ABSTRACT

Particle concentration is a critical unit operation in the biology lab, often accomplished using magnetic beads and centrifugation. In single-phase microfluidic systems, numerous techniques for particle concentration have been reported; however, in droplet-based fluidics, particle concentration is a unit operation missing from the practitioner’s toolkit. This paper reports a technique for particle concentration inside a microfluidic plug using hydrodynamic slug flows. The flows, which naturally occur in the plug, include asymmetric vortices which can trap particles preferentially towards the rear of the plug. The ability to concentrate particles of various size and at various flow velocities is investigated with experiments and CFD simulations. Concentrated particles can be permanently encapsulated using a transverse drop splitter. The ability to concentrate and fractionate particles inside microfluidic plugs could facilitate heterogeneous screening assays in droplet-based fluidics using ultra-low volumes.

KEYWORDS: Concentration, Droplet, Fractionation, Filtration, Vortex, Slug flow

INTRODUCTION

Particle concentration/filtration is a critical unit operation in biochemical assays, commonly used for bead-based detection, cytometry, and hematology. Conventional techniques for particle separation include filters, magnetic beads, and centrifugation. Continuous-flow (single-phase) microfluidic systems can perform particle separation, typically in µL-mL sample volumes, using sieves, dielectrophoresis, deterministic lateral displacement, and inertial forces [1]. Droplet-based (multiphase) microfluidic systems can perform assays in ultra-low sample volumes, ranging from nL to fL [4]; however, despite the many unit operations (including mixing, merging, splitting) which have been demonstrated, particle concentration has remained a significant challenge. Electrowetting devices can localize particles using electric fields [2,3], but this technique requires charged particles, integrated electrodes, and volumes > 100 nL. Here, we report a hydrodynamic method for localizing and fractionating particles within a microfluidic plug. This technique localizes particles inside a low-volume plug by exploiting slug flows which occur due to interactions of the droplet with the surrounding carrier fluid. The paper discusses the geometry of the flow patterns and their ability to concentrate particles under various flow rates and particle size.

CONCEPT

Particle concentration is achieved by exploiting hydrodynamic slug flows which naturally occur inside a plug as it flows through a channel in an immiscible carrier fluid [5-9] (Figure 1). The recirculating flow inside the plug is caused by the plug displacement and the outer flow of the carrier fluid. The carrier fluid acts as a lubricating layer for the aqueous plug, therefore the velocity of the aqueous plug is slightly higher than the carrier fluid. Within the reference frame of the droplet, this causes a reverse-oriented flow on the outer interface, and a forward flow in the interior region. The vortex flow pattern inside the plug is influenced by a several factors including flow velocity, plug length, channel size and viscosity ratio between the plug and carrier fluid [5,7,8]. Previous studies report two recirculating flow regions inside the plug [5]; however, our simulations and experiments have revealed four additional co-rotating vortices located at the front and rear of the plug. These vortices occur at low capillary number flows. The vortices act as hydrodynamic traps, collecting particles into distinct zones near the front and rear of the plug (Figure 1). Moreover, we observe that the vortices are asymmetric; particles tend to aggregate preferentially to the rear of the droplet. Particles concentrated via hydrodynamic concentration can be permanently separated by vertically splitting the plug (see below).

THEORY AND SIMULATION

The formation of the trapping vortices is governed by the capillary number ($Ca=\mu V/\sigma$), where $\mu$ is the viscosity of the carrier fluid, $V$ is the velocity of the plug, and $\sigma$ is the interfacial tension between the fluids. Computational fluid dynamics (CFD) simulations, performed in COMSOL, illustrate the evolution and geometry of the vortices and their depen-
dependence on capillary number (Figure 2). The simulations use a level set method in an axisymmetric geometry to model an aqueous plug flowing through a circular hydrophobic tube filled with 1 cst immiscible carrier fluid. Co-rotating vortices form at the front and rear of the droplet at low capillary number ($Ca < 10^{-3}$). The vortices are most pronounced at low capillary number ($Ca \approx 10^{-6}$). As $Ca$ is increased to $10^{-2}$ and then $10^{-1}$, the vortices move away from the center and polarize towards the front and rear boundaries. In all cases, the rear vortex is stronger than the front vortex due to differences in pressure and flow orientation. At $Ca > 10^{-3}$, the vortices disappear, and are replaced with a bypass flow [8]. In our experiments, we utilized low capillary number $Ca < 10^{-3}$ to generate the vortices and hence the concentration effect. Particles in the droplet tend to recirculate in the vortices, and eventually collect in the stagnant regions. Due to the asymmetry of the vortices, particles preferentially collect in the rear of the drop.

**EXPERIMENTAL**

To characterize the parameters affecting particle concentration, we generated plugs containing various sized polystyrene beads in a 1 centistoke silicone oil as the carrier fluid. The diameter of the polystyrene beads range from 0.1 µm to 10 µm. The experiments are conducted using 500 µm and 200 µm ID Teflon tubing. A syringe pump and a commercial tee junction are used to generate the plugs, and several centimeters of tubing (5-10 cm) provides the region for hydrodynamic concentration. In fractionation experiments, a cross-junction is used to split the plugs into droplets. The localization of particles is measured using a CCD camera and a fiber optic absorbance detector. A plug passing by the detector causes a corresponding drop in the photodetector signal which is exponentially fixed to the tube carrying the droplets [10] (Figure 3). A second fiber, on the opposite end, leads to the optic absorbance detector. The fiber optic detector consists of an excitation LED coupled into an optical fiber which is orthogonally fixed to the tube carrying the droplets [10] (Figure 3). A second fiber, on the opposite end, leads to the photodetector. A plug passing by the detector causes a corresponding drop in the photodetector signal which is exponentially related to the particle concentration. The photodetector signal is amplified by a transimpedance amplifier and recorded by a data acquisition system.

**RESULTS AND DISCUSSION**

When monodisperse beads are present in the plug, several phenomena are observed depending on the particle size and plug velocity (Figure 4). Large particles (10 µm) in a slow-traveling plug strongly aggregate at the two ends due to the trapping vortices (see Figure 1D). This is believed to be due to two reasons: i) the particles are collected in the rear by the asymmetric trapping vortices, and ii) the hydrophobic nature of the polystyrene beads causes them to remain near the water/oil interface. When the plug velocity is increased, the aggregation is reduced, and particles tend to recirculate wider patterns in the drop. A preference toward the rear of the drop is maintained in both cases.

Smaller particles (<10 µm) exhibit a markedly different behavior, forming two distinct regions within the plug. The front region has relatively lower concentration and

![Figure 2: CFD simulations illustrating the formation and geometry of trapping vortices formed inside a microfluidic plug as it flows through a microchannel. Streamlines (A-D) illustrate how the geometry of the vortices change with increasing capillary number. They also show the asymmetry of the vortices – the vortices at the front are weaker than those in the rear.](image)

![Figure 3: (A) Schematic and (B) experimental setup of a fiber optic absorbance detector used to quantify the concentration profiles of particles within the plug.](image)

![Figure 4: Concentration of monodisperse particles in a 500 µm ID plug. (A) Absorbance profiles for various plug velocities and particle size. (B) Micrographs comparing the shape and concentration profiles with different size particles.](image)
assumes a typical spheroid shape, while the rear region has higher concentration and a noticeable tapering in the plug diameter. In between the two regions, there is a transition region marked by a slight bulge in the shape of the plug. With increased flow rate, the forward region lengthens while the rear region shortens, thereby increasing the concentration effect. We believe the shape of the two regions is due to the asymmetry of the vortices as well as the differences in pressure between the front and rear of the droplet; however, more studies will be needed to understand the phenomenon. Past studies have suggested that plugs in Taylor flow can deform due to hydrodynamic interactions with the surrounding fluid [8].

When very small particles are used (<0.1 µm) they exhibit the same ‘binary’ localization, except there is no pinching effect (Figure 5). These experiments are of practical use because many soluble dyes or nanoparticle suspensions can be concentrated in this manner. Two distinct regions of concentration can be clearly seen, and the length of the rear concentrated region expands and contracts proportionally with the flow rate.

Using the hydrodynamic concentration technique, we demonstrate a plug-based fractionation system which can filter particles from supernatant (Figure 6) in a 500 nL volume. The process begins with a tee junction plug generator (not shown) which generates plugs with uniformly distributed particles. In our tests, 10 µm beads are used. As the plugs pass through the length of tubing (several centimeters), the majority of the beads concentrate at the rear end of plug due to the asymmetric trapping vortices. A transverse drop splitter (a cross junction) is then used to vertically split the droplet and therefore permanently separate the concentrated beads from the remainder of the drop. This results in a high bead concentration in the last sequential drop. Therefore, this device provides the functionality of a centrifuge, where the last droplet is the ‘pellet’ separated from the supernatant.

CONCLUSION

This paper demonstrates the ability to perform particle localization inside microfluidic plugs. The technique expands the capabilities of droplet-based systems to include particle filtration, an operation that was previously limited to continuous flow systems. The divergent behavior of small versus large particles suggests that this phenomenon could potentially be used for size-based separation in a plug. Thus, the system can serve as a centrifuge and particle filtration in ultralow volume samples that cannot be accommodated in traditional microfluidic systems. A particle fractionation technique can also enable heterogeneous screening assays (those which require washing steps) in plug-based microfluidic systems.

REFERENCES


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