pH-GRADIENT CHROMATOFOCUSING OF PROTEINS ON A CHIP

Hoon Suk Rho*, Alexander T. Hanke, Marcel Ottens**, and Han Gardeniers***

** Mesoscale Chemical Systems, MESA+ Institute for nanotechnology, University of Twente, THE NETHERLANDS*

*** BioProcess Engineering group, Department of Biotechnology, Delft University of Technology, Faculty of Applied Sciences, THE NETHERLANDS*

ABSTRACT

We present a novel microfluidic system for the pH-gradient focusing of proteins with the integration of 16 parallel micro-mixers, a micro-column, and a multiplexer. In this work we successfully achieved the creation of 16 non-linear gradients and the generation of a solid-phase micro-column for the realization of anion exchange chromatography on a single chip. With the device we demonstrated the separation of a protein mixture of R-phycoerythrin and FITC-BSA based on pH-gradient chromatofocusing.

KEYWORDS: Microfluidics, Chromatofocusing, pH gradient, Micro-column

INTRODUCTION

During the last decade, the potential of microfluidic systems for high-resolution separation was presented in proteomic and pharmaceutical applications [1-4]. Also microfluidic liquid chromatography systems that contain microchannels with patterned microstructures have shown to give higher resolution than classical or conventional chromatography. Combination of the techniques could bring a new powerful analysis tool for separation of biomolecules. Here, we developed a novel microfluidic platform for pH-gradient chromatofocusing of proteins with the advantages of microfluidic systems, including reduced sample volume and time consumption, fast analysis, automation, and integration.

The chip has been designed to integrate all the processes of anion exchange chromatography including pH gradient generation, solid-phase column creation, and fractionation. Figure 1 shows the process flow of pH-gradient chromatofocusing of proteins in conventional methods (A) and our microfluidic method (B). When crude sample of proteins flows through a column where charged resin particles are packed, proteins bind onto the particles because proteins are naturally charged negatively. The bound proteins are released from the particles when there is no net electrical charge between proteins and particles. Since the charges of protein are different from each other, proteins bound to the particles can be separately eluted by controlling the pH of elution buffer. For performing pH-gradient chromatofocusing on a chip, a pH gradient generator, a micro-column, and sampling unit were combined into a single microfluidic device.

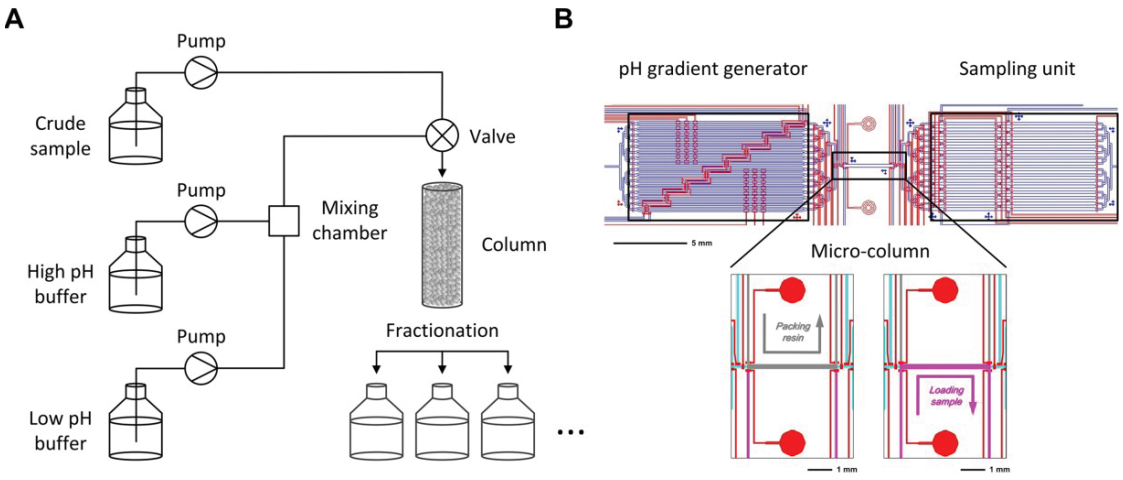

Figure 1: Process flow of pH-gradient chromatofocusing. (A) Conventional method, and (B) On-chip pH-gradient chromatofocusing.

RESULTS AND DISCUSSION

The pH-gradient generator was designed by adapting 16 parallel peristaltic mixers. The elution buffer can be diluted from 4 % to 96 % of the initial buffer concentration, with a buffer of different pH, which leads to 16 points on a non-linear pH-gradient (Figure 2 A). Figure 2 B shows the mixing performance of the mixers by loading blue colored dye and Milli-Q water into two loading sites of the mixers. Figure 2 C presents the on-chip and off-chip standard curves of a fluorescent dye (FITC) created by the mixers to validate the metering capability of the gradient generator. The stepwise elution of various pH solutions and collection of the fractions were controlled by a multiplexer. The buffer solutions eluted from the column were collected separately in a sampling unit which consisted of 16 fraction collectors. Figure 3 shows the generation of a micro-column on a chip. When a micro-particle solution flowed in a microfluidic channel where a sieve valve located at the end of the channel, the particles were captured and packed in the channel. Figure 3 shows the process of packing polystyrene micro-particles (Ø 3 µm) in a channel (A) and solution exchange through the micro-column (B).

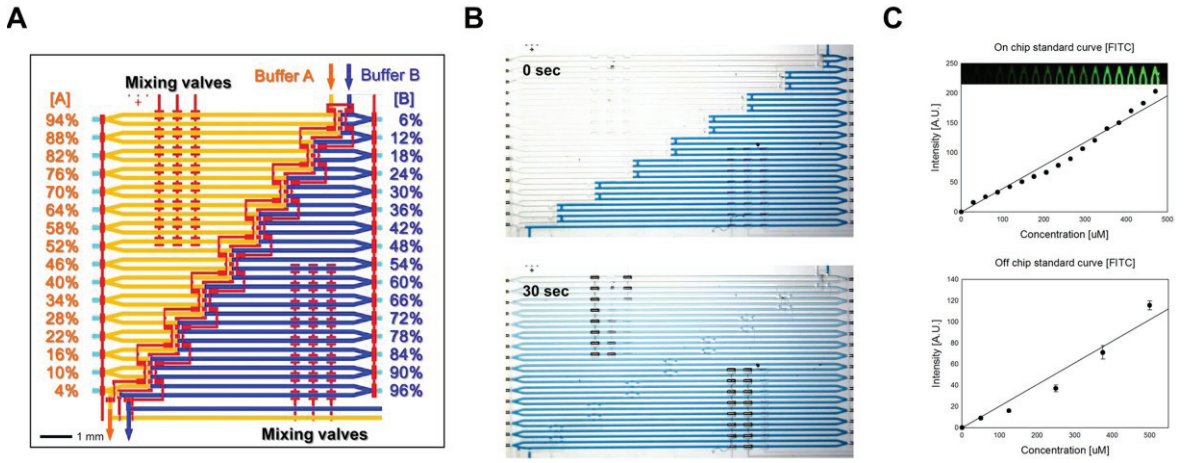

Figure 2: pH gradient generator. (A) Design of 16 parallel mixers for the creation of pH gradient, (B) Mixing performance of the mixer, and (C) Generation of concentration gradient of FITC-BSA.

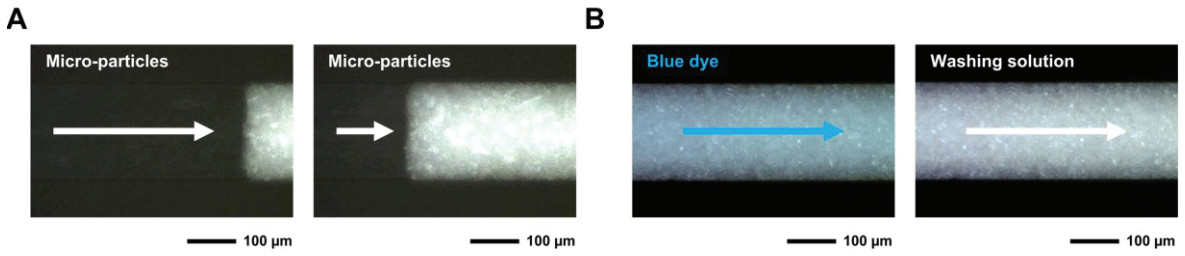

Figure 3: Generation of a micro-column. (A) Packing micro-particles (polystyrene, Ø 3 µm) in a microfluidic channel, and (B) Elution test through the micro-column with a blue dye and Milli-Q.

Figure 4 shows the separation of standard protein mixture, R-phycoerythrin (PE, pI ~ 4.4) and FITC-BSA (pI ~ 4.8). The mixture was loaded on the micro-column which was prepared by microfluidic packing of Source 15Q beads (Ø 15 µm anion exchange resin particles, GE Healthcare) and eluted by buffer solutions which pH ranged from 4.10 to 4.95. Figure 4 C shows the microscope images of 16 fraction collectors by bright field illumination, fluorescent illumination with a N2.1 filter (ex: BP 515 - 560 nm; em: LP 590 nm), and fluorescent illumination with a I3 filter (ex: BP 450 - 490 nm; em: LP 515 nm). By fluorescent detection it was observed that PE was eluted at pH 4.50 and FITC-BSA was eluted at pH 4.67 and pH 4.72.

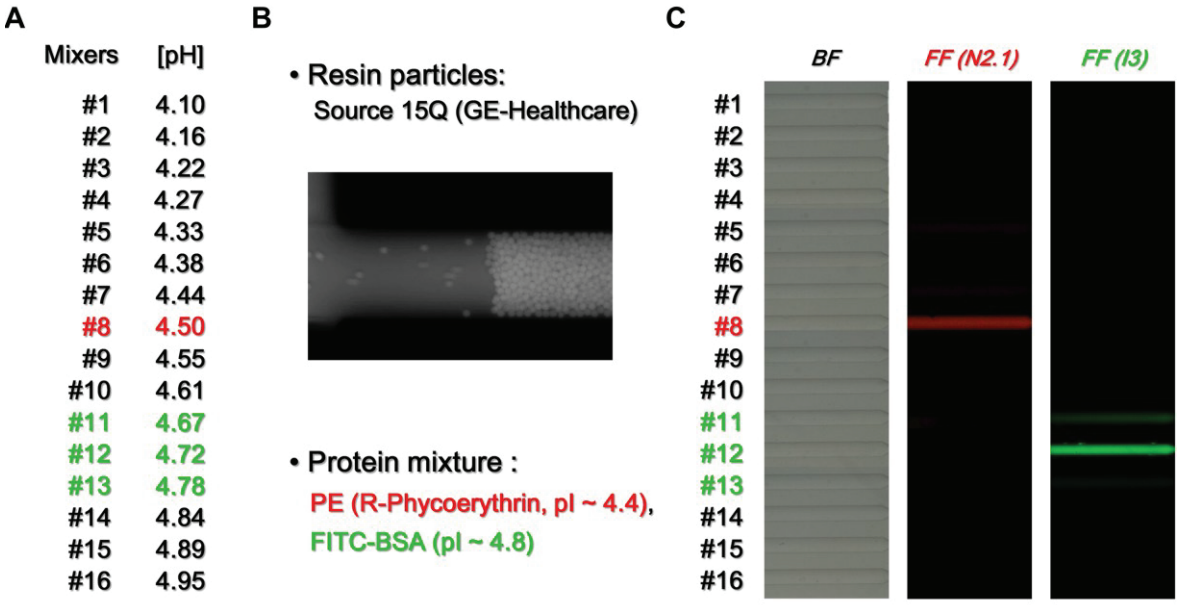

Figure 4: Separation of standard protein mixtures, PE and FITC-BSA. The protein mixture was loaded into an anion exchange micro-column and eluted by buffer solutions which pH ranged from 4.10 to 4.95. PE (pI ~ 4.4) was eluted at pH 4.50 and FITC-BSA (pI ~ 4.8) was eluted at pH 4.67 and pH 4.72.

CONCLUSION

We have developed a new approach for performing pH-gradient chromatofocusing with an integrated microfluidic chip. We believe that the fully automated and integrated microfluidic platforms for high-throughput protein screening could be considered as a promising approach for fast biopharmaceutical process development.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge financial support from Project F2.003 HTPD in BE-Basic Foundation.

REFERENCES

[1] D. Figeys, and D. Pinto, "Proteomics on a chip: Promising developments," Electrophoresis, 22, 208-216, (2001).

[2] D. N. Breslauer, P. J. Lee, L. P. Lee, "Microfluidics-based systems biology," Mol. BioSyst., 2, 97-112, (2006).

[3] J. Huft, C. A. Haynes, and C. L. Hansen, "Microfluidic Integration of Parallel Solid-Phase Liquid Chromatography," Anal Chem, 85(5), 2999-3005, (2013).

[4] J. Huft, C. A. Haynes, and C. L. Hansen, "Fabrication of High-Quality Microfluidic Solid-Phase Chromatography Columns," Anal Chem, 85(3), 1797-1802, (2013).

CONTACT

* H. Gardeniers, tel: +31-(0)53-489 4356; j.g.e.gardeniers@utwente.nl