ABSTRACT
We designed pressure driven chromatography system using 100 nm scale nanofluidic channel (extended nanochannel) for million plate numbers. The chromatography was performed utilizing a channel in an equivalent diameter of 300 nm and 89 mm long (the world’s longest nanochannel) with 35 fL injection volume. The theoretical plate numbers 36,000 were well accorded with theoretically-estimated 39,000. From these results, performance of the system was verified, and it has potential to realize 1,000,000 plates for protein separation. This technique will be a revolutionary tool for comprehensive analysis of ultra-small biological samples such as single cells and bacteria.

KEYWORDS: Liquid chromatography, Extended nanospace, Nanofluidics, Pressure-driven flow

INTRODUCTION
In separation science, separation systems have always been developed seeking for higher efficiency and smaller sample volume. Especially in chromatography, development of packed column with smaller particle and monolith column has been intensively researched. On the other hand, our group developed chromatography system using open extended nanochannels [1] as shown in Figure 1, and a separation efficiency of 440,000 plates/m was verified. However, the total plate number was only 450 due to the short length of nanochannel (1.2 mm). Herein, we designed and developed a new high pressure nanofluidic control system using longer extended nanochannel to realize 1,000,000 plates, and verified its performance.

Figure 1. Concept of (a) conventional HPLC and (b) chromatography using extended nanochannel.
Figure 2. Calculation of theoretical plate number N plotted against channel length and size
THEORY

In order to estimate theoretical plate number N, theoretical equations of plate height in open tubular chromatography [2] was used. Figure 2 showed a color diagram of N plotted against channel diameter and channel length, calculated using applied pressure of 20 MPa and diffusion constant of BSA proteins $2.0 \times 10^{11}$ m$^2$/s in the condition of water with 25% acetonitrile [3]. As a result, plate number for BSA proteins was estimated to reach 1,000,000 in the condition of 300 nm wide and deep and 89 mm long.

EXPERIMENTAL

Based on this design, a new chromatography system was constructed as shown in Figure 3. Air pressure was supplied from a gas cylinder and increased up to 20 MPa using a compressor and a gas booster. The pressure was kept in pressure tanks and controlled by opening/closing solenoid valves with a time resolution of 50 ms. Then, the pressure pushed solutions in liquid reservoirs into a fused silica microchip. In the microchip, channels were fabricated with 300 nm diameter and 89 mm long based on calculated results. Sample was loaded from upper channel to cross-point, and was cut by flow of mobile phase from left channel to cross-point. As a result, cut sample was injected into right channel (separation channel). In order to verify the performance of the system, experiments using fluorescents dyes (Pyrromethene 597 and Coumarine 460) were performed. Based on the designed system, 39,000 plates was estimated with fluorescent dye in application of 8 MPa. The mobile phase and stationary phase are hexane with 10% 2-propanol and silica surface.

RESULTS AND DISCUSSION

The sample was cut and injected into the extended nanochannel and detected using fluorescent microscope. The injected sample volume was reproducible as shown in Figure 4, and its volume was estimated to be 35 fL from fluorescent intensity. As shown in Figure 5, they were separated as well as conventional HPLC. As shown Table 1, the theoretical plate numbers in the extended nanochannel 36,000, which were 10 times higher than a result in HPLC, were well accorded with theoretically-estimated 39,000. From these results, performance of the system was verified, and it has potential to realize 1,000,000 plates for protein separation. Also, the peak resolution was improved from 4.9 (HPLC) to 7.5 (extended nanochannel). The sample volume was decreased 8 orders of magnitude, which promise application to single cell proteomic analysis because the volume is much smaller than a single cell (pL). In the future work, as designed, achievement of 1,000,000 plates using BSA is expected.
CONCLUSION

We designed pressure driven chromatography system using extended nanochannel for million plate numbers. Plate numbers were estimated by theoretical equations in open tubular chromatography, and channel diameter and length to achieve million plate numbers were determined. Based on the design, the chromatography was performed utilizing a channel in an equivalent diameter of 300 nm and 89 mm long (the world’s longest nanochannel). Injection volume was reproducible and its volume was 35 fL. Obtained theoretical plate numbers 36,000 were well accorded with theoretically-estimated 39,000. From these results, performance of the system was verified, and it has potential to realize 1,000,000 plates for protein separation. In the future work, as designed, achievement of 1,000,000 plates using BSA is expected. This technique will be a revolutionary tool for comprehensive analysis of ultra-small biological samples such as single cells and bacteria.

REFERENCES


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