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# Increased size-sorting performance in gravitational SPLITT by using a pinched sample inlet design

Split-flow thin fractionation is a continuous, flow-assisted separation technique for sorting macromolecules and particulate matter on a preparative scale. On reducing the thickness of the sample inlet conduit of a gravitational split-flow thin fractionation channel, size-sorting performance is found to increase since particles that are continuously fed into the channel can be more rapidly compressed toward the upper wall of the channel. Experiments are carried out by measuring the number percentage of particles eluted at each outlet as a function of different thickness values of the sample inlet conduit. The effects that the total thickness of the gravitational split-flow thin fractionation channel and the sample feed concentration have on the size-fractionation performance are examined with the goal of determining the best pinched sample inlet, gravitational split-flow thin fractionation channel design.

**Key Words:** SPLITT; Gravitational SPLITT fractionation; Pinched sample inlet; Continuous particle separation

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## 1 Introduction

Split-flow thin fractionation (SPLITT fractionation or SF) refers to a group of flow-assisted separation techniques suitable for rapid and continuous fractionation of macromolecules, colloids, and particulate materials on a preparative scale [1–6]. Unlike field-flow fractionation (FFF), which is based on a batch sample injection and used for analytical or micro-preparative scale applications, SF techniques operate via a continuous feed of sample dispersion, and they thus provide separation of sample components on a preparative scale. In SF, separation takes place in a thin rectangular channel (300–700 μm thick) where flow splitters are located at both ends of the channel [1, 2]. **Figure 1** shows a side view of an SF channel in which the dispersed sample solution is continuously introduced into the SF channel through the sample inlet *a′* with the carrier liquid that is delivered through the inlet *b′*. The two streams (feed stream and carrier stream) merge together right after the inlet splitter and a virtual plane, the inlet splitting plane (ISP) as represented by the dotted line, is formed due to the difference in the flow stream rates at the two inlets. When a suitable form of field is applied perpendicularly to the flow migration, particles will settle across the channel thickness because of differences in their characteristic transport coefficients, and they are simultaneously transported toward the end of the channel by the bulk flow. When the applied field is gravitational (GSF) or centrifugal, a particle transport coefficient

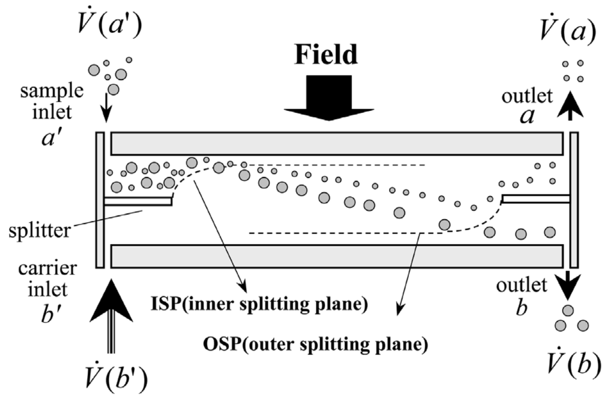


**Figure 1.** Schematic side view of SF channel.

is related to the particle diameter or density [1, 3, 4]. At the outlet splitter, the bulk flow is divided into two parts and an outlet splitting plane (OSP) is formed based on the ratio of the two outgoing flow rates. At the end of the GSF channel, particles are sorted across the channel thickness according to their differences in size (in case of homogeneous density and shape), and they then exit each channel outlet in different proportions according to the flow rate ratio at the GSF channel outlets. In other words, particles are expected to be enriched or depleted in a certain diameter at each GSF channel outlet depending on the channel flow rates at the two outlets. As a consequence, in an ideal GSF experiment fractions collected at outlet *a* and at outlet *b* contain particles which are smaller and larger, respectively, than a certain diameter, the so-called cutoff diameter. The cutoff diameter, $d_c$, is defined as the diameter of particles with a fraction of retrieved particles of 0.5 at each outlet and it is expressed as [3, 7–9]

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$$d_c = \sqrt{\frac{18\,\eta}{bLG\,\Delta\rho}\left\{\dot{V}(a) - \frac{1}{2}\,\dot{V}(a')\right\}} \quad (1)$$

where $\eta$ is the viscosity of carrier fluid, $b$ the channel breadth, $L$ the channel length (distance between the splitters), $G$ the acceleration due to gravity, $\Delta\rho$ the density difference between carrier liquid and particles, $\dot{V}(a')$ the feed flow rate, and $\dot{V}(a)$ the carrier flow rate.

Assessment of performance in particle size-sorting with respect to a certain cutoff diameter is based on the fact that all fed particles could simultaneously start settling from the upper wall of the GSF channel precisely when they leave the inlet splitter. Since the GSF separation axis lies along the channel thickness, for the highest separation efficiency an infinitely thin sample band must be ideally formed at the surface of the upper wall of the channel as the initial sample band in chromatography or FFF should ideally be as narrow as a delta function before the separation begins [10]. In a typical GSF channel, the alignment of fed particles into a thin layer near the upper wall of the channel is, in fact, obtained by applying a high carrier flow rate from the channel inlet $b'$, as illustrated in Figure 1, so that particles are sufficiently pushed toward the upper wall. However, it is impossible to obtain a continuous, extremely narrow starting band since there is, in fact, a finite gap between the upper wall of the GSF channel and the ISP, the extension of which depends on the ratio of the feed rate and of the carrier flow rate. As a consequence, particles of an identical diameter may actually settle from any elevation between the upper wall and the ISP, and difference in the starting points of the settlement may lead to deviations from ideality. This results in co-elution of particles of a certain diameter range at both outlets. In order to reduce the diameter range of co-eluting particles at both SF outlets, it is suggested that a very low feed flow rate, $\dot{V}(a')$, be used or a relatively high carrier flow rate, $\dot{V}(a)$, so that a very thin ISP is initially formed. This can be found from the expression that relates resolution in SF to channel flow rates as [2]

$$\frac{d_{bs}}{\Delta d} = \frac{d_{bs}}{d_{bs} - d_{al}} \cong \frac{2\dot{V}(a)}{\dot{V}(a')} \quad (2)$$

where $d_{bs}$ and $d_{al}$ are defined as the diameter of the smallest particle collected at the outlet $b$ at a retrieval factor of $F_b = 1$, and the diameter of the largest particle fully collected at outlet $a$ at a retrieval factor of $F_a = 1$, respectively given by [1, 10, 11]

$$d_{bs} = \sqrt{\frac{18\,\eta}{bLG\,\Delta\rho}\,\dot{V}(a)} \quad (3)$$

$$d_{al} = \sqrt{\frac{18\,\eta}{bLG\,\Delta\rho}\{\dot{V}(a) - \dot{V}(a')\}} \quad (4)$$

In the above equations, particles with the diameter interval of $d_{al}-d_{bs}$ will exit from both outlets and this size range can not entirely be resolved [4, 11]. Equation (2) also shows that size-sorting power increases when the ratio of $\dot{V}(a)$ to $\dot{V}(a')$ increases. However, utilizing a low feed rate tends to reduce particle throughput. On the other hand, an increase in carrier flow rate increases dilution of sorted samples at the outlet and it also tends to induce deviations from ideality due to the increased effect of hydrodynamic lift forces [14, 15]. Increasing carrier flow rate or decreasing feed rate will eventually lengthen the processing time. In addition, deviations from ideality may arise from the incomplete transportation of particles toward the upper channel wall right after particles leave the inlet splitter. Since particles are hydrodynamically pushed toward the upper wall against gravity, initial compression of particles toward the upper channel wall may not be completed as the channel thickness becomes larger.

In this paper, a simple modification of the GSF channel inlet is described to enhance size-sorting performance by reducing the thickness of the sample inlet conduit of a GSF channel. This modification is analogous to the pinched inlet design already proposed in FFF [14,15]. However, for GSF the sample inlet pathway thickness is here reduced by layering a plastic sheet above the inlet splitter, as shown in Figure 2.a. By reducing the thickness of the sample inlet conduit, particles leaving the inlet splitter can be more efficiently pushed against the upper wall of the GSF channel by the carrier stream. With such a pinched inlet GSF channel, the number of particles leaving away from the ideal trajectory is expected to decrease to some degree. Experiments are carried out by collecting particles at each out let and by measuring the number percentage of silica particles larger than or smaller than cutoff diameter. Size-sorting efficiency tests are performed with the so-designed pinched inlet GSF channels of different inlet and total channel thickness values, and with different feed concentrations.

## 2 Experimental

The GSF channel systems used in this study were built inhouse. Three conventional and three pinched inlet channels were employed. The assembly of the conventional GSF channel is similar to those previously reported [6, 16], while the pinched inlet GSF channel was constructed as shown in **Figure 2**. The pinched inlet channel was made by using multiple layers of Mylar spacers which were cut into different shapes as illustrated in Figure 2.b. A conventional GSF channel design utilizes two identical spacers with both ends cut into triangular end pieces. For a pinched inlet GSF channel, the inlet part of the spacer layered right above the stainless steel splitter as in Figure 2.b was cut to exactly the same shape as the inlet

 

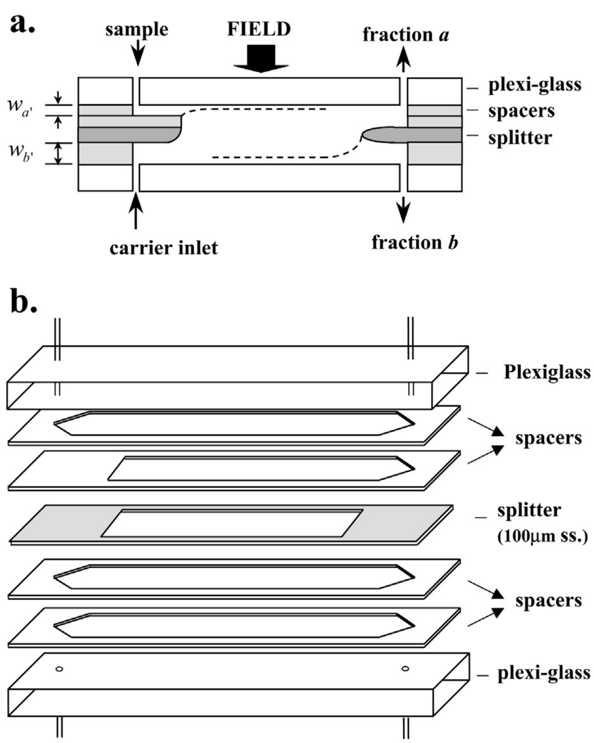


**Figure 2.** Schematics of the pinched inlet GSF channel: a) side view and b) the assembly of a pinched inlet channel.

splitter so that the thickness of sample inlet conduit was decreased but at the outlet end it was shaped as the other spacers. The thicknesses of the sample inlet, $w_{a'}$, and the carrier inlet, $w_{b'}$, were varied by stacking multiple layers of Mylar spacer having a thickness of 50 μm. The channel dimensions are listed in **Table 1**. All channels employed the same 100-μm thick, 4.0-cm wide, 20.0-cm long stainless steel splitter.

The silica particles (0.5–10.0 μm) were purchased from Sigma Aldrich. The density value of the silica particles used in the calculation of the required flow rates to provide a theoretical cutoff diameter of 5.0 μm was 2.65 g/cm$^3$. The carrier liquid used to disperse the silica particles and for the GSF experiments was made up of ultrapure water (>18 MΩ) containing 0.02% $NaN_3$ as bactericide and 0.1% FL-70 from Fisher Scientific (Fairlawn, NJ, USA), which is a mixture of ionic and nonionic surfactants containing 3.0% oleic acid, 3.0% $Na_2CO_3$, 1.8% Tergitol, 1.4% tetrasodium ethylenediamine tetraacetate, and 1.0% polyethylene glycol 400 in water. Silica particles were fed into the GSF channel using a Minipulse3 peristaltic pump from Gilson (Villiers-le-Bel, France) through the inlet *a′*. Feed concentrations were varied from 0.1% to 2.0% (*w/v*). The carrier liquid was delivered to the inlet *b′* by an FMI lab pump from Fluid Metering, Inc. (Oysterbay, NY, USA). For the collection of particles at each outlet and for the simultaneous on-line circulation of the carrier fluid,

**Table 1.** Dimensions of the GSF channels. Same stainless steel splitters (100-μm thick) are employed in all the channels.

| Channel | $w_{a'}$ [μm] | $w_{b'}$ [μm] | $w_{tot}$ [μm] | Type |
|---|---|---|---|---|
| I | 150 | 150 | 400 | conventional |
| II | 100 | 150 | 400 | pinched |
| III | 50 | 150 | 400 | pinched |
| IV | 100 | 100 | 300 | conventional |
| V | 50 | 50 | 200 | conventional |
| VI | 50 | 100 | 300 | pinched |

two PCUUs units (Particle Concentrator with Upstream Ultrafiltration) [6] were used at each outlet. To control flow rates, a fine metering valve from Crawford Fitting Co. (Solon, OH) was placed at the outlet of a PCUU that was directly connected to channel outlet *b*. The collected particle fractions were examined by a model S-4200 Scanning Electron Microscope from Hitachi Ltd. (Tokyo, Japan) and electron micrographs were saved into a PC. A particle count was performed by measuring each particle size from electron micrographs and about 200 particles were counted for each run.

## 3 Results and discussion

The performance of a pinched sample inlet GSF channel was tested by comparing the size-sorting capabilities of a conventional GSF channel with two pinched inlet GSF channels of different inlet thicknesses. Comparison was made by measuring the number percentage of particles at each outlet. **Figure 3** shows electron micrographs of the silica fractions collected at both outlets of channels I–III. Feed concentration of dispersed silica solution was 0.1% (*w/v*). The cutoff diameter for all runs was set at 5.0 μm by adjusting flow rates as $\dot{V}(a') = \dot{V}(b) = 1.0$ mL/min and $\dot{V}(b') = \dot{V}(a) = 11.3$ mL/min. Channel I had conventional design with identical inlet thicknesses, i.e. $w_{a'} = w_{b'} = 150$ μm for both sample and carrier inlet. The particle fraction *a*-I denotes the fraction collected at outlet *a* of channel I. From the micrographs of Figure 3.a, fraction *a*-I, which is expected to contain particles smaller than the cutoff diameter value, appears to be well sorted, while fraction b-I, which is supposed to contain particles larger than the cutoff diameter value, appears to contain a few smaller particles. The number percentage data are determined by counting at least 200 particles for each fraction, and **Table 2** lists the data corresponding to Figure 3. The number percentage for particles smaller than the cutoff diameter ($d_c$) for fraction *a*-I reaches about 95%, while the recovery of particles larger than $d_c$ in fraction *b*-I is about 66.7%. These are typical recovery values that can be expected from a single GSF run using a conventional channel design [6, 16]. When the pinched inlet GSF chan-

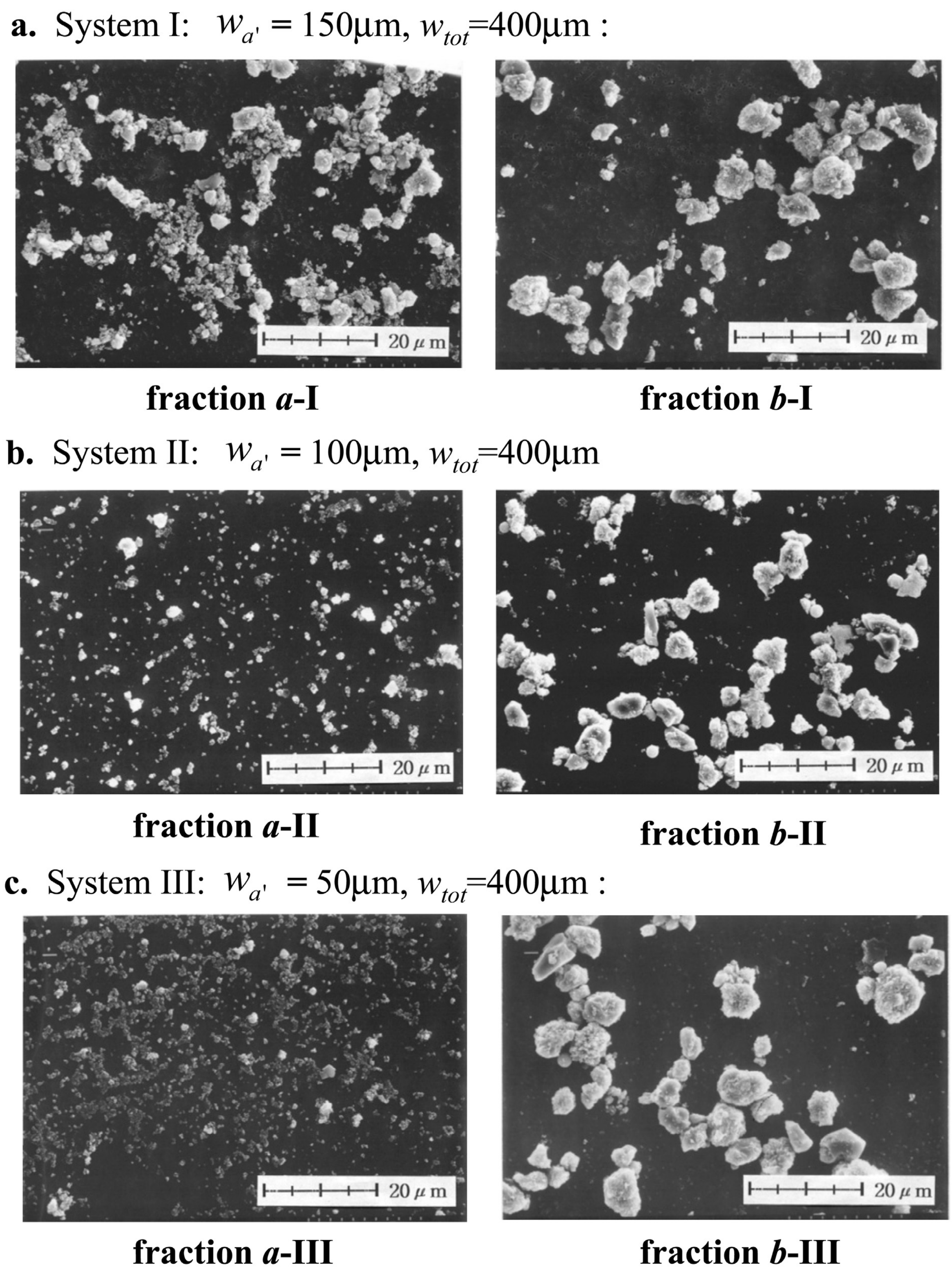


**Figure 3.** Electron micrographs of collected fractions obtained with (channels II, III) or without (channel I) pinched inlet. The total channel thickness ($w_{tot}$) and the sample inlet ($w_{a'}$) values are marked. Flow rates: $\dot{V}(a') = \dot{V}(b)$ = 1.0 mL/min, and $\dot{V}(b') = \dot{V}(a)$ = 11.3 mL/min.

 

**Table 2.** Number percentage values of collected fractions using pinched inlet GSF channels and the conventional SF channel I. Data relevant to experiments in Figure 3.

| $w_{a'}:w_{b'}$ [μm] | System | Fraction *a* | Number % $<d_c(=5.0\ \mu m)$ | Fraction *b* | Number % $>d_c(=5.0\ \mu m)$ |
|---|---|---|---|---|---|
| 150:150 | I | *a*-I | **95.5** | *b*-I | **66.7** |
| 100:150 | II | *a*-II | **98.7** | *b*-II | **70.4** |
| 50:150 | III | *a*-III | **≈99.9** | *b*-III | **83.3** |

**Table 3.** Effect of total channel thickness on number percentage values of particles relevant to Figure 4. Particle counts based on more than 200 for each fraction.

| $w_{tot}$ [μm] | System | Fraction *a* | Number % $<d_c(=5.0\ \mu m)$ | Fraction *b* | Number % $>d_c(=5.0\ \mu m)$ |
|---|---|---|---|---|---|
| 400 | I | *a*-I | **95.5** | *b*-I | **66.7** |
| 300 | IV | *a*-IV | **96.1** | *b*-IV | **81.1** |
| 200 | V | *a*-V | **97.5** | *b*-V | **72.8** |

nel II ($w_{a'}$ = 100 μm) is utilized, the number percentage of fraction *b*-II increases up to 70.4%, which is not, in fact, significantly different from the number percentage of *b*-I. However, when in channel III the inlet thickness is reduced to 50 μm (with total channel thickness maintained constant), the number percentage value increases up to 83.3%. The micrographs of Figure 3.c show that the fraction *b*-III is significantly enriched with more large particles and with fewer small particles. It is noted from Table 2 that a pinched inlet GSF channel can further improve sorting performance as the inlet thickness tends to be reduced. However, it must be also noted that, although the number percentage of fractions collected at outlet *b* can be improved by a pinched inlet design, particle recovery at the outlet *a* is maintained above 95%. This implies that contamination by undersized particles in fraction *b* is more serious than that by large particles in fraction *a*.

For all run conditions in Figure 3, the total channel thickness was fixed at 400 μm. According to SF theory, the plate number is proportional to channel thickness [2]. Therefore, the effect of channel thickness on the separation efficiency has to be examined first. In **Figure 4**, two conventional SF channels of different channel thickness are tested: 300 μm for channel IV and 200 μm for channel V. Both the channels employed splitters made of 100-μm thick stainless steel sheets, and the same channel flow rate was used in the experiments. Electron micrographs of collected fractions at each outlet are shown in Figure 4, and relevant number percentage values are listed in **Table 3**. Recovery values obtained for channel IV and V can be compared with those obtained for channel I. It is shown that number percentage value of fraction *b* significantly increases from 66.7% (fraction *b*-I) up to 81.1% (fraction *b*-IV) when the channel thickness decreases from 400 μm to 300. However, the number percentage decreases down to 72.8% (fraction *b*-V) when channel thickness was further decreased down to 200 μm. The decrease in number percentage observed for the last case is consistent with theory, which states that separation efficiency decreases with decreasing channel thickness [2] Nevertheless, the experiments performed here show that the thickest channel (system I, $w_{tot}$ = 400 μm) has the worst sorting performance. In fact, the low recovery value observed for the fraction *b*-I with the 400 μm-thick channel is likely to be ascribed to deviations from the ideal distribution of sample particles toward the upper wall of the channel at the injection step. One reason for such deviations could lie in the increase of the distance that particles must travel toward the upper wall when particles are initially compressed upward by carrier flow at the end of the inlet splitter. Since linear, bulk flow velocity substantially decreases with increasing channel thickness, particle compression toward the upper wall by a decreased flow velocity will not be strong enough to push them upward Such a decrease in linear, bulk flow velocity in thicker channels could lead to deviations from ideality. This negative effect can be overcome when the linear bulk flow rate is increased. However, an increase of flow rate is always accompanied by dilution of fractionated particles. The observed effects of channel thickness and of pinched inlet modification on fractionation recovery suggest that a pinched inlet GSF channel with $w_{tot}$ = 300 μm is likely to be a good compromise to provide a good fractionation.

With the channel VI, the effect of feed concentration on fractionation recovery was then studied. Sample suspensions were fed into channel VI at four different feed concentrations (0.1, 0.5, 1.0, and 2.0% *w/v*), and the electron micrographs of the collected fractions are shown in **Fig-**

 

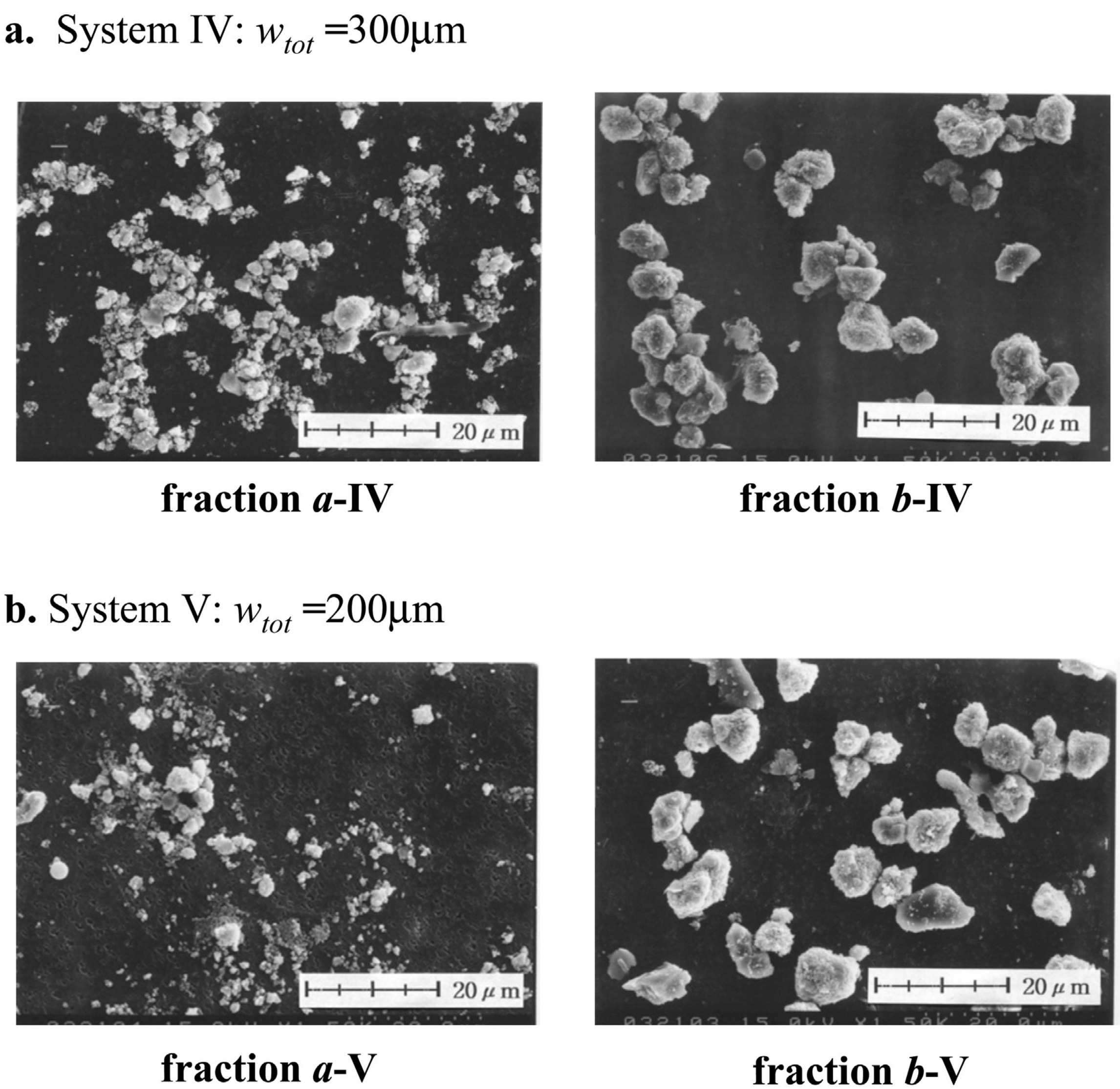


**Figure 4.** Electron micrographs of collected fractions obtained with two conventional GSF channels (channel IV, V): effect of channel thickness on the number percentage values. Flow rate conditions are the same as used in Figure 3.

**Table 4.** Effect of feed concentration on the number percentage values of particles collected at each outlet of the pinched inlet GSF channel VI. Run conditions are the same as those used for Figure 5.

| % [w/v] | Fraction *a* | Number % $<d_c(= 5.0\ \mu m)$ | Fraction *b* | Number % $>d_c(= 5.0\ \mu m)$ |
|---|---|---|---|---|
| 0.1 | *a*-0.1 | **98.5** | *b*-0.1 | **90.1** |
| 0.5 | *a*-0.5 | **98.1** | *b*-0.5 | **82.4** |
| 1.0 | *a*-1.0 | **98.6** | *b*-1.0 | **71.7** |
| 2.0 | *a*-2.0 | **95.9** | *b*-2.0 | **65.7** |

**ure 5**. Flow rate conditions were the same as utilized for experiments shown in Figure 4. According to earlier reports [3, 6], a lower feed concentration provides better particle sorting. The relevant recovery value are listed in **Table 4**. It can be therein observed that, as the feed concentration decreases, recovery values for fraction *b* significantly increase. In the case of the lowest feed concentration (0.1%), the recovery value can reach up to 90.1%,

 

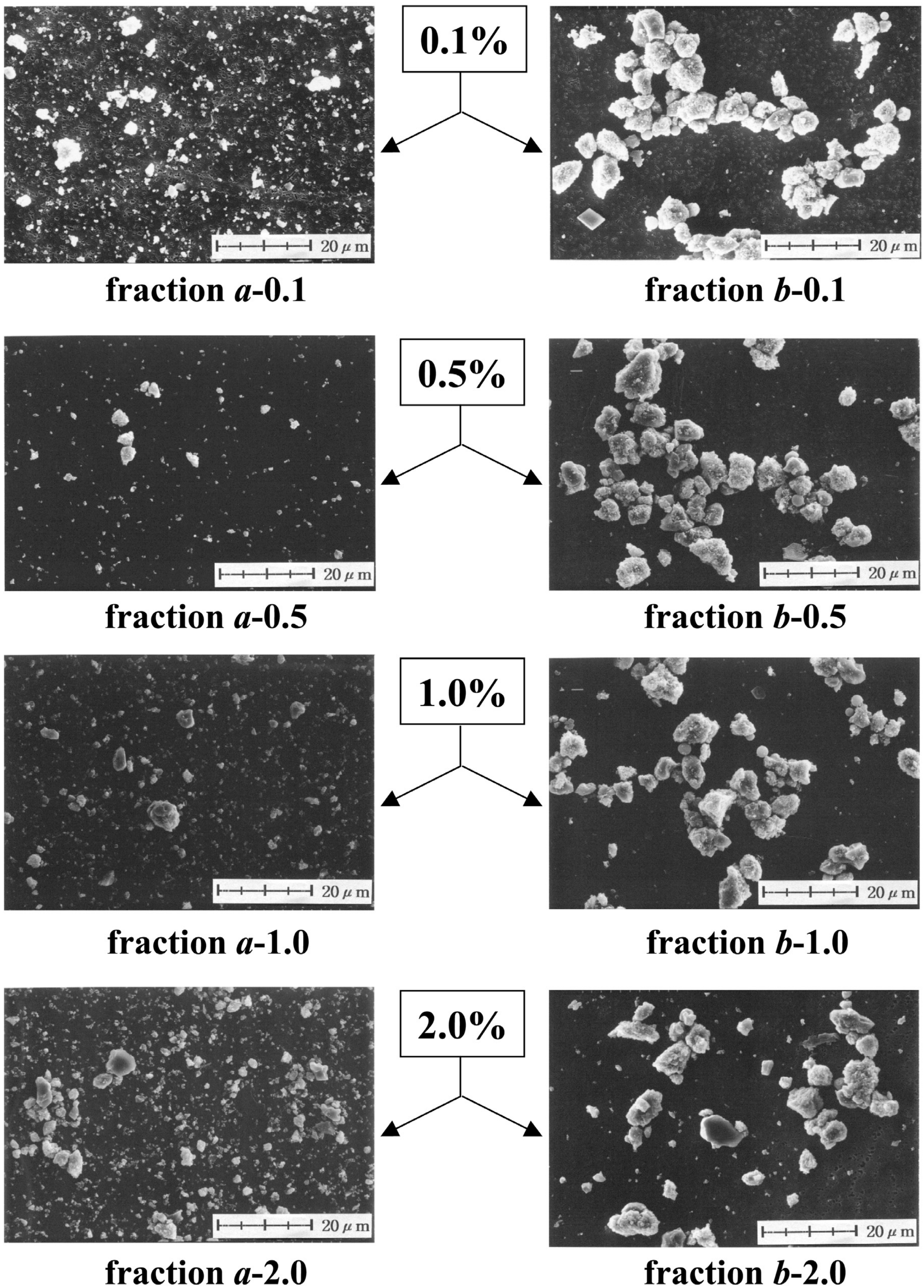


**Figure 5.** Electron micrographs of fractions collected at different feed concentrations using the pinched inlet GSF channel VI. Flow rate conditions are the same used in Figure 4.

 

which is higher than the value obtained with the pinched inlet GSF channel III (83.3% for the fraction *b*-III). Channels VI and III have, in fact, same inlet thickness, $w_{a'}$, but they differ in the total channel thicknesses, $w_{tot}$. It is thus noteworthy that sorting performance of channel VI ($w_{tot}$ = 300 μm) still proves to be higher than that of the thicker channel III ($w_{tot}$ = 400 μm), as in the corresponding cases of conventional GSF channels. Since both pinched inlet GSF channels III and VI have identical sample inlet thickness ($w_{a'}$ = 50 μm), the thicker channel (channel III) could give rise to lower recovery values because the decrease in linear flow velocity with respect to the thinner channel (channel VI) would lower the initial compression of sample particles toward the upper wall of the channel. Otherwise, at a feed concentration of 0.5%, the number percentage value for the fraction *b*-0.5 increases up to 82.4%, which is similar to the value obtained with the conventional channel IV at a feed concentration of 0.1% (81.1% for the fraction *b*-IV). This implies that the pinched inlet modification for channels of $w_{tot}$ = 300 μm allows for an increase in feed concentration to some degree, which can be alternatively used to increase either the sample throughput or increasing sorting capabilities of a single split-flow fractionation experiment.

In conclusion, this work suggests that the pinched sample inlet modification in a GSF channel can be useful to increase the fractionation efficiency. Compared to the conventional methods to enhance fractionation performance, such as multiple-run GSF or the use of a low feed rate [6], a pinched sample inlet GSF channel can increase number percentage values without lengthening total sorting time. In addition, it is found that number percentage values decrease with increasing channel thickness, probably because of the relevant decrease in the linear bulk flow velocity. This effect is known to depend on particle mass or density. As a consequence, deviations from ideal particle trajectories may increase when GSF experiments are set for larger cutoff diameter values.

In this work, sample particles much smaller in size than the inlet thickness, $w_{a'}$, were employed. When particles have a size of more than half the inlet thickness, particle transportation within the GSF sample inlet conduit may not be easily accomplished. As a consequence, the design of the pinched inlet GSF channel should be properly selected according to the size of particles to be sorted.

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