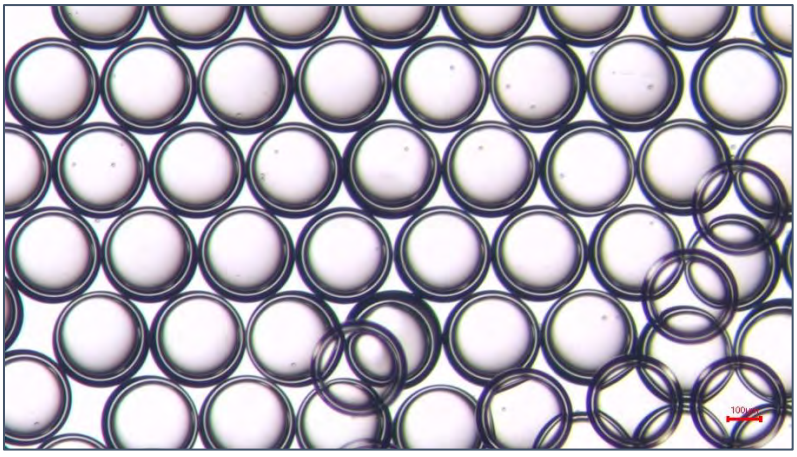
POLYMETHACRYLATE RESIN MICROCAPSULES SYNTHESIS



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 [1]. The encapsulation of Active Pharmaceutical Compounds in core-shell microcapsule 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 the size and the structure. Microfluidics 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, capsules are formed by consolidating shell phase of the resulting double emulsions by UV-crosslinking of polymers [2] and photoinitiator used as shell phase. Core and continuous phases are aqueous phases non-miscible with the shell. Fine control of the fluid flows leads to defined capsule and shell dimensions. In-situ polymerization is achieved, meaning that the droplets are exposed to UV-light while still being moving forward in the output tubing connected to the Raydrop®. Hard shell microcapsules are thus directly collected in the collection vial. The in-situ process allows to avoid coalescence and deformation of the droplets that can arise in an ex-situ process where the droplets are polymerized after collection.

II) Materials and methods

1) Materials

Core phase:

- Water

This application note only presents results for capsules containing water but it is possible to add products such as vitamins, proteins and any other water-soluble compound.

Shell phase:

- Commercial Allnex methacrylate-based resin containing 20% of ethyl acetate (EtOAc, Merck) and 0.1% wt of photoinitiator Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide (TPO, Sigma-Aldrich)

It is important to notice that the photoinitiator reacts with light, so to avoid solidification of the solution, the solution must be protected from light by using a smoked glass container for example.

Continuous phase:

- Water containing 2% Poly(vinyl alcohol) (PVA, Sigma-Aldrich)

Priming and cleaning phase:

- Ethyl acetate (EtOAc, Merck)

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/ .

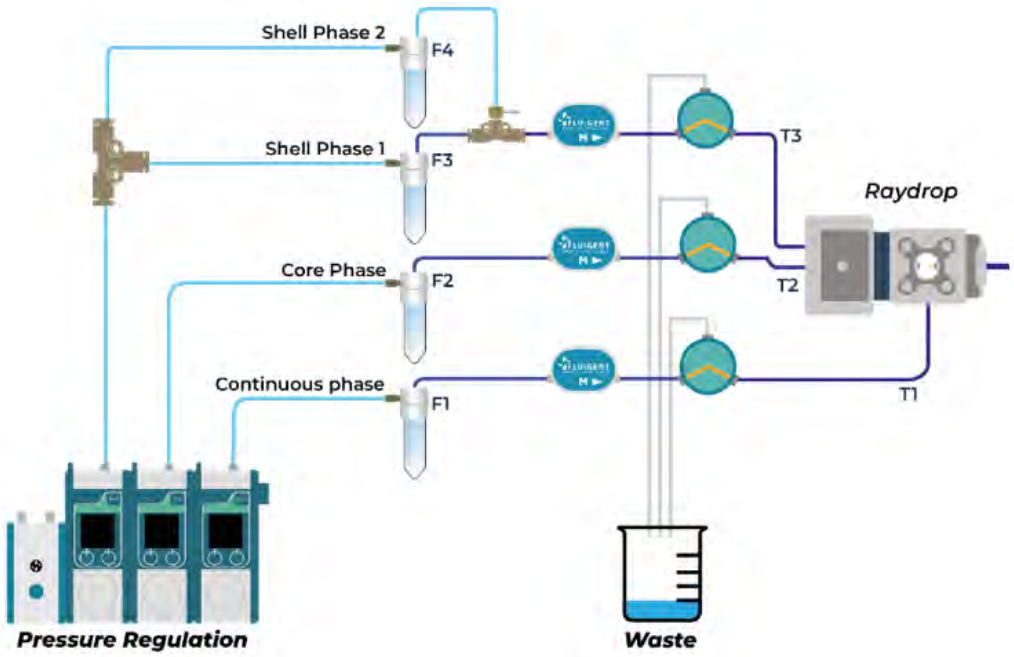


Figure 1: Experimental set-up to produce double emulsion. This flow scheme can be slightly different from the one corresponding to the commercialized version of the platform, where normally two reservoirs for the core phase are included.

Fluid reservoirs

Falcon identification	F1	F2	F3	F4
Volume (mL)	50	50	50	15
Phase[1]	Continuous	Core	Shell (priming and cleaning)	Shell
Composition	Water + 2% PVA	Water	EtOAc	Allnex methacrylate-based resin + 20% EtOAc + 0.1% wt TPO

[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 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).

Double emulsions are formed by pumping the three fluids through the Raydrop® using a pressure controller. The flowrates are monitored using flowmeters. This time, as a polymer double emulsion is formed, the platform configuration changes a bit. We want the cross-linking to only start inside the outlet tubing. This reaction should not begin in the Raydrop® generator, so an UV filter is placed between the LED light and the camera as underlined in Figure 3. This avoids an early shell solution cross-linking during droplet formation.

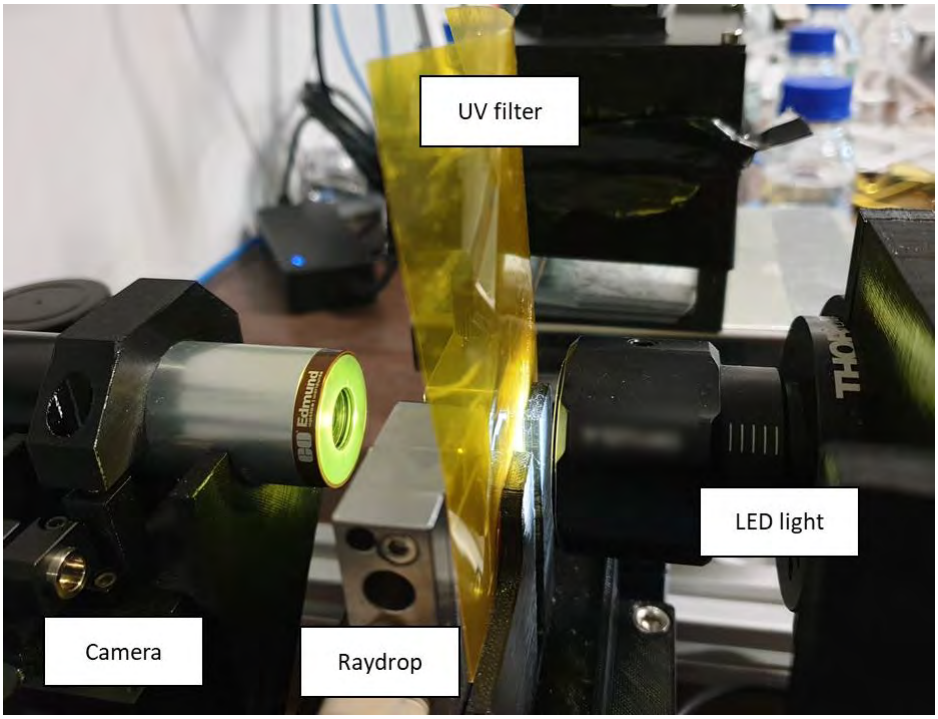


Figure 3: UV-filter which avoid the early cross-linking of the shell solution

An UV-light source is placed above the outlet tubing. It is turned on to crosslink the methacrylate shell thanks to the photoinitiator action. In-situ crosslinking is achieved, meaning that the droplets are exposed to UV-light while still being moving forward in the outlet tubing connected to the Raydrop®. Hard shell microcapsules are thus directly collected in a vial. The in-situ process is useful: it avoids coalescence and deformation of the droplets that can arise in an ex-situ process where the droplets are cross-linked after collection.

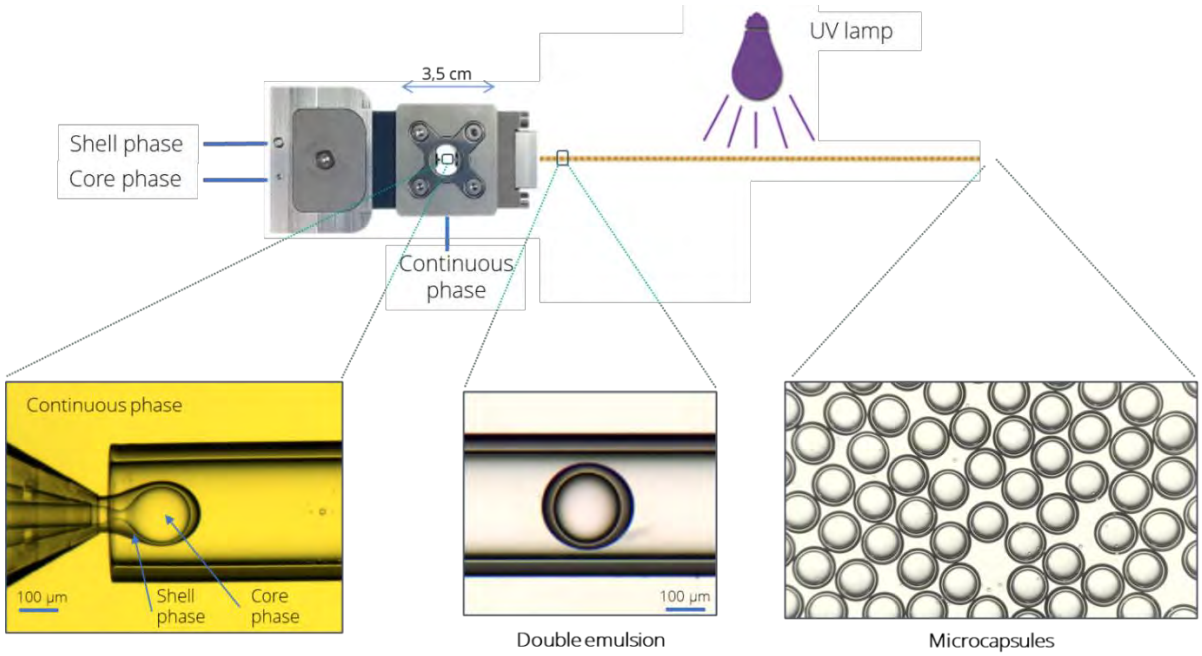
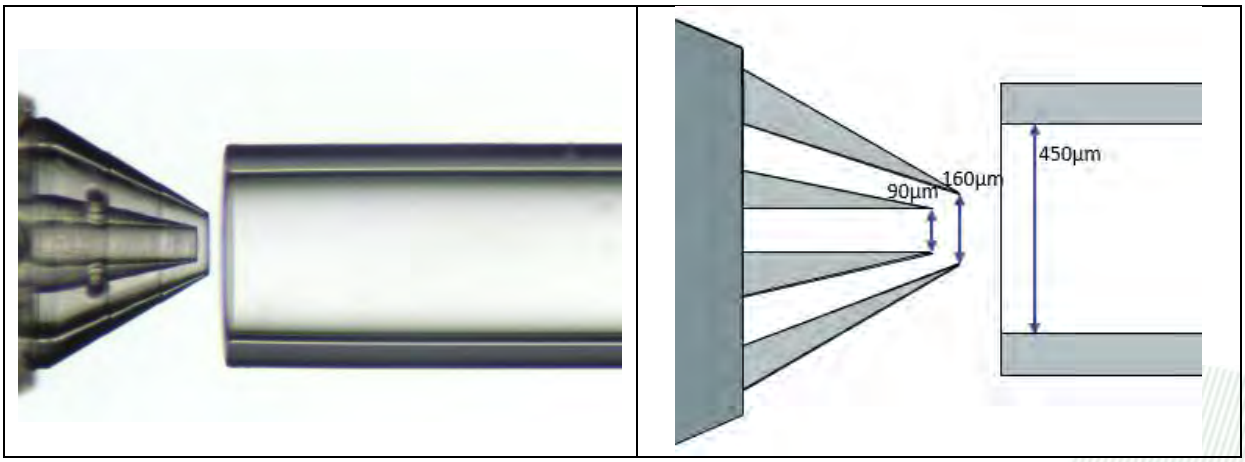


Figure 4: In-situ cross-linking process leads to the formation of monodispersed microcapsules

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 5: Insert and extraction capillary

Nozzle information

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. Once droplet formation is stable, the shell phase is switched to the methacrylate-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 ethyl acetate 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 methacrylate-based shell solution (F4)
7. Wait[2] until the methacrylate solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a methacrylate solution shell and an aqueous core in the continuous phase.

[2] *This can take 3 to 5 minutes, depending on the flow rate of the methacrylate phase and the diameters and length of the tubing.*

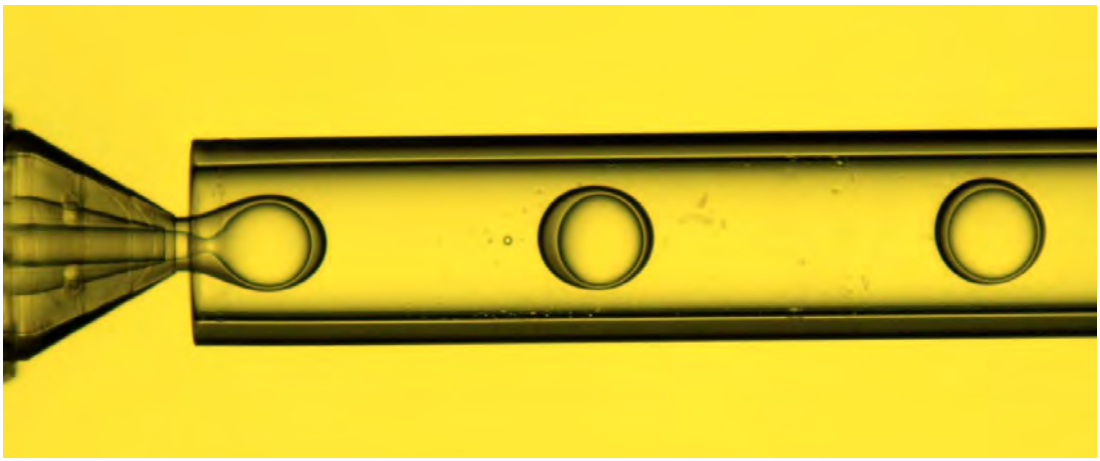


Figure 6: Generation of droplets in the Raydrop®. The picture is yellow due to the UV filter.

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

1) Capsule formation

Now that the double emulsion is produced with the adapted flow rates, the in-situ cross-linking take place. Figure 7 describes the cross-linking phenomena thanks to the UV radiation action.

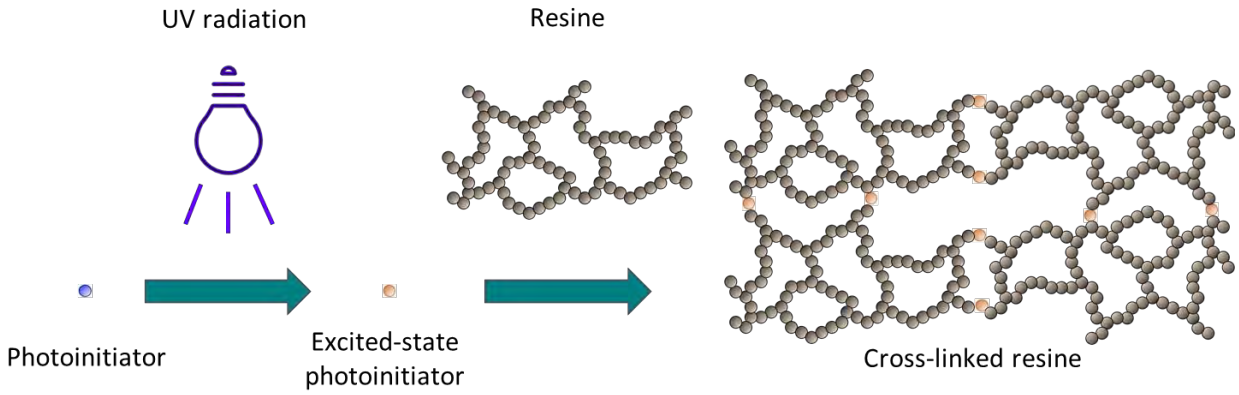


Figure 7: Principle of the cross-linking process of a resin

10. Switch on the UV light
11. Collect the capsules at the outlet of the tubing

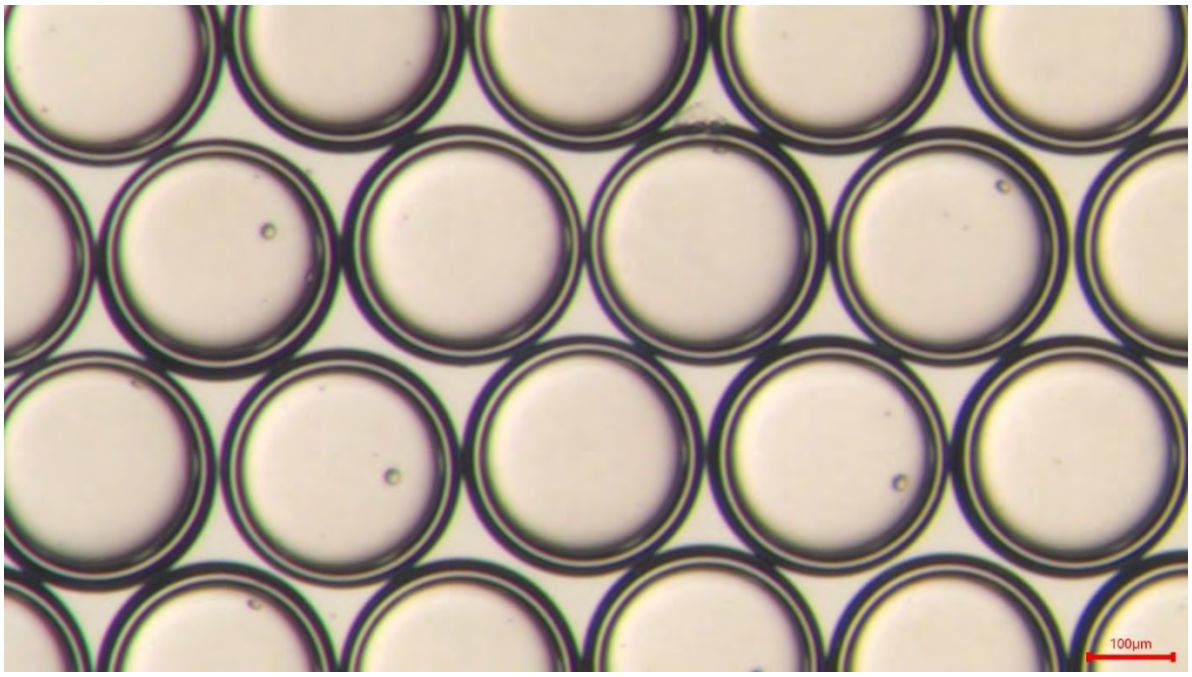


Figure 8: Capsules obtained at the output, observed under the microscope

12. Once that enough capsules have been produced, switch off the UV lamp.

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 allows the evacuation of the methacrylate resin. 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..

13. To flush the methacrylate resin out of tubing and Raydrop®, switch the valve on the priming solution (F3)

14. Wait[2] until the cleaning solution crosses the tubing and reaches the Raydrop® to form a double emulsion with an ethyl acetate shell and an aqueous core in the continuous phase.
15. Cut the flow of the core phase
16. Then, cut the flow of the shell phase
17. Finally, cut the flow of the continuous phase

If a salt solution is used in the core phase, it is recommended to flush the tubing and the Raydrop® with water after experiments. It will prevent salt from crystallizing in the tubing or the Raydrop® and blocking them.

III) Results

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

The outer diameter of the emulsions and thus the capsule size can be varied within a broad range by changing the size of the collector capillary and the nozzle tip dimensions. This is easily achieved by a change of the two inserts.

For a configuration with the nozzle and output capillaries (respectively 90µm and 450µm), as presented in this application note, adjusting the flowrates of the fluids allows for fine control of the capsule dimensions (see Figure 9). With this setup droplet from 200µm to 300µm can be easily produced.

The shell thickness of microcapsules can also be varied by changing the ratio of flowrates of the shell and core phases, as shown in Figure 9. Here shell thickness varies from 10µm to 50µm.

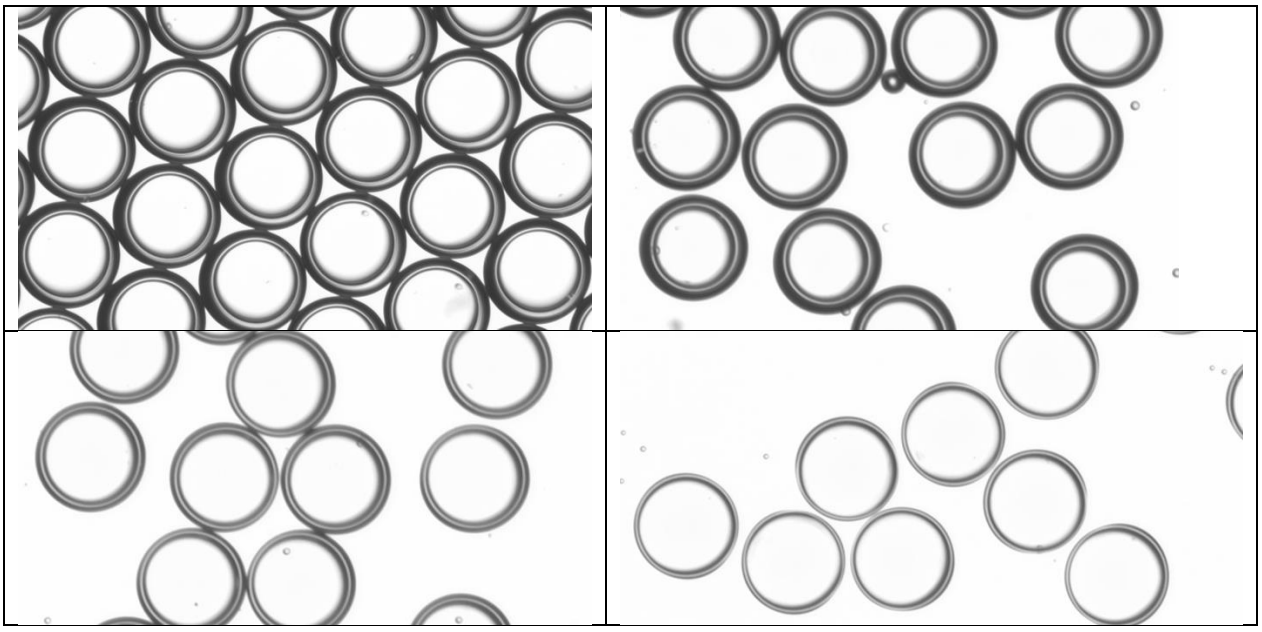


Figure 9: Microcapsule of 250µm with shell thickness adjustment between 10µm to 50µm

2) Particule size distribution evaluation of the monodispersity of the capsules

One of the advantages of the microfluidic platform containing a Raydrop® droplet generator is the high monodispersity of the produced droplets. To underline this fact, numerous capsules have been produced to study the size of those. The production conditions are described in Table 1.

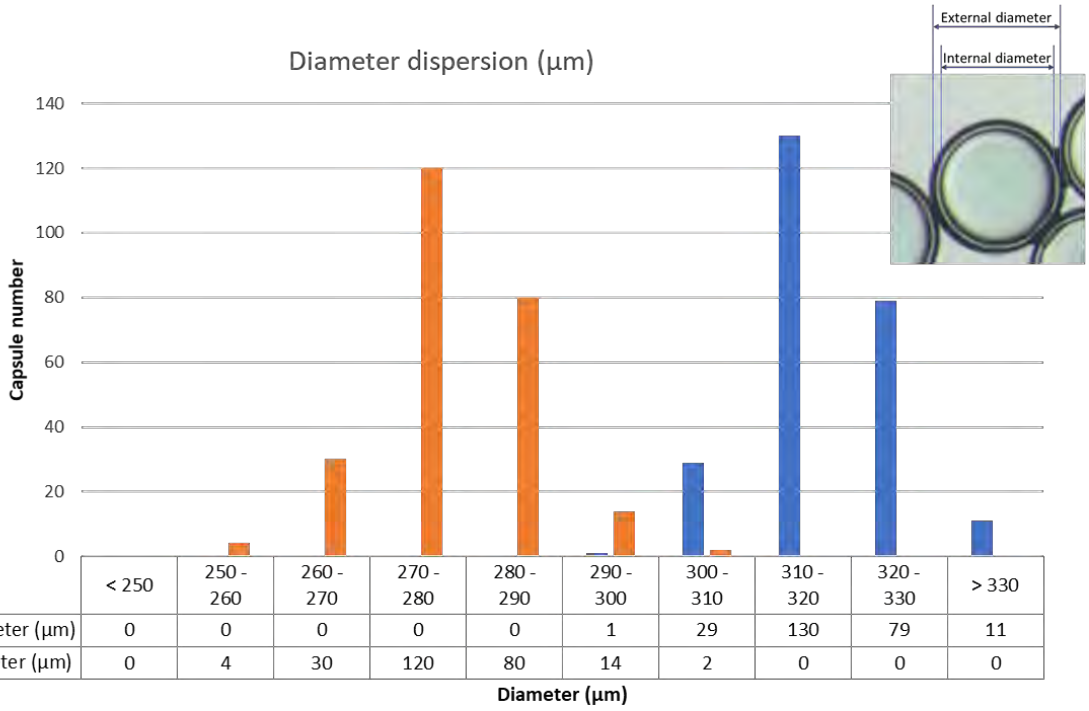
	Continuous phase	Shell	Core
Composition	Water + 2% PVA	Allnex methacrylate-based resin + 20% EtOAc + 0.1% wt TPO	Water
Flow rate (µL/min)	187	20	13

Table 1: Flowrates used to produce a sample of methacrylate resin

Capsules are collected on a microscope slide and different pictures are taken with a microscope. Using an image processing software, different diameters are measured:

- the external or shell diameter, which is the global size of the capsule
- the internal or core diameter, which is the size of the encapsulated material.

On a sample of 250 capsules, a measurement of the internal and of the external diameter of each capsule is performed. Once that all measures are done, a histogram is plotted; Figure 10 highlights the Particle Size Dispersion (PSD) of the internal diameter (in orange) and of the external diameter (in blue).



	< 250	250 - 260	260 - 270	270 - 280	280 - 290	290 - 300	300 - 310	310 - 320	320 - 330	> 330
external diameter (µm)	0	0	0	0	0	1	29	130	79	11
internal diameter (µm)	0	4	30	120	80	14	2	0	0	0

Figure 10: Dispersion of internal and external diameter on a sample of 250 capsules

Thanks to this histogram, both distributions are monomodal and we know that the predominant size of capsules is between 310 and 320 µm for the external diameter and between 270 and 280 µm for the internal diameter. Moreover, by calculating the difference between the external and the internal diameter, a value of the shell thickness is obtained. Here, the shell thickness is regular. Indeed, for each diameter

interval, the number of capsules is globally the same for an external and an internal diameter.

To complete the analysis of the sample, various statistical calculations are also made.

	External diameter (µm)	Internal diameter (µm)	Shell thickness (µm)
Average	318	278	40
Maximum	337	303	59
Minimum	298	257	25
Standard deviation	7	8	6
Coefficient of variation (CV)	2	3	15
D10	309	268	-
D50	318	277	-
D90	327	289	-
SPAN	0,06	0,07	-

Table 2: Statistics concerning the diameters and the shell thickness on the studied sample

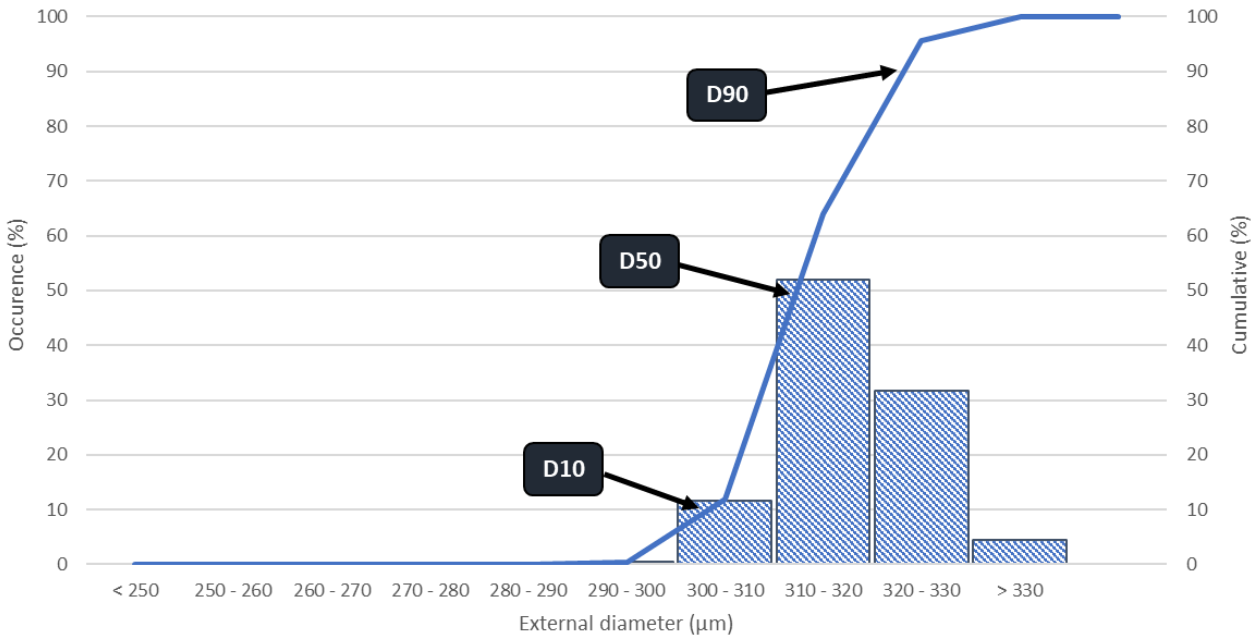


Figure 11: Particle size distribution as a function of the external diameter

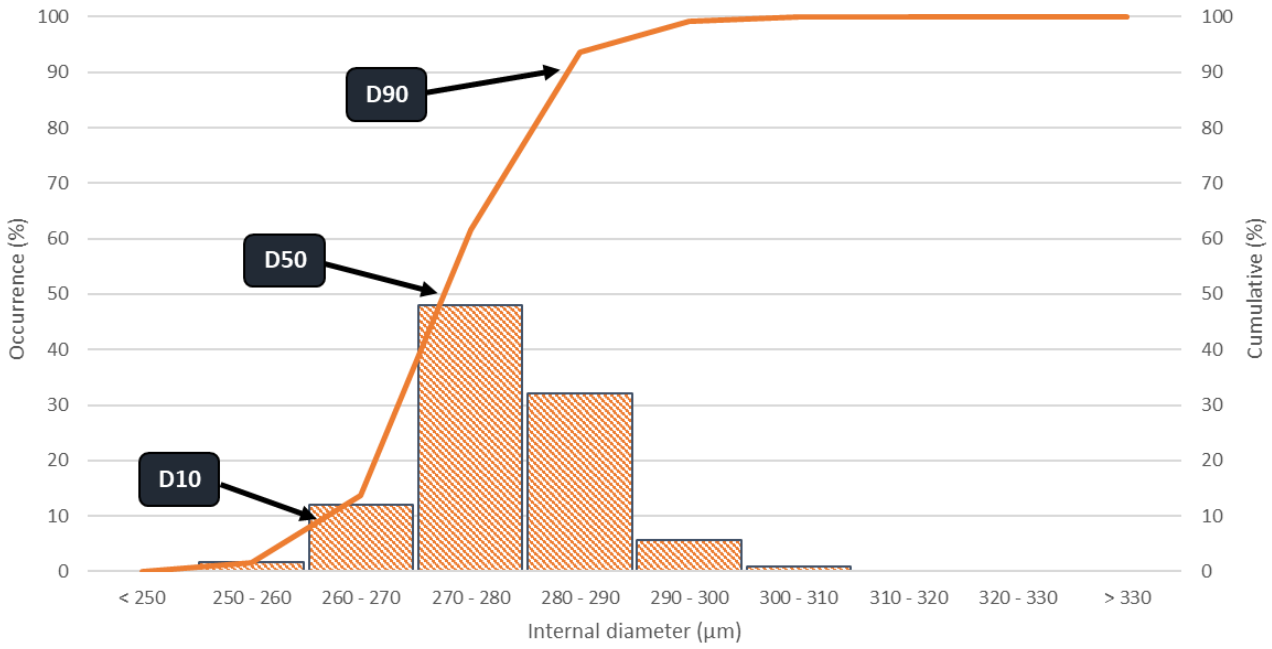


Figure 12: Particle size distribution as a function of the internal diameter

The statistics underscores that the average size of the capsule is 318 µm and the average internal diameter is 278 µm. The polydispersity, defined as the distribution of the particle size, is 2 for the external diameter and 3 for the internal diameter. This means that all particles have very similar sizes.

Calculation of D10, D50, D90 and the SPAN[3] are made for both internal and external diameter. According to the value of D10 for the external diameter, 10% of all capsules have a diameter inferior to 309 µm. D90 equal to 327 µm means that 10% of the capsules have a diameter greater than 327 µm. Finally, the median diameter of external diameters is given by D50, so here 318 µm. The values of D10 and D90 only have a difference of 15 µm, so the external diameter of capsules is very homogeneous.

[3] The SPAN value is defined as the width of the distribution. It also gives an indication of how far the 10 percent and 90 percent points are apart, normalized with the midpoint D50. The SPAN is equal to 0.06 for the external diameter and 0.07 for the internal diameter so the capsules have very similar sizes and the width of the distribution is narrow.

$$SPAN = \frac{D90 - D10}{D50}$$

In the same way for the internal diameter, 10% of all capsules have a diameter inferior to 268 µm according to D10. D90 equal to 289 µm means that 10% of the capsules have a diameter greater than 289 µm. Finally, the median diameter of internal diameters is given by D50, so here 277 µm. The values of D10 and D90 only have a difference of 20 µm, so the internal diameter of capsules is very homogeneous. This is indeed one of the big advantages of using the Raydrop® emulsification device as pointed out in the introduction.

$$CV\ (\%) = \frac{Standard\ deviation}{Average\ diameter} * 100$$

The Coefficient of Variation (CV) is a standardized measurement of the dispersion of the particle distribution. The CV is equal to 2% for the external diameter and 3% for the internal diameter so the dispersion around the average is low.

IV) Conclusion

The production of polymethacrylate-based solid capsules is possible with the use of a microfluidic platform. The aqueous core enables the encapsulation of water-soluble compounds, as vitamins, proteins or any water-soluble compound. The Raydrop® offers the possibility to change easily the shell thickness by varying flowrates. Moreover, a sample of 250 capsules have been studied to analyze the distribution of particles. This is an important criterion which allows to evaluate the dispersity of the capsules. As established in the previous section, the droplets that are produced with a Raydrop® microfluidic generator are highly monodispersed with low SPAN values concerning the internal and the external diameters.

V) References

[1] GALOGAHI, Fariba Malekpour, ZHU, Yong, AN, Hongjie et NGUYEN, Nam-Trung, 2020. Core-shell microparticles: Generation approaches and applications. Journal of Science: Advanced Materials and Devices. 1 décembre 2020. Vol. 5, n° 4, pp. 417-435. DOI 10.1016/j.jsamd.2020.09.001.

[2] CHEN, Philipp W., ERB, Randall M. et STUDART, André R., 2012. Designer Polymer-Based Microcapsules Made Using Microfluidics. Langmuir. 10 janvier 2012. Vol. 28, n° 1, pp. 144-152. DOI 10.1021/la203088u.