VISCOELASTIC BASED DROPLET SORTING IN MICROFLUIDIC CHANNELS
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ABSTRACT
We present a passive droplet sorting technique based on intrinsic viscoelastic fluid properties dependent on inner and outer fluid phase viscosity ratio (k-value), droplet deformability, and viscoelastic properties. We demonstrate sorting rates of >200 Hz of both homogeneous and heterogeneous sized droplet populations between 40-100 µm’s and enrichment rates as high as 99%. We demonstrate sorting viscosity discriminations as low as 2 cPs based on a change from 2 cPs to 4 cPs corresponding to a k-value of 0.4 to 0.8 respectively. Of particular interest for this research, we aim to investigate the ability to sort droplets containing digital PCR amplified DNA.

KEYWORDS: Droplet Sorting, Droplet Microfluidics, Digital Biology, Viscoelastic Fluid, Hydrodynamic Focusing

INTRODUCTION
On-chip droplet-reactors have gained attention in the field of “digital” microfluidics for applications like single-cell analysis and single-molecule DNA detection[4]. Droplet sorting is a desirable function of droplet microfluidic processing for sample enrichment, quantitative detection, or isolation and purification. Recent works have demonstrated size-based droplet sorting[5-7], while others have performed sorting based on fluorescence detection and di-electrophoresis (DEP) based active switching to perform fluorescence activated droplet sorting, or FADS[8]. Greater simplicity and function can be achieved if more intrinsic passive-sorting mechanisms are developed to sort droplets by content alone, and eliminate the dependence on size or fluorescence based separation schemes. The works in [6], [7] utilized deformable based migration to sort droplets and cells of different sizes and stiffness, but have not explored the influence of viscoelastic fluid behavior at relevant viscosities for sorting applications.

We present a method of sorting droplets based on the intrinsic viscosity ratio, k, of inner and outer fluid phases, as well as Newtonian vs. non-Newtonian behavior. This is advantageous as it enables bi-directional droplet migration for sorting monodisperse and polydisperse droplet populations with a reduced dependence on size. In this work, we demonstrate sorting rates of >200 Hz and enrichment rates as high as 99% of both monodisperse and polydisperse droplet sizes having diameters in the range of 40-100 µm’s. We achieve sorting viscosity discriminations as low as 2 cPs based on a change from 2 cPs to 4 cPs corresponding to a k-value of 0.4 to 0.8 respectively. Of particular interest for this research, we aim to investigate the ability to detect droplets having digital-polymerase chain reaction (dPCR) amplified DNA, which theory predicts would elicit a change in viscosity as single nucleotides polymerize into long-chain double-stranded DNA[1-3]. Figure 1 illustrates the behavior of deformable droplet migration as a function of viscosity ratio, k, and viscoelasticity.

THEORY
Based on research by Chan and Leal for second-order fluids [9], deformable droplets propagating in a unidirectional Poiseuille or simple shear fluid flow with low Reynolds numbers and Capillary numbers will experience a transverse shear gradient that induces cross-stream droplet migration. The direction and magnitude of the cross-stream migration, $\mu_\text{oa}$, in Poiseuille flow is described in equation 1 as a function of droplet size, $a$; interfacial tension, $\gamma$; inner fluid phase viscosity, $\mu_i$;
outer fluid phase viscosity, $\mu_o$, droplet position $y$, distance between two parallel plane walls, $d$; and the maximum fluid velocity, $V_m$, defined as $V_m = V_{avg} \cdot 3/2$, which occurs at $y=d/2$.

$$\mu_m = 16 \alpha \frac{\mu_o V_m^2 a^3}{y^2} \left(1 - 2 \frac{y}{d}\right).$$  (1)

The term $\alpha$ depends on the inner to outer fluid phase viscosity ratio, $k = \mu_i / \mu_o$, and is defined for three-dimensional quadratic shearing flow in equation 2 as follows:

$$\alpha = \frac{1}{(1+k)^2(2+3k)} \left[\frac{3}{14} \times \frac{16 + 19k}{(2+3k)} (1-k-2k^2) + \frac{10+11k}{140} (8-k+3k^2)\right].$$  (2)

Their findings described that in the presence of a viscoelastic second-order fluid in one or both fluid phases of an emulsion, a sign inversion of the term, $\alpha$, occurs between the viscosity ratio $0.5 < k < 10$ as illustrated in Figure 2. In this work we interrogate how this technique can be used to sort droplets in rectangular channels with varying flow rates, droplet sizes, viscosity ratios, $k$, Weissenberg, $Wi$, Capillary, $Ca$, and Reynolds, $Re$, numbers.

**EXPERIMENTAL**

Microfluidic devices were fabricated using traditional soft-lithography processing with channel heights ranging from 50-130 µm, widths from 100-500 µm, and lengths from 1-7 cm long, both straight and curved with a radius of curvature of 1 cm. FC-40 (Sigma, USA) with dynamic viscosity, $\mu_o$, of 4.1 cPs @ 25ºC and density, $\rho$, of 1.85 kg/m³ was selected as the continuous oil phase because of its low viscosity. This was desired because a smaller change in absolute viscosity would translate to a larger change in the k value, $\mu_i/\mu_o$. In addition, the value of 4.1 cPs is much closer to that of pure water with a dynamic viscosity of 1 cPs at 20 ºC. The interfacial tension of the two fluids were modified through the use of Krytox 157 FSL (Dupont Chemicals, USA) at 7% wt/wt and 1H,1H,2H,2H-perfluoro-1-Octanol (Sigma Aldrich, USA) at 0.05% wt/wt. The viscosity of the aqueous phase was tuned through the addition of two Newtonian viscous enhancing fluids, glycerol (EMD Chemicals, USA) and Ficoll® 400 with $M_w$ 400,000 (Sigma, USA); and two non-Newtonian additives, poly-ethylene oxide (PEO) or Polyox® WSA N-10 with $M_w$ 100,000 (Amerchol Corp., USA), and trimethyl-glycine (TMG) or betaine monohydrate (Sigma-Aldrich, USA). Fluid viscosities of the aqueous and oil phase solutions were determined using a Physica MCR301 Rheometer with a 25mm CP 25-2/S measuring cone (Anton Parr, USA) at shear rates ranging from 1/100 to 100 s⁻¹. Shear stress vs. shear rate information was characterized using the Rheoplus rheometer software.

**RESULTS AND DISCUSSION**

In figure 3 we interrogate the application of this technique for sorting droplet with viscoelastic properties, and different viscosity ratios, between the inner droplet and outer fluid phases. Viscoelastic sorting was achieved independent of size variations.

**Figure 2:** a) plot of alpha, $\alpha$, as a function of viscosity ratio, $k$, showing both sign and magnitude. Note that between the range of $0.5 < k < 10$, $\alpha$ is negative with a global minimum at $k=1$. b) Projected positive and negative droplet migration of 75 µm droplets of various viscosities. Conditions: 20 µL/min flow rate; 100x200 µm channel; $\gamma=15$ N/m; $Ca=0.008; Re=0.8$.

**Figure 3:** Equilibrium positions of droplets 1.7 cm downstream of injection point in a 100 µm tall x 200 µm wide channel. a) water, $k=0.2$ b) 70% glycerol,$k=3.5$ c) 10% Ficoll $k=1$, d)4% TMG $k=0.2$, e)1.5% Poly-ethylene-oxide (PEO) $k=3$ f)4% TMG w/ 50% glycerol $k=1$, g)10% TMG $k=0.3$, h) 5% PEO w/ 5% Ficoll $k=20$. Oil is FC-40 w/ 7% Krytox flowing at 20µL/min.
In Figure 4 we demonstrate sorting of two viscoelastic droplets containing 4% tri-methyl-glycine (TMG) with a small change in viscosity caused by adding 50% glycerol to the solution of one droplet emulsion solution. Sorting rates of 238 Hz have been achieved for monodisperse and polydisperse sized droplet populations of 40-100 µm with enrichment rates of 90%±10% using FC-40 at 40 µL/min flow rates in 100 µm tall x 400 µm wide channel at 7 cm length.

![Droplet sorting device](image)

**Figure 4:** Separation of viscoelastic droplets with different k-values at positions 1-4 in a 100 µm tall, gradually widening channel at downstream positions of 17 mm, 34 mm, 51 mm, and 70 mm. Light colored droplets contain 4% TMG, k=0.2, dark-ringed droplets contain 4% TMG and 50% glycerol, k=1.2. Equilibrium position is established early on and continues to outlet.

**CONCLUSION**

We demonstrate passive droplet sorting in microfluidic channels at varied droplet diameters, viscosities, and fluid flow rates. We demonstrate viscoelastic based differentiation between droplets having a k-value of 0.5-10 from other k values or non-Newtonian fluids at sorting rates >200 drops/s with efficiencies as high as 99% and 2cPs discrimination. Successful droplet sorting is achieved for a range of droplet sizes, and geometry profiles in both straight and curved rectangular microfluidic channels. We anticipate this sorting method to be useful for applications such as sorting of droplets containing amplified DNA product for digital PCR quantification and enrichment as well as other applications that elicit viscosity changes.

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