Field-Flow Fractionation and Hydrodynamic Chromatography on a Microfluidic Chip

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ABSTRACT: We present gravitational field-flow fractionation and hydrodynamic chromatography of colloids eluting through 18 μm microchannels. Using video microscopy and mesoscopic simulations, we investigate the average retention ratio of colloids with both a large specific weight and neutral buoyancy. We consider the entire range of colloid sizes, including particles that barely fit in the microchannel and nanoscopic particles. Ideal theory predicts four operational modes, from hydrodynamic chromatography to Faxén-mode field-flow fractionation. We experimentally demonstrate, for the first time, the existence of the Faxén-mode field-flow fractionation and the transition from hydrodynamic chromatography to normal-mode field-flow fractionation. Furthermore, video microscopy and simulations show that the retention ratios are largely reduced above the steric-inversion point, causing the variation of the retention ratio in the steric- and Faxén-mode regimes to be suppressed due to increased drag. We demonstrate that theory can accurately predict retention ratios if hydrodynamic interactions with the microchannel walls (wall drag) are added to the ideal theory. Rather than limiting the applicability, these effects allow the microfluidic channel size to be tuned to ensure high selectivity. Our findings indicate that particle velocimetry methods must account for the wall-induced lag when determining flow rates in highly confining systems.

Field-flow fractionation (FFF), a broad class of separation techniques, is achieved by imposing a transverse force $f$ across a channel of height $h$ to a solution of solutes such as colloids, macromolecules, or cells. A concentration gradient is established in response to the competition between the thermal energy, $k_B T$, and the potential energy drop across the channel, $f h$. The system can thus be described in terms of the dimensionless retention parameter, $\lambda = k_B T/f h$. This solute distribution is carried through a channel by a Poiseuille flow with a characteristic retention ratio (average colloid velocity normalized by average fluid velocity, $R = \langle V \rangle/\langle u \rangle$). Colloids of different sizes $r$ have different concentration distributions and therefore different retention ratios, $R$.

This simple system possesses surprisingly rich elution behavior. Previously, we considered the ideal retention theory (ignoring complications such as nonparabolic flow, nondilute concentration effects, slip, or hydrodynamic/lift interactions), and predicted the existence of four distinct operational modes. The transitions between them were mapped; however, only three of the four modes and only a single transition had ever been previously observed experimentally.

For large $\lambda$, thermal energy dominates and solutes diffuse across the entire channel. Only steric interactions with the walls limit their distribution, as shown in Figure 1. This is the hydrodynamic chromatography limit of FFF (HC), where larger particles elute before smaller particles. Ideally, density-matched tracer particles used for micro-particle image velocimetry elute in HC, in the absence of any net external body forces.

If the potential energy dominates over the thermal energy, the concentration profile across the channel height becomes exponential. In this normal-mode field-flow fractionation (nFFF), ensembles of small solutes can loosely be thought of as point particles subject to an external force that increases with particle size $r$ (nFFF in Figure 1). Therefore, larger particles stay close to the accumulation wall where the lower flow velocity causes them to move more slowly than smaller particles. Experimental observation of the transition between HC and nFFF has not previously been reported.

However, nFFF can only continue to exist for a certain size range before the size dependence changes once again. Larger (heavier) particles are pushed against the wall and steric interactions exclude the particles from sampling the slow flow rates in highly confining systems.

Figure 1. Schematic of ideal field-flow fractionation operational modes.

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moving velocity so larger particles elute faster in this steric-mode field-flow fractionation (sFFF), as seen in Figure 1.\textsuperscript{13}

At the largest sizes, a significant portion of each particle’s cross section is subject to varying fluid velocities. Ideally, even large particles in flow profiles with no curvature, such as Couette flow, would possess the same velocity as the fluid at the particles’ centers of mass. However, when the flow profile possesses curvature and the particles’ size is comparable to that curvature, such as large particles in Poiseuille flows, then the velocities can differ significantly from the velocity of the fluid at the particles’ centers of mass. In such situations, a correction to the Stokes’ law is required that causes larger particles to elute more slowly than they would otherwise. Faxén’s law describes this phenomenon. We have recently considered Faxén’s law in the context of FFF and predicted the existence of the Faxe-mode field-flow fractionation (fFFF),\textsuperscript{10} but this new separation mode remains unverified by experiments or simulations. In this work, we experimentally and computationally demonstrate the existence of fFFF but show that Faxén’s law is relatively negligible at all particle sizes. We show that this is because increased hydrodynamic friction due to interactions with the channel walls play a more significant role at large particle-to-channel height ratios. We show that this is particularly important in microfluidic systems.

In field-flow fractionation, an external force is applied perpendicular to the flow direction to create a solute concentration distribution. In a general sense, the external force scales with normalized particle size like \( \frac{f}{r^3} \), where \( r = \frac{r}{h} \) and the power \( \alpha \) differs for different types of FFF external fields. For weight-based subtechniques, \( \alpha = 3 \), whereas \( \alpha = 1 \) for flow- or thermal-based FFF. Therefore, we can write \( \lambda = \Lambda r^{-\alpha} \) where the device retention parameter \( \Lambda \) characterizes the FFF apparatus.\textsuperscript{10} In the case of gravitational field-flow fractionation (GrFFF), \( \Lambda = \frac{3k_BT}{4\pi\rho_0D\eta^3} \) results from gravitational acceleration, \( G \), acting on colloids with a density difference \( \Delta \rho \) between the carrier fluid and particles. We have previously predicted that all four ideal operational modes can be described by a single unified, ideal retention theory formula,\textsuperscript{10} namely

\[
R(\hat{r}, \Lambda) = \frac{6\Lambda}{\hat{r}^3} \left[ 1 - 2\hat{r}^2 \right] \left[ 1 - \frac{2\hat{r}^2}{\Lambda} \right] + \mathcal{F}(\hat{r})
\]

In eq 1, the Langevin function is given by \( \mathcal{L}(x) = \coth(x) - \frac{1}{x} \) and the second term is

\[
\mathcal{F}(\hat{r}) = \begin{cases} 
6\hat{r}(1 - \hat{r}) & \text{if Faxén's law is neglected} \\
6\hat{r}(1 - \frac{4}{3}\hat{r}) & \text{if Faxén's law is accounted for}
\end{cases}
\]

As mentioned, such an ideal theory neglects complications to the flow profile (such as nonparabolic flow\textsuperscript{14} or slip\textsuperscript{15}) and to the mobility of the eluting particles (such as nonspherical particles\textsuperscript{14}). Paramount among these, it does not take into account hydrodynamic interactions that lead to wall-induced increased drag (wall drag).

Experiments utilizing FFF can only avoid ambiguity in determining solute size due to the nonmonotonicity of eq 1, by working in only one of the four modes (usually nFFF for its high selectivity). So far, the only mode transition observed experimentally is the steric-inversion point between nFFF and sFFF.\textsuperscript{15–21} To highlight the validity of our theoretical treatment (eq 1), the results of five different nonmicrofluidic subtechniques of FFF (thermal FFF,\textsuperscript{15} symmetric-flow FFF,\textsuperscript{16} asymmetric-flow FFF,\textsuperscript{17} hollow-fiber FFF,\textsuperscript{18} and GrFFF\textsuperscript{19}) around the steric-inversion point are shown in Figure 2. A least-squares fitting procedure that varies the device retention parameter and the void time were used to determine the best fits shown in Figure 2. Even for multiple scalings as shown (\( \alpha = 1 \) and 3), in all conditions, the ideal retention theory fits the transition from nFFF to sFFF quite well regardless of Faxén’s law: Dotted lines neglect Faxén’s law, while dashed lines account for it. In this range, the dotted and dashed lines overlap perfectly. Thermal FFF utilizes a temperature gradient and \( \alpha = 1 \). The data is for \( \Delta T = 40 \text{ K} \) (*), \( \Delta T = 5 \text{ K} \) (■), Symmetric (▲), asymmetric (■), and hollow-fiber (★) FFF use a cross flow and have \( \alpha = 1 \). For GrFFF, \( \alpha = 3 \) (●). Data reproduced with permission from ref 15. Copyright 2004 Elsevier BV.

![Figure 2](https://example.com/figure2.png)

Figure 2. Fit of eq 1 to a variety of FFF systems. Dotted lines ignore Faxén’s law, while dashed lines account for it. In this range, the dotted and dashed lines overlap perfectly. Thermal FFF utilizes a temperature gradient and \( \alpha = 1 \). The data is for \( \Delta T = 40 \text{ K} \) (*), \( \Delta T = 5 \text{ K} \) (■), Symmetric (▲), asymmetric (■), and hollow-fiber (★) FFF use a cross flow and have \( \alpha = 1 \). For GrFFF, \( \alpha = 3 \) (●). Data reproduced with permission from ref 15. Copyright 2004 Elsevier BV.

The ideal retention theory fits the data in Figure 2 well because the particles are small relative to the channel height (\( \hat{r} < 0.025 \), in all cases). When the particle sizes are larger, the theories differ greatly for the different forms of \( \mathcal{F}(\hat{r}) \) in eq 2.

The theoretically predicted retention curves for \( \alpha = 3 \) and \( \Lambda = 1.6 \times 10^{-3} \) (strong field) and \( \Lambda = 10^{-3} \) (weak field) across the entire range of possible colloid sizes are shown in Figure 3 and Figure 4, respectively. Such theoretical curves have been discussed in detail\textsuperscript{10} and only a few important features must be noted: (1) When Faxén’s law is neglected (dotted lines), the sFFF regime extends to particles with a diameter equal to the channel height and \( R(\hat{r} \to 0.5, \Lambda) \to 1.5 \). However, when Faxén’s law is properly included (dashed lines), FFFF appears, causing the retention ratio to peak and then slowly decrease to \( R(\hat{r} \to 0.5, \Lambda) = 1 \). (2) All four FFFF modes are expected for \( \Lambda = 1.6 \times 10^{-3} \), but when \( \Lambda = 10^{-3} \), only HC and fFFF are predicted. The ideal theory for \( \Lambda = 1.6 \times 10^{-3} \) (dashed line in Figure 3) predicts that HC exists in the range of \( 0 < \hat{r} < 0.023 \). After reaching a maximum, the slope of \( R(\hat{r}, \Lambda) \) then changes to the range of \( 0.023 < \hat{r} < 0.083 \). This represents nFFF, which exists until the steric-inversion point
Figure 3. Experimentally determined retention ratios in a channel of height $h = 18 \pm 1 \mu m$ in a gravitational field ($\alpha = 3$) are presented as closed circles. Density difference between colloids and the carrier fluid is $\Delta \rho = 0.063 g cm^{-3}$. This density difference is equivalent to a device retention parameter of $\Lambda = 1.6 \times 10^{-5}$. Error bars represent the statistical uncertainty on the mean, while the shaded area (bracketed by purple dashed—dotted lines) shows the uncertainty on the experimental parameters about the theoretical prediction. Data points possess multiple colored error bars associated with each pair of eluted colloids. The dotted line shows the ideal retention ratio when Faxen’s law is neglected. The dashed line includes Faxen’s law. The retention ratio that numerically accounts for increased drag coefficients (and Faxen’s law) \cite{22} is shown as a solid line.

Figure 4. Same as Figure 3 but for a small density difference between colloids and the carrier fluid of $\Delta \rho = 0.003 g cm^{-3}$ or equivalently a device retention parameter of $\Lambda = 10^{-3}$.

EXPERIMENTAL SECTION

Experimental Methods. In order to explore the less accessible modes of field flow fractionation, our system must elute ensembles of colloids that are significantly smaller than $h$, as well as others with diameters comparable to $h$. A microfluidic apparatus allows us to investigate the entire range of relative particle sizes. Miniaturized FFF systems have been used previously, \cite{25–28} but never with such a relatively broad range of particle radii or such small channel heights. A section of our microfluidic channel and two eluting colloids can be seen in Figure 5.

The benefits of performing FFF on a microfluidic chip (especially thermal FFF) have been well-documented, \cite{28} and it has been suggested that microfluidic channels may help in purifying, characterizing, and generally processing nanomaterials. \cite{27, 28} This work utilizes microfluidic devices to study the elution of particles that encompass the full range of sizes; however, we can envision FFF in future nanofluidic systems. \cite{29, 30} Current systems commonly utilize electrokinetic \cite{31} or confinement-based techniques, \cite{32} which have been particularly successful in studying DNA, \cite{33–36} but nanofluidic HC \cite{37–40} and electric double layer methods \cite{41–45} have also proven viable. In such systems, one expects nonideal effects to be even more pronounced. Indeed the importance of increased hindrance has long been recognized in the nanofiltration literature. \cite{46–48} As we shall show, although they do not limit the applicability of microfluidic or nanofluidic technology to FFF, nonideal effects of hydrodynamic interactions with channel...
our microfluidic devices consisted of 40 mm long polydimethylsiloxane (PDMS) elastomer (Sylgrad 184 from Dow Corning) channels fabricated using soft lithography based on replica molding. The PDMS was replicated from a master mold (SU8-10 MicroChem), created on silicon wafers. Each silicon wafer was cleaned using piranha etch then baked at 200 °C for 30 min and let cool for 5 min. Spin speed and time were chosen based on final thickess requirements. The wafer was then prebaked at 65 °C for 2 min followed by 95 °C for 6 min and allowed to cool at least 5 min. It was then exposed to UV, with the maximum intensity of 16.18 mW cm$^{-2}$ for 15 s and postbaked at 65 °C for 1 min and 95 °C for 2 min. Finally, SU-8 developer removed the photoresist. All wafers were coated with aminosilane to facilitate PDMS removal.

The PDMS with curing agent (10:1 mixing ratio) was poured on the master mold and baked at 80 °C for 2 h. The cured PDMS was peeled off the mold to create the microchannel structures. They were cut out, inlet holes were punched, and devices were rinsed for 5 min in each of these solutions; soapy water, deionized water, ethanol, and isopropanol and treated in oxygen plasma (Glow Research AutoGlow) at 30 W for 30 s. All wafers were coated with aminosilane to facilitate PDMS removal.

Pressure-driven flow was established through the channel, using pressure regulators (Marsh Bellofram Type 10) and Solenoid valves (SMC S070C-SDG-32), which were controlled by LabView (National Instruments). Experimental data was only recorded once the flow rate stabilized. Stabilization against pressure drift was verified by checking that the difference in mean velocities of a randomly selected sample of particles from the first quarter of a video set and sample from the final quarter were significantly smaller than the standard deviation within either set (typically differences varied by about 2% and were only a tenth of the standard deviation of either set).

Polystyrene microparticles with nominal radii of $r = \{0.100 \pm 0.005; 0.485 \pm 0.005; 1.9 \pm 0.1; 3.0 \pm 0.2; 5.0 \pm 0.4; 7.8 \pm 0.8\}$μm and density of $\rho_p = 1.060 \pm 0.002$ g cm$^{-3}$ (Bangs Laboratories, Inc.) in 1% suspensions were diluted with the base carrier (SMP Laboratories, Inc.) in 1% suspensions to match the carrier density ($\rho = (1.6 \pm 3$). All experiments were conducted at room temperature (25 ± 1 °C). In the base carrier fluid, $\Lambda = (1.6 \pm 0.4) \times 10^{-3}$. When the particles are neutrally buoyant in the glycerol solution, $\Lambda = (3 \pm 2) \times 10^{-3}$. Experiments were performed using pairs of colloids in order to minimize uncertainty related to variability in carrier fluid velocity between experiments, and relative retention ratios were measured. The movement of colloids through the channels was captured using video microscopy, and particle velocities were obtained using custom analysis software. A brief series of representative frames are shown in Figure 5.

A tracking program identified individual particles and generated velocity distribution profiles for each particle size based on the Fourier transform of the position versus time data. Both programs, for capturing and tracking, were controlled by Labview (National Instruments). Between 150 and 200 events were considered in determining each data point. The smallest particles ($r = 0.1$ μm) were analyzed semianually due to automatic tracking difficulties. In this case, captured videos were analyzed frame by frame, by ImageJ (Image Processing and Analysis in Java), and the mean velocity was calculated from the change in position along the channel versus time (Figure 5).

Error bars in Figures 3 and 4 represent the statistical uncertainty on the mean, while the shaded regions represent the uncertainty associated with the experimental parameters about the numerically determined prediction (which will be introduced in Results and Discussion). Because experimental runs were performed using pairs of coeluting colloids, each data point (except the smallest and largest particles) has two error bars representing the statistical uncertainty for each run. The error bar colors identify pairs in Figures 3 and 4.

The aspect ratio, width to height, of the channel is approximately 5:1. Although theory and simulations assume an infinite aspect ratio, as can be seen in Figure 5, the expected flow profile of the carrier fluid in a rectangular microchannel only differs from the Poiseuille flow profile near the lateral walls. Therefore, the instances for which colloids were recorded within 5 μm of the lateral walls were disregarded, in case they interacted with the lateral walls. This near-lateral wall region is marked in Figure 5 by dashed lines. If the entire rectangular channel were considered, the percent difference between the average carrier fluid velocity and the Poiseuille flow assumption would be 11.6%. By disregarding flow within 5 μm of each lateral wall, the percent difference is reduced to nearly half and 84.3% of all points on the cross section of interest are within 10% of the Poiseuille flow profile of the theory and simulations. Increasing the size of this region beyond 5 μm would require more events to be disregarded, resulting in a reduced statistical certainty.

**Computational Methods.** We have performed simulations of spherical colloids embedded in a coarse-grained multiparticle collision dynamics (MPCD) fluid.\textsuperscript{53,54} MPCD is ideal for studying FFF in microfluidic devices because both hydrodynamic interactions and thermal motion are intrinsically present (i.e., MPCD is well-suited for situations involving moderate Péclet numbers\textsuperscript{55}). A nonphysical, multiparticle collision operator, which locally conserves mass, momentum, and energy, replaces a detailed description of the interactions between fluid molecules. This operator ensures a Maxwell–Boltzmann velocity distribution and that the hydrodynamic equations of motion are obeyed over sufficiently long lengths and times.\textsuperscript{56}

Multiparticle collision dynamics simulations integrate the motion of fluid particles in two steps. The position $\vec{x}(t)$ of each MPCD fluid particle $i$ is updated ballistically over a time step $\delta t$ to become $\vec{x}(t + \delta t) = \vec{x}(t) + \vec{v}(t)\delta t$. Second, momentum is transferred between MPCD fluid particles through a multiparticle collision. The system is partitioned into cubic cells of volume $a^3 = 1$, with each cell $j$ containing on average $(n_j) = \rho_{\text{MPCD}} a^3 = 5$ particles. Each cell has a center of mass velocity $\vec{v}_j(t)$ corresponding to the local mesoscopic fluid velocity.
(each MPCD fluid particle has a mass $m$). To ensure Galilean invariance is respected, a random vector shifts the cells each time step. $^{77,58}$ The collision operator implements an Andersen thermostat such that the iterative scheme is

$$\vec{v}_i(t + \delta t) = \vec{g}\delta t + \vec{v}_{CM}(t) + \vec{v}_{\text{RAN}} - \frac{1}{n} \sum_{j \neq i} \vec{v}_{\text{RAN}}$$  

(3)

where $\vec{v}_{\text{RAN}}$ is a randomly generated velocity drawn from a Maxwell–Boltzmann distribution and $g$ is a constant acceleration to generate the flow profile. $^{59}$ Simple bounce-back rules govern collisions between MPCD particles and walls, although phantom fluid particles must be included in the collision operator of cells intersecting walls to ensure that artificial viscous thinning does not occur and that the no-slip boundary conditions are respected. $^{60,61}$ The system is periodic in $\hat{x}$ and $\hat{z}$ with the mean fluid flow in the $\hat{x}$ direction. Planar, channel walls are in the $\pm \hat{z}$ direction.

The hard bead, representing the colloid, is a spherical wall, which also obeys bounce-back rules to create steric interactions with the channel walls. The colloids of density $\rho = (1000m)/(\pi a^3)$ are subject to an external force in the $\hat{y}$ direction to simulate gravitational acceleration $g\hat{y}$. In order to efficiently simulate each operational mode, the box size is varied, requiring that $g\hat{y}$ and $g\hat{x}$ are varied to keep $\Lambda$ strictly constant and $R_p$ roughly constant. In all cases, the length of the system varies as $10 \times r$ and the breadth as $5 \times r$. Four channel heights, $h = \{210, 90, 26, 15\}$ are used with the four device retention parameters $\Lambda = \{\infty, 1.87 \times 10^{-3}, 1.87 \times 10^{-5}, 1.87 \times 10^{-7}\}$. Because the channel height changes, the fluid acceleration must change in order to keep $R_p$ small and roughly constant. For each channel height, we use the fluid accelerations $g = 10^{-3}, 5.44 \times 10^{-4}, 6.52 \times 10^{-3}, 1.96 \times 10^{-2}$, respectively.

However, we found that for the strongest forces used the bead could become pinned to the wall with no fluid between the particle surface and the plane. When this occurs, the bead did not translate because bounce-back conditions ensure a no-slip boundary condition between the two surfaces. This unphysical situation is due to the discrete particle nature of MPCD and demonstrates a limitation of the method. For this reason, large beads in strong fields that had a null average velocity were rejected from the analysis.

## RESULTS AND DISCUSSION

### Experimental Results.

The experimentally determined retention ratios for colloids with $\Delta \rho = 0.063$ g cm$^{-3}$ are shown as circles in Figure 3. Since the absolute average carrier velocity is irrelevant and only relative changes in retention ratio are significant, the experimental values between size pairs were normalized. To compare these data to the theoretical predictions, we chose one data point and normalized it to the theoretical value. In this case, $R(\tau = 7.8 \mu m, \Delta \rho = 0.063$ g cm$^{-3}$ = 0.622) was chosen in order to produce the minimum $\chi^2$. In scaling the retention curve in this way, the retention ratio values for $\tau = 0.0056$ and $\tau = 0.0269$ appear to be less than expected. The measured velocity difference between $\tau = 0.0269$ and $\tau = 0.106$ is likely the most indefinite value because the fastest of the smaller particles may go unrecorded due to the experimental challenges in tracking small, fast colloids. If the measured difference is systematically less than the actual difference, then the $\tau = 0.0056$ retention ratio will be artificially shifted downward.

The data in Figure 3 is quite different from the ideal theory, using either $f(\tau)$ from eq 2 (dotted and dashed lines), especially in the case of large colloids. The experimentally observed retention ratio initially rises for HC then drops rapidly for nFFF. This is the first published observation of the theoretically expected transition between HC and nFFF. The minimum occurs at a larger particle size compared to the expectations of ideal theory. Past nFFF and the steric-inversion point, the retention ratio is significantly less than predicted by eq 1 (dashed lines). In fact, the retention ratio remains relatively constant such that it is almost impossible to discern sFFF, and fFFF is not experimentally observed.

As shown in Figure 4, when $\Delta \rho = 0.003$ g cm$^{-3}$ and the transverse field is all but removed, the retention behavior is much different. In this weak-field case, the values of the experimental retention ratio (squares) are set by normalizing the $\tau = 0.2761$ value to $R = 0.800$. Without a strong transverse field, neither nFFF nor sFFF were predicted to exist, and this is indeed what is qualitatively observed in Figure 4. In fact, the retention ratio rises slightly to the global maximum at $\tau \approx 0.1075$ through the HC regime and slowly decreases, thus qualitatively verifying the previously predicted FFFF operational mode. However, the ideal, theoretical curves (dashed lines) fail to predict the measured retention ratios in any quantitative sense. Just as in Figure 3, at all but the smallest particle sizes, eq 1 severely overpredicts the retention ratio.

Interestingly, eq 1 represents the literature data for FFF channels with larger channel heights extremely well, as we saw in Figure 2, but fails for large colloids eluting through microfluidic channels. This inadequacy arises because hydrodynamic effects that are neglected in eq 1 must be considered when the channel heights and particle diameters are comparable. Accounting for wall-drag will be considered in detail in the Discussion and Interpretation of Results.

Obviously, microfluidic systems include further nonideal factors that can complicate the retention behavior, such as wall roughness or sticking events, the finite width of the channel, varying flow rates, nonspherical or nonmonodisperse particles, and variation in PDMS channel heights. Therefore, at this point we cannot be positively certain that the observed suppression of the retention ratio is definitively due to hydrodynamically mediated colloid–wall interactions. To corroborate our experimental findings, we now consider our mesoscopic simulations that quite naturally encompass complications due to the confinement of mobile colloids in a flowing, thermal fluid but yet are ideal in the sense that they do not possess the experimental issues mentioned above.

### Computational Results.

Simulations of neutrally buoyant colloids ($\Lambda = \infty$, blue circles in Figure 6) demonstrate that the retention ratio is indeed greater than unity for the vast majority of particles (i.e., $R > 1$ in the hydrodynamic chromatography limit). Simulations show two regions: for small particles, $R$ increases, while for large particles, $R$ decreases. MPCD simulations agree with experimental findings and substantiate the existence of fFFF in the weak fields. Furthermore, simulations of elution in weak external fields ($\Lambda = 1.87 \times 10^{-4}$, black squares in Figure 6) corroborate the experimental observation of the transition from HC to nFFF.

When the field is nonnegligible, the retention curves possess an nFFF region of steep descent after a brief HC climb. The simulation data for both $\Lambda = 1.87 \times 10^{-7}$ and $1.87 \times 10^{-5}$ (red
Analytical Chemistry

Figure 6. Simulation results for $\alpha = 3$ and $\Lambda = (\infty, 1.87 \times 10^{-3}, 1.87 \times 10^{-4}, 1.87 \times 10^{-5})$ are shown as blue circles (○), black squares (■), gray diamonds (◆) and red triangles (▲), respectively. The solid lines show the predicted retention curve for each $\Lambda$, numerically taking into account Faxén’s law and more significantly hydrodynamic interactions with both channel walls.22

▲ and gray ◆ in Figure 6) show a steric-inversion point at a lower retention ratio and larger particle size than predicted by the ideal theory (not shown). For strong fields and large colloids, the discrete particle nature of MPCD cannot adequately model a continuous fluid, and simulations do not span the entire range of sizes. On the other hand, when $\Lambda = 1.87 \times 10^{-5}$ (■), MPCD provides prediction across the entire range of sizes. The simulations for $\Lambda = 1.87 \times 10^{-7}$ convincingly verify the conclusions of Figures 3 and 4; even under simplified prototypical conditions, sFFF and fFFF are significantly suppressed in microfluidic channels due to wall-induced hydrodynamic effects, leaving an extended range of particle sizes over which the retention ratio does not rise. There is little selectivity or resolution between particles larger than $r \gtrsim 0.2$ when $\Lambda = 1.87 \times 10^{-7}$.

Discussion and Interpretation of Results. Neither the video microscopy data (Figures 3 and 4) nor the MPCD simulations (Figure 6; explicit comparison not shown) of field-flow fractionation in a microfluidic channel are well-predicted by the ideal retention theory encapsulated in eq 1. The most significant complication omitted from ideal retention theory is hydrodynamic interactions with the microfluidic channel walls, leading to an increased effective friction coefficient. Hydrodynamic interactions can be divided into forces acting antiparallel (drag) and forces acting perpendicular (lift) to the wall. Inertial lift forces arise from a combination of symmetry, breaking of the wake by the wall, and the shear-gradient generated force.52 In our experiments and simulations, we keep the particle Reynolds number below $Re_p = 6\pi \rho \eta / \alpha < 0.01$, such that lift forces remain negligible.52 Increased drag forces, however, can no longer be neglected. Drag forces are generated because the no-slip condition on the surface of the mobile colloid and on the channel wall causes greater shearing and greater friction than in the absence of boundaries.

Correcting the friction coefficients accurately enough over all heights requires the multipole expansion method utilized by Pasol et al. to include the effects of both walls.22 Only by numerically calculating the retention ratios using the results of Pasol et al., while also allowing the retention parameter to vary as a function of radius ($\Lambda = \Lambda r^{-\alpha}$), do we find agreement between theory and measured experiments and simulations, as discussed below. Many simple forms for the friction coefficient of a sphere moving near a planar surface exist in the literature, notably, the expansion solution of Faxén,63 the near and far wall approximations of Goldman et al.,64 and the empirical form of Williams et al.65 While each of these forms slows the particles and reduces the retention ratio for large particle sizes, none adequately resembles simulations or experiments. This is because each is accurate only over a limited range of distances from a single wall, while the retention ratio results from weighted integration over all possible heights. The form given by Pasol et al. includes the effects of hydrodynamic interactions with both walls, as well as the effects of Faxén’s law, and so is accurate at large $r$ values.22

For all device retention parameters, $\Lambda$, the modified retention ratios numerically calculated using the friction coefficients found by Pasol et al.22 quantitatively agree with both the microfluidic video microscopy and MPCD simulation data. In Figures 3, 4, and 6, solid lines represent the numerically predicted retention ratios that take into account both Faxén’s law and more importantly wall-drag effects. Retention ratios for negligible fields are the least affected by frictional effects and remain relatively parabolic, as seen in the solid line in Figure 4 and the blue line in Figure 6 ($\Lambda = 10^{-3}$ and $\Lambda = \infty$, respectively). Even in strong fields as in Figure 6 (red line; $\Lambda = 1.87 \times 10^{-5}$), HC is predicted to still exist for a brief size range and is relatively unaffected by wall-induced drag effects. Likewise, qualitatively, nFFF remains a region with a steep negative slope of the retention ratio. In weak and intermediate fields ($\Lambda = 1.87 \times 10^{-4}$ and $\Lambda = 1.87 \times 10^{-5}$ in Figure 6), the slope is nearly zero for all larger colloids. In extremely high fields ($\Lambda = 1.87 \times 10^{-7}$ in Figure 6), a slow climb remains such that sFFF is predicted to exist but with a significantly reduced slope compared to the ideal theory. Evidently, fFFF only exists for weak fields and is suppressed in larger fields. Thus, in microfluidic devices, frictional effects due to the walls are predicted to reduce resolution above the steric-inversion point compared to what would otherwise be predicted in the absence of increased drag. Finally, as $r \to 0.5$, the retention ratio drops suddenly, indicating that the effective friction coefficient has increased considerably.

Solid lines in Figures 3 and 4 represent the numerically predicted $R(r, \Lambda)$ based on the experimental parameters, while the shaded areas represent the experimental uncertainty on the curves (error bars on the data points represent the standard deviation on the mean of video microscopy data). We are able to quantitatively predict the retention ratios for microfluidic separation devices across the entire range of particle sizes investigated by accurately including the effect of hydrodynamic friction. Figures 3 and 4 show that regardless of $\Delta \rho$, wall effects only become significant once the colloid radius is increased above $\sim 5\%$ of the channel height, $r \gtrsim 0.05$.

CONCLUSIONS

We have experimentally and computationally investigated the utility of field-flow fractionation for fractionating the entire range of spherical particle sizes possible on a microfluidic chip. Working on a microfluidic chip allows us to experimentally observe the transition from the hydrodynamic chromatography limit of FFF to the normal-mode FFF. The existence of this transition has been hypothesized for many years, but experimental verification has not been previously reported in the literature. Our results show that normal-mode FFF remains a high-selectivity technique in microfluidic channels that can be
implemented with a simple channel design. By measuring the retention ratio of neutrally buoyant colloids in a microfluidic device, we have verified the existence of Faxén-mode FFF, though our results demonstrate that both fFFF and steric-mode FFF are substantially diminished in strong external fields. In strong fields, Faxén-mode FFF becomes an inappropriate eponym because hydrodynamic interactions with the channels produce wall drag that increases the friction coefficients of the colloids and dominates over the effect of Faxén’s law. While the ideal retention theory works well for samples with hydrodynamic radii significantly smaller than the separation between plates, video microscopy demonstrates that the ideal theory fails for large colloids in microfluidic devices. Simulations verify that the ideal theory that neglects hydrodynamic complications overestimates colloid velocity, particularly in the near-wall region. Although including Faxén’s law is a simple improvement to the ideal retention theory, hydrodynamic forces acting antiparallel to the velocity of the colloids dominate in precisely the regimes in which Faxén’s law would otherwise have been significant (steric- and Faxén-mode FFF). These wall interactions increase drag and so decrease the retention ratio below the Faxen prediction. Moving in the vicinity of microchannel walls causes the colloids to lag behind their ideally predicted speeds, which may have important consequences for particle tracking velocimetry techniques in microfluidic systems. Micro-particle image velocimetry implicitly models the fluid velocity as equivalent to tracer particles’ velocities; however, it is clear from the present study that this assumption holds a significant risk of underestimating fluid velocities, especially in micro- and nanoscale systems. Frictional effects must be rigorously accounted for in order to convert microbead velocities into accurate solvent velocity profiles, in particular when dealing with particles greater than ∼5% of the channel height. We find that the multipole expansion method produced values that accurately predict the retention ratio, while more traditional approximations fail.

Although it seems at first glance that performing field-flow fractionation in a microfluidic channel introduces more complications, there is a very clear benefit of incorporating FFF components into microfluidic and into future nanofluidic devices. When the external transverse field is strong, normal-mode FFF drops sharply to the steric-inversion point offering an operational range with significant selectivity. However, rather than rapidly rising in steric-mode FFF and thus introducing ambiguity through pronounced nonmonotonicity, as occurs in traditional, macroscopic FFF devices, the retention ratio is suppressed and nearly flat for all larger colloidal particle sizes. This quality can give researchers a clear signal that the sample is no longer eluting in the microfluidic device’s exclusive operating range.

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Notes
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■ ABBREVIATIONS

FFF, field-flow fractionation; HC, hydrodynamic chromatography; nFFF, normal-mode field flow fractionation; sFFF, steric-mode field flow fractionation; fFFF, Faxén-mode field flow fractionation; GrFFF, gravitational FFF; PDMS, polydimethylsiloxane; SMR, suspended microchannel resonator; MPCD, multiparticle collision dynamics

■ REFERENCES


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