of 40 times can be achieved using a straight microchannel. By contrast, the preconcentration factor increases to 50 times when using a convergent channel. The practical feasibility of the proposed device is demonstrated by performing the preconcentration, separation, and collection of a mixed FITC-BSA and Tetramethylrhodamine sample. © 2016 AIP Publishing LLC.

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I. INTRODUCTION

Microfluidic systems have many benefits compared to their macroscale counterparts, including a faster detection time, a lower power consumption, an improved portability, a reduced reagent and sample consumption, and a lower cost. Consequently, the realization of microfluidic platforms for the manipulation and analysis of biological and chemical samples has attracted significant interest in recent decades.^{1–4} Human blood samples include many proteins with relevance to disease diagnosis and therapeutic monitoring. However, biochemical samples such as blood typically comprise many different components, including deoxyribonucleic acid (DNA), proteins, cells, and even ions. As a result, effective methods for separating multi-component samples are urgently required. Capillary electrophoresis^{5,6} is a well-established technique for separating DNA, proteins, cells, and peptides. However, for human blood samples, the target proteins required for diagnosis purposes are usually present in only very low quantities in early stage of a disease, and hence the diagnosis process represents a significant challenge.⁷ Thus, in addition to sample separation, how to focus protein concentration from low concentration samples is also a major concern.

Many methods have been proposed for sample preconcentration, including field-amplified sample stacking (FASS),⁸ isotachopheresis,^{9–12} electrokinetic trapping,^{13–15} isoelectric focusing,¹⁶ micellar electrokinetic sweeping,¹⁷ chromatographic preconcentration,^{18,19} and membrane

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preconcentration.^{20,21} Of these various techniques, electrokinetic trapping is particularly attractive since it is applicable to any charged molecule or particle. Moreover, electrokinetic trapping requires no temporal or spatial buffer changes and is compatible with other analytical techniques such as capillary electrophoresis.²² Kim *et al.*²³ presented an electrokinetic protein preconcentration chip consisting of polydimethylsiloxane (PDMS) microfluidic channels reversibly bonded to a glass substrate. The experimental results showed that a 10^3 – 10^6 -fold protein concentration could be achieved within approximately 30 min due to the formation of an ion exclusion-enrichment effect between the PDMS and glass layers under the application of an external electrical field. In addition, various electrokinetic trapping methods based on nanoporous charge-selective membranes^{24–29} or nanofluidic channels^{30–34} have been proposed. Khandurina *et al.*³⁵ presented an on-chip DNA concentration technique based on a porous membrane structure and showed that the injection scheme enabled polymerase chain reaction (PCR) product detection to be performed after just 10 thermal cycles. Huang and Yang³⁶ presented a microfluidic concentration device consisting of two microchannels connected by a nanochannel. It was shown that through an appropriate switching of the external electrical potentials, a 50-fold (around the conductivity ratio between buffer solution and that in depletion region) sample concentration could be achieved within approximately 1 min.

Lee *et al.*³⁷ proposed a high-throughput protein preconcentration device comprising a PDMS microfluidic chip and a glass substrate patterned with a submicron thick Nafion ion-selective membrane. The experimental results showed that a concentration factor as high as 10^4 -fold could be obtained in approximately 5 min. Kim and Han³⁸ developed a protein preconcentration device based on self-sealed vertical polymeric nanoporous-junctions. The feasibility of the device was demonstrated by performing the concentration of beta-phycoerythrin proteins. Chen and Yang³⁹ fabricated preconcentration devices incorporating straight or convergent-divergent microchannels and showed that for an equivalent width of the main microchannel, the convergent-divergent microchannel yielded a faster concentration effect than the straight channel. Chao *et al.*⁴⁰ presented sample preconcentration devices consisting of a microchannel (straight or convergent) and a Nafion membrane. The performance of the two devices was compared using fluorescein dye and fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) samples. It was shown that the convergent microchannel obtained a greater preconcentration effect than the straight microchannel. Ko *et al.*⁴¹ presented a nanofluidic protein concentration device consisting of a straight microchannel with integrated anode and cathode channels and a Nafion membrane. The performance of the proposed device was demonstrated by performing the immunoassay of C-reactive protein (CRP). Song *et al.*⁴² presented a sample preconcentration device consisting of a Nafion membrane and a microchannel for the concurrent preconcentration and separation of composite DNA-containing samples. The experimental results showed that a binary mixture of free and bound 102-mer DNA could be preconcentrated and separated within 6 min.

This paper presents a microfluidic device consisting of a PDMS microchannel and a Nafion membrane for the concurrent preconcentration, separation, guidance and collection of mixed-species samples. In the proposed device, the sample is concentrated at the anodic side of the microchannel/membrane junction as a result of the ion concentration polarization (ICP) effect. A separation of the sample components then takes place due to the difference in the electrophoretic mobilities of the species. Finally, the species of interest is guided to a collection reservoir and trapped in place by means of a magnetically actuated valve. The preconcentration performance of the proposed device is evaluated for two different microchannel configurations, namely, straight and convergent. Finally, the practicality of the proposed device is demonstrated by performing the preconcentration, separation, and collection of a mixed sample comprising FITC-BSA and Tetramethylrhodamine (TAMRA).

II. EXPERIMENTAL

A. Principle and design

Figure 1 illustrates the basic principle and design of the proposed nano-microfluidic device. When a voltage (V_1) is applied to the inlet reservoir of the microchannel, an ICP effect is

FIG. 1. Schematic illustration of Nafion-membrane-based nano-microfluidic device containing a negatively charged mixed-species sample consisting of FITC-BSA and TAMRA. The sample accumulates at the junction of the microchannel and membrane as a result of the ICP phenomenon and EOF effect and is separated due to the different electrophoretic mobilities of the sample components.

generated on both sides of the Nafion membrane. Moreover, an electro-osmotic flow (EOF) is induced from left to right (as indicated by the arrow), which drives the mixed-species sample continuously from the reservoir toward the Nafion junction. As a result of the ICP phenomenon and EOF effect,⁴³ the mixed-species sample accumulates on the anodic side of the Nafion membrane. Furthermore, due to a difference in the electrophoretic mobilities of the sample components (FITC-BSA and TAMRA in the present case), a separation of the mixed-species sample also occurs.

B. Materials and methods

Figure 2 shows the geometries and dimensions of the straight- and convergent-microchannel devices fabricated in the present study. In both designs, the PDMS microchannel has a depth of $20\text{ }\mu\text{m}$, while the Nafion-filled nanochannel has a width of $100\text{ }\mu\text{m}$ and a depth of $6\text{ }\mu\text{m}$. Furthermore, both designs incorporate three independent electrical connections, namely, V_1 , V_2 , and Ground, to manipulate the sample during the concentration, separation, and collection phases. As shown in Fig. 2(a), the straight microchannel has a length and width of 8.5 mm and $150\text{ }\mu\text{m}$, respectively. The convergent channel has a length of 2.5 mm and a width of $75\text{ }\mu\text{m}$ (see Fig. 2(b)). Figure 2(c) illustrates the configuration and dimensions of the convergent-channel microchip fitted with a magnetic valve to prevent backflow of the separated sample following the collection process.

Figure 3 presents an overview of the microchip fabrication process. The basic steps in the fabrication procedure can be summarized as follows: (1) microfluidic channels with the dimensions shown in Fig. 2 are patterned on PDMS substrates using a photolithography technique with SU-8 negative photoresist.¹⁵ (2) Silicone elastomer and an elastomer curing agent (Sil-More Industrial Ltd., USA Sylgard 184A and Sylgard 184B) are mixed in a ratio of 10:1 and poured onto silicon wafer substrates. The SU-8 microstructure mold is spin-coated with PDMS at 1500 rpm for 60 s creating a thin-film membrane. The silicon wafer substrates and spin-coated SU-8 mold are cured at $80\text{ }^\circ\text{C}$ for 4 h . (3) The PDMS inverse structure not containing microfluidic channels is mechanically peeled off the silicon wafers and a hole is drilled in the PDMS layer. (4) The PDMS layer is plasma oxidized and sealed permanently to the SU-8 mold. (5) The sealed PDMS structure is mechanically peeled off the template. (6) The PDMS structure is bonded to a glass substrate patterned with Nafion by means of an oxygen plasma process. (7) A powerful magnet (length of 3 mm and height of 9 mm) is inserted into the hole when the valve need to close and fixed in place via an interference fit.

FIG. 2. Schematic illustrations of nano-microfluidic chips with: (a) straight microchannel, (b) convergent microchannel, and (c) convergent microchannel and magnetic valve.

Two buffer solutions were used in the present experiments, namely, phosphate buffered saline (PBS) with a concentration of 10^{-3} M (pH = 7.4) and Tris(hydroxymethyl)aminomethane (Tris), also with a concentration of 10^{-3} M (pH = 7.4). The mixed-species sample consisted of FITC-BSA with a concentration of 10^{-6} M (pH = 7.4) and TAMRA with a concentration of 10^{-5} M (pH = 7.4). The FITC-BSA sample was mixed with PBS buffer solution and maintained at pH 7.4, while the TAMRA sample was mixed with Tris buffer solution and also maintained at pH 7.4.

Direct current (DC) measurements were obtained as a function of the applied voltage using a Keithley 2400 source meter (Keithley Instruments, USA) and nF EC1000S (California Instruments, USA) with LabTracer 2.0 software. Images of the experimental concentration, separation, and guidance process were captured using an optical microscope (ECLIPSE 50Ti, Nikon, Japan) integrated with a charge coupled device (CCD) camera (DBK 41BU02, The Imaging Source, Germany, IC Capture 2.0 software). The camera was fitted with two different lenses (in different experiments), namely, a mono filter lens (Nikon, Japan) designed to receive wavelengths in the range of 450–490 nm and a dual filter lens (Nikon, Japan) designed to receive wavelengths in the range of both 450–490 nm and 510–590 nm.

Prior to the experiments, the microfluidic chips were treated with NaOH for 1 min and then rinsed in deionized (DI) water. The mixed-species sample was then injected into the microchip until the main microchannel was completely filled. The chip was positioned on the microscope stage, and an electrode was placed in both reservoirs on the anodic side (i.e., V_1 and V_2) and

FIG. 3. Schematic illustration showing major steps in fabrication process of proposed microchip.

connected to the power supply to drive fluid through the chip. In establishing the EOF flow, a voltage of 90 V was applied to the anodic side of the reservoir, while the cathodic side was grounded. The preconcentration phenomenon within the microfluidic chip was then observed using a mercury-lamp-induced fluorescence technique. The fluorescence intensity of the captured images was analyzed quantitatively in terms of integrated optical density arbitrary units (A.U.) using ImagePro Plus 6.2 software (Media Cybernetics, Silver Spring, USA).

III. RESULTS AND DISCUSSION

A. Concurrent preconcentration and guidance of FITC-BSA sample in straight and convergent microchannels

The effect of the microchannel geometry (straight or convergent) on the preconcentration performance of the proposed device was evaluated using a single-species FITC-BSA sample. The sample was loaded into the three reservoirs (i.e., V_1 , V_2 , and G) of each device and driven into the main channel under a configured voltage of $V_1 = 90$ V and $V_2 = 90$ V. In general, the applied electric field (E) is equal to $E = V/L$, where V is the magnitude of the applied voltage (in volts) and L is the total length of the channel (in centimeters). In the microchips developed in the present study, the inclined separation microchannel leading to the collection reservoir has a higher electric field than the horizontal concentration channel due to its shorter length (see Fig. 2). A higher electric field results in a greater electrophoretic velocity and is thus instrumental in guiding the sample into the collection chamber following the preconcentration process.

The accumulation of the FITC-BSA sample in the horizontal and inclined channels of the two microchips was observed over time using the filter lens with a wavelength range of 450–490 nm. The corresponding results are presented in Figs. 4(a) and 4(b) for the straight- and convergent-microchannel devices, respectively. For both devices, the intensity of the fluorescence signal in the separation channel increases over time as a result of the difference in the

electric field strengths in the inclined and horizontal microchannels, respectively. Thus, the ability of both devices to guide the concentrated sample to the collection chamber is confirmed. However, comparing the results presented in Figs. 4(a) and 4(b), a difference in the measured fluorescence intensity is observed in the straight- and convergent-channel devices, respectively. For example, in the straight microchannel device, the fluorescence intensity has a value of approximately 170 AU after 35 min. According to the calibration curve shown in Fig. 4(c), a fluorescence intensity of 170 AU is equivalent to a FITC-BSA sample concentration of 4×10^{-5} M. In other words, a preconcentration factor of approximately 40 times is obtained in the straight microchannel. By contrast, the fluorescence intensity in the convergent microchannel device is equal to approximately 210 AU after the same preconcentration time. Thus, the FITC-BSA concentration is close to 5×10^{-5} M, corresponding to a preconcentration factor of

FIG. 4. Continuous accumulation of FITC-BSA sample over time ($t=5, 15, 25$, and 35 min). Experimental fluorescence images (upper) and fluorescence intensity plots along channel center line (lower) in: (a) straight microchannel and (b) convergent microchannel. (c) Calibration curve for determination of FITC-BSA concentration. Data points represent the mean of five measurements, and the error bars represent one standard deviation from the mean. The insets (a-1)–(a-4) and (b-1)–(b-4) are zoomed at preconcentration locations.

50 times. In other words, the convergent microchannel yields a better preconcentration performance than the straight microchannel.

B. Concurrent preconcentration, separation, and guidance of mixed-species sample

The preconcentration, separation, and guidance performance of the proposed device was evaluated using a mixed-species sample consisting of negatively charged FITC-BSA (10^{-6} M) and TAMRA (10^{-5} M). The experiments were performed using the convergent-channel device shown in Fig. 2(c) with a magnetically actuated valve. To collect the targeted sample in the separation channel following the concentration process, the collection reservoir (i.e., V_2) was loaded with PBS buffer (10^{-3} M). The mixed-species sample was then loaded into the reservoirs (i.e., V_1 and G) of the horizontal microchannel. Figure 5(a) shows the variation over time of the fluorescence intensity of the mixed-species sample in the horizontal and separation channels given a voltage configuration of $V_1 = 90$ V and $V_2 = 0$ V. (Note that the experimental process was observed using the dual filter lens with receiving wavelengths of 450–490 nm (BSA) and 510–560 nm (TAMRA).) A sample concentration effect is clearly observed after 3 min. However, the individual components of the sample cannot be clearly distinguished. For a preconcentration time of 6 min, however, two separate fluorescence signals are observed. In other words, a separation of the sample occurs. The fluorescence peak corresponding to the FITC-BSA component becomes increasingly distinct and intense as the preconcentration time increases. Thus, the concentration and separation of the two species within the microchannel is confirmed. Figures 5(b) and 5(c) show the calibration curves for the FITC-BSA and TAMRA concentrations, respectively.

FIG. 5. (a) Experimental images and fluorescence intensity plots showing accumulation and separation of FITC-BSA and TAMRA samples in convergent microchannel over time ($t = 3, 6, 9$, and 12 min). (b) Calibration curve for determination of FITC-BSA concentration. (c) Calibration curve for determination of TAMRA concentration. Data points represent the mean of five measurements, and the error bars represent one standard deviation from the mean. The insets (a-1)–(a-4) are zoomed at preconcentration locations. The separation distance between the BSA and TAMRA is 2 pixel, 15 pixel, 21 pixel, and 30 pixel, respectively. One pixel is approximately equal to $13 \mu\text{m}$ which is analyzed by ImagePro Plus 6.2 software.

FIG. 6. Variation of current over time when using magnetic valve to guide separated samples. During initial accumulation and separation phase, voltages $V_1 = 90$ and $V_2 = 0$ are applied. The magnetic valve is then opened and voltages of $V_1 = 90$ and $V_2 = 90$ are used to guide one of the concentrated samples into the separation channel.

From these curves, the FITC-BSA concentration is determined to be around 3×10^{-5} M (corresponding to a preconcentration factor of 30 times), while the TAMRA concentration is close to 4×10^{-4} M (corresponding to a 40-fold preconcentration factor).

In Fig. 5(a), the insets (a-1)–(a-4) show separation distance between BSA and TAMRA at different instants. The separation distance is 2 pixel, 15 pixel, 21 pixel, and 30 pixel, respectively. One pixel is approximately equal to $13 \mu\text{m}$ for this case. These pixels are measured by ImagePro Plus 6.2 software. The resolving power of our optical device is calculated by $R = \frac{0.61\lambda}{\text{N.A.}}$, where λ is the emission wavelength (λ is 520 nm for BSA and 590 nm for TAMRA) and N.A. is the numerical aperture of the objective lens from our device, which is 0.06. Therefore, the resolving power is $5.3 \mu\text{m}$ – $6.0 \mu\text{m}$. The resolution is able to resolve our measured images.

Figure 6 shows the variation over time of the measured current during the magnetic valving operation. As described above, the mixed-species sample is concurrently concentrated and separated in the horizontal microchannel given a voltage configuration of $V_1 = 90$ V and $V_2 = 0$ V. When the sample of interest approaches the intersection of the horizontal microchannel and inclined separation microchannel, the valve is opened and a voltage configuration of $V_1 = 90$ V and $V_2 = 90$ V is applied to guide the sample into the separation microchannel. As shown in Fig. 6, when $V_2 = 90$ V is first applied, the transient current in the separation channel is higher than that in the horizontal microchannel. However, once the concentrated sample has entered the separation microchannel, the current in the horizontal microchannel exceeds that in the separation microchannel. The unsteady conductivity gradient near the interface complicates the calculation of the electric field since it involves unsteady electroosmosis on the fluid flow and field.^{44,45} However, the conductivity increases as the ion concentration increases since the electrical current is transported in solution by the ions. Consequently, the conductivity of the mixed-species sample and PBS buffer solution was measured using an electrical conductivity meter (EC meter). The mean electrical conductivity value for the mixed-species sample was found to be $1140 \mu\text{S}/\text{cm}$, while that for the PBS buffer solution was equal to $210 \mu\text{S}/\text{cm}$. The lower electrical conductivity of the PBS buffer (10^{-3} M) results in a lower current, and hence a poor preconcentration phenomenon is observed.

Figure 7 presents a series of schematic illustrations and experimental images showing the concentration, separation, and collection of the mixed-species sample in the convergent microchannel device. In Fig. 7(a), the sample is concentrated in the horizontal microchannel under the effects of a voltage configuration of $V_1 = 90$ and $V_2 = 0$. (Note that the valve is closed.) As the preconcentration time increases, a separation of the species components occurs, as shown in Fig. 7(b). When the FITC-BSA component approaches the intersection of the horizontal microchannel and inclined separation channel, the valve is opened and a voltage configuration of $V_1 = 90$ V and $V_2 = 90$ V is applied to guide the sample into the separation microchannel (see Fig. 7(c)).

TABLE I. Summary of experimental conditions for each channel.

Channel	Convergent channel	Voltage (V ₁ ,V ₂)	Separation	Solutions	Valve	Time (min)	Intensity (concentration factor)
No	(90,90)	No	10 ⁻⁶ M FITC-BSA + 10 ⁻³ M PBS	No	35	170 (40×)
Yes	(90,90)	No	10 ⁻⁶ M FITC-BSA + 10 ⁻³ M PBS	No	35	215 (50×)
Yes	(90,90)	Yes	10 ⁻⁶ M FITC-BSA + 10 ⁻³ M PBS + 10 ⁻⁵ M TAMRA + 10 ⁻³ M Tris	Yes	12	BSA: 115 (50×) TAMRA: 130 (90×)

FIG. 7. Preconcentration, separation, guidance, and collection steps in convergent microchannel with magnetic valve. (a) Given voltages of $V_1 = 90$ and $V_2 = 0$, FITC-BSA, and TAMRA are concentrated in horizontal microchannel. (b) FITC-BSA and TAMRA are separated in horizontal microchannel. (c) Magnetic valve is opened and voltages $V_1 = 90$ and $V_2 = 90$ are applied to guide FITC-BSA into separation microchannel. (d) FITC-BSA is collected in reservoir and magnetic valve is closed to prevent backflow.

Finally, the FITC-BSA sample is collected in the separation channel reservoir and the valve is closed to prevent backflow into the main channel, as shown in Fig. 7(d). An inspection of the experimental image in Fig. 7(d) shows that the FITC-BSA concentration is close to 5×10^{-5} M (as determined from the calibration curve in Fig. 5(b)), while the TAMRA concentration is close to 9×10^{-4} M (see Fig. 5(c)). Thus, the preconcentration factor for the FITC-BSA sample is determined to be approximately 50-fold, while that for the TAMRA sample is found to be approximately 90-fold. The parameters used in experiments are summarized in Table I.

IV. CONCLUSIONS

This study has presented a Nafion-membrane-based micro-nanofluidic device equipped with a magnetically actuated valve for the concurrent preconcentration, separation, guidance, and collection of mixed-species samples. In the proposed device, the sample is concentrated on the anodic side of the Nafion membrane via the ICP phenomenon and an EOF exclusion effect. As the preconcentration time increases, a separation of the sample components occurs due to a difference in their electrophoretic mobilities. Finally, the sample species of interest is guided into a separation channel through an appropriate manipulation of the external voltage configuration and the magnetic valve is closed to prevent backflow of the sample to the main microchannel. The performance of the proposed device has been evaluated using both a single-species sample (FITC-BSA) and a mixed-species sample (FITC-BSA and TAMRA). The main contributions and findings of this study can be summarized as follows: First, the preconcentration performance of the proposed device was evaluated for two different microchannel configurations,

namely, straight and convergent. In both cases, the preconcentration factor was evaluated using the single-species sample (FITC-BSA). The sample concentration in the straight microchannel device was found to be around 4×10^{-5} M following a preconcentration time of 35 min. Thus, the preconcentration factor was determined to be approximately 40-fold. By contrast, in the convergent microchannel device, the sample concentration was close to 5×10^{-5} M; corresponding to a preconcentration factor of approximately 50-fold. Second, the concentration, separation, guidance, and collection performance of the convergent-channel microchip was evaluated using the mixed-species sample (FITC-BSA and TAMRA). The experimental results showed that an FITC-BSA concentration of close to 5×10^{-5} M was obtained in the collection chamber (corresponding to a 50-fold preconcentration factor), while a TAMRA concentration of close to 9×10^{-4} M was obtained in the main microchannel (corresponding to a 90-fold preconcentration factor). In the future, the proposed device will be applied to clinical cases for practical detections.

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