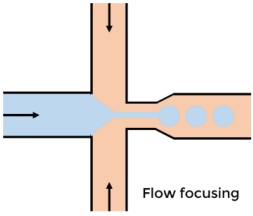
Tutorial 01
Droplet generation and Thermostability

Straightforward production of monodisperse water-in-oil droplets stabilized by FluoSurf surfactant and evaluation of thermostability.

Context

Water-in-oil emulsion droplets produced with microfluidic devices have become widely used as screening tools for biological and chemical applications including material synthesis, single-cell analysis, digital polymerase chain reaction assays (dPCR) or in vitro diagnostics. Droplet microfluidics enable chemical and biological experiments to be performed at high-throughput in a confined and controlled environment with enhanced efficiency and using minimal amount of starting material.



Droplet generation in microfluidics is based on the use of two immiscible phases that are most commonly an oil phase and an aqueous phase. In the case of water-in-oil droplets, a surfactant is added to the oil phase to allow droplet stabilization. In the present tutorial, we report on the production of water-in-oil emulsion droplets, using **FluoSurf** surfactant in a non-commercial flow-focusing PDMS device.

Material and Methods

Continuous and dispersed phase preparation

The oil continuous and extraction phases were prepared by dissolving 4wt/wt% neat FluoSurf surfactant in FluoOil-7500.
The dispersed aqueous phase used for this experiment was ultrapure water.

Microfluidic set-up

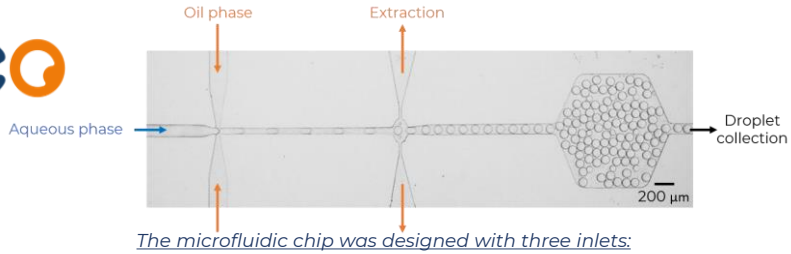
The microfluidic chip was connected to 1mL plastic syringes containing the different phases via PTFE tubing (0.3x0.76MM) and the outlet was connected to a plastic vial to collect the generated emulsion. Syringe pumps were used to control the flow rate of the different phases in the microfluidic device,:

- Oil continuous phase: 300 µL/hr
- Aqueous dispersed phase: 100 µL/hr
- Oil extraction phase: - 150 µL/hr

Droplet generation was monitored using an inverted microscope.

PDMS chip preparation

A microfluidic polydimethylsiloxane (PDMS) flow-focusing device was fabricated according to standard protocols using soft lithography. The device was then permanently bonded to a microscope glass side after activation with plasma treatment.
After plasma activation, the PDMS is oxidized and thus becomes slightly hydrophilic. As channels must be non-wetting to the inner aqueous phase, a surface treatment with FluoST was performed to ensure surface hydrophobicity. Briefly, the channels were purged with argon and then filled with FluoST via a glass syringe. FluoST was then flushed from the channels with argon and the device was left to heat at 65°C for a minimum of two hours before use.



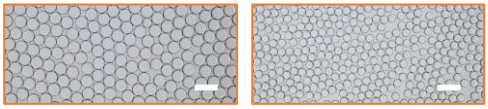
The microfluidic chip was designed with three inlets:

- *Aqueous dispersed phase*
- *Oil continuous phase (FluoSurf 4w/w% in FluoOil-7500)*
- *Extraction oil phase (optional): remove the excess of oil and concentrate the droplets.*

Droplet generation

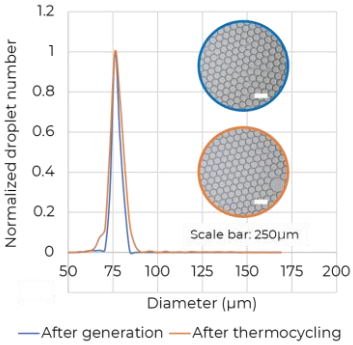
1) **Stabilization of the system** : the different phases are flowed through the device to make sure air bubbles are completely extracted from the chip.
2) **Droplet generation** : Once the flow is stabilized and droplets start to be homogeneously generated, a vial can be placed under the outlet tubing for collection. With the flow parameters defined in the material and methods and thanks to FluoSurf surfactant (4 wt/wt%), stable monodisperse droplets can be generated and collected for as long as there is a supply of continuous and dispersed phase through the inlet syringes.
3) **Size tuning :** droplet size can be tuned by varying the oil and aqueous phases flow rates:

125µm (1.0nL) droplets
Aqueous phase: 100µL/hr
Oil phase: 300µL/hr
Extraction phase: -150µL/hr



75µm (0.2nL) droplets
Aqueous phase: 50µL/hr
Oil phase: 900µL/hr
Extraction phase: -700µL/hr

Thermostability of microfluidic droplets



Two different emulsion samples were collected. The first one was directly re-injected into a non-commercial glass chamber. The second one was submitted to a classical PCR thermocycling process with 30 heating cycles [98°C 10s, 50°C 5s, 72°C 10s] to evaluate droplet thermo-stability. A population of about 10000 droplets was statistically analyzed with the ImageJ software to determine the average size before and after heating cycles.

➔ Droplet size distribution before and after thermocycling remains homogeneous, with the exception of few larger droplets. The average mean diameter remains the same (125 µm (1.0 nL)). From the statistical analysis, it was calculated that after heating cycles, only 2.5% of the overall droplet population had a size that differed by more than 10% of the average initial droplet diameter.

Conclusion

FluoSurf surfactant allows to generate stable, monodisperse droplets that can be used for PCR-based applications (or any other microfluidic application that requires production of stable droplets) and stored for weeks without destabilization. FluoSurf has proven to be a reliable ally for reproducible droplet-based microfluidic experiments.

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For any questions or additional information on microfluidic droplet generation or on our products, please do not hesitate to contact us at contact@emulseo.com.