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INTEGRATION OF A PDMS/SU-8/QUARTZ MICROFLUIDIC CHIP WITH A NOVEL MACROPOROUS POLY DIMETHYLSILOXANE (PDMS) MEMBRANE FOR ISOELECTRIC FOCUSING OF PROTEINS USING WHOLE-CHANNEL IMAGING DETECTION

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Introduction

Capillary isoelectric focusing (CIEF) is a high-resolution capillary electrophoresis (CE) technique for separating zwitterionic biomolecules, such as proteins and peptides. In this method, by generating a stable pH gradient along the length of the capillary and under the influence of a constant electric field, samples can be separated according to their different isoelectric points (pI). For identifying the focused zones in CIEF, the whole column imaging detection (WCID) is more reliable than any other single point detection methods since it avoids the need of focused peak mobilization, presenting several advantages such as lower detection time, minimized peak dispersion and consequently higher resolution. Capillary-based IEF-WCID has been invented by Convergent Bioscience Inc. (iCE280 analyzer) for separation of proteins and biomarkers [1-2]. In the iCE280 analyzer, hollow fibers are glued to the capillary to separate electrolytes from the samples and a metal slit with a 65 μm opening is glued to the top of the capillary to improve detection sensitivity by blocking stray light. However, this method has several limitations because of the use of capillary such as low throughput, difficulty to be integrated with other separation modes and low detection sensitivity.

Microfluidic chips provide a viable alternative to the capillary format as they offer a number of advantages including low cost, high speed, high throughput, potential for integration, flexibility and portability for a wide range of applications in analytical and bioanalytical chemistry [3-4]. In this study, a microfluidic chip is developed and successfully applied for separating complex samples of proteins using the IEF-UV-WCID technology. This design uses SU8 channels for UV blocking, capillaries for sample injection and macroporous membranes for avoiding undesired hydrodynamic flow and can be extended to multi and complex channel structures for more complex analysis.

Fabrication of the chip

The integrated isoelectric focusing chips were fabricated using conventional methods of soft lithography. The top and bottom layers of the chips were fabricated separately and bonded together by oxygen plasma. The top layer is composed of a flat 2mm thick 10:1 PDMS (Dow Corning, Midland, MI, USA) layer and two macroporous membranes. Fabrication of the macroporous membrane is detailed in a previous publication of the authors [5]. The bottom layer was formed by patterning an SU-8 (Microchem, Newton, MA, USA) layer on 3 × 1 inch quartz substrate (Ted Pella Inc. Redding, CA, USA). The microchannel was designed as 100 μm thick, 100 μm wide and 5 cm long. To assist the bonding of SU-8 bottom layer with PDMS top layer, additional holes were punched on the PDMS layer. Following the oxygen plasma bonding, partially cured PDMS prepolymer (mixed with 10:1 ratio and cured for 7 min at 65 °C) was introduced in these holes to strengthen the bonding. The bonded chip was baked at 65 °C overnight to improve the bonding and complete the curing of PDMS. Finally, two capillaries with 40 μm i.d. and 100 μm o.d. (Polymicro Technologies, Tucson, AZ) were injected to the tapered ends of the chip under microscope. The capillaries were glued to prevent leakage around the end of the channel. The fabrication of the chip was completed with the placement of the reservoirs aligned with the electrolyte injection holes.

Results and discussion

IEF experiments were performed using an iCE280 analyzer (Convergent Bioscience), which consists of a deuterium (D2) 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 fibers and a cylindrical lens. The UV adsorption image of the whole-

channel was captured by a CCD camera. Sample solutions were prepared in deionized water containing 1.0% PVP, 2% pharmalytes (3-10), and bovine albumin (Helena, Beaumont, TX). The anolyte and catholyte were 0.1 mol L^{-1} phosphoric acid and 0.1 mol L^{-1} sodium hydroxide, respectively. The IEF was performed at room temperature. All of the solutions were filtered using a 0.2 μm pore size cellulose acetate filter (Sartorius, Gottingen, Germany) prior to use. The separation channel was preconditioned with a PVP solution (1%, w/v) for 30 min to suppress electro-osmotic flow that may cause peak broadening. Following the preconditioning, the sample mixture was injected into the channel. Focusing was performed by first applying a voltage difference of 1500 V for 4 min and then applying a voltage difference of 3 kV for the rest of the separation. Between each run, the channel was flushed with the 1% PVP solution for 5 min. IEF experiments was repeated three times for each sample. Prior to any separation experiments, the UV-blocking performance of each chip is tested. Figure 1 shows a comparison of the UV light transmitted through the channel region and SU-8 region. The UV intensity is almost equal all over the separation channel and it is six orders of magnitude higher than the UV intensity transmitted through the SU-8 layer. These results demonstrate the efficiency of 100 μm thick SU-8 layer as an optical slit that blocks the stray UV light. The separation results for bovine albumin are shown in figure 2. As it is seen in this figure, three isoforms were separated very well.

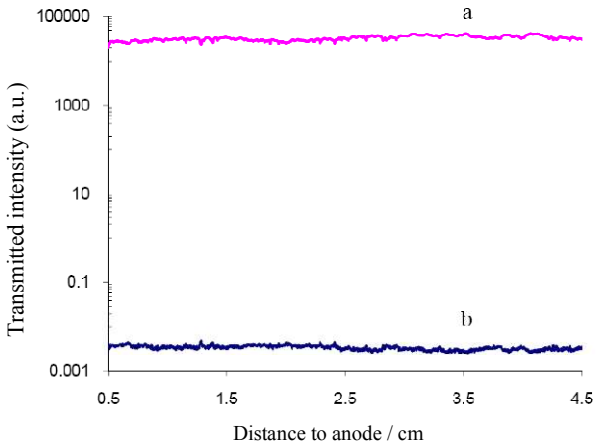

Figure 1: Transmitted UV intensity (a) along the separation channel (b) along SU-8 layer

Conclusion

A PDMS/SU-8/quartz chip with SU-8 optical slit, integrated PDMS membranes and capillary injections was developed and successfully applied for separating complex samples of proteins using the IEF-UV-WCID technology. This new design exhibits several advantages. It was demonstrated that SU-8 layer is very effective as an optical slit to reduce the stray light for improved detection sensitivity. Elimination of the metal slit improves the yield of the fabrication and provides consistent results between different chips. Using PDMS membrane, the hydrodynamic flows causing peak-broadening were effectively reduced and accuracy of the results are significantly improved. Capillary injection provides easy introduction of the samples and facilitates the preconditioning and cleaning of the chip. The separation performance of the chip was validated using bovine albumin. Further investigation of the chip performance evaluating the pH linearity and repeatability are under development. With all the explained futures, the presented chips can be extended to multi-dimensional protein separation experiments.

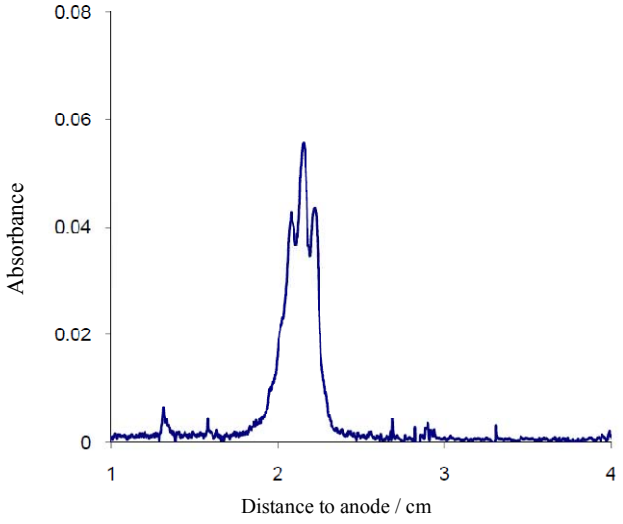

Figure 2: IEF profile of bovine albumin using the integrated chip. Separation channel is 100 μm× 100 μm× 5cm. The sample contains 0.1 mg mL^{-1} bovine albumin, 2% pharmalytes (pH 3–10) and 1% PVP.

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