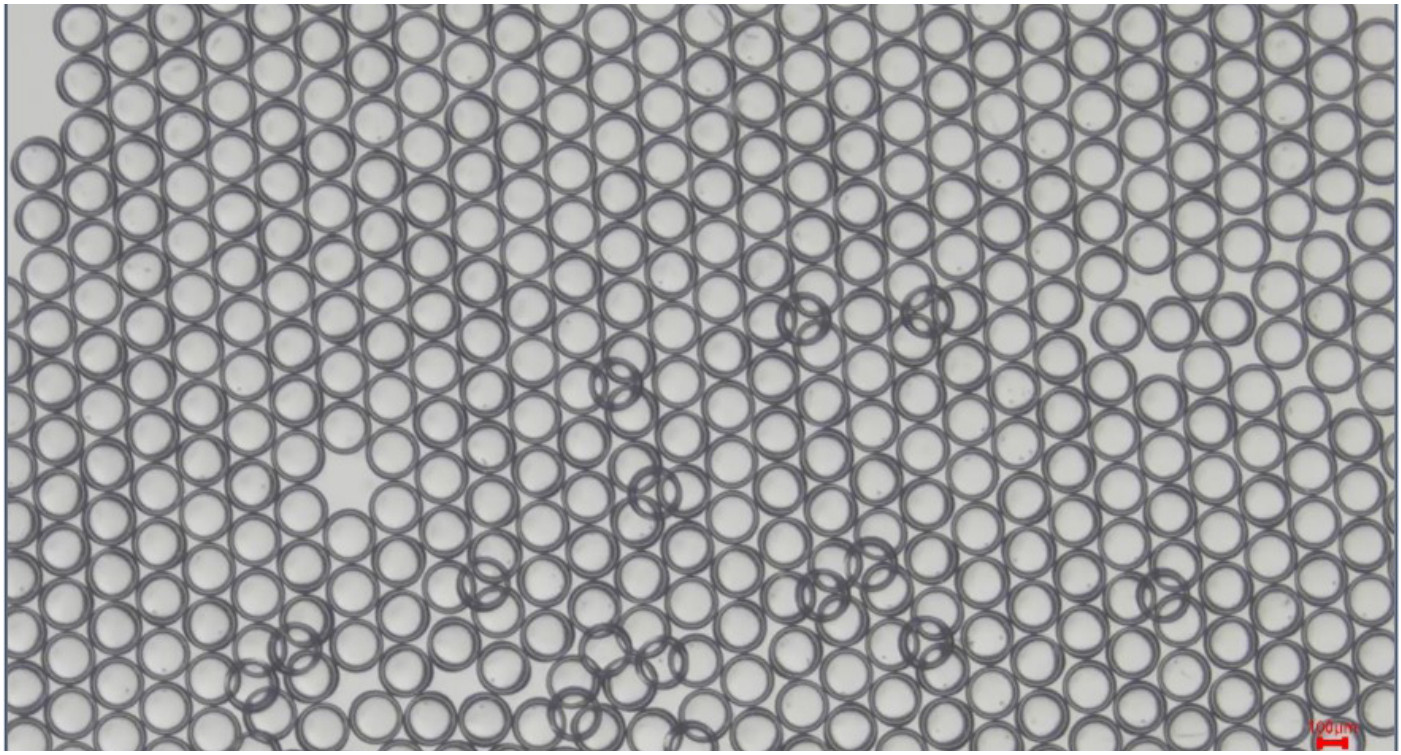
PEGDA MICROCAPSULE CREATION



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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 Ingredient (API) in core-shell microcapsule is of great interest for several purposes: taste and odor masking, controlled release of drugs... In pharmaceutics, the possibility of encapsulating drugs, nutrients, and living cells that can be protected by a solid biocompatible shell to target a specific site is an intense field of research.

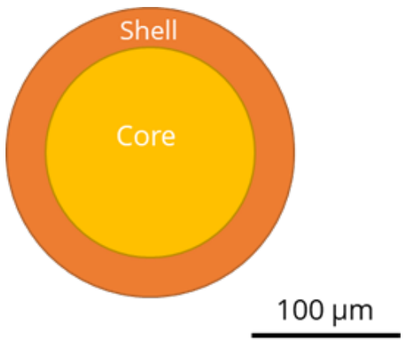


Figure 1: Definition of a solid core-shell

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

In contrast, microfluidics allows the production of monodisperse double emulsions which lead to monodispersed microcapsules with a high control over both the size and the structure. Microfluidic tools are also used 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 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 by acid-triggered gastric delivery or other delivery methods depending on the composition of the shell.

In this Application Note, poly(ethylene glycol) diacrylate (PEGDA) is used to produce capsules. PEGDA is part of the photo-crosslinkable hydrogel matrices well-known for their biomedical applications, as well as tissue engineering and regenerative medicine, drug delivery, cancer therapies, and biosensing [1]. A study of photoinitiators [2], chemicals added to the PEGDA that initiate the cross-linking reaction (here under the effect of a UV irradiation), is made to understand their influences on the solidification of the PEGDA. Moreover, it is shown that the use of PEGDA with a molecular weight of 250 Da (PEGDA-250) allows the encapsulation of either water or oil thanks to the solubility

MATERIALS AND METHODS

Materials

Reagents:

Core phase:

- Distillated water

Shell phase:

- Pure Poly(ethylene glycol) diacrylate Mw = 250 g/mol (PEGDA-250, Sigma-Aldrich) with various photoinitiators, depending on the experiment:

Photoinitiator 1 (PI1): 1% wt of photoinitiator 2-Hydroxy-2-methylpropiophenone (DAROCUR 1173, Sigma-Aldrich)

Photoinitiator 2 (PI2): 0.1% wt of photoinitiator Diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (DAROCUR TPO, Sigma-Aldrich)

Photoinitiator 3 (PI3): 0.1% wt of 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (IRGACURE 2959, Sigma-Aldrich)

The choice of a photoinitiator depends on its properties (biocompatibility, ...) but also on its irradiation wavelength and solubility in the solvent used.

Here, three different radical-generating photoinitiators have been tested.

The PEGDA-250 is selected because it is insoluble in water, unlike PEGDA-575 or PEGDA-700 [3].

It is important to notice that the photoinitiator reacts with light, so to avoid solidification of the shell solution, the solution must be protected from light by using an opaque container.

Continuous phase:

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

Priming and cleaning phase:

- Ethyl acetate (EtOAc, Merck)

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. Three 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. Three Flow Units M are used here to monitor and control the flow rates of the dispersed and continuous phase

Platform device: **PEGDA microcapsule creation**

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.

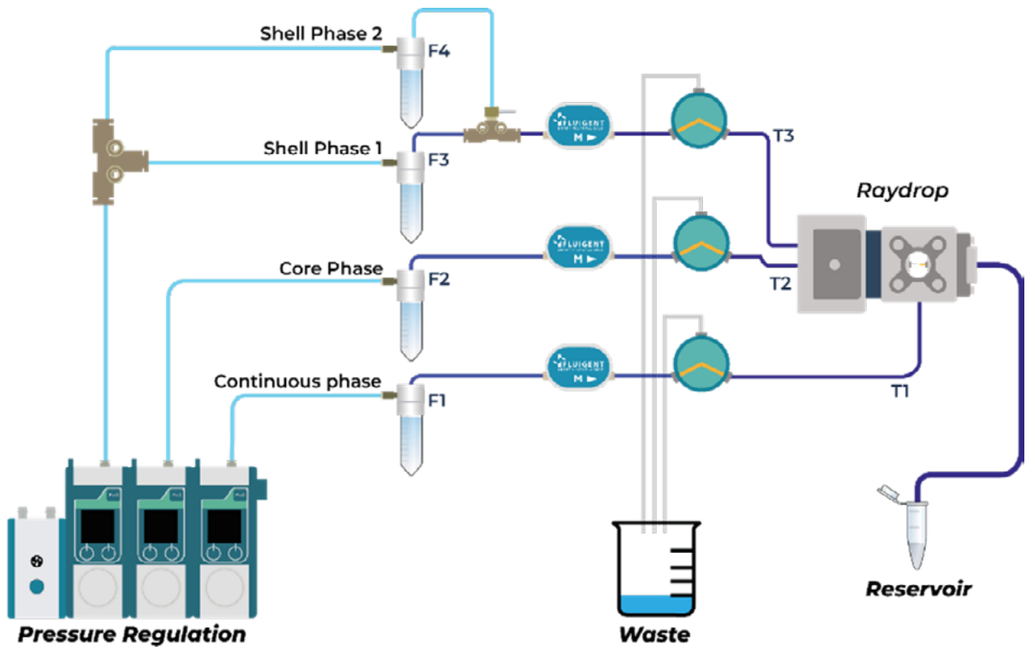


Figure 2: Experimental set-up to produce double emulsions.

Fluid reservoirs:

Falcon identification	F1	F2	F3	F4
Volume (mL)	50	50	50	15
Phase*	Continuous	Core	Shell (priming and cleaning)	Shell
Composition	Water + 2% PVA	Water	EtOAc	PEGDA-250 + DARO-CUR 1173 OR TPO OR IRGACURE 2959

* Each phase is filtered in order to avoid contamination from solid particles (dust, precipitate,...). 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.

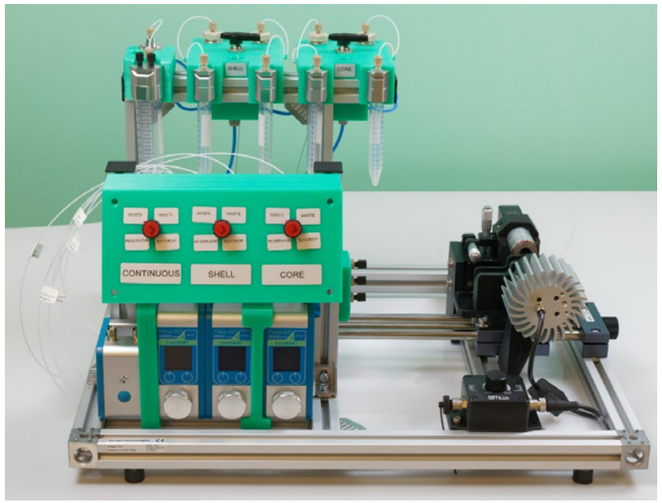


Figure 3: Raydrop Platform

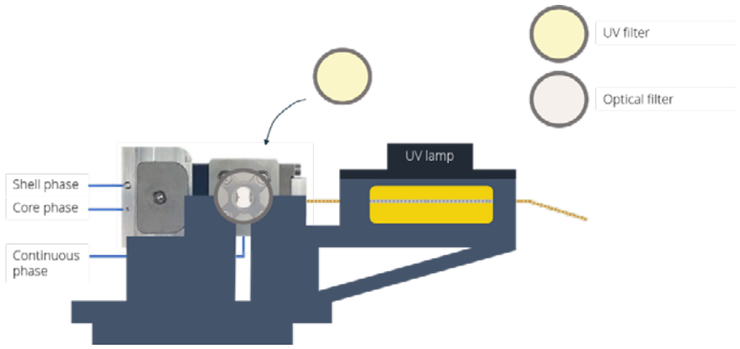
- **Mechanics:** The mechanical part includes x-y-z displacement plates that allow the adjustment of the focus and the observation window in the Raydrop®.

- **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®.

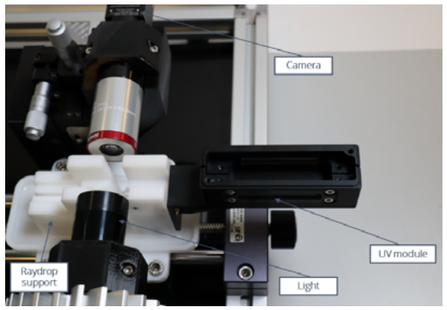
- **Optics:** The optical part of the platform contains an LED light source and a color USB 3.0 camera. This camera is connected to a computer to observe the droplet formation, control the stability of the emulsion, and measure the size of interest (core, shell).

Double emulsions are formed by pumping the three fluids through the Raydrop® using a pressure controller. The flow rates are monitored using flowmeters. This time, as a polymeric double emulsion is formed, the platform configuration changes a bit.

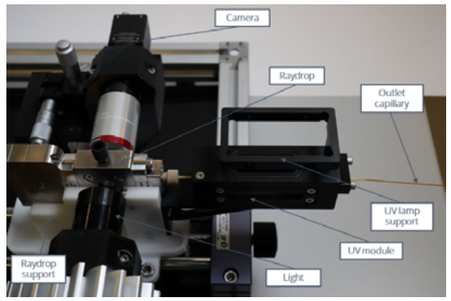
A module integrating a UV lamp is added to the platform to precisely control the cross-linking of the resin shell, as shown in Figure 4.



Scheme of the module. On the left, the Raydrop® generates double emulsion droplets that are transported in a capillary submitted to UV illumination in the box on the right. Note that the capillary is coated with a UV opaque coating except in the UV box.



Module integrated into the platform.



Raydrop® and outlet tubing were placed in the module. A UV lamp has to be fixed in the lamp holder.

Figure 4: The module integrated into the Raydrop Platform.

This module is movable using a displacement plate, which allows us to observe not only the formation of the emulsion in the Raydrop® but also to check at the exit of the Raydrop® that the emulsion remains stable (essential step during the formation of double emulsion) in the outlet tubing. The observation is made with the camera of the platform, by moving the module (see Figure 5) so that the camera can follow the emulsion progression in the outlet tubing.

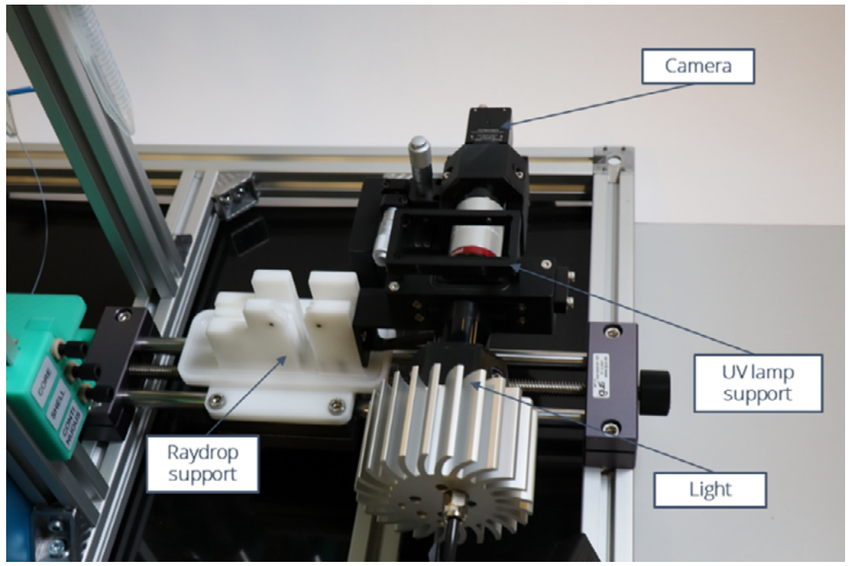


Figure 5: : Module in observation position in the outlet tubing.

Two windows with an anti-UV glass filter guarantee a safe observation for the user.

The UV light source irradiates the tubing at a wavelength of 385nm to initiate the cross-linking of the resin. Hard shell capsules come out at the end of the tubing, which can be more or less inclined to simplify the collection in a vial.

The *in-situ* cross-linking of the emulsion (see Figure 6) 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.

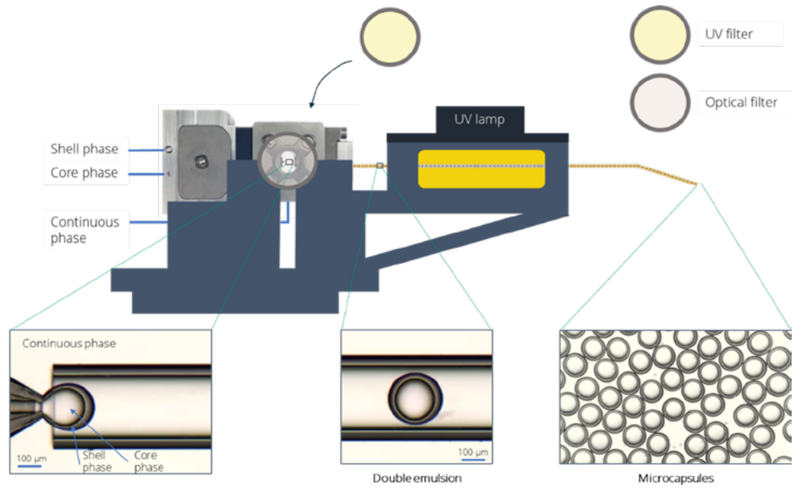
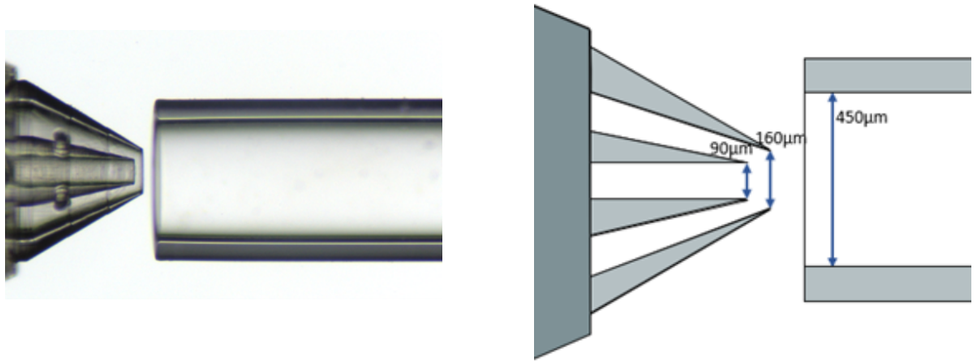


Figure 6: : In-situ cross-linking process leads to the formation of monodispersed microcapsules .

Raydrop® configuration

The Raydrop® is Secoya's microfluidic droplet generator for simple and double emulsion. A single emulsion is produced with a nozzle carrying one liquid (the droplet phase) while using a nozzle with two liquid entries (see Figure 7), stable double emulsions can be produced. It is also possible to produce simple emulsion using the double emulsion nozzle with an inner (core) phase set to zero. Thanks to its cylindrical geometry, this system doesn't require coatings to be applied on the wall of the collection capillary in front of the nozzle because the single or double droplet is surrounded by the continuous phase and therefore never in contact with the wall of the capillary; to avoid fast coalescence after droplets collection, surfactants are usually added to the different phases.



Picture of the Raydrop® Scheme of the Raydrop®

Figure 7: Double emulsion nozzle in front of the extraction capillary.

Nozzle informations:

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

Emulsion generation

To generate a double emulsion, the system must first be primed with pure solvent in the shell phase. Once a stable droplet formation is reached, the shell phase is switched to the PEGDA solution. This priming procedure avoids problems caused, for example, by the presence of bubbles or undesired contact between the core and continuous phase during the transient phase before the droplet generation stabilized. The user should follow the steps beside.

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 PEGDA solution (F4).
7. Wait until the PEGDA solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a PEGDA solution and an aqueous core in the continuous phase, in the same way as in Figure 8.

Figure 8: 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.

Particle formation

To form PEGDA particles, whereas beads (using simple emulsion) or capsules (using double emulsion), the emulsion is irradiated by UV light. The photoinitiator reacts to the light and initiates a cross-linking with the PEGDA. A chain-growth mechanism is enabled, leading to the formation of a three-dimensional network. This phenomenon is underscored in Figure 9 with three photoinitiators frequently used with PEG polymers.

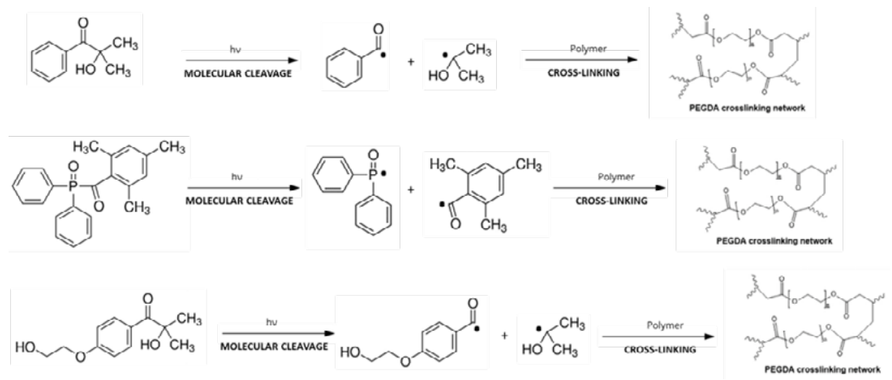
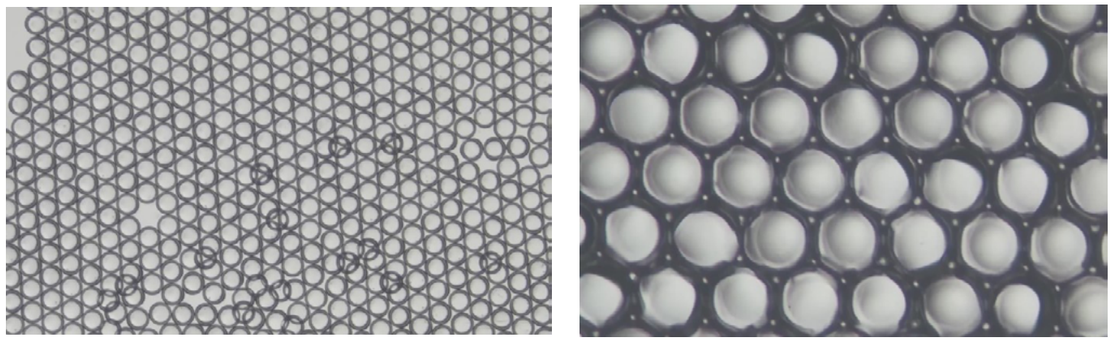


Figure 9: Chain-growth mechanisms [4].

Practically, the triggering of the shell solidification (to form microcapsules) is done according to the following steps:

10. Translate the module so that the camera shows the double emulsion in the outlet tubing
11. Check that all droplets are of the same size and that the droplet train is regular with a constant space between each drop. If the droplet train is not regular, modify the flow rates to stabilize the production.
12. Once the drop train is stable, switch on the UV light.
13. Collect the capsules at the outlet of the tubing (Figure 10).



Picture of the Raydrop® Scheme of the Raydrop®

Figure 10: PEGDA capsules containing water, observed under the microscope. On the left, capsules were collected in water. On the right, dried capsules. Both scale bars correspond to 200µm.

14. Once 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 PEGDA. In this way, the tubing stays clean and clogging is avoided.

15. First, translate the module so that the camera displays the nozzle of the Raydrop®
16. To flush the PEGDA out of tubing and Raydrop®, switch the valve on the priming solution (F3)
17. Wait 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.
18. Cut off the flow of the core phase
19. Then, cut off the flow of the shell phase
20. Finally, cut off the flow of the continuous phase

RESULTS

Once the production of PEGDA particles is established, it is possible to produce either beads or capsules. In the following, both possibilities are presented, using various photoinitiators.

Bead formation

Firstly, PEGDA beads are formed in the Raydrop® (the core phase flowrate is set to 0 µL/min). The droplets are transported in the glass capillary out of the Raydrop® and irradiated in the UV module. After the UV radiation, solid beads are collected in a vessel and the pictures of Figure 11 are taken. As underlined in Figure 12, the size of beads can be chosen by fixing a specific shell flowrate (also called dispersed phase).

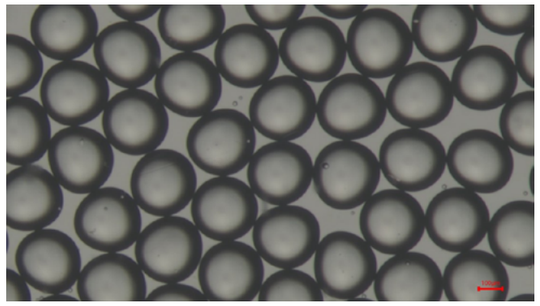


Figure 11: Various sizes of PEGDA beads. The scale bar corresponds to 100µm.

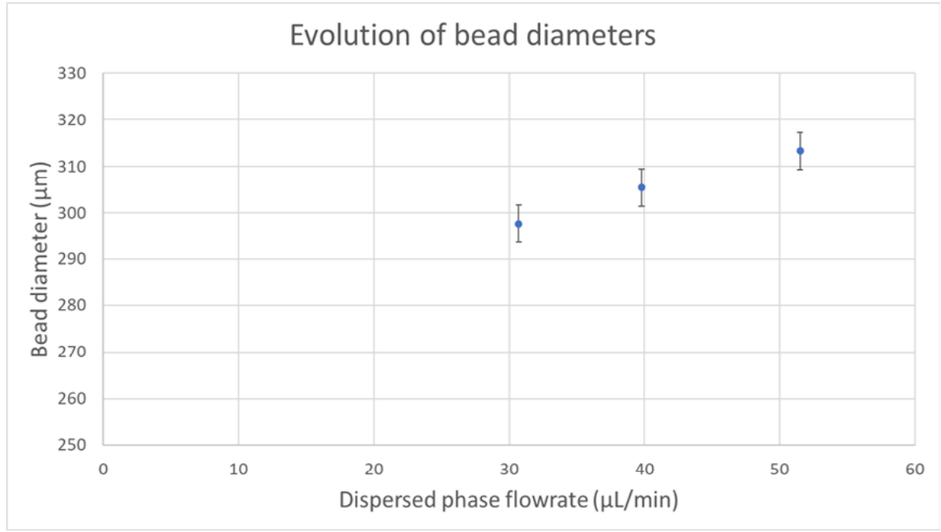


Figure 11: Various sizes of PEGDA beads. The scale bar corresponds to 100µm.

Remark: to produce beads, a simple emulsion of PEGDA in water is needed. To do that, we advise you to use an insert with a nozzle for simple emulsion and not an insert for double emulsion. It will simplify the production of the emulsion and it also reduces the difficulty degree of production.

Capsule formation: photoinitiator comparison

A study of the influence of the photoinitiator on the capsule formation is performed using the three following photoinitiators: Darocur 1173, TPO, and IRGACURE 2959. The UV lamp used irradiates at 385nm and its intensity can be tuned from the minimum intensity Imin (corresponding to 3.8 mW/cm²) to maximum intensity Imax (corresponding to 39.3 mW/cm²).

Use of DORACUR 1173 for cross-linking

The Darocur 1173 is dissolved in PEGDA at a concentration of 1% wt. As described in paragraph 4), a double emulsion of water in PEGDA in water is formed inside the Raydrop®. The double emulsion is then irradiated inside the UV module to make the photoinitiator react and form solid microcapsules.

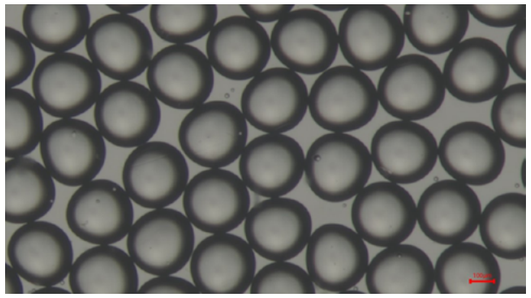


Figure 13: PEGDA particles with Darocur 1173 irradiated at Imin. The scale bar corresponds to 100µm.

When the double emulsion is irradiated at Imin, the capsules are not stable, and the shell explodes within 1 minute of their formation (see Figure 13).
When the double emulsion is irradiated at higher intensity Imax, solid capsules are obtained (see Figure 14) and they are brittle when pressed with tweezers.

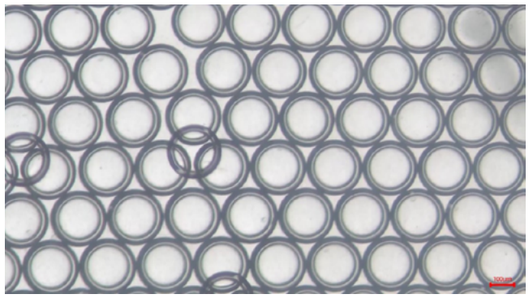
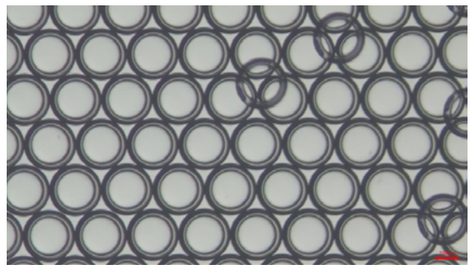


Figure 14: PEGDA microcapsules with Darocur 1173 irradiated at Imax. The scale bar corresponds to 100µm.

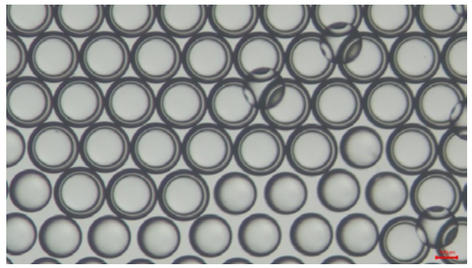
Finally, the PEGDA cross-linking requires a certain amount of UV to be induced. There is therefore a certain intensity limit below which the capsules will not solidify.

Use of IRGACURE 2959 for cross-linking

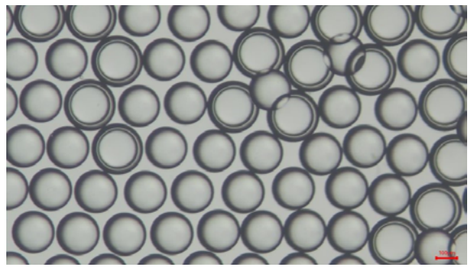
IRGACUR 2959 is dissolved in PEGDA at a concentration of 0.1% wt. Here again, the double emulsion is irradiated at Imax to form capsules.



PEGDA particles are collected and observed under a microscope. The shell is clearly visible.



Around 30 seconds after the first observation, shells explode because the cross-linking is not achieved.



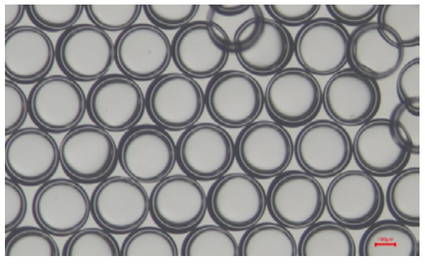
Quite every shell has exploded, and any solid capsule has been produced.

The production of solid microcapsules with IRGACUR 2959 as a photoinitiator is not possible (see Figure 15). Whatever the intensity of the UV lamp, the shell does not become solid. This is probably due to the irradiation length of the UV lamp used which does not correspond to the excitation length of IRGACUR 2959 (274 nm [2]).

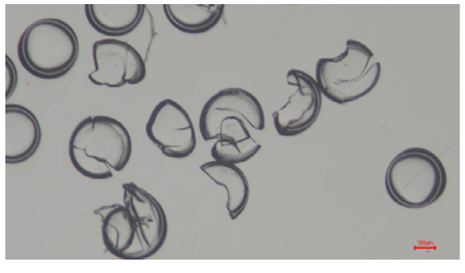
Figure 15: PEGDA particles with IRGACUR 2959. The scale bar corresponds to 100µm.

Use of TPO for cross-linking

The third photoinitiator to be tested is TPO. A double emulsion is generated and irradiated by the UV light at Imin. As a result, solid microcapsules are obtained (see Figure 16).



PEGDA capsules



Broken PEGDA capsules

Figure 16: PEGDA particles with TPO photoinitiator. The scale bar corresponds to 100µm.

The TPO allows the creation of hard microcapsules. It is possible to break them with tweezers (see Figure 16 right).

CONCLUSION

PEGDA microcapsules are of interest because of their biocompatibility. In this application note, we demonstrated that it is easy to produce beads and capsules of PEGDA with the Raydrop platform including a UV module. The study and comparison of three different photoinitiators highlight the influence of the photoinitiator on the final robustness of the particles.

According to experiments, we recommend using the TPO photoinitiator to produce PEGDA particles because of its good compatibility with PEGDA and because of its irradiation length which corresponds to the UV lamp used. TPO has also fast kinetics, which allows it to quickly form strong and brittle beads and capsules.

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