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Pinched Flow Fractionation: Continuous Size Separation of Particles Utilizing a Laminar Flow Profile in a Pinched Microchannel

Masumi Yamada,[†] Megumi Nakashima,[†] and Minoru Seki*,[‡]

Department of Chemistry and Biotechnology, School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan, and Department of Chemical Engineering, Graduate School of Engineering, Osaka Prefecture University, 1-1 Gakuen-cho, Sakai, Osaka 599-8531, Japan

A concept of "pinched flow fractionation" for the continuous size separation and analysis of particles in microfabricated devices has been proposed and demonstrated. In this method, particles suspended in liquid were continuously introduced into a microchannel having a pinched segment and were aligned to one sidewall in the pinched segment by another liquid flow without particles. The particles were then separated perpendicularly to the flow direction according to their sizes by the spreading flow profile inside the microchannel. Polymer microbeads were successfully separated, and the effects of the flow rate and channel shapes on the separation performance were examined. Also, separated particles were collected independently by making branches at the end of the pinched segment. Since this method utilizes only the laminar flow profile inside a microchannel, complicated outer field control could be eliminated, which is usually required for other kinds of particle separation methods such as field flow fractionation. Also, this method can be applied both for particle size analysis and for preparation of monodispersed particles, since separation can be rapidly and continuously performed.

Accurate size separation and size measurement of various kinds of particles, including polymer beads, ceramics, cells, and pharmaceutical emulsions, are one of the most important technologies in the fields of industrial production, environmental assessment, and chemical or biological research. Many studies have focused on particle separation, using field flow fractionation (FFF),[1−5] hydrodynamic chromatography (HDC),[6−9] capillary hydrodynamic fractionation (CHDF),[10−11] or split-flow thin (SPLITT) fractionation.[12−15] With these methods, not only particles but also macromolecules whose diameters are typically from 1 nm to 100 μm, can be separated. Also, with the recent progress in microfabrication technology,[16−19] some researchers have reported on miniaturized particle separation systems using these methods.[5,9] However, these methods have advantages and disadvantages.

FFF, HDC, and CHDF are used mainly for particle or macromolecule analysis, which utilizes the flow rate distribution in a thin channel or capillary. FFF requires outer fields such as a gravitational, centrifugal, electrical, thermal, or cross-flow field, while they are not required for CHFD and HDC. Although the separation accuracy is high in these methods, particle separation requires a relatively long time. Also, since the separation process is not continuous, it is not suitable for the preparation of particles. In addition, complicated outer devices are required for injecting a small amount of particles into the separation field.

On the other hand, SPLITT fractionation is a continuous particle separation method and is used mainly for the preparation of particles with uniform characteristics. However, it requires outer fields as in the case with FFF. Therefore, a continuous and simple method, which can be performed without the help of an outer fields, is required and desirable for particle separation.

In the present study, we propose a new and simple method for the size separation of particles, pinched flow fractionation (PFF), in a microchannel that has a pinched segment and whose

* To whom correspondence should be addressed. Tel.: +81-72-254-9296. Fax: +81-72-254-9911. E-mail address: seki@chemeng.osakafu-u.ac.jp.

[†] The University of Tokyo.

[‡] Osaka Prefecture University.

(1) Giddings, J. C. *Science* **1993**, *260*, 1456−1465.
(2) Kim, W. S.; Park, Y. H.; Shin, J. Y.; Lee, D. W.; Lee, S. *Anal. Chem.* **1999**, *71*, 3265−3272.
(3) Lee, W. J.; Min, B. R.; Moon, M. H. *Anal. Chem.* **1999**, *71*, 3446−3452.
(4) Wang, X. B.; Yang, J.; Huang, Y.; Vykoukal, J.; Becker, F. F.; Gascoyne, P. R. C. *Anal. Chem.* **2000**, *72*, 832−839.
(5) Edwards, T. L.; Gale, B. K.; Frazier, A. B. *Anal. Chem.* **2002**, *74*, 1211−1216.
(6) Small, H. *J. Colloid Interface Sci.* **1974**, *48*, 147−161.
(7) Thornton, A. W.; Olivier, J. P.; Smart, C. G.; Gilman, L. B. *ACS Symp. Ser.* **1987**, *332*, 256−271.
(8) Williams, A.; Varela, E.; Meehan, E.; Tribe, K. *Int. J. Pharm.* **2002**, *242*, 295−299.
(9) Chmela, E.; Tijssen, R.; Blom, M. T.; Gardeniers, H. J. G. E.; van den Berg, A. *Anal. Chem.* **2002**, *74*, 3470−3475.
(10) Silebi, C. A.; Dosramos, J. G. *J. Colloid Interface Sci.* **1989**, *130*, 14−24.
(11) Miller, C. M.; Sudol, E. D.; Silebi, C. A.; El-Aasser, M. S. *J. Colloid Interface Sci.* **1995**, *172*, 249−256.
(12) Giddings, J. C. *Sep. Sci. Technol.* **1985**, *20*, 749−768.
(13) Jiang, Y.; Kummerow, A.; Hansen. M. *J. Microcolumn Sep.* **1997**, *9*, 261−273.
(14) Dondi, F.; Contado, C.; Blo, G.; Martin, S. G. *Chromatographia* **1998**, *48*, 643−654.
(15) Fuh, C. B. *Anal. Chem.* **2000**, *72*, 266A−271A.
(16) Reyes, D. R.; Iossifidis, D.; Auroux, P. A.; Manz, A. *Anal. Chem.* **2002**, *74*, 2623−2636.
(17) Auroux, P. A.; Iossifidis, D.; Reyes, D. R.; Manz, A. *Anal. Chem.* **2002**, *74*, 2637−2652.
(18) Hong, J. W.; Fujii, T.; Seki, M.; Yamamoto, T.; Endo, I. *Electrophoresis* **2001**, *22*, 328−333.
(19) Yamada, M.; Seki, M. *Anal. Chem.* **2004**, *76*, 895−899.

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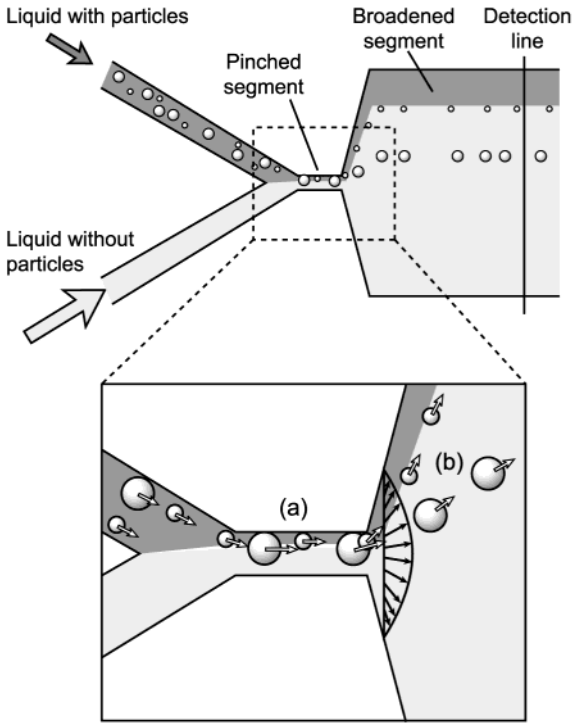

Figure 1. Principle of pinched flow fractionation. (a) In the pinched segment, particles are aligned to one sidewall regardless of their sizes by controlling the flow rates from two inlets; (b) particles are separated according to their sizes by the spreading flow profile at the boundary of the pinched and the broadened segments. The liquid containing particles is dark-colored.

cross sectional shape is rectangular. This method utilizes only the laminar flow profile in a specific microchannel geometry, not necessitating any kinds of outer field. Also, since this method is continuous, not only analytical but also preparative applications will be easily achieved without complicated sample injection schemes. In this study, separation of polymer microbeads was demonstrated, and the influences of operating conditions and microchannel geometries on separation performance were examined. On the other hand, separated particles were successfully collected by making branches at the end of the pinched segment.

PRINCIPLE

The principle of PFF is shown in Figure 1. First, liquids with and without particles are introduced into the microchannel continuously from each inlet. At this time, the liquid flow containing particles is focused on one sidewall in the pinched segment by controlling flow rates from both inlets. This operation triggers the alignment of particles to the sidewall regardless of their sizes (Figure 1a). This particle alignment is similar to the technique used in flow-FFF, in the sense that particle positions are forced to be uniform by another liquid flow.[1] Next, at the boundary between the pinched and the following broadened microchannel segments, a force toward the center of the microchannel is exerted mainly on the larger particles by the spreading flow profile, whereas a force toward the sidewall is exerted mainly on the smaller particles (Figure 1b). Consequently, the slight difference of the particle positions in the pinched segment is significantly amplified in the broadened segment, so the particles are separated perpendicularly to the flow direction according to their sizes.

Certain factors would affect separation performance. First, the ratio of flow rates from two inlets would be the crucial precondition, since this ratio determines the width of the particle-containing liquid in the pinched segment, which relates to the alignment of particles to the sidewall. Also, microchannel geometries (especially the width of the pinched segment and the boundary angle between the pinched and the broadened segments) would affect the separation performance, since particle movement is dominated by flow profiles inside the channel. In addition, flow rate would have some effects on separation performance, since the inertial force of particle movement increases as particle speed increases. So in this study, these four factors were taken into consideration, and their influences on separation performance were examined.

In addition, when applying this method for particle size analysis, particle sizes should be determined from their effluent positions along the detection line. To model the relation between the particle sizes and their effluent positions, it is required to figure out the flow profiles inside microchannel. Therefore, the flow profile was visualized using small fluorescent microspheres, and the results obtained form the experiments were compared to theoretical values.

EXPERIMENTAL SECTION

Materials. Si (100) wafer was obtained from Furuuchi Chemical Corp. Negative photoresist, SU-8 50, was obtained from MicroChem. Corp.. PDMS (Sylgard 184) was obtained from Dow Corning. Poly(styrene/divinylbenzene) beads (Source 30Q and Source 15Q) and Dextran T500 (M_w ~500 000) were obtained from Amersham Biosciences Corp. Green fluorescent polymer microspheres (G0100) were obtained from Duke Scientific Corp.

Microdevice Fabrication. Microdevices were fabricated using the usual rapid prototyping and replica molding methods,[20,21] and the material was PDMS. PDMS is highly transparent, so it is suitable for optical detection. First, a negative photoresist (SU-8 50) master was formed on a silicon wafer. PDMS prepolymer was then cast on the master and cured. After curing, the PDMS replica was peeled off from the master and then bonded with a flat PDMS plate after O_2 plasma treatment. Using this method, a microchannel whose cross sectional shape is rectangular can be easily and rapidly fabricated, which is difficult to obtain from the usual glass or silicon microdevice fabrication method using wet etching techniques.

Microchannel Design. Microchannel design is shown in Figure 2. Figure 2a shows the microdevice for particle separation and size measurement (type 1 microdevice), and several types of microdevice were designed and fabricated in order to examine the influence of microchannel geometry on separation performance. The width of the pinched segment w_p was 47, 56, or 82 μm, and the angle θ of the boundary between the pinched and the broadened segments was 60° or 180°. The width of the broadened segment was 1 mm, and the depth of the microchannel was between 45 and 55 μm for all microdevices. Figure 2b shows the microdevice for particle separation and collection (type 2 microdevice). Five branch channels, whose widths were 1 mm,

(20) Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M. *Anal. Chem.* **1998**, *70*, 4974−4984.
(21) Hong, J. W.; Hosokawa, K.; Fujii, T.; Seki, M.; Endo, I. *Biotechnol. Prog.* **2001**, *17*, 958−962.

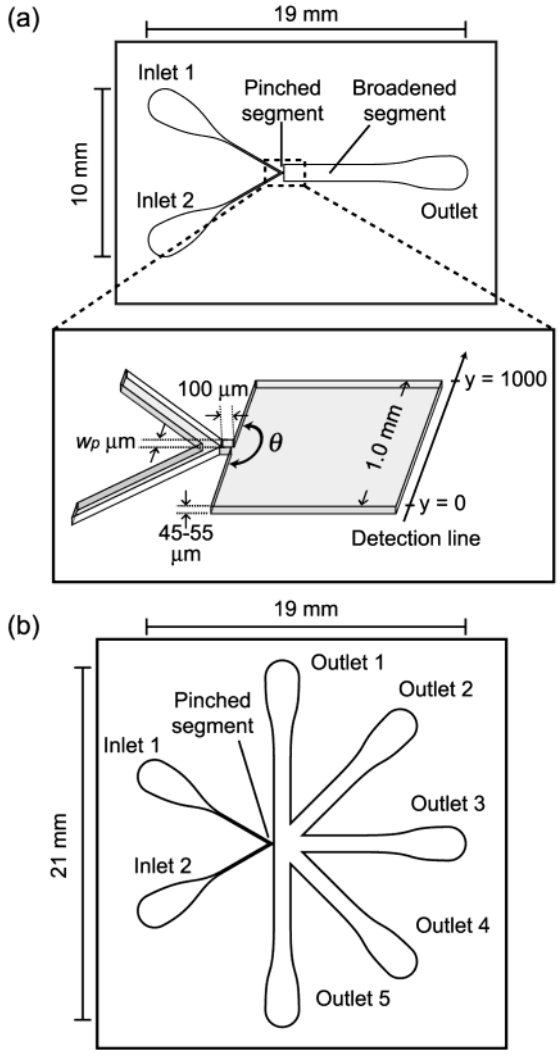

Figure 2. Schematic diagrams of microdevice and microchannel. (a) is the microdevice for particle separation and size distribution measurement (type 1 microdevice); (b) is the microdevice for particle separation and collection (type 2 microdevice).

were connected to the pinched segment. The pinched segment width was 50 μm, and the microchannel depth was ~50 μm.

Particle Separation and Measurement. Before performing particle separation, the microchannel was treated with O_2 plasma in order to avoid the adhesion of particles onto the PDMS surface. In this study, the mixture of poly(styrene/divinylbenzene) beads, whose diameters were either 15 (Source 15Q) or 30 μm (Source 30Q), was separated as model particles. From our measurement with image processing, variation coefficients of particle diameters were 1.5% for Source 15Q and 1.0% for Source 30Q, respectively. These beads were suspended in 10% (w/w) dextran solution whose viscosity was relatively high. By using such a viscous solution, the sedimentation of particles onto the syringe wall before introduction into the microchannel could be prevented. The concentrations of 15-μm beads and 30-μm beads were ~500/1 μL of solution. Dextran solutions with and without particles were introduced into the microchannel from inlet 1 and inlet 2 as seen in Figure 2, respectively, using syringe pumps (KDS200, KD Scientific Inc.). Flow rates were controlled independently.

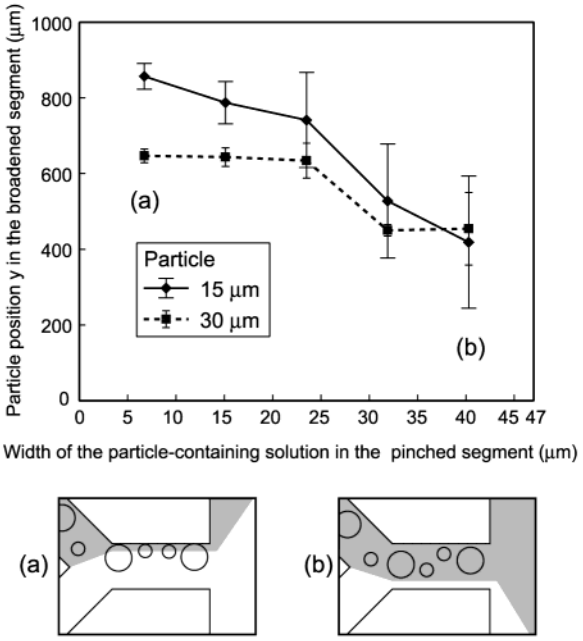

Figure 3. Relation between the width of the particle-containing solution in the pinched segment and the particle position in the broadened segment. Particle position *y* corresponds to the *y*-axis in Figure 2. (a) and (b) are the schematic diagrams of particle alignment in the pinched segment when the widths of the particle-containing solution were 6.7 and 40.3 μm, respectively. Particle-containing solution is dark-colored.

Separation phenomena were observed under an optical microscope, and moving images were captured using a CCD camera (DXC-151, Sony Corp.). In type 1 microdevices, the number of particles was counted every 10 or 100 μm along the detection line in the broadened segment, and the frequency was calculated. On average, 75 beads were counted for each condition.

Visualization of Flow Profile. To visualize the flow profile inside the microchannel, green fluorescent polymer spheres, whose diameter was 1.0 μm, were used, and their movements were observed using a fluorescent microscope. Moving images were captured using an ICCD camera (C2400-89V, Hamamatsu photonics K. K.), and the tracks of sphere movement were delineated computationally.

RESULTS AND DISCUSSION

Effect of the Ratio of Flow Rates. First, we tested whether particles of different diameters could actually be separated and how the ratio of flow rates from the two inlets, which relates to the focusing of the solution and the aligning of particles on the sidewall, would affect the separation performance. A type 1 microdevice, whose pinched segment width and boundary angle were 47 μm and 180°, respectively, was used. The flow rate of the particle-containing solution for inlet 1 was varied from 120 to 20 μL/h, and that of the solution without particles for inlet 2 was varied from 20 to 120 μL/h, while the total flow rate was kept constant. The 120 and 20 μL/h corresponded to the 40.3- and 6.7-μm widths of the solutions, respectively, in the pinched segment.

Figure 3 shows the relation between the width of the particle-containing solution in the pinched segment and the position of particles in the broadened segment. When the width of the particle-containing solution was larger than 30 μm, i.e., the flow

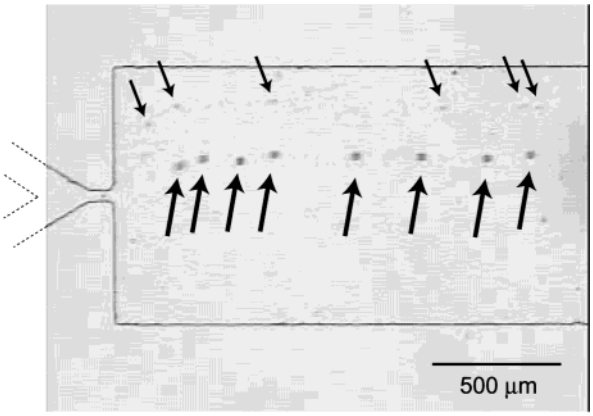

Figure 4. Photograph of separated particles. Particles indicated by small arrows are 15 μm in diameter, and those indicated by large arrows are 30 μm in diameter (see Supporting Information for a movie of the separation).

rate of the particle-containing solution was higher than 90 μL/h, both large and small particles were not aligned to the sidewall in the pinched segment (Figure 3b), and the particle positions in the broadened segment were not constant, which made the separation impossible. However, when the width of the particle-containing solution was decreased to 15.1 μm, large particles were aligned to the sidewall, and the distribution of positions in the broadened segment was decreased. In addition, by further decrease of the width (6.7 μm), the difference in the average positions of large and small particles became significant and the distribution of particle positions decreased, since both large particles and small particles were aligned to the sidewall in the pinched segment (Figure 3a). Figure 4 shows an actual photograph of separated particles, when the width of the particle-containing solution was 6.7 μm. These results showed that the separation of particles according to size could be carried out using this simple method. Also, it can be said that, for effective separation, the width of the particle-containing solution in the pinched segment should be smaller than the diameter of the smallest particles.

Effect of the Pinched Segment Width. Next, the influence of the pinched segment width on separation performance was examined, since it could easily be considered that the change in the pinched segment width would trigger the flow profile change inside microchannel. In this experiment, three types of microchannel, whose pinched segment widths *wp* were 47, 56, and 82 μm, respectively, were tested. Flow rates for inlets 1 and 2 were 20 and 120 μL/h, respectively, so the widths of the particle-containing solution in the pinched segment were 6.7, 8.0, and 11.7 μm for 47-, 56-, and 82-μm widths of the pinched segment, respectively. These values were smaller than the diameter of small particles. The operation conditions in regard to the pinched segment width of 47 μm were the same as those shown in Figure 3a.

The results are shown in Figure 5. As can be seen, when the pinched segment width was 47 μm, the distance between large and small particle peaks was the greatest. However, as the pinched width increased, both large and small particle peaks shifted toward the channel wall, and the distance became smaller. This can be explained as follows; when the pinched segment width became larger, the relative difference in the positions of large and small particles became smaller, degrading the separation performance.

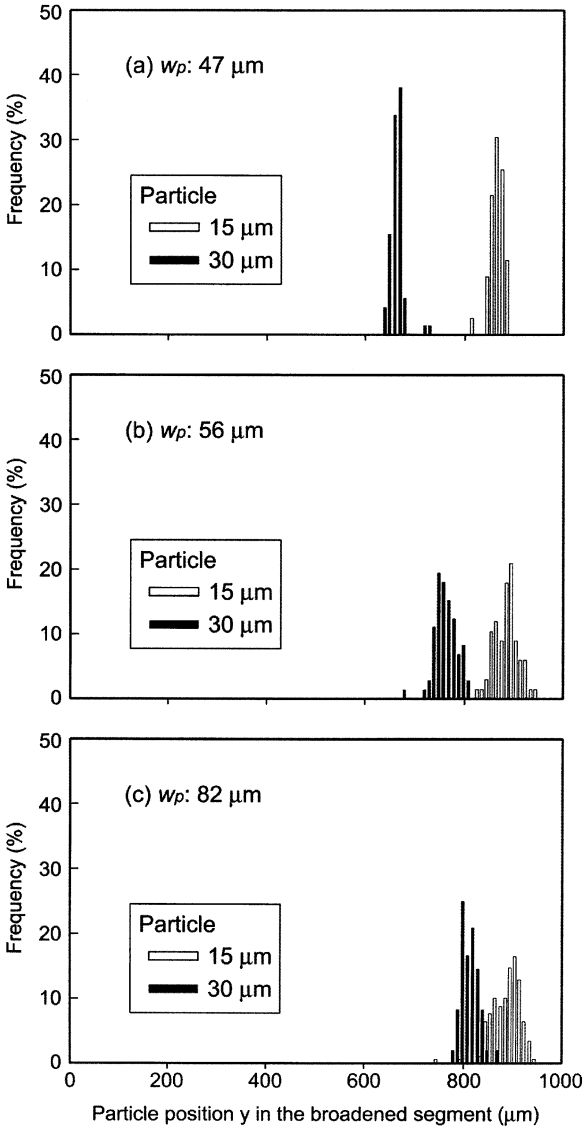

Figure 5. Effect of the pinched segment width w_p on separation performance.

Also, it was observed that better separation performance, i.e., larger distance between both particle peaks, was accompanied with narrower spatial distribution of particle peaks. For an explanation of this phenomenon, imperfection of alignment could be considered. In short, when the relative particle size is large compared to the pinched segment width, particles are aligned to the wall properly. On the other hand, when the relative particle size is small, particle initial position would affect the position in the pinched segment more significantly, and the aligned positions would not become uniform. Therefore, it can be said that the narrower pinched segment is desirable for effective separation, on the condition that the segment is not clogged with particles.

Visualization of Flow Profile. To make a model of the particle movement, and to explain the effect of the pinched segment width more quantitatively, we examined how the flow was amplified in the microchannel. A type 1 microdevice, whose pinched segment width was 47 μm, and which showed the best separation performance in the previous experiment (Figure 5a), was used. Flow speed, ratio of flow rates from both inlets, and solution introduced into the microdevice were the same as those shown in Figure 5,

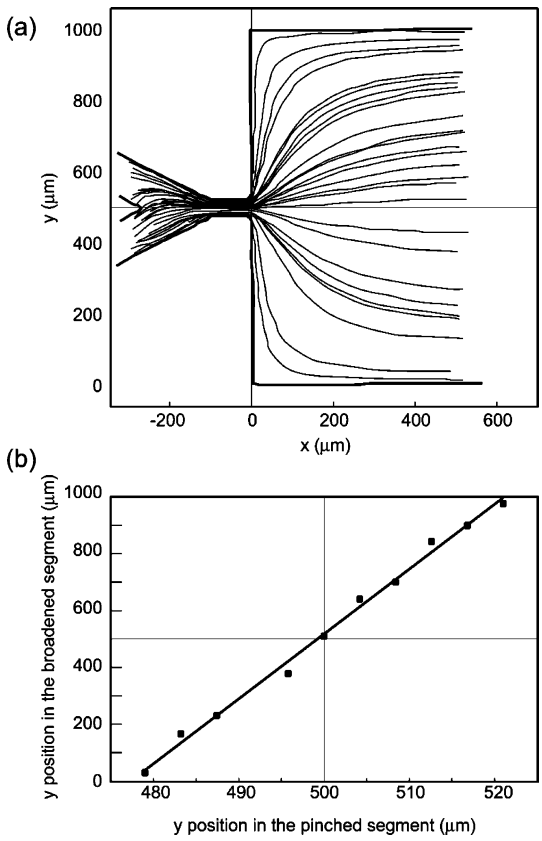

Figure 6. Flow profile in microchannel. (a) is the delineated tracks of microsphere (1.0 μm in diameter) movement, and (b) is the plot of microsphere position y in the broadened segment (x = 500 μm) against that in the pinched segment ($x = -50$ μm).

while both liquid flows contained fluorescent microspheres, whose diameter was 1.0 μm.

The visualized microsphere movement is shown in Figure 6a. Since the microsphere diameter was sufficiently small compared to microchannel scales, it can be said that these lines show the almost actual streamlines. Figure 6b shows the plot of microsphere position y in the broadened segment ($x = 500$ μm) against that in the pinched segment ($x = -50$ μm). From this result, it was revealed that the stream in the pinched segment was almost uniformly and linearly amplified in the broadened segment. Also, it was confirmed that the slope of the fitting line shown in Figure 6b was 22.8, which was nearly the same as the ratio of the pinched and the broadened segment width, 21.3.

Considering this flow amplification, the particle effluent position in the broadened segment can be determined by the relation of the particle diameter and both segment widths. Assuming that the flow width in the pinched segment is uniformly amplified, and neglecting the flow rate distribution, the particle effluent position y in the broadened segment can be expressed simply as follows:

$$y = (w_P - D_P/2)w_B/w_P \quad (1)$$

where w_P and w_B are the widths of the pinched and the broadened segments, respectively, and D_P is the particle diameter. Theoretically, when the particle diameter is equal to the pinched segment width, particles would go along the centerline in the broadened segment.

Table 1. Comparison of Particle Effluent Positions between Measured and Calculated Values (μm)[a]

	15 μm		30 μm	
	measured	calculated	measured	calculated
a	869.6 ± 13.8	840.4	664.4 ± 13.9	680.1
b	890.4 ± 24.2	866.1	764.2 ± 22.7	732.1
c	893.6 ± 28.5	908.5	814.8 ± 17.8	817.1

[a] (a), (b), and (c) correspond to those in Figure 5, respectively.

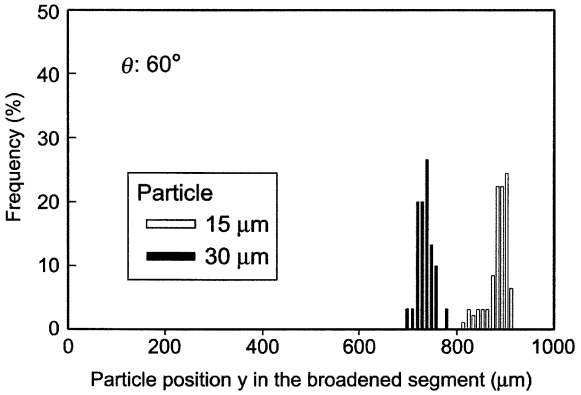

Figure 7. Separation performance when a microchannel with 60° boundary angle was used. The distance of two peaks became smaller compared to that of Figure 5 (a). The effluent positions of particles were 891.6 ± 22.3 and 736.3 ± 16.6 μm, for small (15 μm in diameter) and large (30 μm in diameter) particles, respectively.

Table 1 shows the comparison of particle effluent positions between measured and calculated values. (a), (b), and (c) in Table 1 correspond to those in Figure 5, respectively (w_p for (a), (b), and (c) are 47, 56, and 82 μm, respectively). Comparing the calculated and measured values, it can be said that particle size can be determined by detecting the effluent position. However, there are slight differences between these values, which may be due to the flow rate distribution, and the surface roughness of the microchannel. Flow rate distribution would make the particle position toward center, since the flow rate in the center of a microchannel is faster than that near the channel wall. On the other hand, the slightly rounded end corner of the pinched segment would make the effluent position toward the sidewall, which is an effect similar to the broadening of the pinched segment. For more accurate modeling of particle movement, the influence of these two factors should be taken into consideration.

Effect of the Boundary Angle. Since this separation method utilizes the spreading flow profile at the boundary between the pinched and the broadened segments, the effect of the angle at the boundary between the two channel segments on separation performance was examined. A type 1 microdevice whose boundary angle was 60° was fabricated, and its separation performance was compared to that of the microchannel having a 180° boundary angle. The pinched segment width was also 47 μm, and the flow rates for inlets 1 and 2 were 20 and 120 μL/h, respectively.

The result is shown in Figure 7. When a microchannel having a 60° angle was used, the peak distance between small and large particles became closer as compared to that of a 180° angle microchannel (Figure 5a). It could be considered that when the

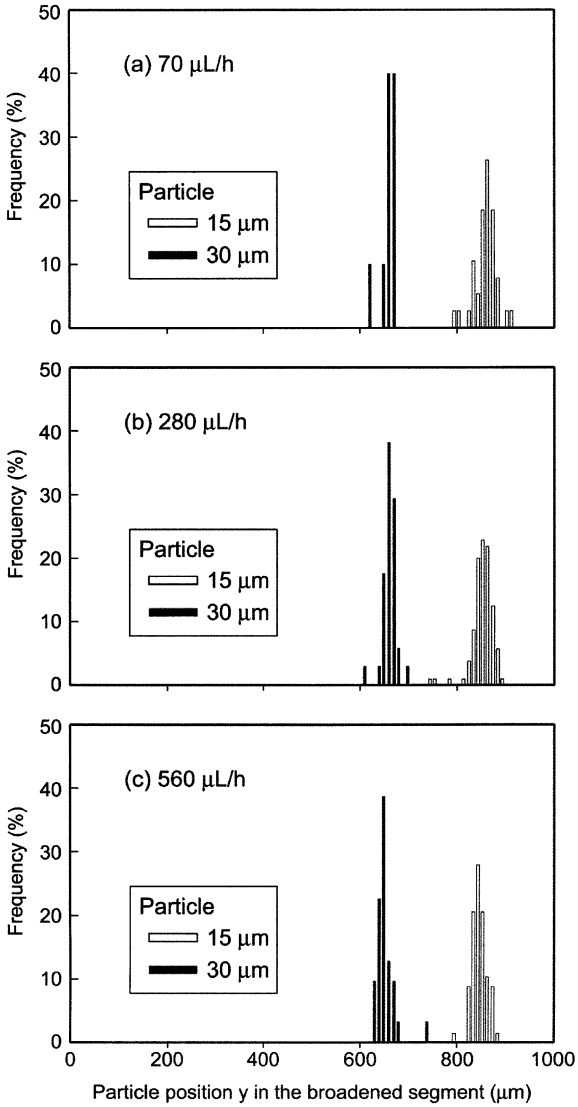

Figure 8. Effect of total flow rate on separation performance. The effluent positions of small particles (15 μm in diameter) were 865.3 $\pm$ 23.8, 858.9 $\pm$ 22.3, and 852.8 $\pm$ 15.8 μm, and those of large particles (30 μm in diameter) were 658.0 $\pm$ 15.4, 661.5 $\pm$ 14.2, and 652.9 $\pm$ 19.9 μm, for (a), (b), and (c), respectively.

boundary angle decreased, the flow profile change at the boundary between the pinched and the broadened segments became gentle. And then, this gentle profile would have an effect similar to that of the broadening of the pinched segment or the dull shape of the boundary corner. Therefore, a steep change in the flow profile was desirable for effective separation.

Effect of the Total Flow Rate. Next, we examined whether the flow rate (flow speed) would affect the separation performance. The total flow rate was changed from 70 to 560 μL/h, while keeping the ratio of the flow rate from inlets 1 and 2 constant (1:6) and keeping the width of the particle-containing solution in the pinched segment constant at 6.7 μm. A type 1 microdevice, whose pinched segment width was 47 μm, and whose boundary angle was 180° was used.

The result is shown in Figure 8 (and in Figure 5a with the flow rate of 140 μL/h). It was observed that effluent positions slightly shifted toward the channel center as the flow rate increased, caused by the inertial force of particle movement. However, the influence of flow rate on separation efficiency was small, even in the highest flow rate condition. This suggests that the flow profiles were almost the same for every flow rate condition. Therefore, it was also suggested that rapid particle separation and analysis could be achieved using this method, since the separation performance would be little affected by flow rate.

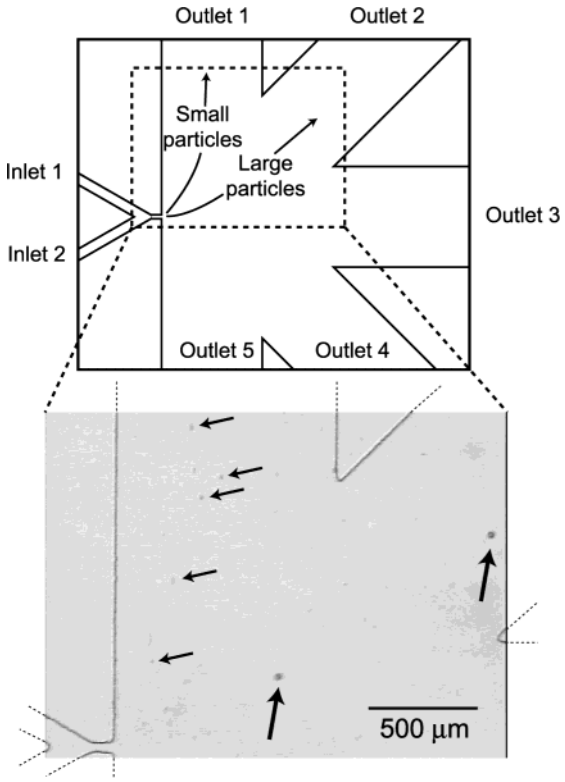

Figure 9. Photograph of separated and collected particles. Particles indicatied by small arrows are 15 μm in diameter, and those indicated by large arrows are 30 μm in diameter (see Supporting Information for a movie of particle collection).

Collection of Separated Particles. Finally, we performed particle collection using the type 2 microdevice shown in Figure 2b, to demonstrate the ability of this separation method for the preparation of uniform-sized particles. The pinched segment width was 50 μm, and the flow rates for inlets 1 and 2 were 50 and 300 μL/h, respectively. The numbers of particles flowing into each outlet were counted.

A photograph of particle separation and collection is given in Figure 9. As can be seen, large particles moved mainly toward outlet 2, while small particles moved toward outlet 1. As measured, 99.0% of small particles went to outlet 1, while the rest went to outlet 2. In the case of large particles, 91.6% went to outlet 2, while the rest went to outlet 3. This imperfection of collection, especially in the case of large particles, may be due to the difference in flow distribution to each outlet, which was caused by the slight difference in pressure losses. However, the possibility of monodispersed particle preparation was successfully shown in this experiment.

CONCLUSIONS

We have developed a new method for the particle sizing, pinched flow fractionation, using microfluidic devices. The results

confirmed that particles of different sizes could be continuously and accurately separated using this method, and particle size could be determined by the effluent position. Also, it was revealed that the geometry of the microchannels influenced separation performance, which corresponded to our theory that the behavior of particles is dominated by the specific flow profile.

This method can easily be applied both for the size analysis and for the size-dependent separation of various kinds of particles, such as cells, drugs, emulsions, gels, and nanometer-sized particles, since the separation principle would not be affected by the nature of particles and fluid and would be valid even when the scale becomes much smaller. Using this method, large-scale treatment will be achieved by arranging the same structures in parallel, while more precise separation can be achieved by connecting microchannels with different shapes in series.

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SUPPORTING INFORMATION AVAILABLE

Addtional information as noted in text (movie, AVI). This material is available free of charge via the Internet at http://pubs.acs.org.

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