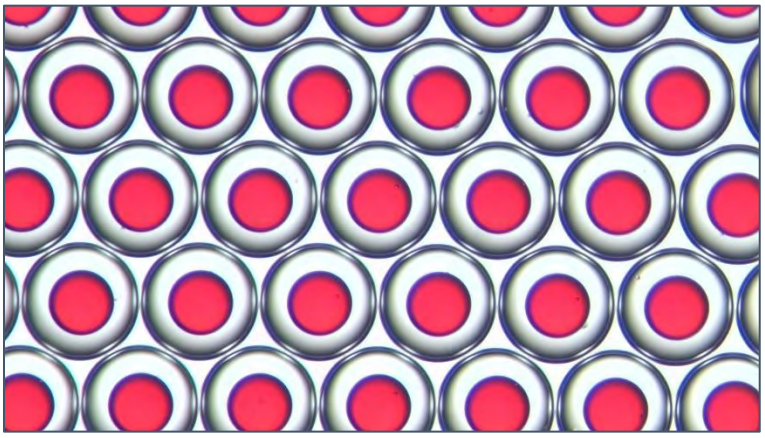
# CHITOSAN MICROCAPSULES CREATION



Application note

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# I) Introduction

Over the past few decades, core-shell microcapsules have been extensively used for the delivery and release of materials in the pharmaceutical, cosmetic, and food industries. The encapsulation of Active Pharmaceutical Compounds in core-shell microcapsules is of great interest for several purposes: taste and odor masking, controlled release of drugs... In pharmaceutics the possibility to encapsulate drugs, nutrients, and living cells that can be protected by a solid biocompatible shell in order to target a specific site is an intense field of research.

However, classical methods of microencapsulation like coacervation, spray drying, solvent evaporation, etc, require complex process and equipment and make difficult to control the size and load of the microcapsules.

In contrast, microfluidics allows to produce monodisperse double emulsions which lead to monodispersed microcapsules with a high control over both size and structure. Microfluidic tools are also used in order to create capsules of varying compositions. With this technology, it is possible to encapsulate aqueous or oily phases. The encapsulation of aqueous phases allows the capsule to contain proteins or active pharmaceutical ingredients (APIs). On the other hand, oily phases containing lipophilic or poorly water-soluble drugs can also be encapsulated. Moreover, capsules can be used for drug delivery or acid-triggered gastric delivery depending on the composition of the shell.

In this Application Note, chitosan-shell/oily-core microcapsules are obtained using the Raydrop® double emulsion generator, a capillary based microfluidic device. Indeed, microcapsules consisting of a chitosan shell and an oily core have been extensively studied as chitosan - a cationic polysaccharide - exhibits numerous benefits: excellent biological activity, good biocompatibility [1] and biodegradability and pH sensitivity [2] for acid-triggered gastric delivery. The method of production as well as the influence of the fluidic parameters on the size and the release from the oil across the shell are studied and presented.

# II) Materials and methods

## 1) Materials

**Core phase:**

- Soybean oil (Sigma-Aldrich) containing red dye Sudan IV (Sigma-Aldrich)

*Here, adding a dye will increase the contrast. This allows to differentiate well the core and the shell of the double emulsion. Moreover, the dye was useful to measure the encapsulation rate of capsules.*

**Shell phase:**

- Water containing 2% chitosan (viscosity 30-100 mPa·s, Glentham Life Sciences UK), 2% acetic acid (Sigma-Aldrich), 1% Pluronic® F-127 (Sigma-Aldrich)

*It is important to notice that the chitosan is very viscous. Therefore, the preparation of this solution should be done in the following order: add droplets of acetic acid to reach an acid medium (pH close to 4) so that the chitosan dissolves easily. Then add Pluronic® F-127, a non-ionic copolymer surfactant, chosen according to the publication of Liu and al. [2], to the water while stirring with a bar magnet. Once the PLURONIC® F-127 is dissolved, gradually add chitosan.*

**Continuous phase:**

- 1-octanol (Glentham Life Sciences UK) containing 2% Span 80 (Sigma-Aldrich)

*Here, the Span 80 is also a nonionic surfactant that was chosen according to the publication of Du and Al. [2]*

**Collect phase:**

- Heptane (VWR) containing 2% Span 80 (Sigma-Aldrich) and 0,3 wt% glutaraldehyde (50% in $H_2O$, Glentham Life Sciences UK)

*The choice of the products forming the collect phase is based on the publication of Du and Al. [1] The glutaraldehyde is the cross-linking reagent. It reacts with the chitosan to create a hydrogel. [3]*

## 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® device. This platform is divided into three parts: mechanics, fluidics and optics. More information about this platform cand be found on the platform webpage available on our website at [https://secoya-tech.com/technologies/emulsifications/](https://secoya-tech.com/technologies/emulsifications/) .

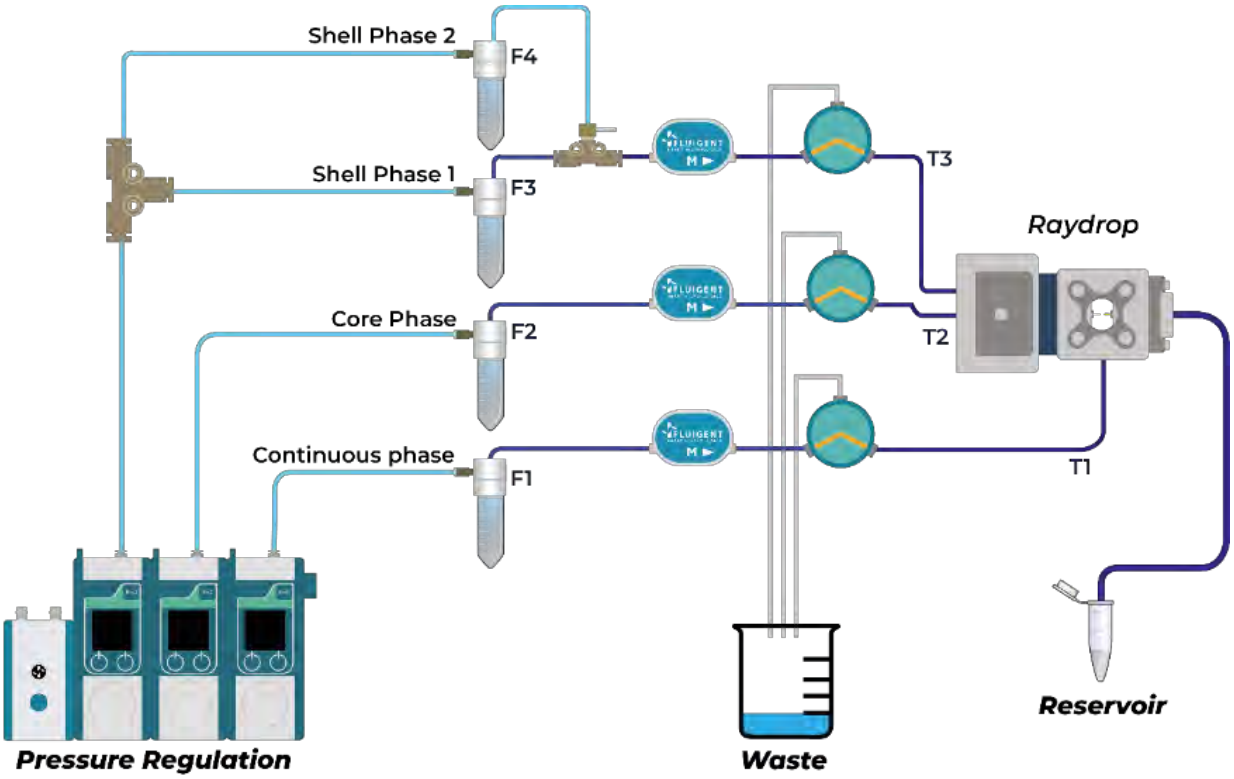



*Figure 1: Experimental set-up used to produce double emulsion. This flow scheme can be slightly different from the one corresponding to the commercialized version of the platform, where normally two reservoirs for the core phase are included.*

## Fluid reservoirs

| Falcon identification | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| Volume (mL) | 50 | 50 | 50 | 15 |
| Phase[1] | Continuous | Core | Shell (priming and cleaning) | Shell |
| Composition | 1-octanol + 2% Span80 | Soybean oil | Water + 2% acetic acid | Water + 2% chitosan 30-100 cps + 1% PLURONIC® F-127 + 2% acetic acid |

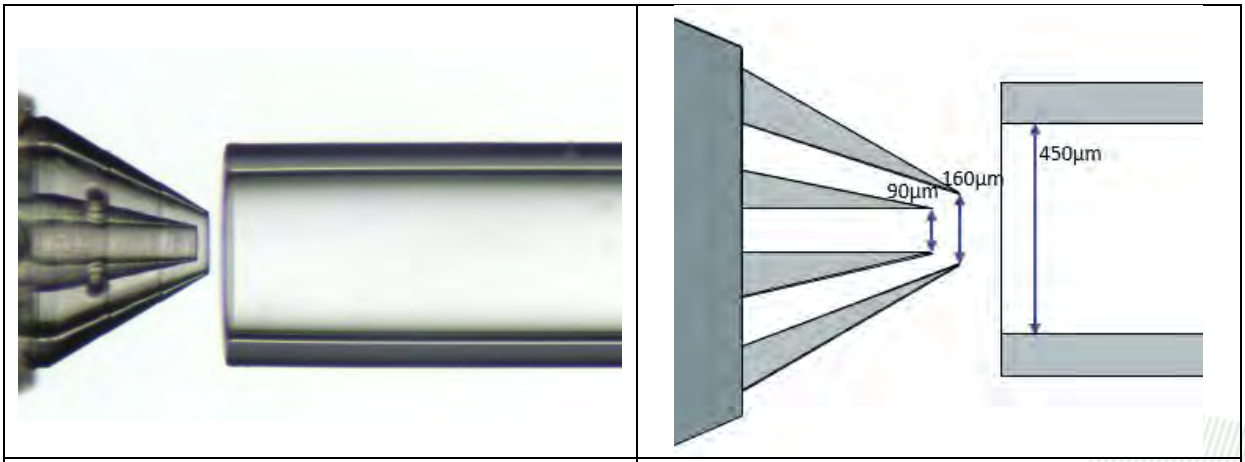

*Figure 2: Raydrop® Platform*

[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.*

- **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 double emulsions are generated. After each reservoir, a filter is included that eliminates impurities that could plug 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 technology produces controlled simple emulsions. By changing an insert of the Raydrop®, stable double emulsions can be produced without coating of oily and water phases; for stability reasons usually surfactants are added to the different phases.




| Picture of the Raydrop® | Scheme of the Raydrop® |
|---|---|

*Figure 3: Insert and extraction capillary*

**Nozzle information for this application note**

| Part | Core nozzle | Size-shell nozzle | Size-extraction capillary |
|---|---|---|---|
| **Inside diameter (µm)** | 90 | 160 | 450 |

## 4) Emulsion generation

To generate droplets, the system must first be primed with pure solvent in the shell phase (here water with acetic acid). Once droplet formation is stable, the shell phase is switched to the chitosan-based solution. This permits to avoid clogging issues during the transient phase. The user should follow the steps below:

1. Set the valve on the Falcon F3 (priming solution)
2. Fill the Raydrop® with the continuous phase
3. Set the continuous phase (F1) to the desired flow rate
4. Set the shell phase (F3) to the desired flow rate. At this point, a co-flow of 1-octanol and water is generated.
5. Set the core phase (F2) to the desired flow rate to generate double emulsions
6. Once the double emulsion production is stabilized – which can be observed with the camera images- switch the valve to the chitosan-based shell solution (F4)
7. Wait[2] until the chitosan solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a chitosan solution shell and a soybean oil in the 1-octanol continuous phase.

[2] *This can take 10 to 15 minutes, depending on the flow rate of the chitosan phase and the diameters and length of the tubing.*

| | Continuous phase | Shell | Core |
|---|---|---|---|
| **Composition** | 1-octanol + 2% Span 80 | Water + 2% chitosan 30-100cps + 1% PF127 + 2% AcOH | Soybean oil |
| **Flow rate (µL/min)** | 130 | 15 | 12 |

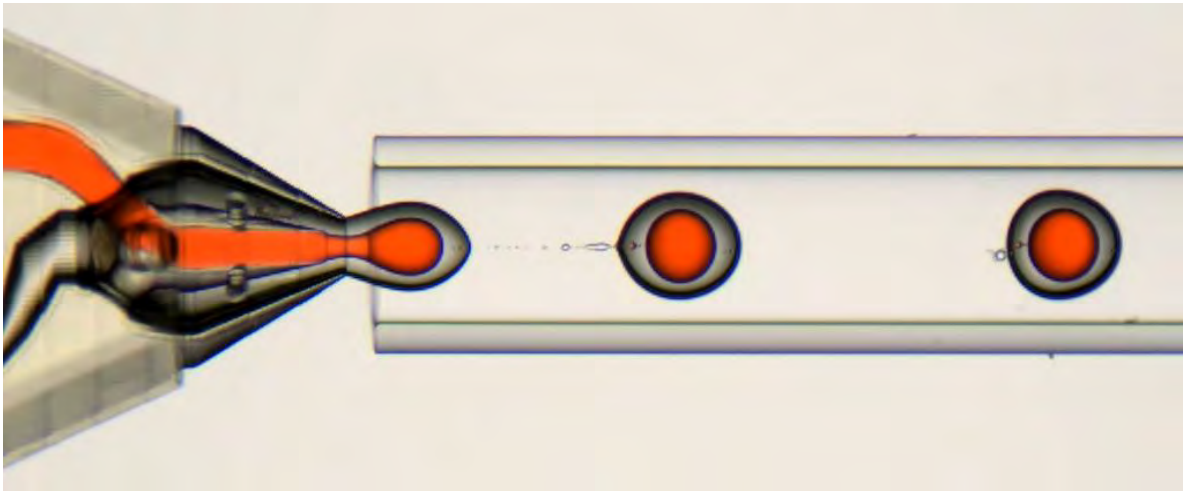


*Figure 4: Generation of droplets in the Raydrop®*

8. If necessary, stabilize the double emulsion by varying the flow rates
9. Adjust the flow rates to obtain the desired droplet diameter and shell thickness
10. Collect the droplets at the outlet of the Raydrop®

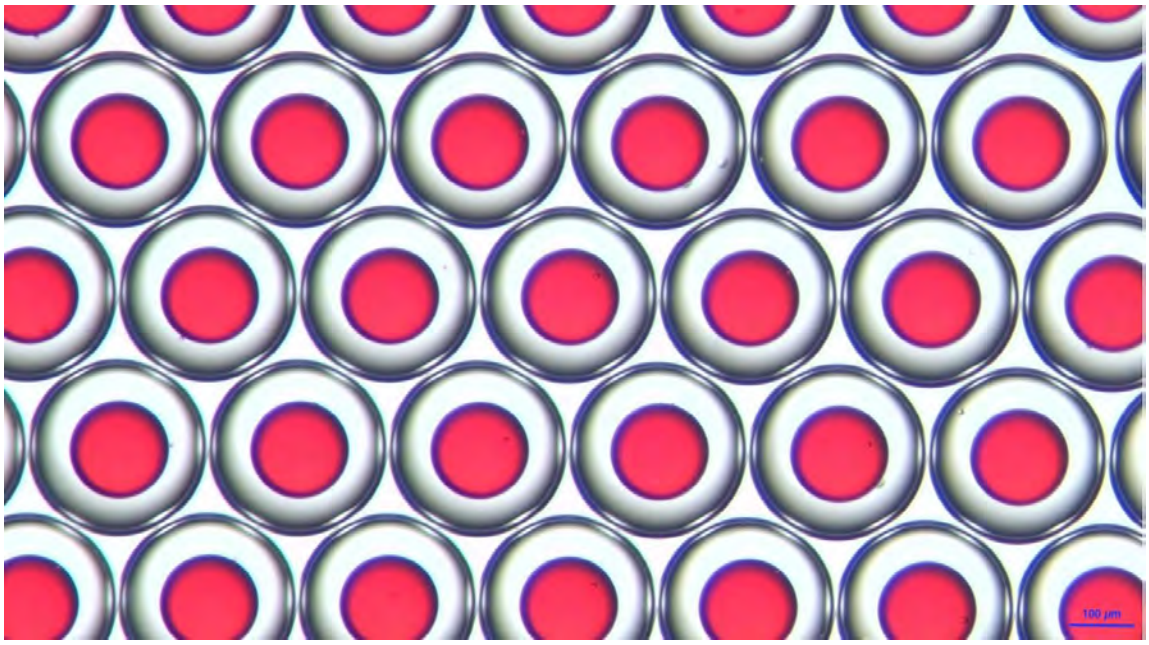



*Figure 5: Double emulsion obtained at the output, observed under the microscope*

Before stopping the experiment, it is important to flush the shell tubing (T3) and the nozzle of the Raydrop® with the priming solution (F3). This priming and cleaning solution only contains water and acetic acid, which allows the evacuation of the chitosan. In this way, the tubing stays clean and clogging is avoided, enabling immediately the user to perform a next experiment on a different core and shell composition e.g..

11. To flush the chitosan out of tubing and Raydrop®, switch the valve on the priming solution (F3)
12. Wait[3] until the cleaning solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a water solution shell and a soybean oil in the 1-octanol continuous phase
13. Cut the flow of the core phase
14. Then, cut the flow of the shell phase
15. Finally, cut the flow of the continuous phase

[3] Please refer to the footnote 2

## 5) Capsule formation

After being generated, the droplets are collected in a coagulation bath. The chitosan contained in the shell phase is in contact with the glutaraldehyde from the coagulation bath. As a result, chitosan reacts with glutaraldehyde by solvent extraction and chemical cross-linking based on the Schiff base reaction [3]. The droplets are solidified and become glutaraldehyde cross-linked chitosan microcapsules.

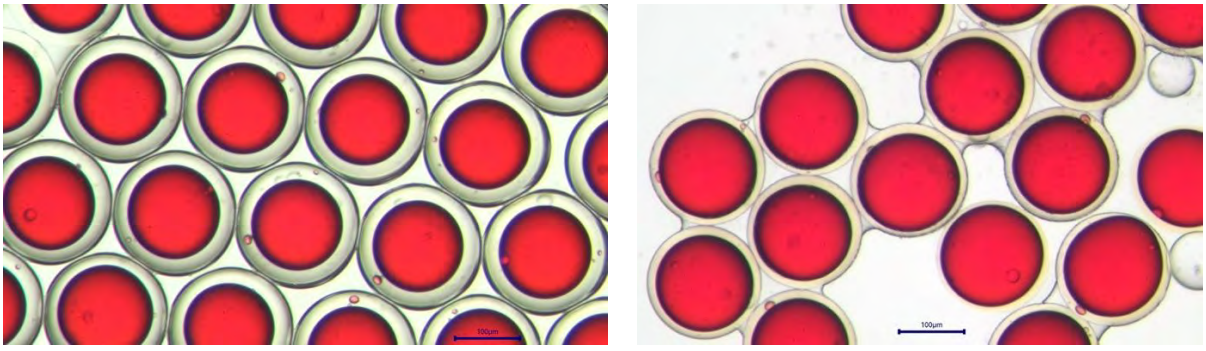


*Figure 6: Glutaraldehyde cross-linked chitosan microcapsules on the cross-linking bath. On the left, after 4 minutes in the cross-linking bath. On the right, after 1h in the cross-linking bath. The shell thickness decreases and becomes progressively yellow, as a part of its water content diffuses in the continuous phase. Expelled water is clearly visible wetting the capsules.*

# III) Results

Now that the products used have been mentioned and the formation of the double emulsion and capsules has been described, let's move on to the analysis of the capsules and the results.

## 1) Evolution of the droplet diameter during the cross-linking process

For this test series, we have used the operating conditions shown in Table 1, the droplets are collected into the collection bath. An analysis of the size of the capsules is performed using a microscope and a measurement software. For a given sample, several measurements of the capsule diameter are made at different times after production. The evolution of the diameter is highlighted in Figure 7.

| | Continuous phase | Shell | Core |
|---|---|---|---|
| **Composition** | 1-octanol + 2% Span 80 | Water + 2% chitosan 30-100cps + 1% PF127 + 2% AcOH | Soybean oil |
| **Pressure (mbar)** | 164 | 1650 | 1384 |
| **Flow rate (µL/min)** | 162 | 16,4 | 13,6 |

*Table 1: Selected operating conditions for the evaluation of the diameter of capsules*

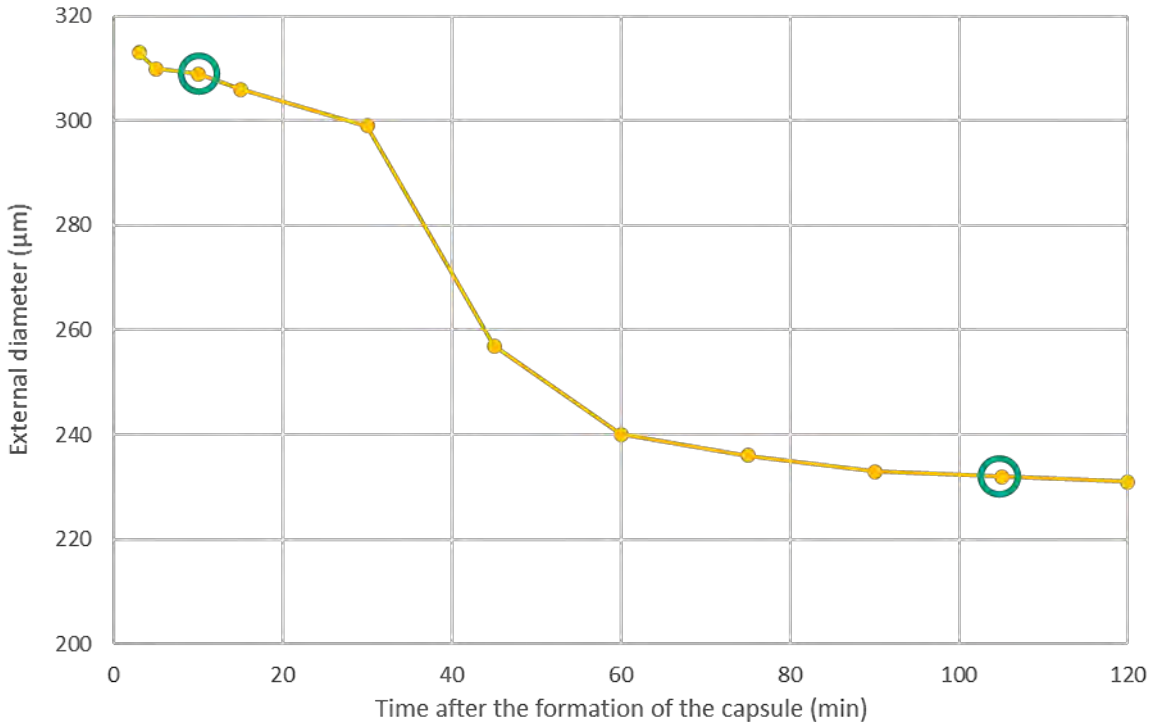



*Figure 7: Size of chitosan capsules as a function of time*

The capsules have a diameter ranging from 315 µm to 230 µm. Moreover, we observe that during crosslinking, the diameter of the capsules decreases. Indeed, a diameter of 310 µm is obtained 10 minutes after droplet formation, while a diameter of 230 µm is obtained 105 minutes after droplet formation. Moreover, this result agrees with the publication of Du et al. [1] and can be explained by the fact that during crosslinking, water in the shell phase is extracted.

## 2) Influence of the middle phase flow rate

After having analyzed the size of the capsules over time, the influence of the middle phase flow rate is observed. We varied the shell flow rate at fixed continuous and core phases flow rates. For each shell phase flow rate, a video was recorded, and the diameter of the shell and the core were measured using a measurement software. The evolution of these two diameters is underlined in Figure 8 and the operating

conditions are shown in Table 2. In Figure 9 the evolution of the thickness of the droplet with the evolution of the shell flow rate is presented.

| | Continuous phase | Shell | Core |
|---|---|---|---|
| **Composition** | 1-octanol + 2% Span 80 | Water + 2% chitosan 30-100cps + 1% PF127 + 2% AcOH | Soybean oil |
| **Pressure (mbar)** | 174 | variable | 1378 |
| **Flow rate (µL/min)** | 180 | variable | 13,6 |

*Table 2: Operating conditions for the evaluation of the size of capsules for a middle flow rate variation*

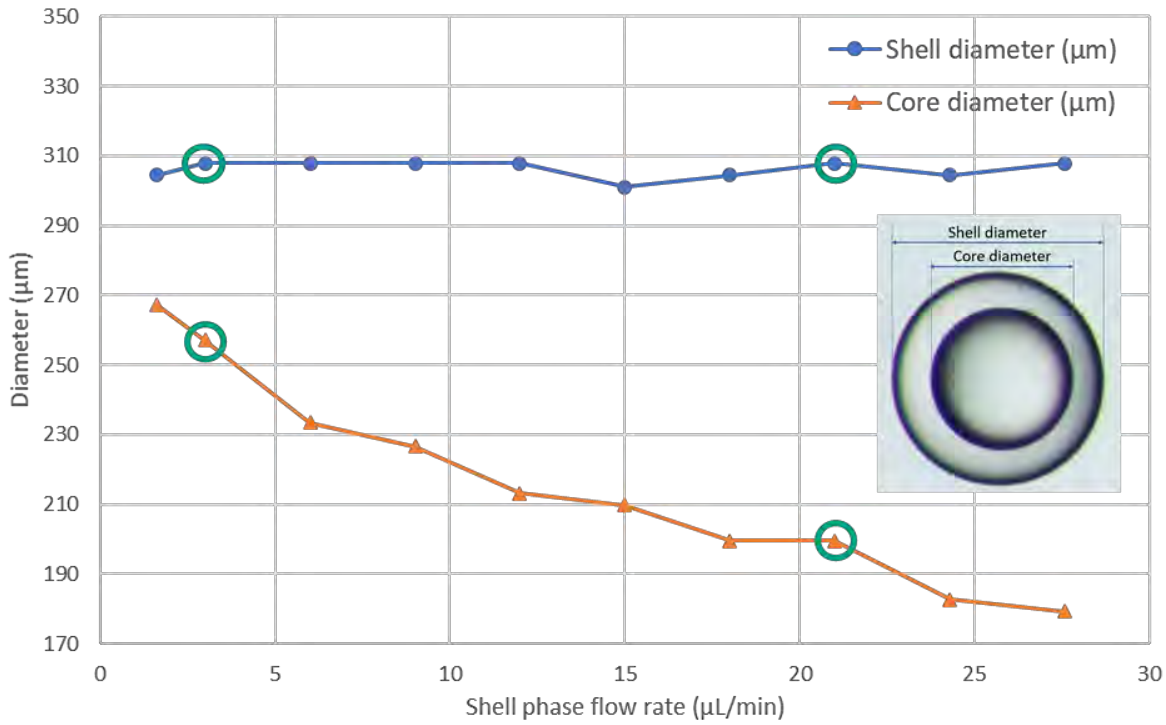



*Figure 8: Core size and shell size as a function of the shell liquid flow rate*

We observe that the shell diameter remains relatively constant when varying the shell liquid. As an example, a shell diameter of 310 µm is obtained using a shell flow rate of 3 µL/min, while a shell diameter of 305 µm is obtained using a shell flow rate of 24 µL/min. However, we notice that when the flow rate of the shell phase increases, the

diameter of the core decreases. Indeed, a core diameter of 255 µm is obtained using a shell flow rate of 3 µL/min, while a core diameter of 185 µm is obtained using a shell flow rate of 24 µL/min. This phenomenon can be explained by the fact that the size of the shell is constrained in a limited range by the geometry of the system when the continuous phase flow is constant.

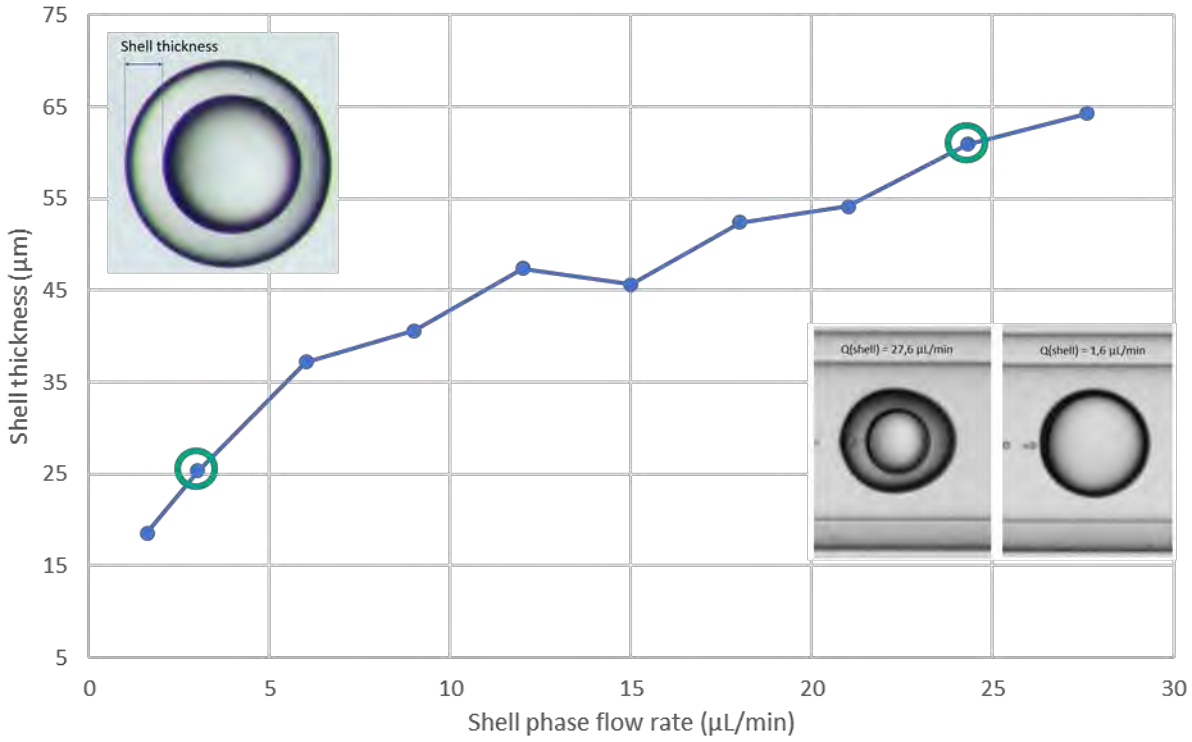



*Figure 9: Thickness of the shell as a function of the shell phase flow rate*

Figure 9 underscores the increase in shell thickness when the shell phase flow rate increases. The shell thickness increases with the shell phase flow rate. Indeed, a shell thickness of 25 µm is obtained when the shell phase flow rate is 3 µL/min, while a shell thickness of 61 µm is obtained when the shell phase flow rate is 24 µL/min. The droplet generation frequency increases and the core size decreases by two effects: as the shell phase increases in flow rate but particle diameter is fixed due to the invariant continuous flow rate and velocity, more pinching off events occur, leaving less core phase liquid inside every droplet.

## 3) Influence of the outer phase flow rate

Previously, the continuous phase flow rate and core flow rate remained constant and the shell flow rate was varying. In this part, the shell flow rate and core flow rate are fixed to the values shown in Table 3. The continuous phase flow rate is now altered in between different tests. Once again, for each continuous phase flow rate, a video was recorded and the diameter of the shell and the core were measured. The shell and core diameter evolution is shown in Figure 10, as well as the evolution of the thickness of the droplet as a function of continuous phase flow rate.

| | Continuous phase | Shell | Core |
|---|---|---|---|
| **Composition** | 1-octanol + 2% Span 80 | Water + 2% chitosan 30-100cps + 1% PF127 + 2% AcOH | Soybean oil |
| **Pressure (mbar)** | variable | 1553 | 1378 |
| **Flow rate (µL/min)** | variable | 16,4 | 13,6 |

*Table 3: Operating conditions for the evaluation of the size of capsules for a continuous flow rate variation*

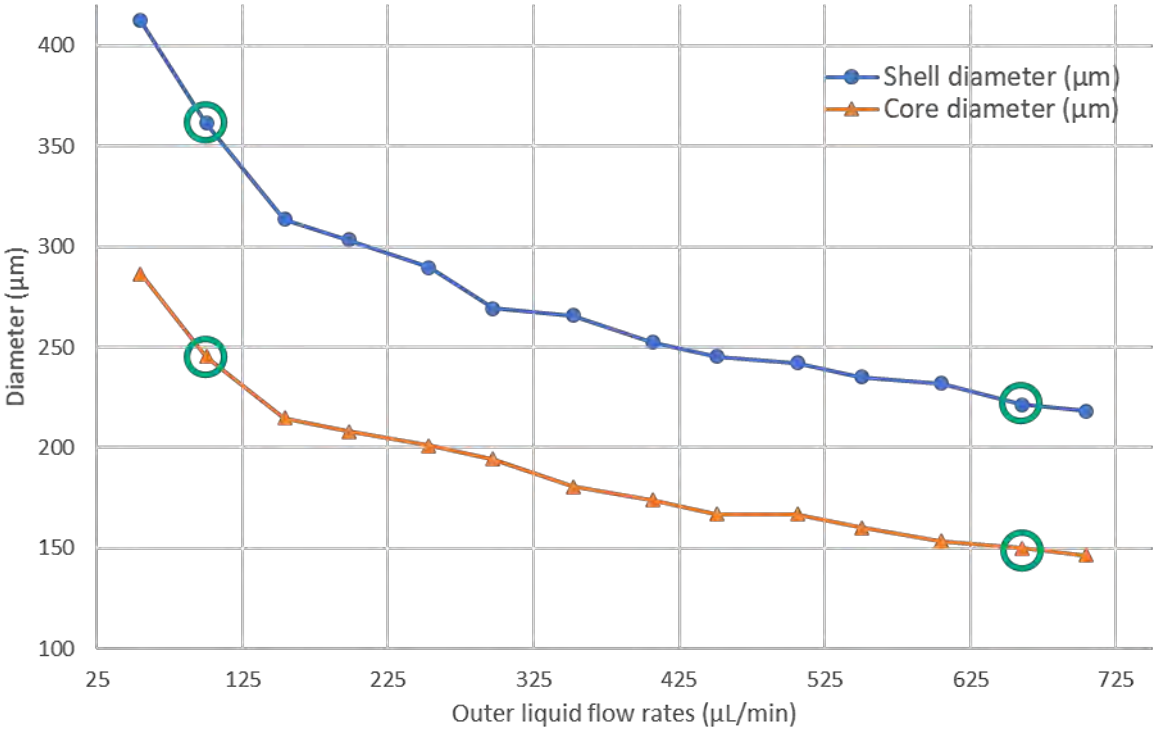



*Figure 10: Core size and shell size as a function of the outer phase flow rate*

We observe that when the continuous phase flow rate increases, both shell and core diameter decrease. A shell diameter of 360 µm is obtained using a shell flow rate of 100 µL/min, while a shell diameter of 222 µm is obtained using a shell flow rate of 660 µL/min. For the core of the droplet, the diameter is 245 µm using a shell flow rate of 100 µL/min, while a diameter of 150 µm is obtained using a shell flow rate of 660 µL/min. The size decrease of both shell and core diameters can be explained by the increase of the stress applied on the pinched jet at the nozzle outlet, which leads to the formation of smaller droplets. As the flow rates of the shell and core phases are maintained constant, the droplet generation frequency increases.

## 4) Capsule release analysis

Collected droplets are separated in two samples with different crosslinking times inside the glutaraldehyde bath. The first sample contains droplets that have cross-linked for 17 minutes and the second contains droplets that have cross-linked for 45 minutes. Each sample is washed with a buffer solution at pH 7.3 and then stored in the same buffer to keep the capsules in the conditions of stability.

An analysis of the release of red dye contained by the capsules is performed using a spectrophotometer. This allows to determine the absorbance of the Sudan IV dye of the solution containing the capsules according to a 520nm wavelength. The evolution in absorbance can be directly correlated with loss of material inside the solidified droplets before and after production, but also during storage.

For each sample, several measurements of the absorbance are made at different times. In total, 15 measurements were made per sample over a period of 24 hours. The obtained results are presented in the Table 4. No significative difference was observed between the two samples and in both cases about 95% of the encapsulated oil remained after 24h when stored inside the buffer solution, demonstrating that a compact shell structure preventing oil leakage is formed in less than 20 minutes of cross-linking.

| | **Capsules cross-linked for 17 minutes** | **Capsules cross-linked for 45 minutes** |
|---|---|---|
| **Release rate of the red dye in the buffer solution containing the capsules after 24 hours** | < 5% | < 5% |

*Table 4: Release rate of capsules*

## IV) Conclusion

The production of stable microcapsules with a solid chitosan shell and a liquid oily core using a microfluidic system consisting of pressure-based flow controllers and the Raydrop® microfluidic tool has been successfully achieved. The microfluidic platform also allows to preselect not only the core diameter but also the shell thickness adjusting the flow rates of the different fluids. These microcapsules can be used in a wide range of applications, like the encapsulation of volatile products like mint oil [1] as well as specific drugs, which will be delivered according to the pH acidity [2].

## V) References


[1] DU, Yuhan, MO, Liangji, WANG, Xiaoda, WANG, Hongxing, GE, Xue-hui and QIU, Ting, 2020. Preparation of mint oil microcapsules by microfluidics with high efficiency and controllability in release properties. Microfluidics and Nanofluidics. June 2020. Vol. 24, no. 6, p. 42. DOI 10.1007/s10404-020-02346-2.

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[3] KILDEEVA, N. R., PERMINOV, P. A., VLADIMIROV, L. V., NOVIKOV, V. V. and MIKHAILOV, S. N., 2009. About mechanism of chitosan cross-linking with glutaraldehyde. Russian Journal of Bioorganic Chemistry. 1 May 2009. Vol. 35, no. 3, p. 360–369. DOI 10.1134/S106816200903011X.