Field-free particle focusing in microfluidic plugs

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Particle concentration is a key unit operation in biochemical assays. Although there are many techniques for particle concentration in continuous-phase microfluidics, relatively few are available in multiphase (plug-based) microfluidics. Existing approaches generally require external electric or magnetic fields together with charged or magnetized particles. This paper reports a passive technique for particle concentration in water-in-oil plugs which relies on the interaction between particle sedimentation and the recirculating vortices inherent to plug flow in a cylindrical capillary. This interaction can be quantified using the Shields parameter (θ), a dimensionless ratio of a particle's drag force to its gravitational force, which scales with plug velocity. Three regimes of particle behavior are identified. When θ is less than the movement threshold (region I), particles sediment to the bottom of the plug where the internal vortices subsequently concentrate the particles at the rear of the plug. We demonstrate highly efficient concentration ($\sim 100\%$) of $38\text{ }\mu\text{m}$ glass beads in $500\text{ }\mu\text{m}$ diameter plugs traveling at velocities up to 5 mm/s . As θ is increased beyond the movement threshold (region II), particles are suspended in well-defined circulation zones which begin at the rear of the plug. The length of the zone scales linearly with plug velocity, and at sufficiently large θ , it spans the length of the plug (region III). A second effect, attributed to the co-rotating vortices at the rear cap, causes particle aggregation in the cap, regardless of flow velocity. Region I is useful for concentrating/collecting particles, while the latter two are useful for mixing the beads with the solution. Therefore, the two key steps of a bead-based assay, concentration and resuspension, can be achieved simply by changing the plug velocity. By exploiting an interaction of sedimentation and recirculation unique to multiphase flow, this simple technique achieves particle concentration without on-chip components, and could therefore be applied to a range of heterogeneous screening assays in discrete nl plugs. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.3700120>]

I. INTRODUCTION

Particle concentration is a key operation in biochemical assays. In single-phase microfluidic devices, high-throughput continuous particle separation can be achieved using several techniques which typically focus particles within selected streamlines in a channel (see topical reviews^{1,2}). Active focusing techniques exploit the interaction of particles with external electric, magnetic, and acoustic fields. Passive techniques generally rely on the interaction of particles with laminar flows in microfluidic structures; these include pinched flow fractionation, lateral displacement, and hydrophoretic separation. Inertial microfluidic techniques utilize particle migration and secondary flows to focus and separate particles with high throughput.^{3,4} Gravity-driven particle concentration has also been demonstrated using a hydrodynamic amplification principle to improve separation fidelity.⁵

By comparison, the emerging field of multiphase microfluidics has a relatively few particle concentration techniques available. In general, the existing approaches utilize active electric or magnetic fields. Using local electric fields, Cho and Kim segregated charged particles in a

droplet with 83% efficiency in an electrowetting platform.⁶ Valley and Wu demonstrated a similar approach using an optoelectrowetting platform.⁷ Magnetic fields can be used to localize paramagnetic beads in a droplet, and when functionalized, the beads can capture biomolecules in the droplet. This approach was used to purify proteins,⁸ DNA,⁹ and virus particles.¹⁰ All of these techniques are promising; however, the requirement of external field generators can add additional complexity to the microfluidic system, which may be undesirable.

In this paper, we report a multiphase particle concentration technique which requires no external fields and has high concentration efficiency. It relies on the interaction of particle sedimentation forces and the recirculating vortices which naturally occur in the plug as it flows through the channel. Through the combined interaction of the two phenomena, particles are segregated to the rear of the plug with high efficiency. Section II presents a theoretical framework for the circulation velocity and particle sedimentation. The non-dimensional Shields parameter is used to categorize the interactions between the two phenomena into 3 distinct flow regimes. Section III describes the experimental setup. The model is supported by experimental data (Section IV), which demonstrates efficient concentration of 38 μm glass beads in a 500 μm circular channel.

II. THEORETICAL

A. Multiphase plug flow

Aqueous plugs of uniform length can be generated by combining an aqueous sample and an immiscible carrier fluid (typically a hydrocarbon or fluorocarbon oil) in a tee or cross junction.^{11,12} In a laminar flow environment, the plug breakoff process occurs in a repeatable manner, resulting in monodisperse plugs. Each plug is separated from the channel walls by a thin film of carrier fluid (i.e., a wetting film) which spontaneously forms if the surface tension between the plug and the wall is greater than that of the carrier fluid and the wall.^{13,14} The thickness of the wetting film h scales according to Bretherton's law, $h \propto R_C Ca^{2/3}$, where R_C is the capillary radius.¹⁵ The capillary number Ca is given by $\mu_C V_P / \gamma$, where μ_C is the viscosity of the carrier fluid, V_P is the plug velocity, and γ is the interfacial tension. Although Bretherton's law was derived specifically for gas-liquid flow, the same scaling holds true in liquid-liquid plug flows when the viscosity of the carrier exceeds the viscosity of the plug and $Ca < 0.01$.¹⁶ This is generally the case in plug-based microfluidics and has been experimentally verified in prior experimental results from Ghaini and Agar¹⁷ and our group.¹⁸ At low Ca , the thickness of the wetting film is generally 1%–5% of the channel radius,¹⁶ and the velocity at the plug's exterior surface is close to zero.

B. Microvortices in liquid-liquid plug flow

In Taylor's classic model of gas-liquid plug flow in a cylindrical channel, shear forces exerted by the wetting film generate axisymmetric vortices within the plug.¹⁹ The same holds true for liquid-liquid plugs.^{16,20–22} Within the reference frame of the plug, the internal vortices are oriented backward near the plug's outer surface, and forward along its axis (Figure 1(a)). If the viscosity ratio of plug and carrier fluid μ_P / μ_C is $\ll 1$, there are additional co-rotating vortices at the front and rear cap of the plug.²² Computational fluid dynamics simulations of the caps have been shown in our prior publication.¹⁸ Both the internal and cap vortices are responsible for the concentration phenomena to be discussed later.

A first order analytical model we reported previously¹⁸ is useful for understanding the parabolic flow profile which occurs in the internal vortices (Figure 1). It should be noted that the multiphase flow profile is influenced by a wealth of factors, including the capillary number, channel radius, cross sectional geometry, and the viscosity ratio between the plug and the carrier fluid.^{16,23–25} However, if $Ca \ll 1$ and $\mu_P / \mu_C \leq 1$, which is often the case in droplet microfluidics, the internal vortices follow a simple model where the circulation velocity V_C scales proportionally with the plug velocity V_P .²² At small Ca , the wetting film is $< 5\%$ of the channel radius, and we can reasonably assume the axial velocity at the plug's exterior surface is negligible.²² Under this boundary condition, the flow follows the classic Poiseuille profile in a

FIG. 1. Liquid-liquid plug flow. (a) Schematic of microvortices which develop in a plug as it moves through the channel at a velocity V_p . The plug radius is R_p , the channel radius is R_c , and the wetting film has a thickness h . (b) Analytical model illustrating forward and reverse flow in the reference frame of the plug. The flow can be approximated as a classic Poiseuille profile.

cylindrical pipe (Figure 1(b)). The parabolic axisymmetric flow profile is given by $v(r) = (V_C/R_p^2)(R_p^2 - r^2)$, where r is the radius, $V_C = 1/(4\mu_p)(dP/dx)R_p^2$ is the circulation velocity, dP/dx is the axial pressure gradient, and R_p and μ_p are the radius and viscosity of the plug, respectively. The circulation velocity (V_C) is the difference between the forward and reverse flows, which is important to this work, because it defines the strength of the microvortices. The plug velocity (V_p) can be derived by taking the average flow velocity along a cross section, and is found to be $V_p = V_C/2$. Therefore, within the reference frame of the plug, the direction of flow transitions from forward to reverse when $v = V_p = V_C/2$, which occurs at a radius $r = (\sqrt{2}/2)R_p$. The maximum shear stress at the plug surface ($r = R_p$), used later for calculating the shear velocity, is given by $\tau = 2\mu_p V_C/R_p$.

C. Particle sedimentation

The sedimentation velocity of a spherical particle with radius a can be found by equating the gravitational force $F_G = (4/3)\pi a^3 \Delta\rho g$ with the Stokes drag force $F_D = 6\pi\mu_p a v$. Here, $\Delta\rho$ is the difference in density between the particle and fluid, g is the gravitational constant, μ_p is the plug viscosity, and v is the velocity of the fluid relative to the particle. The sedimentation velocity is

$$V_S = \frac{2}{9} \frac{a^2 (\Delta\rho) g}{\mu_p}. \quad (1)$$

Table I compares the wide range of sedimentation velocities for several particles used in our experiments. For example, a $1\ \mu\text{m}$ polystyrene particle ($\rho = 1.03\ \text{g/cm}^3$) in water sediments at $16\ \text{nm/s}$. By contrast, a $38\ \mu\text{m}$ glass bead ($\rho = 2.52\ \text{g/cm}^3$) sediments at $1.19\ \text{mm/s}$, nearly $100\,000\times$ larger. In the latter case, particle sedimentation is a useful mechanism which can be exploited for particle concentration. As a rule of thumb, particles will sediment if the sedimentation velocity exceeds the fluid velocity.

D. Particle concentration model

Under appropriate flow conditions, the interaction of particle sedimentation with the recirculating microvortices can be used to concentrate particles in the rear of the plug. Their combined effect results in two distinct particle concentration phenomena (Figure 2): (1) the localization of particles in a circulation zone (circulation effect) and (2) the aggregation of particles

TABLE I. Particle sedimentation velocities.

Particle type/diameter	Sedimentation velocity (V_S)
1 μm polystyrene	16 nm/s
10 μm polystyrene	1.63 $\mu\text{m/s}$
10 μm glass	81 $\mu\text{m/s}$
38 μm glass	1.19 mm/s

near the rear cap (aggregation effect). Scaling of both phenomena can be suitably described by the Shields parameter θ , a dimensionless number which describes the ratio of the drag force exerted by the fluid on a particle to the particle's weight.²⁶ There are two forms useful for our analysis:

$$\theta = \frac{\text{Particle Drag}}{\text{Particle Weight}} = \frac{\tau}{a\Delta\rho g} = 9 \frac{\mu_p}{a^2\Delta\rho g} V_p. \quad (2)$$

Here, τ is the shear stress exerted by the liquid on the particle, and the other parameters are as described earlier. The first form, commonly used in sedimentation engineering, is useful for determining the minimum shear force needed to resuspend a bed of sedimented particles.^{26,27} If θ exceeds the movement threshold, the particles are suspended in the moving fluid, otherwise they sediment. If a particle is spherical, we can recast the Shields parameter into a 2nd form, which is simply the ratio of Stokes drag to weight. This is found by taking F_D/F_G as described earlier, and recalling that $V_p = V_c/2$. The 2nd form is equivalent to the ratio of circulation velocity to sedimentation velocity V_c/V_S , which is useful for calculating the trajectory of particles in circulating flows. In our analysis, we will use the 2nd form, because it conveniently depends on the plug velocity and the particle properties.

E. Circulation effect

We first consider the circulation effect in three different flow regimes, governed by the Shields parameter (Figure 2, column 2). When θ is less than the movement threshold θ_M , the particle's gravitational force exceeds the drag force exerted by the fluid. In order for particle circulation to occur, particles at the rear of the plug must be lifted vertically to the interior streamline by drag forces. When the lift force is insufficient, particles remain in a sediment bed at the rear of the plug, similar to conventional sedimentation.^{27,28} The tensile interface between

FIG. 2. Schematic of the 3 flow regimes, described by the dimensionless Shields parameter θ (column 1). In each flow regime, the circulation effect and the aggregation effect are illustrated in columns 2 and 3, respectively. The combined behavior is shown in column 4. Note: all the schematics show a side view of the plug.

the aqueous plug and oil phase keep the particles within the plug. As more particles are added to the sediment bed, the velocity profile in this region vanishes, which in turn draws in additional particles. As a consequence of the positive feedback, all particles in the plug ultimately collect in the rear of the droplet, thus providing virtually 100% collection efficiency. In region II, when $\theta \sim \theta_M$, gravitational and drag forces are comparable. Here, particles circulate within a well-defined zone which begins at the rear of the plug. To understand why this occurs, we consider a particle at the rear of the plug which is entering the interior, forward-directed streamline. Ignoring relaxation time for simplicity, we assume that the particle travels with a horizontal velocity V_C . Simultaneously, it also experiences a sedimentation velocity V_S , directed to the bottom of the channel. Combining both velocity components, the particle follows a trajectory which ultimately brings it to the lower region of the plug, where the reverse-oriented flow then returns it to the rear of the plug. This process repeats for all particles, forming a circulation zone where particles are confined. The length of the circulation zone, denoted L_C , can be found using the Poiseuille model shown in Figure 1(b). The time required for a particle to reverse direction is $V_S/(0.707R_P)$, where V_S is the sedimentation velocity and R_P is the plug radius. During this time, the particle travels a horizontal length L_C , which can be found by integrating the velocity profile over this time period. Scaling L_C by R_P gives the aspect ratio of the circulation zone, and the dimensionless form can be expressed as a function of the plug velocity or the Shields parameter.

$$\frac{L_C}{R_P} = \frac{5\sqrt{2} V_P}{24 V_S} = \frac{5\sqrt{2}}{48} \theta. \quad (3)$$

When $\theta \gg \theta_M$ (region III), drag forces greatly exceed the particle's weight, and sedimentation is negligible. In this regime, particles circulate uniformly throughout the plug, and no concentration occurs. We note, however, that after an asymptotically long period of time, particles subject to any gravitational force will eventually migrate to the lower vortex and remain there. The time required for this to occur is simply the particle's sedimentation velocity divided by the plug radius.

F. Aggregation effect

The aggregation effect (Figure 2, column 3) refers to the collection of particles in the rear cap. This effect is believed to be due to the interplay between the cap vortices (Figure 1) and the internal vortices. Particles at the rear of the plug which enter the cap will remain there because it is an independent, co-rotating vortex. As additional particles collect in the cap, the effective viscosity will increase, forming a stagnant region which, in turn, will collect additional particles. In dilute suspensions, the effective viscosity scales according to Einstein's relationship, $\mu_P = \mu_{P0}(1 + k_e\phi)$, where μ_{P0} is the initial plug viscosity, k_e is a constant ~ 2.5 , and ϕ is the volume fraction of particles.^{29,30} At higher concentrations, other models (such as Mooney's equation) must be used, and the linear scaling described by Einstein's relationship generally does not hold.³⁰ As particles pack into a sufficiently high volume fraction, the viscosity can become very large, leading to a positive feedback effect where a nucleated particle bed can quickly grow by stagnating the local fluid. In addition to the viscosity effects, particle aggregation can also occur by colloidal interactions such as electrostatic double layers, van der Waals interactions, hydration effects, and steric interactions.³¹ In our experiments, which use particles with diameters of 10–50 μm , such colloidal effects typically are not observed. However, if the technique was to be scaled down, a suitable model would need to consider these effects and their dependence on the physicochemical properties of the fluid and the particle. Like the circulation zone, the aggregation effect also scales with the Shields parameter. Gravitational forces cause the particle to segregate to the lower half of the cap, while fluidic drag induced by the internal vortices (F_{D1} and F_{D2}) seek to maintain a symmetric aggregation in the cap. Thus, when θ is small, the particles gather toward the lower half of the cap, and when θ is large, particles distribute symmetrically within the cap.

FIG. 3. Experimental setup.

III. EXPERIMENTAL

The experimental setup is shown in Figure 3. Plugs are generated by combining an aqueous bead suspension with oleic acid ($\mu_c = 0.027 \text{ Pa} \cdot \text{s}$) in a $500 \mu\text{m}$ bore PEEK T-junction (IDEX Health and Science). Microcapillary Teflon tubing with $500 \mu\text{m}$ ID (IDEX) are used as the microchannel as well as the syringe interconnect. The bead suspension consists of deionized water containing soda-lime solid glass beads with mean diameter $38 \mu\text{m}$ and a D50 distribution ranging from $34\text{--}42 \mu\text{m}$ (Cospheric). Each syringe is controlled by an independent syringe pump to maintain relative flow rates. The syringe containing the beads is periodically agitated to maintain suspended particles. The flow rates for oil and water range from 0.6 to $10 \mu\text{l/s}$, which generates plug velocities between 1 and 50 mm/s . These conditions correspond to Reynolds number <25 , well within the laminar regime. To image the particle concentration profiles, we use a standard inverted microscope with a $4\times$ objective. The objective is placed several cm downstream the drop generator to allow sufficient time for particle profiles to form. Equilibration typically occurs in the time it takes for the plug to travel several plug lengths, typically $<1 \text{ s}$. Digital videos are recorded with a high speed camera (Casio EX-F1) at 1200 frames per second (fps) with 336×96 pixel resolution, at 600 fps with 432×192 resolution, and 30 fps with 1280×720 resolution. To obtain grayscale intensities vs. time, an image analysis program implemented in MATLAB extracts grayscale pixel values on a point on the channel centerline.

IV. RESULTS AND DISCUSSION

A. Effect of particle size

To illustrate the importance of particle size, Figure 4 compares concentration profiles of beads with a small and large settling velocity V_s when both are subject to identical plug velocity of 3 mm/s . In the case of the $1 \mu\text{m}$ polystyrene bead ($\rho = 1.03 \text{ g/cm}^3$), the Shields parameter is $\theta \sim 3.7 \times 10^5$. As expected with large θ , the particles are uniformly distributed throughout

FIG. 4. Top view comparison of experimental concentration profiles with small, light particles versus large, heavy particles at an identical flow rate of 3 mm/s in a $500 \mu\text{m}$ diameter circular channel. Both experiments use a high particle loading of $>10^5/\text{ml}$.

FIG. 5. Particle concentration profiles as a function of flow velocity and particle loading ($38\text{ }\mu\text{m}$ glass beads). Images shown are high speed video (1200 fps) taken in a circular, $500\text{ }\mu\text{m}$ capillary tubing at the indicated flow velocity. Both the top view and side view are shown. High concentration is defined as $>10^5/\text{ml}$, and low concentration is defined as $<10^4/\text{ml}$ (enhanced online). [URL: <http://dx.doi.org/10.1063/1.3700120.1>] [URL: <http://dx.doi.org/10.1063/1.3700120.2>] [URL: <http://dx.doi.org/10.1063/1.3700120.3>] [URL: <http://dx.doi.org/10.1063/1.3700120.4>] [URL: <http://dx.doi.org/10.1063/1.3700120.5>] [URL: <http://dx.doi.org/10.1063/1.3700120.6>] [URL: <http://dx.doi.org/10.1063/1.3700120.7>] [URL: <http://dx.doi.org/10.1063/1.3700120.8>]

the plug. In contrast, with $38\text{ }\mu\text{m}$ glass beads ($\rho = 2.52\text{ g/cm}^3$), $\theta \sim 5$. In this case, the particles do not circulate and sediment near the rear of the plug. Under each of the images is a line plot which gives the grayscale intensity along the length of the plug. The graphs are inverted, and they are provided as a means to compare the length of the circulation zone (L_C) with the total length of the plug (L_P). The two peaks at the front and rear of each graph represent the shadow at the front and rear each plug. The ratio of L_C/L_P indicates the enrichment factor.

B. Effect of flow velocity

Figure 5 compares the effect of flow velocity on particle circulation patterns. All experiments are conducted with $38\text{ }\mu\text{m}$ glass beads at high or low concentration (experimental parameters are given in the caption). Both the top view and side view are shown to fully illustrate the particle behavior in each flow regime. The non-dimensionalized experimental data are shown in Figure 6, where 3 distinct regimes can clearly be identified. In region I, which occurs at low plug velocity (3 mm/s), the Shields parameter θ is sufficiently small such that the particles are not suspended by the flow. Instead, they sediment in a single bed at the rear of the plug as a result of the lack of circulation as well as the aggregation effect. This flow regime is most suitable for high efficiency particle concentration, since virtually all particles collect near the rear cap (see multimedia). Region II begins when θ exceeds the movement threshold, which is experimentally found to be at $\theta \sim 10$, or a flow velocity of $\sim 6\text{ mm/s}$. This can be compared to previous sedimentation data described by Miller.²⁷ According to Miller's modified Shields diagram, a $38\text{ }\mu\text{m}$ particle would require a minimum shear velocity of 8 mm/s . Taking the shear at the plug's lower surface, this correlates to a minimum plug velocity of about the same, 8 mm/s . Therefore, the movement threshold obtained in our experiments is consistent with basic sedimentation theory. In region II, the particles are suspended, and they circulate in a well-defined zone which begins at the rear of the plug. The length of the circulation zone (L_C) scales linearly with flow velocity, increasing from 0.4 to 1.4 mm over plug velocities ranging from 9 to 25 mm/s ($\theta \sim 15 - 42$). The measured L_C values closely match the theoretical values calculated using Eq. (3). In region III, which occurs at flow velocities greater than 25 mm/s , the predicted L_C is $>1.5\text{ mm}$, which is longer than the plugs generated in the experiment. As a result, the

FIG. 6. Variation in the length of the circulation zone as a function of plug velocity. The data are given in nondimensional form by scaling L_c by the plug radius (which gives the aspect ratio of the circulation zone), and expressing plug velocity in terms of the Shields parameter. The dotted lines demarcate the 3 flow regimes. The data are taken with experimental conditions identical to Figure 5.

circulation zone is constrained by the plug length, and particles circulate through the entire length of the plug. The concentration profile is therefore independent of flow rate.

C. Parabolic particle profiles in circulation zones

In the top view (Figure 7), the circulation zones appear to have a roughly parabolic boundary. This highlights the point that particles may travel streamlines other than the center axis. Particles which occupy streamlines away from the axis will have a slower axial velocity, and therefore will sweep a smaller circulation length before being swept to the rear of the plug. Conversely, particles close to the axial streamline will attain faster velocities and sweep a longer trajectory before returning. The parabolic shape reflects the radial Poiseuille flow profile described earlier. The parabolic circulation zones are especially apparent in long plugs at large flow velocities. Figures 7(b) and 7(c) show the top view of plugs containing $38\ \mu\text{m}$ glass beads, traveling at 10 mm/s and 30 mm/s, respectively.

D. Effect of particle loading

Figure 5 also shows the effect of particle loading on concentration profiles. In these experiments, a high concentration is defined as $>10^5/\text{ml}$, and a low concentration is defined as $<10^4/\text{ml}$, respectively. High particle loading favors the formation of the aggregation zone in the rear cap, regardless of flow velocity. As described earlier, high particle loading contributes to a positive feedback effect, where the deposition of particles reduces the local flow velocity, which in turn leads to further aggregation. This is particularly apparent in the side view images at high concentration, where the aggregate fills the cap. At low plug velocity (3 mm/s, $\theta = 5$), the distribution is

FIG. 7. (a) Schematic of multiple possible trajectories for circulating particles. (b) and (c) Parabolic particle distribution in at 10 and 30 mm/s flow velocity, respectively (top view) (enhanced online). [URL: <http://dx.doi.org/10.1063/1.3700120.9>]

FIG. 8. Very high particle loading ($>10^7/\text{ml}$) causes the aggregate in the rear cap to extend into the plug, extending the length of the stagnation zone. The channel diameter is $500\ \mu\text{m}$ (top view).

biased toward the lower half of the cap because gravitational forces dominate. As the plug velocity increases, the balanced drag of the symmetric internal vortices becomes significant, and the particles become evenly distributed. The shape of the cap is hemispherical at low plug velocity, whereas at larger velocity, the plug adopts bullet shape similar to what is observed in gas-liquid plug flow at high Ca .³² Future research is needed to understand how aggregation in the cap affects its shape. At very large particle concentration ($>10^7/\text{ml}$), the aggregate can extend well beyond the cap region (Figure 8). Since the flow stagnates wherever the aggregate exists, these particles are essentially dragged by the motion of the plug. The particles are held within the plug due to the tensile interface between the two phases. We have found this to be the case even when the interfacial tension is reduced to $2\text{--}5\ \text{mN/m}$ by adding surfactants.

V. CONCLUSIONS

This paper demonstrates a field-free, gravitationally driven approach to perform particle concentration inside microfluidic plugs. The first notable advantage of this technique is that it is passive, requiring no external components, and it can be controlled simply by changing the flow velocity. Second, whereas prior approaches have reported 83% particle collection efficiency,⁶ this technique can achieve an efficiency of nearly 100%. One important requirement is that particles must have a sufficiently large settling velocity. Since V_S scales as the square of the radius, it is best to use dense particles in the $10\text{--}50\ \mu\text{m}$ range. Although this is larger than the magnetic beads typically used in biological assays, there are nonetheless a wide variety of commercial beads available in this range. Such sizes would be compatible with microchannels with diameters $100\text{--}500\ \mu\text{m}$. Many such beads, including the glass beads used here, can be functionalized to capture biomolecules.

On a broader note, particle concentration techniques expand the capabilities of plug-based microfluidic systems to include particle filtration, an operation that was previously limited to continuous flow systems. An advantage of plug-based microfluidics is that it can perform batch-mode processing of small assay volumes. The ability of this technique to switch between circulation and aggregation has promise in bead-based assays, which require resuspension and collection of beads in solution. High plug velocities, where beads circulate throughout the plug, are useful for mixing and agitating the particles with the sample. Decreased flow velocities, where beads sediment, can then be used for collecting the functionalized particles. As described in an earlier paper,³³ particle collection could be immediately followed by plug splitting, thus extracting the beads from the sample (solid phase extraction). Future work will utilize this concentration technique for heterogeneous mixing and extraction assays.

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