FLUIGENT

DOUBLE EMULSION

INTRODUCTION

A double emulsion is defined as a droplet encapsulated within a larger droplet of different immiscible phases. Making double emulsions is a promising method for many researchers[1] [2] as industrial fields such as in cosmetics[3] [4] [5], in food applications, food supplement encapsulation[6] [7], in pharmaceutics for drug delivery and others[8] [9].

Though this is a promising method for many applications, conventional technologies to make double emulsion, such as batch methods suffer from various limitations[6] [8].

Microfluidics is a promising technology for making double emulsions as it allows one to have better control over the sizes and mixtures. This leads to better size distribution, provides automation possibilities.

Historically, the physical properties of the chip materials have been a limitation for double emulsions as they have required chemical coatings on the chip or multiple chips. This can lead to additional cost as well as reduce the useful life of the chip.

In this application note we present you a method which allows one to easily produce double emulsions with any type of solutions, without chemical surface treatments needed , on one single device (Raydrop).

Different examples of double emulsions (w/o/w and o/w/o droplets) are presented to show the versatility and use of the Raydrop.

This application note was made in collaboration with Secoya

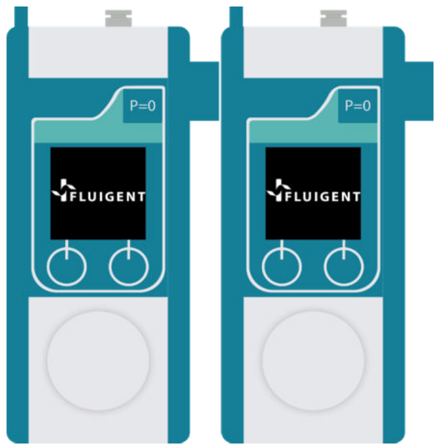
MATERIAL

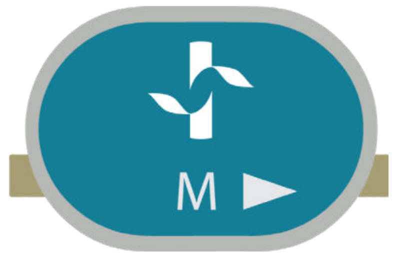
Reagents:

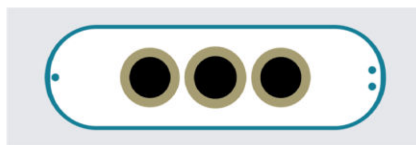
- **Dispersed phase:** Distilled Water and Fluorescein (Sigma aldrich).
- **Continuous phase:** 2Different continuous phases have been used during each experiment depending on the type of emulsion desired:
  - HFE-7500 (Fluorochem)
  - Mineral oil (Sigma Aldrich)
  - dSURF (Fluigent)
  - Tween 20 (Sigma aldrich)
  - Span 80 (Sigma aldrich)

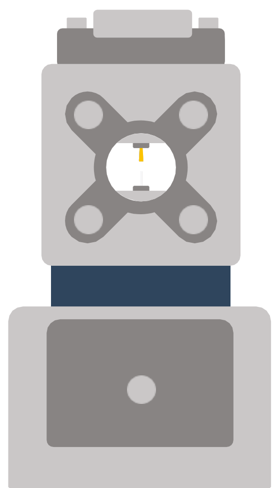
Products/Instrument:

- **Microfluidic flow controller:** The Flow EZ is the most advanced flow controller for pressure-based fluid control. It can be combined with a Flow Unit to control pressure or flow rate. It can be used without a PC. Two Flow EZ with 2 bar of full scale pressure are used in the setup presented here.
- **Flow sensor:** The Flow Unit is a flow sensor that allows real time flow rate measurement. By combining a Flow Unit with the Flow EZ, it is possible to switch from pressure control to flow rate control, allowing for the generation of highly monodispersed droplets over a long period of time. Two Flow Units M are used here to monitor and control the flow rates of the dispersed and continuous phase during the run.
- **2-Switch:** Easy to use 3-port/2-way microfluidic valve is used to switch between water and alginate solution. This allows to have better reproducibility by priming the system and add cleaning step during the experiment.
- **Droplet generator:** The RayDrop droplet and emulsion chip is used to control the generation of alginate droplets. This is based on the alignment of two capillaries immersed in a pressurized chamber containing the continuous phase. The dispersed phase exits one of the capillaries through a 3D-printed nozzle, placed in front of the extraction capillary for collecting the droplets. This non-embedded implementation of an axisymmetric flow-focusing is referred to co-flow-focusing. This geometry which is leading the droplet formation removes all wettability issues that could appear in other chips. It allows one to generate highly monodispersed emulsions with any kind of fluids including alginate.









APPLICATION NOTE

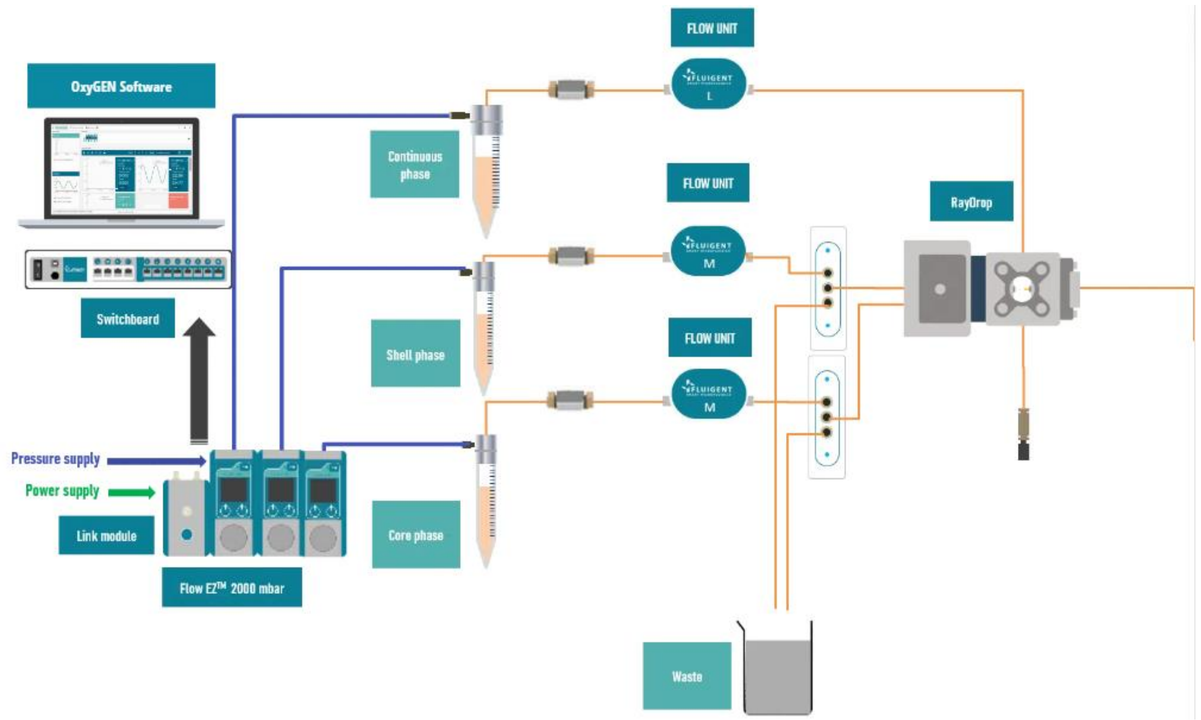


Figure 1: Experimental set-up toproduce double emulsion

Flow EZ™ are used to pressurize the different fluids.

The flow rate of the continuous phase is measured using an L flow-unit from Fluigent while the Shell phase and the Core phase flow rates are measured using M flow-units. Flow-units are calibrated by weghing method.

To facilitate the start-up of the double emulsion process, 2 switch™ automatic valves are placed on the Shell phase channel and on the Core phase channel.

To avoid any clogging of the double-nozzle, an inline filter A-430 from IDEX with a porosity of 2 µm is installed on each phase channel. Double emulsion production is observed with an IDT Y3-pro high-speed camera.

Before doing any experiments, fluids are filtered with a 0.2 µm syringe filter.

A specific setup has been designed to be able to generate double emulsion of fluids presented. However, depending on the combination of fluids used, the hydraulic resistance on each channel has to be adapted in order to maintain a larger pressure drop to the Continuous phase than on the two others and a larger pressure drop on the Shell phase than the Core phase.

RESULTS

Water in oil in water (W/O/W)

During the experiments, the different flow rates are changed to produce either core-shell double-emulsion or double-emulsion with a large shell. The maximum thickness for the shell will strongly depends on the fluid configuration. Similarly, the production rates attainable will also strongly depend on the fluid configuration.

Even though it is possible to produce double emulsion without any surfactant, it's recommended to add it to the Continuous and Shell phases in order to recover the double emulsion produced after formation with good stability. Surfactant can also be used within the core phase, but it is rarely needed.

dSURF Shell

In this first series of experiment, the fluids used are:

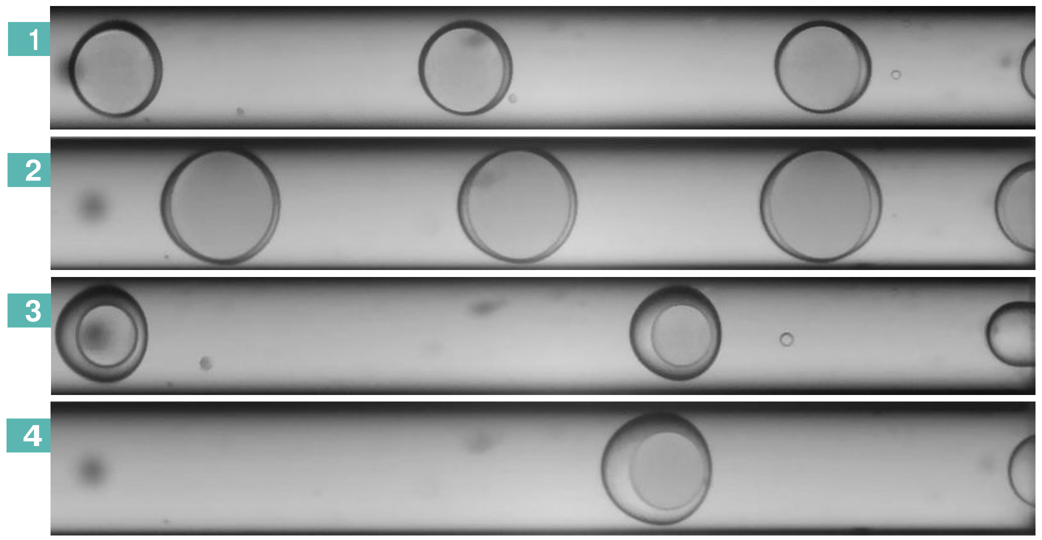
Continuous phase	Water+1% Tween 20
Shell phase	dSURF (HFE +2% Fluigent surfactant)
Core phase	Water+0.5% Fluorescein

Core-shell double emulsion

	Droplet size	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Frequency (Hz)	Production Core phase (mL/h)
1	120 µm (min)	558	1600	9.7	551	75.6	593	1938	4.5
2	155 µm (max)	116	520	4.0	360	33.9	290	600	2.0

Large Shell double emulsion

	Droplet size	Core size (µm)	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Freq. (Hz)	Production Core phase (mL/h)
3	122 µm (min)	78	560	1580	18.5	760	16	347	1063	1
4	134 µm (max)	98	327	1010	6.9	415	7.2	220	267	0.43



Mineral oil light shell

In this second series of experiment, the fluids used are:

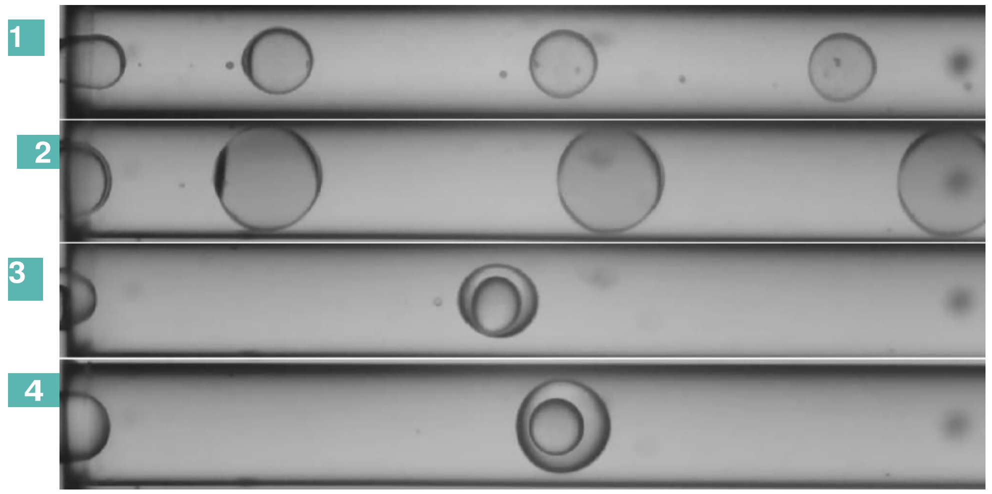
Continuous phase	Water+1% Tween 20
Shell phase	Mineral oil light + 2% Span 80
Core phase	Water+0.5% Fluorescein

Core-shell double emulsion

	Droplet size	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Frequency (Hz)	Production Core phase (mL/h)
1	96 (min)	913	1640		1797	75.6	797	508	4.5
2	152 (mw)	83	160		322	12.6	130	217	0.76

Large Shell double emulsion

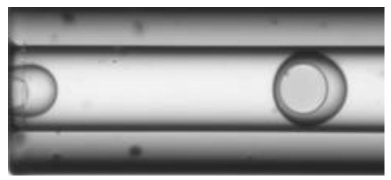
	Droplet size	Core size (µm)	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Freq. (Hz)	Production Core phase (mL/h)
3	108 (min)	77	327	570		816	3	158	306	0.18
4	135 (max)	78	85	154		421	1.0	50	73	0.06



Other example of shell phase

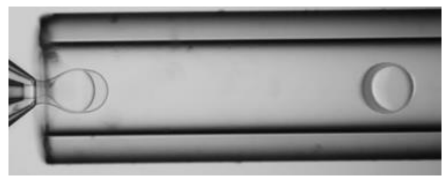
FC40

Continuous phase	Water+1% Tween 20
Shell phase	FC40+2% Krytox 157FSH
Core phase	Water+0.5% Fluorescein



Ethyl Acetate

Continuous phase	Water+1% PVA
Shell phase	Ethyl Acetate
Core phase	Water



APPLICATION NOTE

Oil in water in oil (W/O/W)

In this first series of experiment, the fluids used are:

Continuous phase	Mineral oil light + 1% Span 80
Shell phase	Water+2% Tween 20
Core phase	Mineral oil light

Core-shell double emulsion

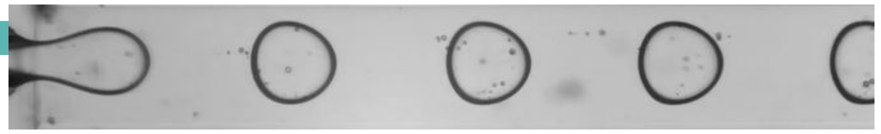
	Droplet size	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Frequency (Hz)	Production Core phase (mL/h)
1	115	72.3	790	1.3	530		2100	292	4.5

Large Shell double emulsion

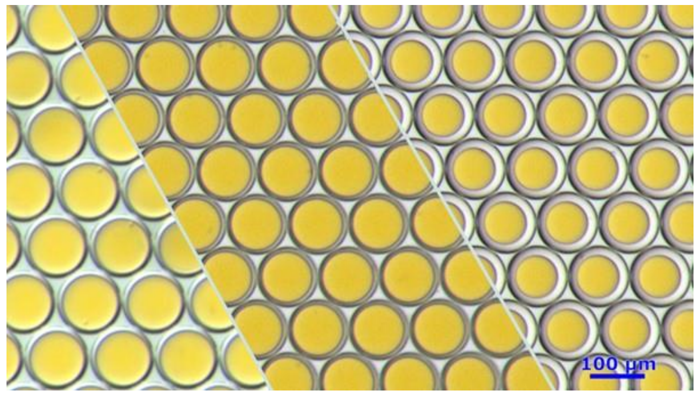
	Droplet size	Core size (µm)	Qc (µl/min)	P (mBar)	Qs (µl/min)	P (mBar)	Q_{core} (µl/min)	P (mBar)	Freq. (Hz)	Production Core phase (mL/h)
2	122	76	43.2	400	2.6	301		600	109	0.18

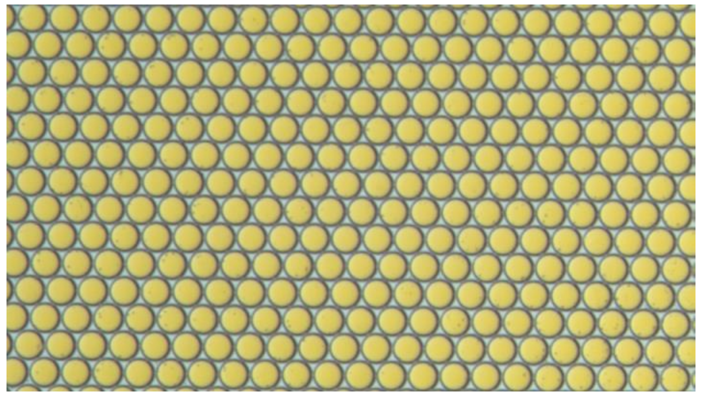
1

2



Monodispersity Analysis





After generation, double emulsion have been recovered for size measurements and monodispersity analysis.

Double emulsions have been produced with different fluids and different core and shell sizes while always maintaining a high monodispersity with a coefficient variation lower than 2%.

CONCLUSION

In this application note we have demonstrated how we can produced in a robust different type of double emulsion with a single device.

Precisecontroloftheflowallowsalsotogeneratehighmonodispersity (≈2% size dispersion) double emulsion at different frequency to target different volume production.

Example with common fluids such as water, mineral oils, fluorocarbon oils and solvents have been demonstrated here. This method can also be used for production of double emulsion with specific fluids such as polymer (PLGA, PLA,…), hydrogel (Alginate, agarose,…) and other.

As a consequence this method is a promising method for different market and could be used in order to get rid of limitation of current technologies such as batch methods and other for different application such as in pharma, food or cosmetic.

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