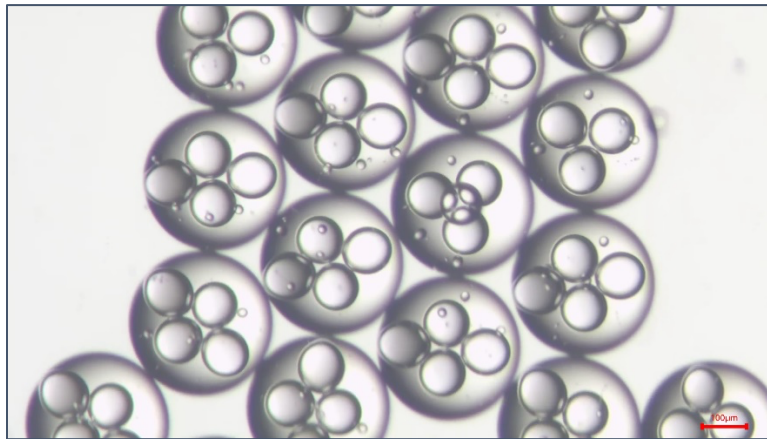# MULTIPLE EMULSIONS CREATION

Application note

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I) Introduction

Traditionally, batch methods are used to produce emulsions in industry. The use of bulk mixing allows to produce huge quantities of emulsions but to the detriment of quality. Indeed, the shear distribution in a bulk mixer is various, leading to numerous particles sizes and a low encapsulation rate in the case of API encapsulation or double emulsion production (core-shell particles). [I] A fortiori, batch method makes the control of multiple emulsions more complex, i.e. the encapsulation of a precise number of droplets of liquid A in a droplet of liquid B.

In general, the use of microfluidics helps to reach low size dispersity and so monodispersed emulsions with a high control over both the size and structure can be obtained. [II] Microfluidic tools are also used to create emulsions of varying compositions. With this technology, it is possible to produce water–in-oil–in-water (W/O/W) emulsions or oil–in-water–in-oil (O/W/O) emulsions. A microfluidic device developed by Secoya Technologies - called the RayDrop® - allows to easily produce such highly controlled emulsions. Examples of applications in double emulsion can be found in the white paper entitled Generation of microcapsules, available on our website https://secoya-tech.com/documents/ .

Inspired by the publication of LI, Er Qiang and al. [III], we wanted to demonstrate the possibility to produce multiple emulsions using the RayDrop®. For instance, these multiple emulsions are precursors in the creation of solid microcapsules used for triggered release. [I] Furthermore, these multicompartmental microspheres are interesting to co-encapsulate incompatible solutions (which would react if they were in contact). [IV]

In this Application Note, aqueous droplets (called "core") in an oily droplet (called "shell") are obtained using the combination of two RayDrop® devices placed in series. The influence of the fluidic parameters on the number of cores contained in the oily shell is underlined in this application note.

II) Materials and methods
1) Materials

Core phase:

- o   Water containing 2% Tween® 20 (viscous liquid, Sigma-Aldrich)

Shell phase:

- o   Mineral oil light (Sigma-Aldrich) containing 2% ABIL® EM 90 (Evonik) and 0.08% Bromocresol Purple (Sigma-Aldrich)

Here, the bromocresol is used as a dye.

Continuous phase:

- o   Solution of 70% wt glycerol (>99.5%, Sigma-Aldrich) in water with 2% Tween® 20 (Sigma-Aldrich)

Glycerol is added in the continuous phase to increase its viscosity. The viscosity of the continuous phase influences the droplet formation in the microfluidic device and so the number of cores in shell droplets. It is also possible to work with pure water at low viscosity, but the reachable number of cores would be decreased.

2) Platform device

The production of droplets is performed with the RayDrop Platform, a lab equipment integrating all the components needed to produce simple and double emulsions using the RayDrop®. This platform, presented Figure 2, is divided into three parts: mechanics, fluidics and optics. More information about this platform can be found on the platform webpage available on our website at https://secoya-tech.com/products/raydrop-platform/. In Figure 1 as schematic representation is shown of the particular setup used for this work. Please notice the exceptional placement of a second RayDrop® – R2 – next to the first RayDrop® R1.
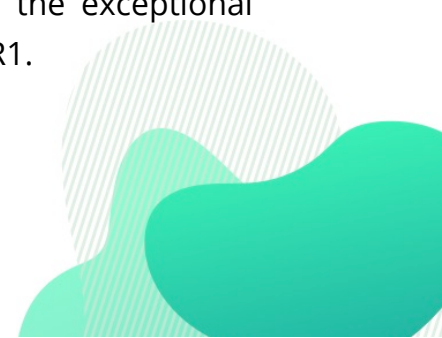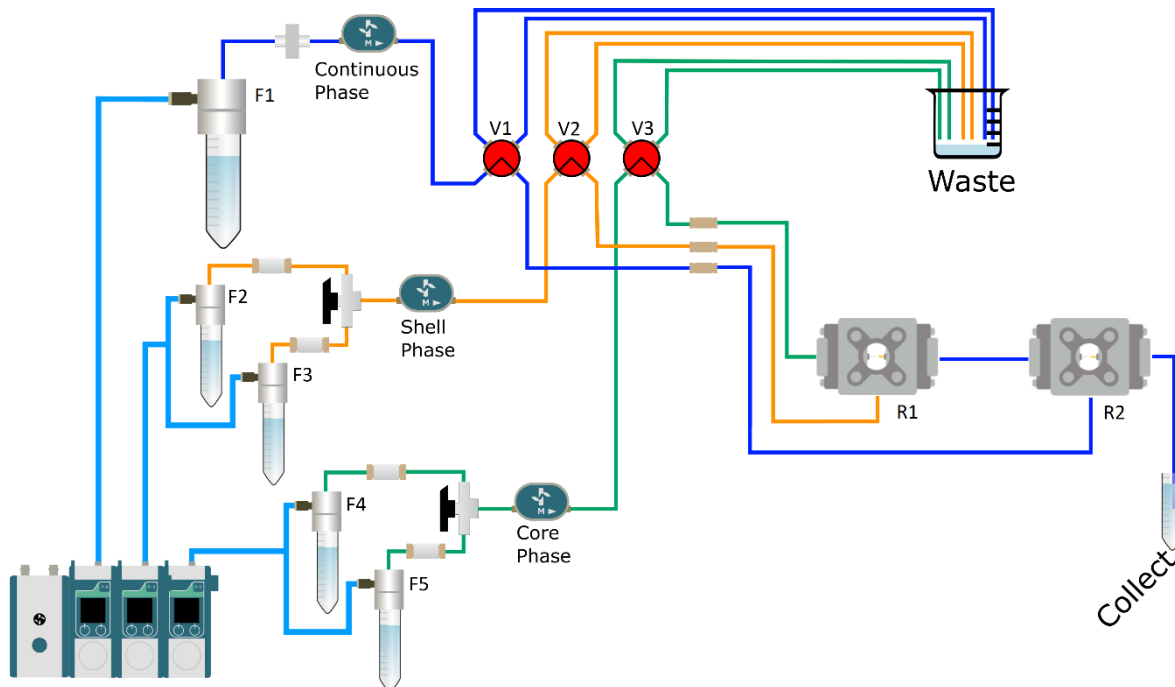
Figure 1: Experimental set-up to produce double emulsion with multiple cores inside a single shell

Fluid reservoirs

Falcon identification	F1	F2	F3	F4	F5
Volume (mL)	50	25	25	25	25
Phase[1]	Continuous	Shell (priming and cleaning)	Shell	Core (priming and cleaning)	Core
Composition	70% glycerol + 30 water + 2% Tween 20	- Not used in this application note -	Mineral oil light + 2% ABIL 90 + 0.08% Bromocresol	- Not used in this application note -	Water + 2% Tween 20

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[1] *Each phase is filtered in order to avoid clogging the tubing or the nozzle of the RayDrop®. Therefore, there is an integrated filter after each Falcon on the platform. In this case, the continuous phase filter has a 10 µm filter pore size and the shell and core filters have a 2 µm filter pore size.*
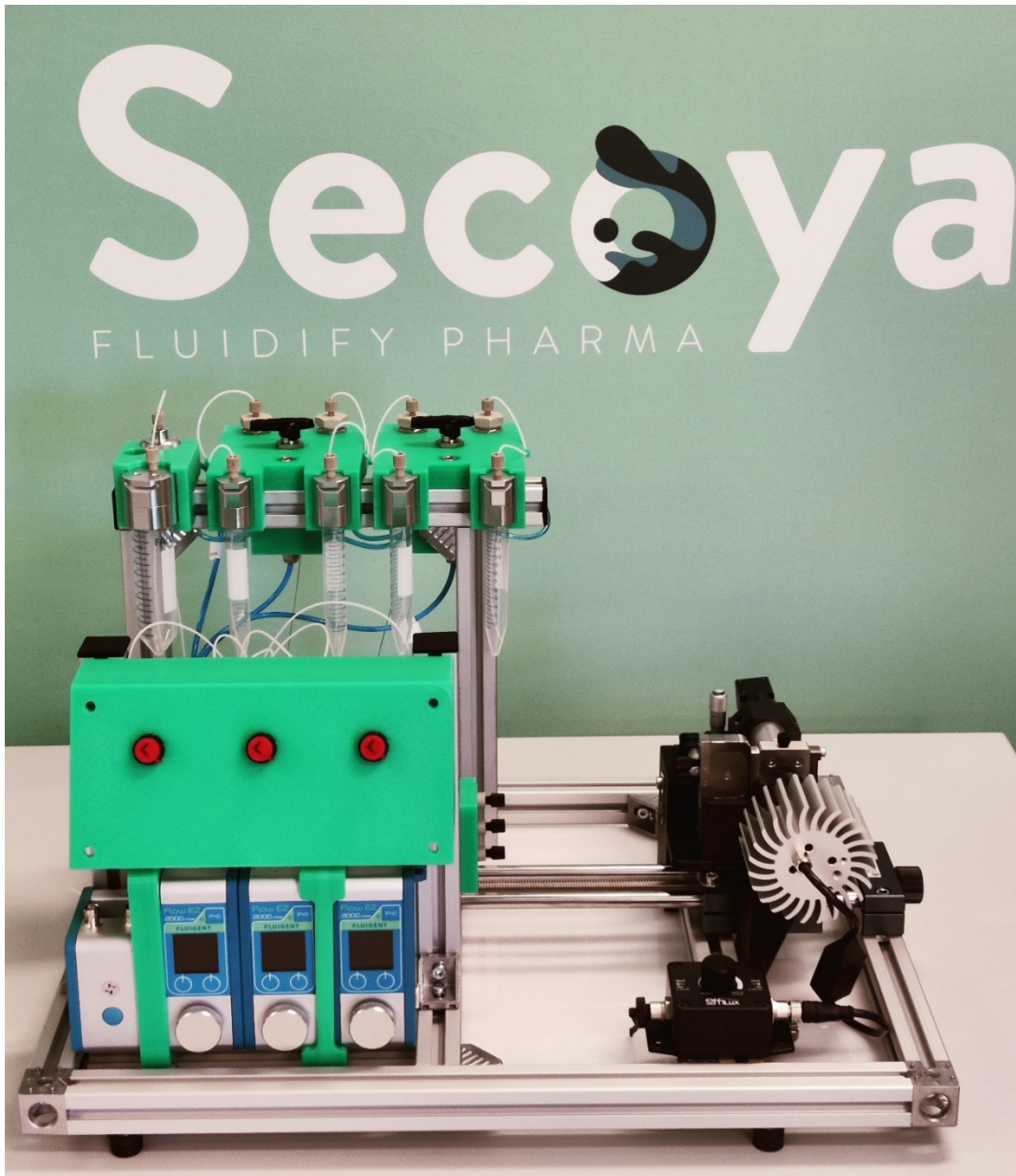
Figure 2: RayDrop Platform

- **Mechanics:** The mechanical part includes x-y-z displacement plates that allow to adjust the focus and the observation window in the RayDrop®.
- **Fluidics:** The fluidic part consists of flowrate controllers along with the required tubing and valves, allowing for automated fluidic injection. A pressure is set on each reservoir, and fluids are injected into the microfluidic chip. It also

includes Falcon reservoirs and the RayDrop®, in which emulsions are generated. After each reservoir, a filter prevents impurities from reaching the RayDrop®.

- **Optics:** The optical part of the platform contains a LED light source and a color USB 3.0 camera. This camera is connected to a computer to observe the droplet formation in live, control the stability of the emulsion and measure the size of interest (core, shell).

3) RayDrop® configuration

The RayDrop® is Secoya's microfluidic droplet generator. This emulsification device is based on the alignment in a metallic cavity of two glass capillaries: the first one is terminated by a 3D printed nozzle and injects the droplet phase in the second one (see Figure 3). At the junction of both capillaries, the continuous phase filling the cavity pinches the jet of droplet phase, leading to the formation of droplets in a highly reproducible way. More information on the RayDrop® can be found in the publication of Dewandre Adrien and al. [V].
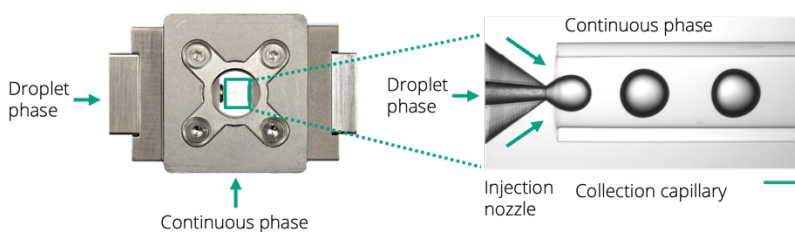

Figure 3: Working principle of the RayDrop® simple emulsion device

Specifically for this application note, two RayDrop® are placed in series. The first RayDrop® is placed on the platform as usual and the second is added after, as shown in Figure 1 and in more detail in Figure 4. To control the formation of droplets inside the second RayDrop®, an additional camera and an additional LED light source are used.

The droplet formed in RayDrop® 1 (R1) are transported in the "shell" phase (see Figure 4) in RayDrop® 2 (R2) where the "shell" phase is pinched by the continuous phase, leading to the formation of a shell droplet encapsulating multiple core droplets.

The range of droplets size formed in a RayDrop® depends on the dimension of both the injection nozzle and collection capillary. The dimensions used for this application note are reported in Table 1.
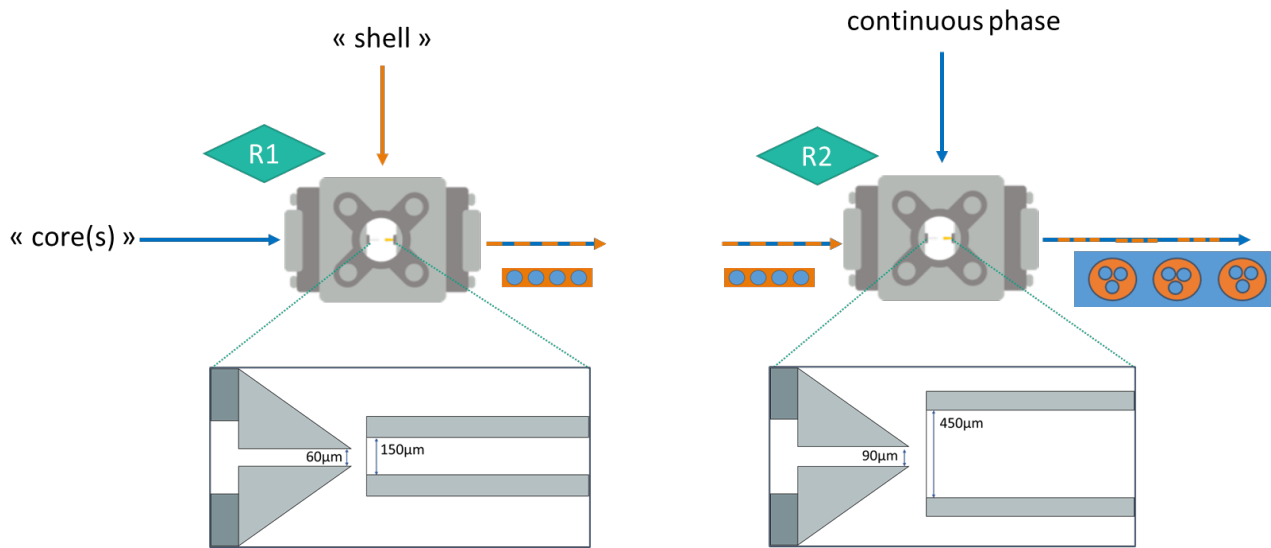

Figure 4: Two RayDrop® in series with nozzles dimensions

Nozzle information

Part	RayDrop®	Nozzle size	Collection capillary size
Inside diameter (µm)	1	60	150
	2	90	450

Table 1: Nozzles and collection capillaries dimensions

4) Emulsion generation

In this application note, liquid double emulsions with multiple cores are formed. To generate droplets, the protocol below can be followed.

Let the two RayDrop® separated, which means that there is no tubing between the outlet of the first RayDrop® and the second RayDrop®, to fill them in one by one.

1. Set the valve V2 on the Falcon F3 which contain the shell solution
2. Set the valve V3 on the Falcon F5 which contain the core solution
3. Fill the RayDrop® R1 with the shell phase (refer to the user guide for more precisions)
4. Fill the RayDrop® R2 with the continuous phase (refer to the user guide for more precisions)
5. Connect the two filled RayDrop® together with a tubing
6. Carefully set the continuous phase to a low flow rate (for example Q_{conti}=40 µL/min) and check that there is no backflow in the first RayDrop®
7. Set the shell phase to a low flow rate (for example Q_{shell}=7 µL/min with $Q_{shell}<Q_{conti}$) to create a simple emulsion oil in water observable in the second RayDrop®
8. Set the core phase to a low flow rate (for example Q_{core}=1 µL/min) to generate a simple emulsion in R1 which leads to a multiple emulsion in R2

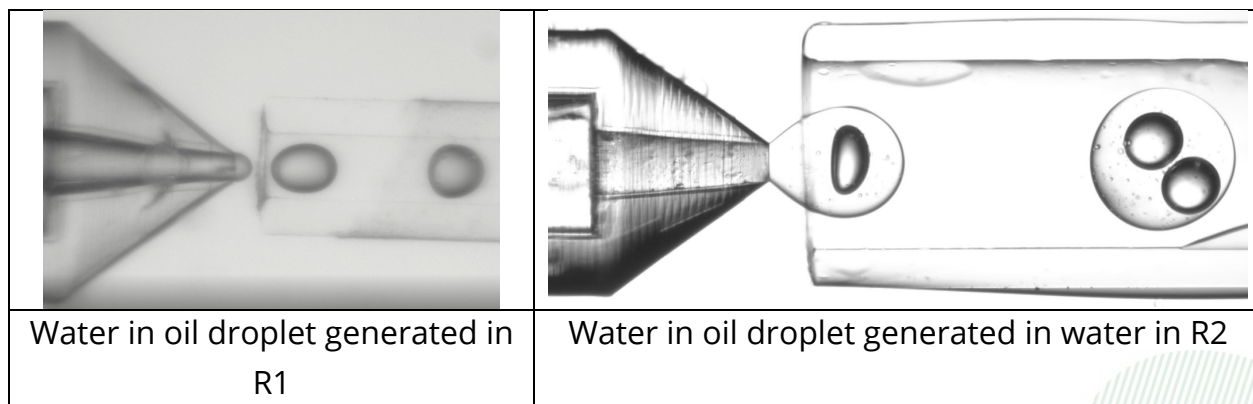| Water in oil droplet generated in R1	Water in oil droplet generated in water in R2

Figure 5: Inserts and extraction capillaries to produce multiple emulsions

9. The multiple emulsion is now produced. The dripping mode, as shown in Figure 5, provides a high stability on the droplets' formation. Thus, generated droplets have good reproducibility. It is possible to vary the flowrates to change the size of the emulsion as well as the number of cores (see the following part for more details)

III) Results

Overview: various number of cores in a droplet

By changing the flowrates of the phases, it is possible to choose the number of cores in one droplet. Figure 6 shows an overview of the achievable configurations: from a unique core to six cores.
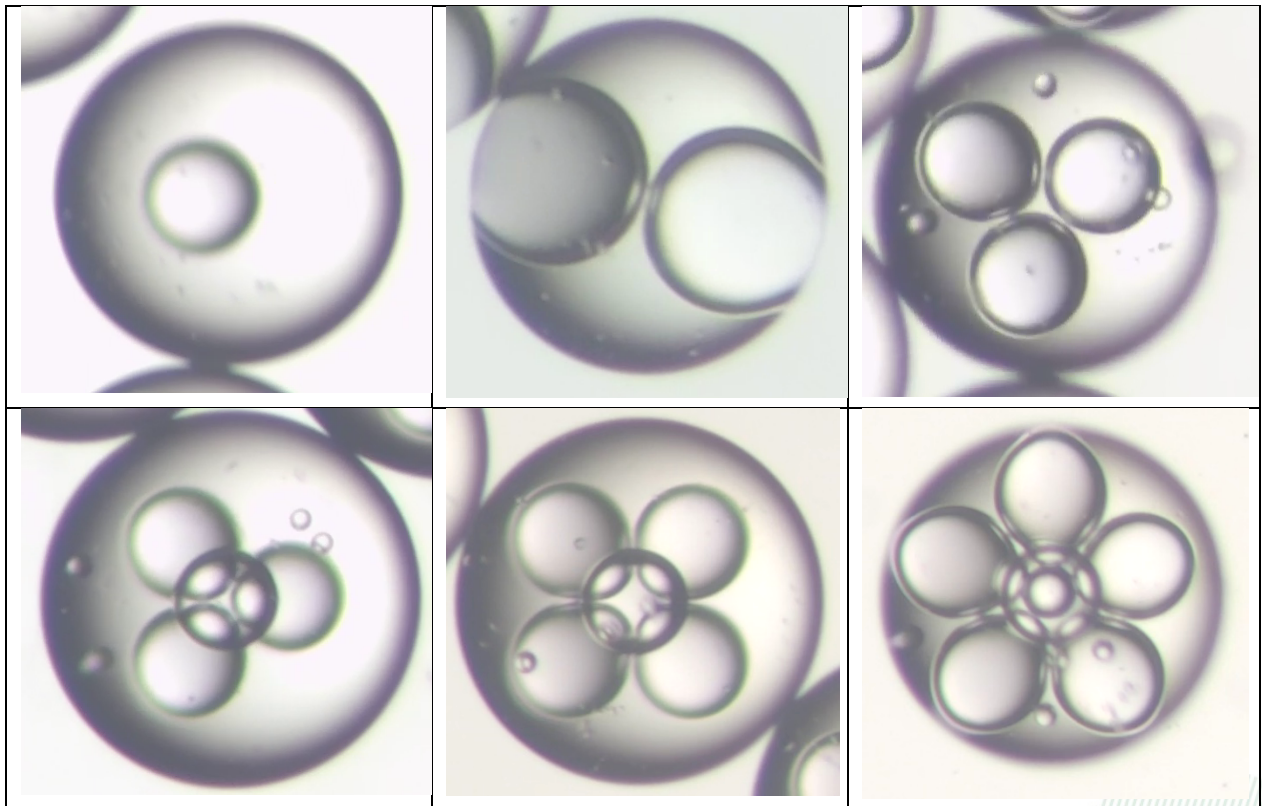

Figure 6: Images of multiple emulsions obtained at different fluids flowrates to vary the number of cores in each droplet

Evolution of the number of cores in each droplet depending on the core flowrate

During the formation of the multiple emulsions in the second RayDrop®, the flowrates of the shell and continuous phases are fixed at values reported in Table 2. Thus, the core phase flowrate is changed. For each flowrate, an analysis of the number of cores in around 50 droplets is performed and these measurements lead to the histogram presented in Figure 7.

	Continuous phase	Shell	Core
Composition	glycerol 70% + water 30% + 2% Tween 20	mineral oil + Abil 90 2% + 0.08% Bromocresol	Water + 2% Tween 20
Pressure (mbar)	6666	800	various
Flowrate (µL/min)	38.6	6	Various

Table 2: Operating conditions for the evaluation of the number of cores in droplets
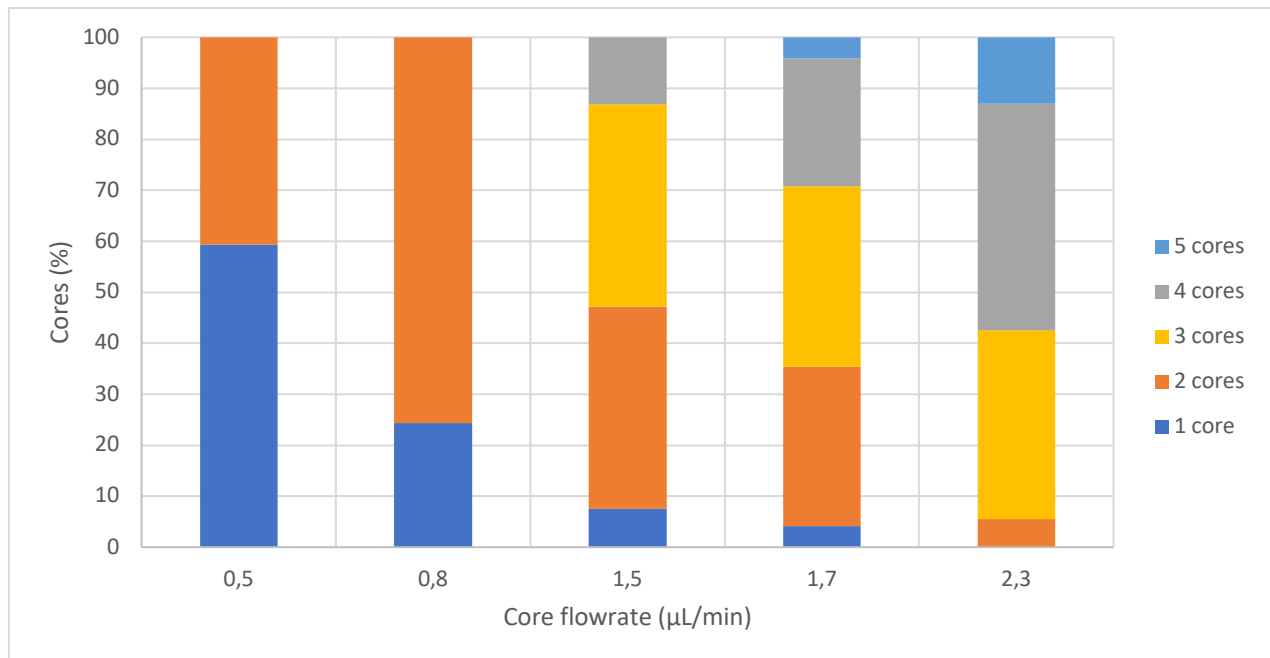

Figure 7: Histogram of the repartition of the number of cores depending on the core flowrate

Figure 7 highlights the fact that by increasing the core flowrate, the number of encapsulated core droplets increases. For a core flowrate of 0.5 µL/min, most droplets contain one core while most droplets contain four cores when the core flowrate is increased at 2.3 µL/min. This trend is also highlighted in Table 3.

Core flowrate (mL/min)	Majority population
0.5	1 core
0.8	2 cores
1.5	2 and 3 cores
1.7	3 cores
2.3	4 cores

Table 3: Majority of cores for various core flowrates

IV)   Conclusion and perspectives

In this application note, we demonstrated the possibility to produce multiple emulsions using two RayDrop® in series integrated in the RayDrop® platform. Then we showed how to drive the number of core droplet by simply varying the core phase flowrate.

Using a RayDrop® equipped with a nozzle carrying two phases for the production of double emulsions (visit our website https://secoya-tech.com/products/raydrop-droplet-generator/ for more details), various type of emulsions with multiple cores could be produced.

For example, by using a double emulsion RayDrop® on R1, it would be possible to have multiple double emulsions in one droplet, as shown in Figure 8.
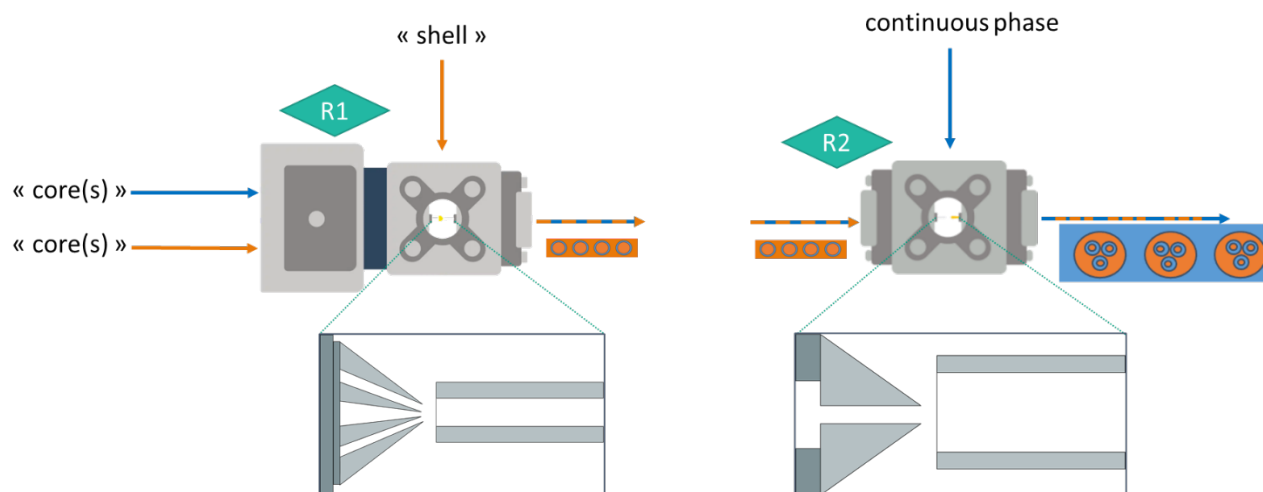
Figure 8: A RayDrop® double emulsion followed by a Raydrop® simple emulsion

In a same way, a setup that uses a simple emulsion RayDrop® on R1 and a double emulsion RayDrop® on R2 would produce multiple droplets in one double droplet as schematically shown in Figure 9.
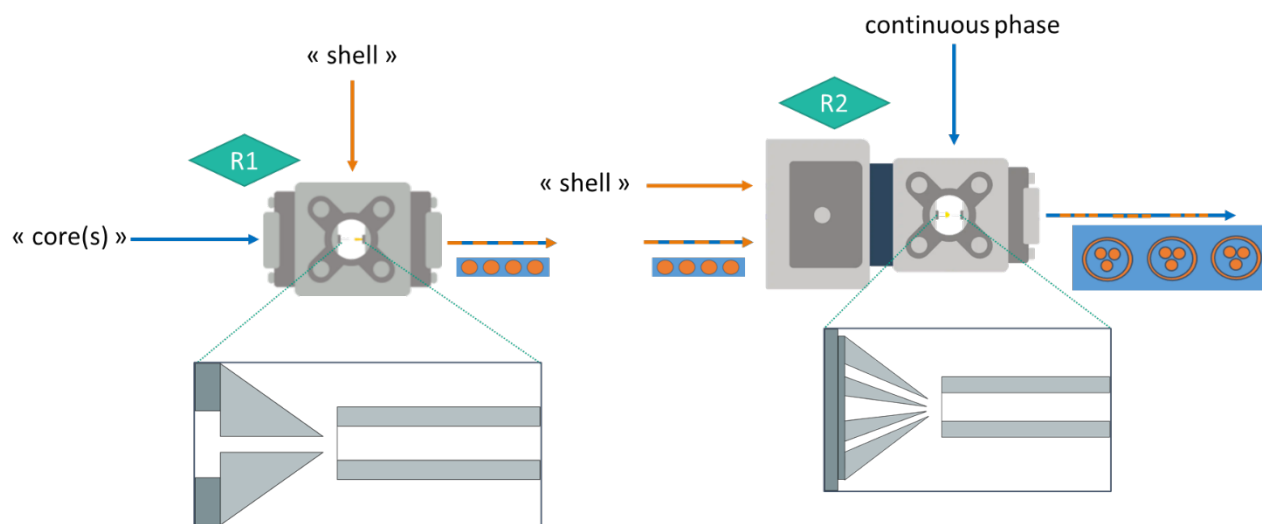

Figure 9: A RayDrop® simple emulsion followed by a RayDrop® double emulsion

Another interesting application of this microfluidic setup would be the co-encapsulation, i.e. the encapsulation inside the core of different fluids in a shell droplet. It can be for example used as synergistic delivery systems or chemical microreactors for incompatible actives or chemicals. [IV]

The configuration shown in Figure 10 would produce multiple emulsions containing different core compositions in the same shell with a microfluidic setup ensuring a high control over the number of cores.
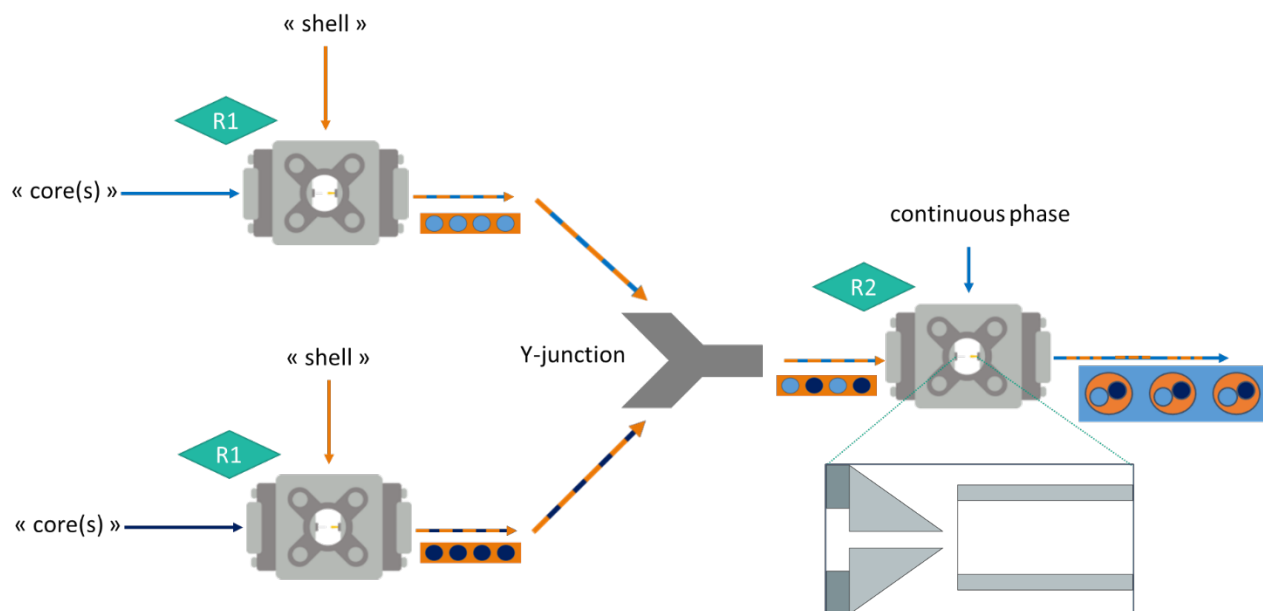

Figure 10: Set-up for the co-encapsulation of two liquids

V)   References

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