# Fast Track

**Seyed Mostafa Shameli**[1]
**Caglar Elbuken**[1]
**Junjie Ou**[1,2]
**Carolyn L. Ren**[1]
**Janusz Pawliszyn**[2]

[1]Department of Mechanical and Mechatronics Engineering, University of Waterloo, Waterloo, ON, Canada
[2]Department of Chemistry, University of Waterloo, Waterloo, ON, Canada

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Research Article

## Fully integrated PDMS/SU-8/quartz microfluidic chip with a novel macroporous poly dimethylsiloxane (PDMS) membrane for isoelectric focusing of proteins using whole-channel imaging detection

A fully integrated polydimethylsiloxane (PDMS)/modified PDMS membrane/SU-8/quartz hybrid chip was developed for protein separation using isoelectric focusing (IEF) mechanism coupled with whole-channel imaging detection (WCID) method. This microfluidic chip integrates three components into one single chip: (i) modified PDMS membranes for separating electrolytes in the reservoirs from the sample in the microchannel and thus reducing pressure disturbance, (ii) SU-8 optical slit to block UV light (below 300 nm) outside the channel aiming to increase detection sensitivity, and (iii) injection and discharge capillaries for continuous operation. Integration of all these components on a single chip is challenging because it requires fabrication techniques for perfect bonding between different materials and is prone to leakage and blockage. This study has addressed all the challenges and presented a fully integrated chip, which is more robust with higher sensitivity than the previously developed IEF chips. This chip was tested by performing protein and p*I* marker separation. The separation results obtained in this chip were compared with that obtained in commercial cartridges. Side-by-side comparison validated the developed chip and fabrication techniques.

**Keywords:**
Microfluidics / Protein separation / Whole-channel imaging
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## 1 Introduction

Capillary isoelectric focusing (CIEF) is a powerful technique for separating proteins, peptides, and other zwitterionic biomolecules that have subtle differences in their isoelectric point (p*I*), pH value at which the molecule has no charge. In this method, an axial pH gradient needs to be set up along a capillary. Under the influence of an applied electric field, samples are separated according to their differential p*I*s [1–3]. Several commercial CIEF instruments have been developed and are available in the market as powerful separation systems. For example, the iCE280 analyzer invented by Convergent Bioscience is such an instrument utilizing CIEF for the separation of proteins and biomarkers [4–6]. This instrument has been widely adopted by the pharmaceutical industry.

Despite the success that CIEF has received from both the academy and industry, the use of capillary for separation has limited its throughput and its integration with other mechanisms for the separation of complex samples. In addition, capillaries have a round cross-section that results in lens effects in detection [7]. These limitations have prompted the development of microfluidic chips for IEF taking advantage of the advances in photolithography technology. There have been a number of studies that successfully demonstrated IEF in chip format. Readers are referred to a recent review article by Shimura [8] for more information.

Most of these studies rely on single-point detection, which requires the focused samples to be mobilized after

**Correspondence:** Professor Carolyn L. Ren, Department of Mechanical and Mechatronics Engineering, University of Waterloo, 200 University Ave. West, Waterloo, ON, N2L 3G1, Canada
**E-mail:** c3ren@mecheng1.uwaterloo.ca
**Fax:** +1-519-885-5862

**Abbreviations: WCD,** whole-column detection; **WCID,** whole-channel imaging detection

**Colour Online:** See the article online to view Figs. 1–6 in colour.

separation. Hydrodynamic flow has been commonly used for pumping the focused sample bands, which cause sample dispersion well documented previously [9]. Therefore, efforts have also been made to explore novel pumping techniques [10]. Others have also investigated new chip materials [11] and methods for multi-dimensional separation, where IEF is one of the dimensions [12–14].

The dispersion due to mobilizing focused sample bands to a detecting point can be avoided by monitoring the entire channel or column during the separation process. In addition to no need for mobilization, whole-column detection (WCD) also provides several other advantages such as shorter detection time, minimized peak dispersion, and consequently higher resolution. Earlier studies on WCD were started by several groups such as Rowlen et al. [15], Wu and Pawliszyn [16], and Wang and Hartwick [17]. Rowlen et al. [15] employed a series of diodes to detect the separation peaks of a zone on a chromatographic column and also introduced the term of WCD which was adopted later by Wang and Hartwick in their study of CIEF coupled with a whole capillary scanning method. Wu and Pawliszyn [16] constructed a concentration gradient imaging detector in which a segment of capillary was illuminated by a defocused laser beam. In their study, the term of whole-column imaging detection (WCID) was introduced and later UV absorbance-based WCID method was invented by Convergent Bioscience in their instrument, iCE280 analyzer. Recently, WCID has also been employed in a chip format for IEF applications. For example, Das and Fan [18] evaluated the effects of separation length and voltage on IEF in a plastic microfluidic chip, where fluorescence-based WCID technology was used for detection. Qiu's group [19, 20] has also developed microfluidic chips for IEF using fluorescence-based WCID technology for detection. Each method has pros and cons. Fluorescence-based detection has a much higher sensitivity than UV absorbance-based detection; however, it requires derivatization that is time consuming, expensive, and more importantly may alter the p*I* of the analytes [21, 22].

UV-based WCID that the iCE280 analyzer employs for detecting protein or biomarker separation does not need derivatization, which will avoid any potential effects due to labelling samples. Considering the success that the iCE280 analyzer has received, the cartridge used in this analyzer therefore is employed in this study as the basis for comparing and developing chip-based IEF technology. The key features incorporated in the iCE280 analyzer include: (i) a metal slit with a 65 μm opening aligned with the separation capillary to block the light outside of the separation capillary region, (ii) two short hollow fibre membranes with one end connected with the separation capillary to separate the electrolytes in the reservoirs and the samples in the separation capillary, and to reduce hydrodynamic flow, and (iii) two capillaries connected to the other end of the hollow fibre membranes for continuous sample injection and discharge. This cartridge is capillary based and thus has all the disadvantages pertaining to CIEF as mentioned above. In addition, the cartridge assembly is currently done manually which often results in poor alignment between the separation capillary and the opening of the metal slit which in turn will lead to low detection sensitivity or even failure of the cartridge. Manually gluing cylindrical hollow fibre membranes with capillaries all of which have a 100 μm diameter is challenging too.

As a natural progression of replacing CIEF with chip-based IEF, several studies have been reported to address the limitations of this cartridge. For example, Liu et al. [23] first proved that IEF can be performed in a PDMS microfluidic chip coupled with UV-based WCID. Later, Ou et al. [24] demonstrated IEF in microfluidic chips with integrated dialysis membranes in replacement of the hollow fibre membrane used in the commercial cartridge. In that design, the same metal slit was used for blocking the UV light as that in the commercial cartridge. In addition, the dialysis membranes were also manually glued to the chip substrates that proved to be very difficult. In another study presented by the same group, the metal slit was replaced by a built-in SU-8 optical slit which eliminates challenging alignment between the metal slit and separation channel and manual gluing processes [25]. In this study, membranes or hollow fibres were not used which results in diffusion of electrolytes into the separation channel and hydrodynamic flow causing low repeatability and low separation resolution.

The above problems can only be resolved if all the functional components can be integrated into one single chip which includes the membranes for separating electrolytes from protein samples, the SU-8 optical slit for preventing stray light and the injection/discharge capillaries that allow continuous operation. Such a chip can potentially replace the cartridge currently being used in the iCE280 analyzer while maintaining comparable separation performance to the commercial cartridge. In addition, it opens up the opportunity for multi-dimensional separation where IEF is one of the dimensions benefiting from the continuous flow control provided by microfluidic chips. The integration is very challenging because it involves the fabrication of different chip materials at different thicknesses, which are prone to leakage and blockage and thus result in low separation performance. Addressing this challenge becomes the focus of this study – developing techniques, which enable a fully integrated chip to be fabricated and validating the developed chips using the iCE280 analyzer.

## 2 Materials and methods

### 2.1 Materials

PDMS prepolymer and the curing agent were purchased from Dow Corning (Midland, MI, USA). SU-8 (2075) photoresist and propylene-glycol-monoether-acetate (PGMEA) developer were obtained from Microchem (Newton, MA, USA). Polystyrene (MW, 192 000) and polyvinylpyrrolidone (PVP) (MW, 360 000), and analytical-reagent

 

grade toluene, acetone, and ethanol were obtained from Aldrich (Milwaukee, MI, USA). Human haemoglobin control containing HbA, HbF, HbS, and HbC were purchased from Helena (Beaumont, TX, USA). The p*I* markers (4.22, 5.85, 7.00, 8.18, 9.46) were from Convergent Bioscience. Fused-silica capillaries with 50 µm id and 100 µm od were purchased from Polymicro Technologies (Tucson, AZ, USA). Water was purified with an ultra-pure water system (Barnstead/Thermolyne, Dubuque, IA, USA) and was used to prepare all solutions. Quartz slides (3 × 1 in.) were purchased from Ted Pella (Redding, CA, USA).

### 2.2 Fabrication of the chip

As shown in Fig. 1, the fabrication of a fully integrated chip is done through three steps. In the first step (Fig. 1A), an open microchannel is fabricated on a quartz substrate with two side walls made of SU-8. In the second step, a PDMS substrate is fabricated with two holes (reservoirs for IEF) around which two modified porous PDMS membranes are bonded on one side of the PDMS substrate (Fig. 1B). Finally, the two substrates – Quartz substrate with an open channel made of SU-8 and PDMS substrate with modified PDMS membranes – are bonded and sealed, followed by the injection of capillaries and gluing of large glass reservoirs (Fig. 1C). The details of fabrication are given below.

### 2.3 Fabrication of quartz substrate with SU-8 side walls

The quartz substrate was prepared by RCA cleaning [26] and then dehydrated on a hot plate at 200°C for 30 min. A 100 µm-thick SU-8 (2075) layer was spun onto the quartz substrate with a spin speed of 500 rpm for 25 s followed by a spin speed of 1800 rpm for 75 s. The substrate was subsequently soft baked on a hot plate at 65°C for 5 min and at 95°C for 12 min to remove the photoresist solvent. After the substrate was cooled to room temperature, UV exposure was performed in a UV mask aligner with a 920 mJ/cm$^2$ dose at a wavelength of 365 nm. In the exposure, a negative photo mask with a

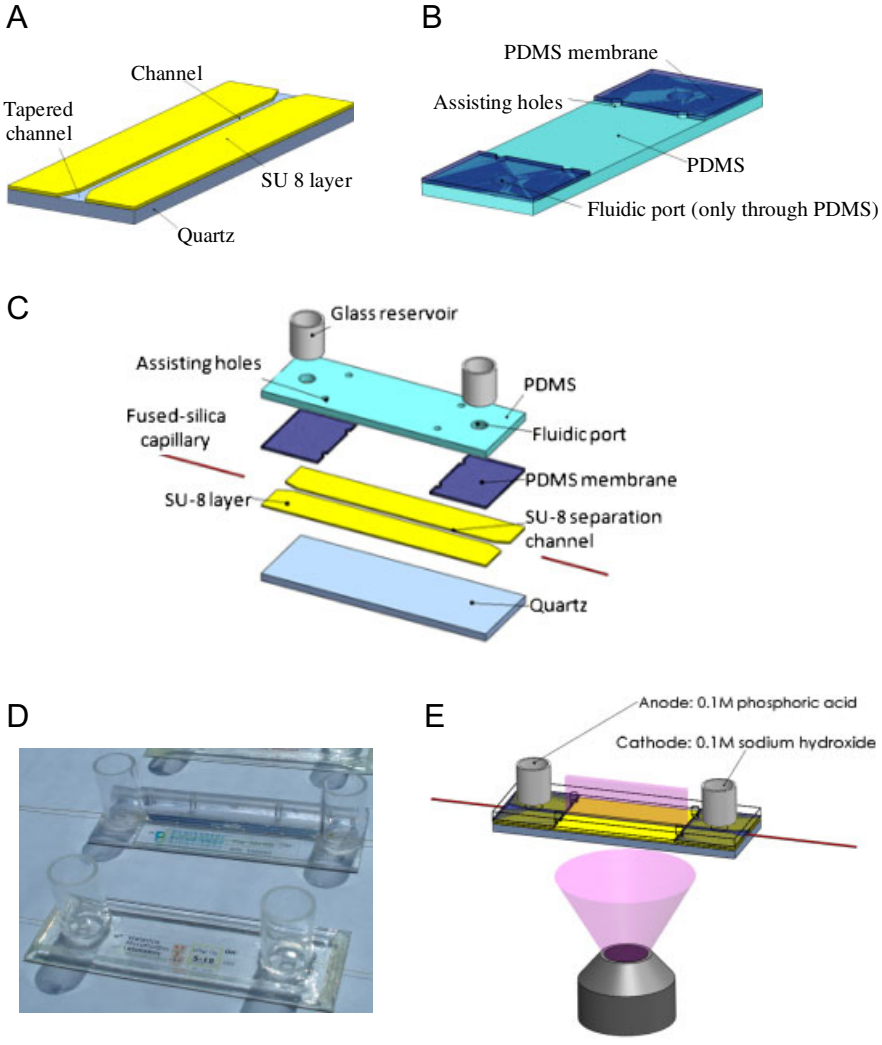


**Figure 1.** (A) Quartz substrate with SU-8 channel walls, (B) PDMS substrate with membranes, (C) assembly of chips, (D) (bottom) image of the final chip (with University of Waterloo logo) and (top) commercial cartridge (courtesy of Convergent Bioscience), and (E) assembled chip with light path.

 

100 μm-wide, 5 cm-long channel design (CAD/Art Services, Bandon, OR, USA) was used for pattern transfer. In this design, the inlet and outlet have a gradual converging and diverging geometry for integrating the injection and discharge capillaries. Post-exposure bake was performed on a hot plate at 65°C for 7 min and at 95°C for 12 min successively. After cooling slowly to room temperature, the photoresist was developed in the propylene-glycol-monoether-acetate developer for 10 min to dissolve the unexposed SU-8 completing the fabrication of the bottom substrate.

### 2.4 Fabrication of PDMS substrate with modified PDMS membranes

The PDMS substrate partly bonded with the modified PDMS membranes was fabricated using the procedure developed previously [27]. Briefly, PDMS prepolymer was thoroughly mixed with curing agent at a weight ratio of 10:1 and degassed for 30 min under vacuum. The mixture was poured onto a blank silicon wafer and cured for 1 h at 80°C. After curing, the PDMS layer with a thickness of 2 mm was peeled off from the silicon wafer and cut into $3 \times 1$ in. pieces. Then, two holes with a 3 mm diameter were punched through the PDMS layer with a distance of 2 in. from each other for the reservoir locations.

In order to prepare the PDMS membrane, one gram of polystyrene was thoroughly dissolved in 10 mL toluene. The obtained viscous solution was spun-coated on a glass slide ($1 \times 3$ in.) at a speed of 500 rpm for 10 s followed by a speed of 2000 rpm for 30 s. Then, the glass slide was baked at 95°C for 10 min to deposit the polystyrene film and this slide will serve as the substrate for the next step. To fabricate the porous membrane, a mixture of PDMS prepolymer base and curing agent 10:1 was thoroughly mixed and diluted with toluene and the previously obtained viscous solution (containing polystyrene and toluene) by the ratio of 10:10:1 respectively. After degassing, the PDMS mixture was spun-coated onto the glass side obtained previously at a speed of 500 rpm for 10 s followed by a speed of 1000 rpm for 30 s as shown in Fig. 2. Then, the slide was soft-baked on a hot plate at 95°C for 30 min. In order to bond the PDMS membrane on the holes of the PDMS substrate, two of the resulted glass slides and the PDMS substrate obtained previously were pre-treated by air plasma and brought into contact as shown in Fig. 2. Only the reservoir areas of the PDMS substrate were bonded with the membranes. Then, the membranes were shaped to fit the width of the PDMS substrate and then the whole structure was immersed into toluene, acetone, and ethanol to dissolve the layer made of the viscous solution so that the PDMS substrate with the membranes could be peeled off from the glass slide. Finally, the PDMS substrate integrated with the PDMS membranes on its reservoir areas was dried in an oven under vacuum for 12 h at 55°C.

### 2.5 Sealing at the PDMS membrane boundary regions

Leakage has proven to be the major challenge when fabricating multilayer microfluidic chips involving multiple different materials which is the situation of this study involving the fabrication of PDMS, modified PDMS membrane, quartz, SU-8, and capillaries. For the initial attempt of avoiding leakage, the entire PDMS substrate was coated with the modified PDMS membrane, which indeed avoided leakage problems; however, it caused detection problems. This is because the separation channel is also covered by the membrane that caused light scattering leading to low detection sensitivity (data not shown here). Therefore, the membrane was designed to cover the reservoir area of the PDMS substrate in order to eliminate light scattering. Because of the definite thickness of the membrane (~10 μm thick) which made a gap between the top PDMS substrate and the bottom SU-8 layer as shown in Fig. 3B, leakage occurred cross the chip width along the edge of the membrane as shown in Fig. 3D.

To solve this leakage problem, a technique involving the use of half-cured PDMS to seal the gap was developed and briefed below. After the PDMS substrate is fabricated with its reservoir areas bonded with the modified PDMS

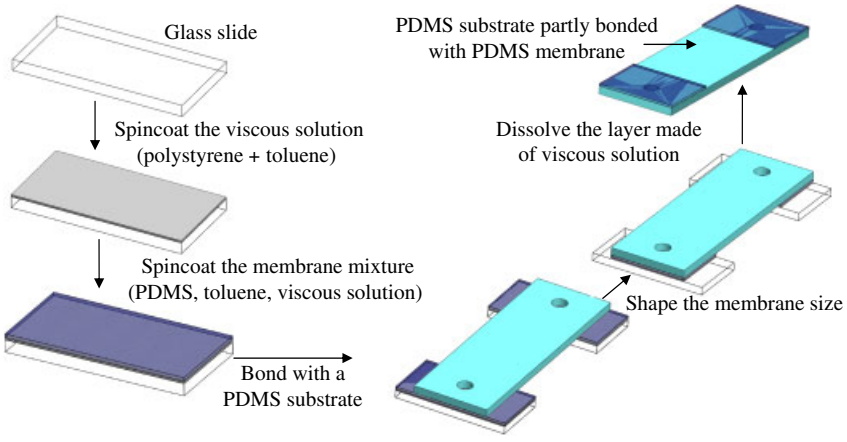


**Figure 2.** Procedure for fabricating a PDMS substrate partly bonded with the modified PDMS membranes.

 

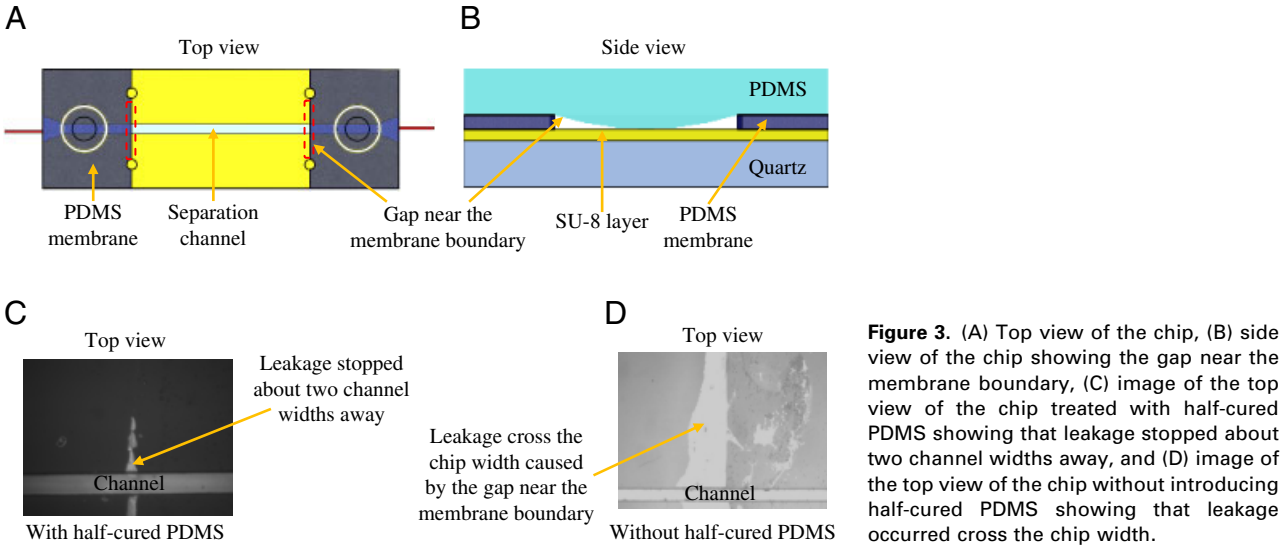


**Figure 3.** (A) Top view of the chip, (B) side view of the chip showing the gap near the membrane boundary, (C) image of the top view of the chip treated with half-cured PDMS showing that leakage stopped about two channel widths away, and (D) image of the top view of the chip without introducing half-cured PDMS showing that leakage occurred cross the chip width.

membranes, four assisting holes with a diameter of 2 mm were punched through the PDMS substrate and PDMS membrane at the membrane boundary as shown in Figs. 1B and C. Then, the PDMS and quartz substrates obtained above were irreversibly bonded together via oxygen plasma at 29.2 W, 300 mTorr pressure for 20 s. Immediately after bonding, half-cured PDMS (previously cured at 65°C for 7 min) was injected into the four assisting holes, which fills the gap caused by the definite thickness of the membrane. Caution has been paid when filling the half-cured PDMS so that it seals the gap, but does not block the channel. As a result, there is still a small gap (its length is about two channel widths) as shown in Fig. 3C, where the liquid from the channel can fill in. It should be noted that this small gap does not affect the separation since it neither causes appreciable liquid losses (maximum 2 nL which is around 0.4% of the total liquid volume) nor affects the applied electrical field which has been validated previously in a similar geometry (a straight channel with a wedge along the channel length) for different applications [28]. The combined microfluidic chip was baked at 65°C for 30 min to harden the bonding and complete PDMS curing.

The fabrication was followed by the insertion of injection and discharge capillaries from the two tapered ends of the SU-8 channel, which was done under a microscope. After the insertion of capillaries, a layer of glue was applied to the capillaries around the edges of the channel to prevent leakage. Finally, the glass reservoirs were glued on top of the chip using room-temperature vulcanizing silicone glue. An image of the fully integrated chip is shown in Fig. 1D that was put side by side with a commercial cartridge from Convergent Bioscience.

## 3 Results and discussion

IEF experiments were performed using an iCE280 analyzer (Convergent Bioscience), which consists of a deuterium ($D_2$)

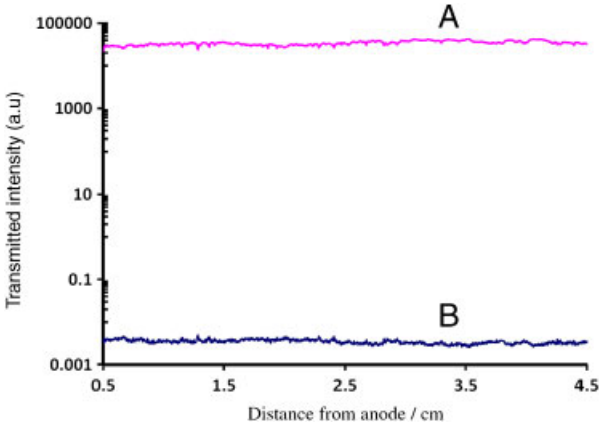


**Figure 4.** Transmitted UV intensity (A) along the separation channel and (B) along SU-8 layer.

lamp as a light source and a whole-channel optical absorption imaging detector operated at 280 nm. During sample focusing, the light beam from the lamp was focused onto the separation channel by a bundle of optical fibres and a cylindrical lens as illustrated in Fig. 1E. The UV abesorption image of the whole-channel was captured by a CCD camera. All the sample solutions were prepared in deionized water containing 1% PVP, 2% pharmalytes (3–10), and desired p*I* mixture or proteins. The anolyte and catholyte were 0.1 mol/L phosphoric acid and 0.1 mol/L sodium hydroxide, respectively. The experiments were performed at room temperature. All the solutions were filtered using a 0.2 μm pore size cellulose acetate filter (Sartorius, Gottingen, Germany) prior to use. The channel inside the chip was preconditioned using a PVP solution (1%, w/v) for 30 min to suppress electro-osmotic flow that may cause peak relocation. Following the preconditioning, the sample mixture was injected into the channel. Focusing was performed by first applying a voltage difference of 1.5 kV for 4 min for pre-focusing and then applying a

 

voltage difference of 3 kV for the rest of the separation that was roughly 10 min. Between each run, the channel was flushed with a 1% PVP solution for 5 min. IEF experiments were repeated three times for each sample.

Prior to separation experiments, the UV-blocking performance of each chip was tested. As explained previously [25], SU-8 contains eight benzene rings in its chemical structure that give SU-8 high absorptive property in the UV range (<300 nm). The absorbance of the SU-8 layer is expected to follow the Beer–Lambert law and increases with thickness. However, thick SU-8 layers (i.e. >100 μm using our available facility) tend to have curved side walls due to the process of multiple coatings of SU-8 (each coating gives a certain thickness). Since the focus of this study is to develop integration techniques, 100 μm-thick SU-8 layer was chosen in this study. Figure 4 shows a comparison of the UV light transmitted through the channel region and the 100 μm-thick SU-8 region. The UV intensity is almost uniform across the separation channel length and it is six orders of magnitude higher than the UV intensity transmitted through the SU-8 layer. These results demonstrate the efficiency of the 100 μm-thick SU-8 layer as an optical slit that blocks the stray UV light.

The fully integrated chip was evaluated based on its separation performance of p*I* markers and protein samples. Figure 5 shows the separation of five p*I* markers (4.22, 5.85, 7.00, 8.18, and 9.46) on the fully integrated chip using the IEF-UV-WCID technology and commercial cartridge, separately. As one can see, these five p*I* markers were successfully separated on the microfluidic chip and the peak heights and peak locations are in perfect agreement with those of the commercial cartridge. The comparison of the peak locations with the p*I* points of the sample mixture indicates the linear pH gradient achieved using the integrated hybrid microchip. The pH linearity was found to be 0.9978 and 0.9981 for the commercial cartridge and fully integrated chip, respectively. This also validates that the integrated membrane successfully prevents hydrodynamic flow and diffusion of electrolytes into the separation channel. Details of the membrane functions can be found in our previous work [27]. The peak height achieved in the fully integrated chip (around 0.23 for 4.22 p*I* marker on the left axis scale) is less than the commercial cartridge (around 0.33 for 4.22 p*I* marker on the right axis scale). This may be due to the fact that the 100 μm-thick SU-8 optical slit cannot block 100% of stray UV light as the metal slit.

To further validate the separation performance of the fully integrated chip, it has been tested for the separation of human haemoglobin control AFSC, which contains four definite isoforms (A, 7.0; F, 7.1; S, 7.3; C, 7.5) with very close p*I* values. The separation results are shown in Fig. 6 that also shows the separation results of the commercial cartridge. Four isoforms were separated very well. Based on these results, it can be concluded that the microfluidic chip can achieve separation as effective as the commercial cartridge.

Repeatability and reproducibility of the fully integrated chip were also examined and presented in Table 1. Run-to-

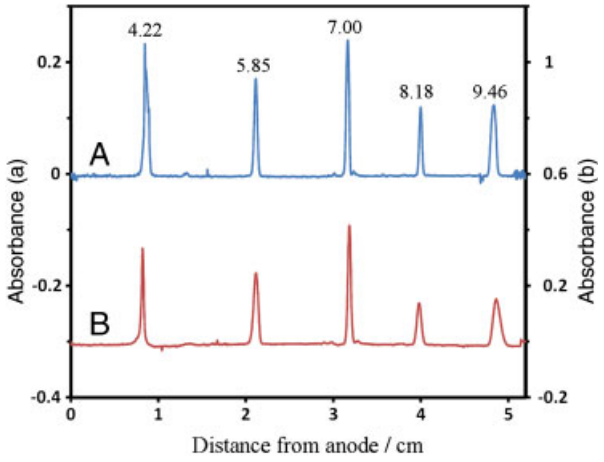


**Figure 5.** IEF profile of PI markers using (A) the fully integrated chip and (B) capillary cartridge. Separation channel: (A) 100 μm width × 100 μm depth × 5 cm length; (B) 100 μm ID × 5 cm length. The sample is a mixture of five p*I* markers (4.22, 5.85, 7.00, 8.18, 9.46, 2% of their original concentration), pharmalytes (pH 3–10) and 1% PVP. The applied voltage was set at 1500 V for the first 4 min and then at 3000 V for the rest of separation.

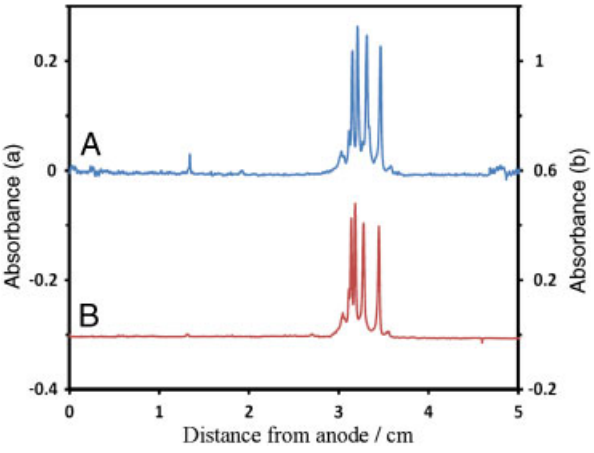


**Figure 6.** IEF profile of human haemoglobin control AFSC using (A) the fully integrated chip and (B) commercial cartridge. Separation channel: (A) 100 μm width × 100 μm depth × 5 cm length; (B) 100 μm id × 5 cm length. The sample contains 0.1 mg/mL human haemoglobin control, 2% pharmalytes (pH 3–10) and 1% PVP. The applied voltage was set at 1500 V for the first 4 min and then at 3000 V for the rest of separation.

**Table 1.** Repeatability and reproducibility of fully integrated chips for IEF-WCID

| Parameter | Peak height[a)] | | | Run-to-run | Chip-to-chip |
|---|---|---|---|---|---|
| | Run 1 | Run 2 | Run 3 | RSD (%) | RSD |
| Chip 1 | 0.2336 | 0.2296 | 0.2329 | 0.92 | |
| Chip 2 | 0.2326 | 0.2341 | 0.2371 | 0.97 | 1.80% |
| Chip 3 | 0.2404 | 0.2387 | 0.2384 | 0.45 | |

a) The peak height was the absorbance of p*I* 7.00 in the p*I* mixture samples. The IEF conditions are the same as in Fig. 5.

run repeatability test was performed for the fully integrated chip by comparing the peak height of p$I = 7.00$ in the p$I$ mixture for three runs under the same operating conditions. The chip-to-chip reproducibility was also investigated by comparing the peak height of p$I = 7.00$ in three different chips. As shown in Table 1, the relative standard deviations (RSD) are less than 1% ($n = 3$) for run-to-run repeatability and less than 2% ($n = 3$) for chip-to-chip reproducibility.

## 4 Concluding remarks

A PDMS/modified PDMS membrane/SU-8/quartz hybrid chip was developed and successfully applied for separating a protein sample and a mixture of p$I$ markers using the IEF-UV-WCID technology. The main contributions of this study are twofold.

First, this fully integrated microfluidic chip fully realizes all the functions of the commercial cartridge used in the iCE280 analyzer in a chip format, while avoiding the challenging alignment and gluing procedures associated with the commercial cartridge. In brief, in this new design, the modified PDMS membranes which can be fabricated using standard micro-fabrication techniques replace the hollow fibres eliminating the need to glue two 3 mm-long, 100 μm-diameter hollow fibres with 100 μm-diameter capillaries including the separation capillary and sample injection and discharge capillaries, the built-in SU-8 layer replaces the metal slit avoiding the alignment needed for gluing the metal slit with the cartridge, and the square (could be rectangular) microchannel replaces the capillary reducing lens effects. In summary, the developed chip is robust and can be employed in the iCE280 analyzer with a lower cost than its current best practise. In addition, this chip format IEF opens up the opportunity for multi-dimensional separation of complex samples, which cannot be realized easily using capillary-based separation.

Second, the fabrication techniques developed in this study can be employed in other chip applications, which involve the fabrication of multilayer structures using multiple different materials. For example, the developed half-cured PDMS with assisting holes for preventing leakage problems can be applied to other fabrications where leakage is prone to happen. In addition, the modified PDMS membranes and associated fabrication techniques can be employed in other separation studies as well.

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