PASSIVE, LABEL-FREE DROPLET SORTING BY CHEMICAL COMPOSITION USING TENSIOPHORESIS

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ABSTRACT

Label-free sensing and sorting is a current challenge in droplet-based high throughput screening. Sorting droplets by chemical composition currently involves fluorescence activated sorting, which requires chemical labeling, physical actuators, and feedback control signals. This paper demonstrates passive, label-free microfluidic drop sorting based on chemical contents using tensiophoresis, the migration of droplets in an orthogonal surfactant microgradient imposed in the oil phase. Studies conducted using Bovine Serum Albumin (BSA) and sodium dodecyl sulfate (SDS) indicate that the droplet migration velocity depends on the concentration of these species in the droplet phase. Pristine droplets (Φ=500 µm) migrate at 1.9 mm/s while droplets containing 1 µM BSA concentration have negligible migration. The same trend has been observed with droplets containing SDS. On the basis of their migration, droplets can be sorted to respective outlets. The results suggests a simple and passive method which can simultaneously sense and sort droplets based on their chemical payload and thereby could be applied for screening proteins and other surface active biomolecules on a quantitative basis.

KEYWORDS

Interfacial tension, droplet, sorting, label-free detection, microgradient, surfactant

INTRODUCTION

Label-free sensing and sorting are key operations for biological screening in microreactors. With regards to droplet sorting, size-based sorting has been demonstrated using channel bifurcations [1] and a pillar array [2]. However, the only known technique for sorting based on chemical contents is fluorescent activated sorting using dielectrophoretic [3] or piezoelectric [4] actuators. These are both sensitive and fast, but they require fluorescent labeling, along with on-chip structures and an active feedback to control sorting signals. With regard to detection, absorbance detection (colorimetric) techniques are label-free but have poor sensitivity due to short optical path lengths. Label-free approaches like Raman spectroscopy have recently been applied to droplets [5] but require complex instrumentation and long integration times. This paper reports a passive, label-free droplet sorting technique which has the unique ability to sort droplets by their interfacial tension (IFT), without chemical labels or on-chip actuators. IFT based sensing and sorting has significant potential in biological assays, as interfacial properties are sensitive to protein concentration, enzymatic activity, pH, and other chemical parameters.

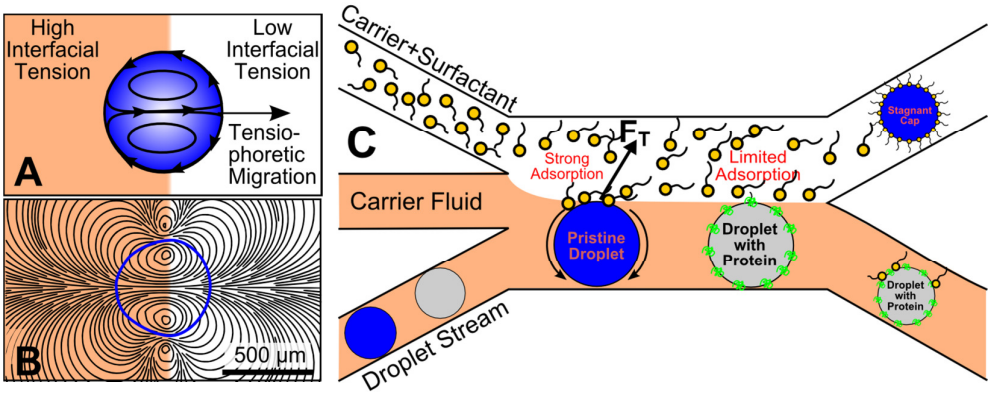

Fig. 1: *Concept of label-free sorting using tensiophoresis*. (A) Droplet migration in an IFT gradient. (B) CFD simulation. (C) Schematic of chip used for sorting droplets based on chemical contents.

THEORY

When a droplet encounters an interfacial tension (IFT) gradient, it migrates towards the region of low IFT to minimize its overall surface energy [6] (**Fig. 1A&B**). The nonuniform IFT across the droplet generates interfacial Marangoni flow which drives migration opposite the IFT gradient vector. In a microfluidic channel, a sharp, controlled profile in IFT can be generated by flowing two parallel streams of oil with different surfactant concentration [7] (**Fig. 2A**). When a droplet is introduced into the high IFT (low surfactant) stream, it will migrate to the low IFT (high surfactant) stream at a rate proportional to its surface energy. Proteins, biomolecules, salts, or other surface active agents present in the droplet adsorb to the droplet interface, reducing its surface energy [6] and its migration velocity. The adsorbed species compete with adsorption of surfactants from the oil, thus reducing the migration velocity. This can also be described in terms of the droplet's IFT: a surface active molecule not only

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reduces droplet's IFT, but also reduces its sensitivity to a surfactant gradient. Therefore, when placed in a surfactant concentration profile, droplets containing surface active agents (low IFT) will migrate slower than pristine droplets (high IFT). If the surface active agent is at a sufficiently large concentration, it will inhibit migration of the droplet completely. In this way, the droplet's migration velocity can serve as a physical indicator of its chemical composition. Depending on their migration velocity, the droplets can be passively sorted in the separation channel and captured in separate outlets at the far end (**Fig. 1C**).

EXPERIMENTAL SETUP

To characterize the label-free sorting of droplets, a tertiary microfluidic junction with an 800 µm wide sorting channel (**Fig. 2A**) was fabricated in polydimethylsiloxane (PDMS) using soft lithography. Droplets with a range of concentrations of BSA/SDS were injected through the lower inlet while the middle and top inlets were used to inject pure oil (oleic acid) and oil-surfactant (Span 80) mixture, respectively. Droplet migration was visualized in fluorescence or bright field using a high speed digital camera. The flow velocity, migration profile, settling position and deformation of the droplet were measured using droplet tracking velocimetry (DTV), a custom image processing software described in [8].

RESULTS AND DISCUSSION

Droplet Migration. **Fig. 2** illustrates the quantitative analysis of droplet migration in a binary surfactant gradient. The migration velocity depends on the surfactant concentration in the upper stream as well as the size of the droplet. To demonstrate this, we track the trajectories of a 350 µm droplet subjected to various surfactant concentrations in the upper stream (**Fig. 2C**). When the concentration is less than the critical micelle concentration (CMC, 20% v/v), the droplets migrate completely into the upper stream. Beyond the CMC, the formation of the stagnant cap prevents complete migration, which results in the droplet settling at the interface [7]. The variation of drop migration velocity and deformation with surfactant concentration is shown in **Fig. 2D&E,** respectively. Migration velocity increases rapidly with surfactant concentration until the CMC, beyond which it remains almost constant **(Fig. 2D)**. The deformation of the droplet during migration also increases with surfactant concentration, due to the non-uniform capillary pressure build up across the droplet **(Fig. 2E)**.

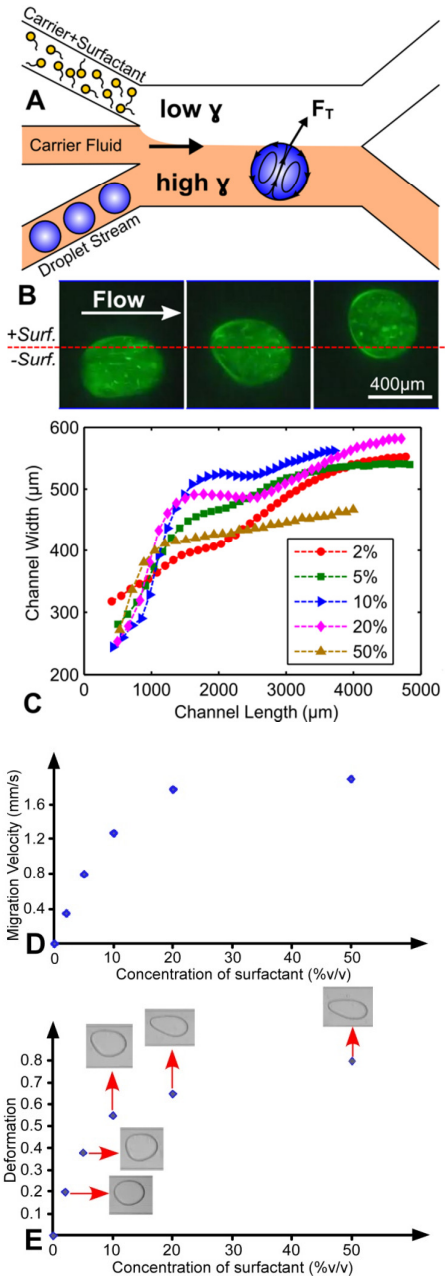

Fig. 2: *Capillary migration of droplets in a binary IFT gradient*. (A) Chip schematic. (B) Fluorescent image of migrating droplet. (C) Droplet trajectories at various surfactant concentrations. (D) Migration velocity and (E) droplet deformation at various surfactant concentrations.

Since the migration velocity is proportional to the IFT gradient, this technique can be used to sort the droplets based on the presence of surface active agents. We have demonstrated this concept using water-in-oleic acid droplets containing sodium dodecyl sulfate (SDS) **(Fig. 3)** and bovine serum albumin (BSA) **(Fig. 4 & 5)**.

Droplet Sorting Based on SDS Concentration. **Fig. 3** illustrates the label free sorting of droplets based on SDS concentration. Droplets containing DI water or 2.3mM SDS solutions are injected through the lower inlet at the flow rate of 1-3 mL/hr. The upper half of the sorting channel contains oleic acid + 5% Span 85, while the lower half contains the droplets in pure oleic acid. Pristine droplets initially migrate slowly towards the interface, and upon contact they quickly traverse the boundary to the upper stream. By contrast, droplets containing SDS do not migrate. The putative cause for the difference in migration profiles is shown in Fig. 3A. SDS (green) adsorbs to the interface, preventing adsorption of the external surfactant (orange). This precludes the formation of an IFT gradient across the droplet, which is necessary for migration. Based on their migration, pristine droplets and SDS droplets collect in outlets A and B, respectively.

Droplet Sorting Based on BSA Concentration. **Fig. 4** illustrates droplet sorting by protein concentration. In this experiment, we inject ~550 µm aqueous droplets containing various concentrations of BSA in an 800 µm sorting channel. The sorting channel contains two parallel streams of oleic acid, the upper with 10% v/v Span 85 surfactant, and the lower stream with no surfactant. The total flow rate of all three phases is 25 µL/s. Droplet

migration profiles are imaged by overlaying subsequent frames. Droplets containing no protein experience sharp migration at velocities up to 2 mm/s, while droplets containing 125 nm BSA experience slower or more gradual migration. The reason for the difference is the same as described for SDS because both molecules adsorb to the interface. **Fig. 5** shows the decrease in migration velocity with increasing protein content (blue). Also shown is the IFT of a bulk protein solution. The migration velocity becomes zero at about 50 nM, close to the knee of the IFT curve. This defines the upper working range of this technique. It is interesting to note that this corresponds to 7 femtomoles of protein in the droplet.

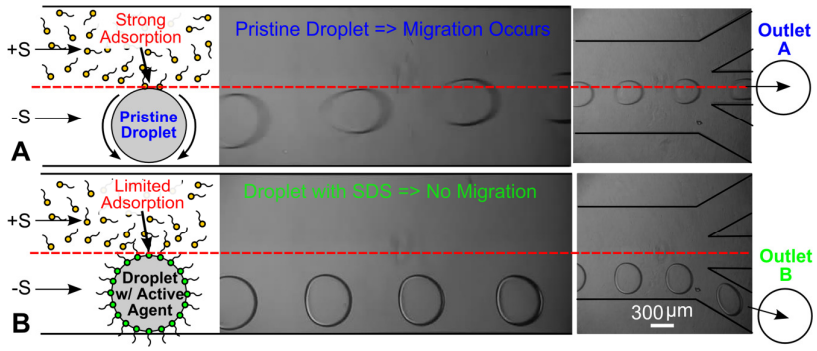

Fig.3: *Label-free droplet sorting by chemical composition*. (A) Pure droplets migrate steadily and collects at outlet A. (B) Droplet containing SDS (2.3mM) do not migrate and are sorted to outlet B.

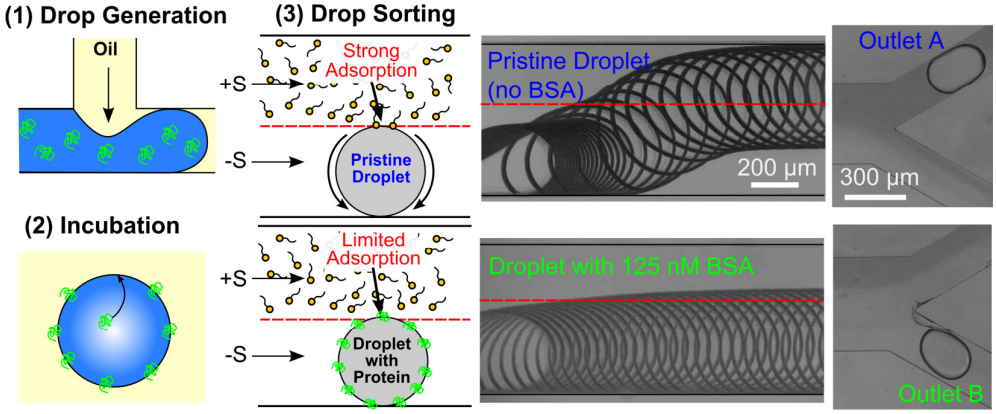

Fig 4*: Label free droplet sorting by protein concentration*. Protein sorting is accomplished in 3 steps (drop generation, incubation, and sorting). The images show the migration profile of a pristine droplet (top) versus a droplet containing 125 nM BSA in a binary surfactant profile. The migration velocity is measured near the inlet using image processing software. The two types of droplets are sorted into respective outlets A and B.

CONCLUSION

In this paper, we used tensiophoresis as label-free method to sense protein and other surface active agents in droplets based on their migration velocity in a surfactant gradient. Since many biomolecules are known to adsorb to interfaces, tensiophoresis can potentially serve as a simple, passive, and sensitive method for sensing and sorting in in a variety of biochemical screening assays.

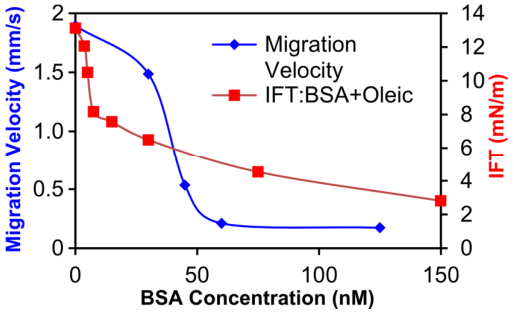

Fig. 5: Droplet migration velocity as a function of protein content within the droplet (Blue) and interfacial tension vs. protein concentration measured using a traditional pendant drop method (Red).

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