WHITE
PAPER

CONTROLLED DOUBLE EMULSIFICATION PROCESS FOR ENCAPSULATION

Generation of microcapsules with a microfluidic platform containing a Raydrop® generator

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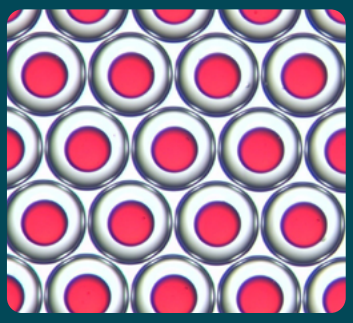
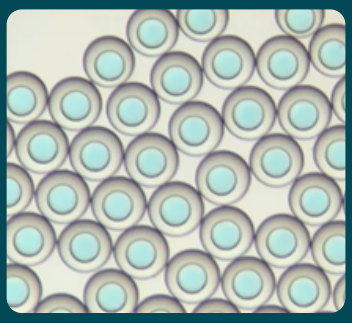
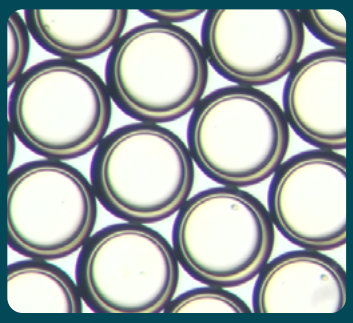


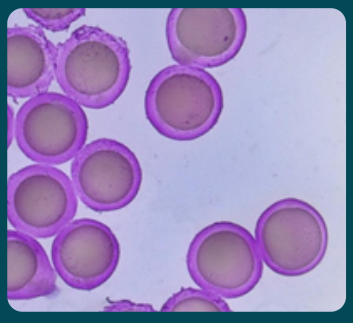
Chitosan



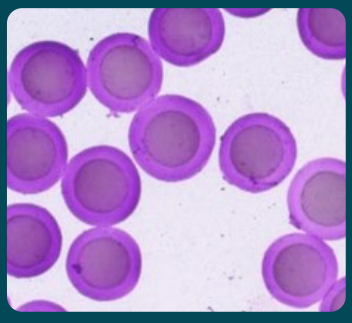
Poly(D,L-lactide-co-glycolide) (PLGA)



Polymethacrylate resin



Alginate



Agarose

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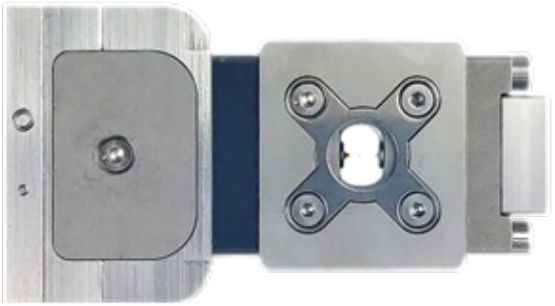
FLUIDIFY PHARMA

FLUIGENT
SMART MICROFLUIDICS™

Executive summary

- *The Raydrop® generator is compatible with a wide variety of materials*
- *Particle size from 40 to 250µm*
- *Encapsulation yields > 90%*



TERMINOLOGY

Emulsion: a dispersed, multiphase system consisting of a minimum of two immiscible liquids. The liquid that forms droplets is called the dispersed phase, while the liquid in the bulk surrounding the droplets is called the continuous phase. [1]

Double emulsion: one droplet of one material in a droplet of another material in a continuous phase.

Microcapsule and microsphere: created by enclosing the active compound within a cavity surrounded by a semipermeable membrane or within a solid matrix. [2]

Encapsulation efficiency: the concentration of the incorporated material (such as active ingredients, drugs, fragrances, proteins, pesticides, antimicrobial agents, etc.) detected in the formulation divided by the initial concentration used to make the formulation. [3]

INTRODUCTION

Emulsions are usually manufactured in batch processes on a large scale. These produced emulsions require large amounts of energy and have wide size distributions with low reproducibility. Moreover, when it comes to encapsulating active pharmaceutical ingredients (APIs) in these drops, the process is complex, and losses are high. Indeed, the APIs must be encapsulated in a material that can then deliver APIs in a delayed and controlled manner. In high value-added areas such as the pharmaceutical industry, the use of microfluidic emulsion systems allows for users to obtain monodisperse emulsions and to improve the quality of the product, with notably reduced API losses due to higher encapsulation efficiency than batch processes.

In this context, Secoya has developed a device named Raydrop®, which is a **microfluidic droplet generator** that facilitates the production of emulsions. Raydrop® technology aims for a more robust production with less wear than most current microfluidic emulsification devices. Depending on the configuration of the Raydrop®, it is possible to **create either simple or double emulsions.** In this white paper, we discuss the Raydrop® and it's invovlement in the production of double emulsions.

Furthermore, the Raydrop® is now a part of a user-friendly microfluidic platform that contains all elements needed to produce a reproducible and high-quality emulsion, such as fluidic elements, mechanic compounds, and optical materials.

EQUIPMENT

1. The Raydrop®

The Raydrop® enables the production of simple emulsions (oil-in-water and water-in-oil) as well as double emulsions (oil-in-water-in-oil or water-in-oil-in-water) without coating treatment. This microfluidic device allows for the generation of controlled double emulsions and production of highly monodispersed microcapsules. The Raydrop® is composed of a stainless-steel chamber in which two inserts are placed. A schematic illustration of its construction is shown in Figure 1 for a double emulsion construction - for a single emulsion, the inlet insert is exchanged on the same Raydrop® unit.

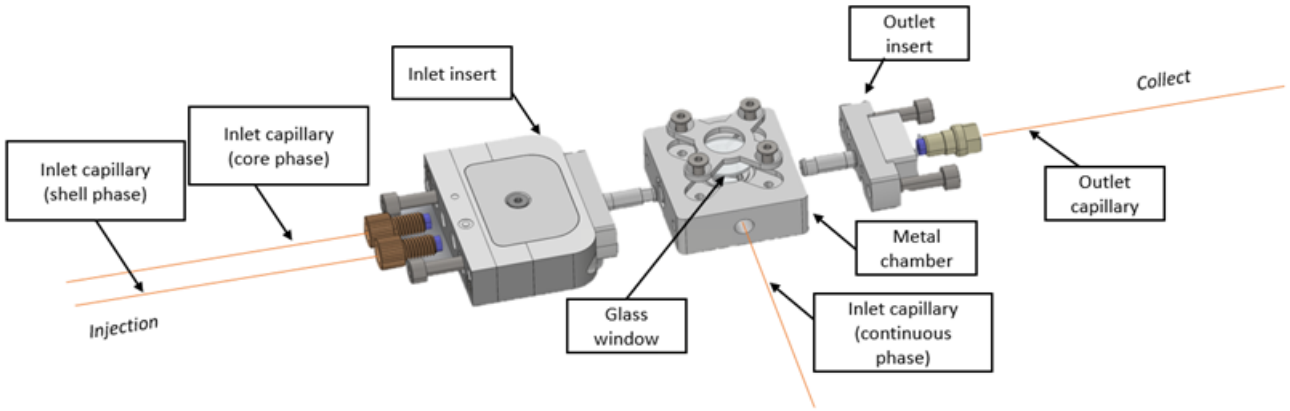


Figure 1: Exploded view of the Raydrop®, non-embedded capillary device developed by Secoya Technologies.

Two standard microfluidic connections (Standard Upchurch fitting ¼-28) are connected to the inlet insert for the shell and the core phases. One microfluidic connection is linked to the chamber to make the continuous phase flow and another microfluidic connection is used on the outlet insert to link an outlet capillary to collect the emulsion. Two glass windows are placed on the metal chamber for visibility of the double emulsion formation inside the device. Both inserts can easily be disassembled from the chamber to clean the device. This can be done when changing fluids.

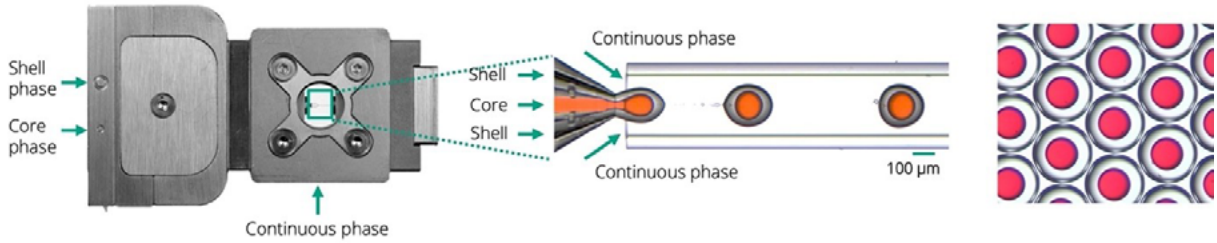


Figure 2: Internal structure of the Raydrop® [4].

The Raydrop's® design is based on the alignment of a 3D-printed nozzle and an extraction capillary immersed in a pressurized chamber containing the continuous phase. The shell and the core phases disperse through the nozzle placed in front of the extraction capillary. Then, the double emulsion is created by a pinching off mechanism where the continuous phase isolates particles. The droplets can be collected at the outlet.

The device is based on axisymmetric flow focusing geometry which removes wettability issues, meaning no coating is required. Moreover, the axisymmetric design induces inherently highly monodispersed emulsions (particle size distribution, PSD < 2 %).
The droplet size is easily controlled with a production frequency up to the kilohertz range. The size of produced double emulsion ranges from 80 to 150 µm, depending on the size of the nozzle. The shell thickness can be adjusted by applying different variations of flowrates to all fluids.

2. Microfluidic double emulsion platform

To circulate fluids inside the Raydrop®, a microfluidic platform is used. This platform is composed of the elements (pumps, controllers, optics, filters) needed to produce an emulsion and is designed for research and development projects.

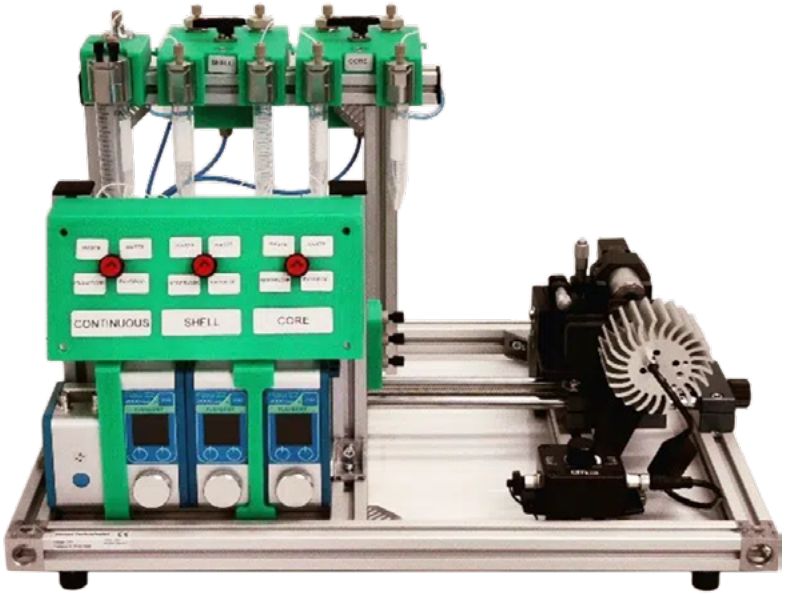


Figure 3: Raydrop® platform.

3. Microfluidic flow control

To circulate the aqueous and oil phases inside the microfluidic chip, three Flow EZ 7 bar (Fluigent) are used (one for each phase) and connected to a Link module to allow full control via OxyGEN software.
Each Flow EZ is also connected to a microfluidic flow sensor: A flow unit L for the continuous phase, a flow unit S for the shell phase, and a flow unit M for the core phase, to ensure an accurate flow-rate measurement and allow direct flow-rate control on the flow-controller.

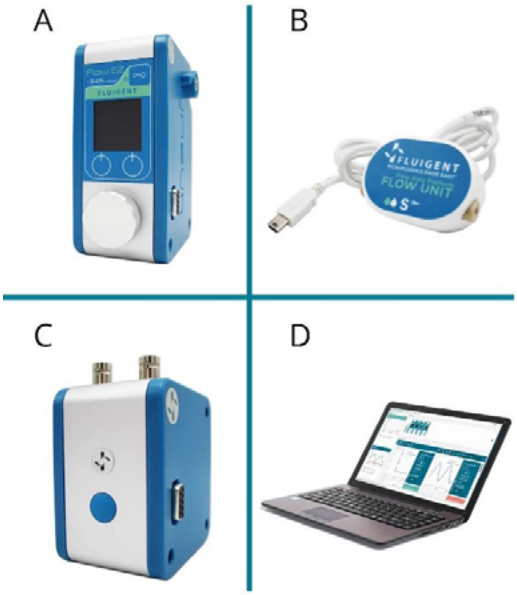


Figure 4: Main flow control equipment: Flow EZ (A), flowmeter (B), Link module (C) and OxyGEN software (D).

4. Microfluidic accessories

To prevent clogging in the fluidic path, filters are used for each phase: a 10 µm filter for the continuous phase and 2 µm filters for the shell and core phases.

A manual valving system is used to allow maximum control over the delivery of liquids to the microfluidic chip. Two-way valves are used for the core and shell phases - between the pressurized reservoir and the flowmeter - to allow switching between a priming solution and the hydrogel.

After the flowmeter and before the microfluidic chip, a four-way valve is added to each phase to ensure control over the liquid destination, either waste or the microfluidic chip.

Finally, PFA and PEEK tubing are used with adapted connectors.

5. Optical system

The embedded optical system, including a camera and an LED light source, enables the viewing of the droplet formation and video acquisition.
The mechanical part groups together the displacement plates, which allows the camera to adjust and to translate the Raydrop®.

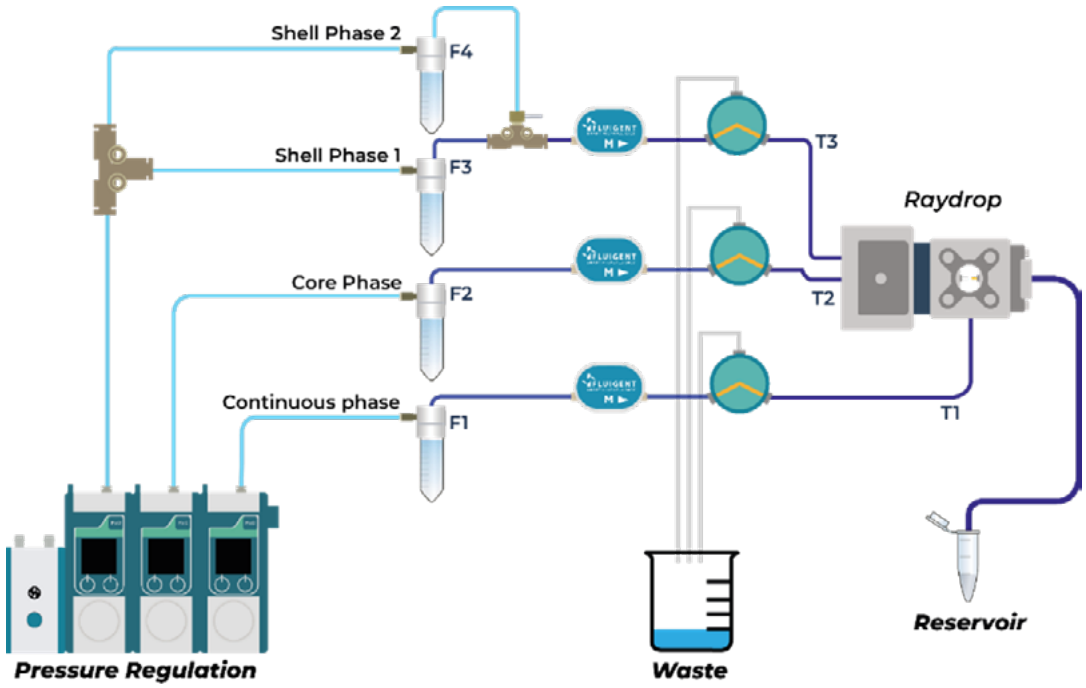


Figure 5: Fluidic system diagram corresponding to the platform. This flow scheme can be slightly different from the one corresponding to the commercialized version of the platform, where normally two reservoirs for the core phase are included.

In the following document, five applications made with the microfluidic platform containing a Raydrop® present an overview of the abilities of this platform. The first three cases concern oil encapsulation in different polymeric shells.

First chitosan, a water-soluble polysaccharide crosslinkable to ex situ in the presence of a polyaldehyde (here glutaraldehyde) is used to create a soybean oil encapsulation.

Next, Medium Chain Triglycerides oil (MCT) is encapsulated in agarose, and then alginate shells. In the following cases, another encapsulation mode is underscored to encapsulate an aqueous core: first in a poly(lactic-co-glycolic acid) (PLGA) shell for the encapsulation of proteincapsule, and finally, in a commercial polymetacrylate resin shell crosslinked by UV irradiation.

USE CASE 1

Chitosan microcapsules

The first application of the Raydrop® is the production of an oil-in-water-in-oil emulsion. This emulsion is collected in a bath containing a cross-linking agent to solidify the shell and form capsules containing an oily phase. The encapsulation of oil contains lipophilic drugs or volatile oils. [5] Chitosan is biocompatible and has a pH sensitivity that can be useful for acid-triggered delivery [6].

To form a double emulsion, three solutions are needed. In this case, the core phase is soybean oil containing red dye, the shell phase is 2% of chitosan in water, and the continuous phase is 1-octanol containing 2% of the surfactant Span 80.

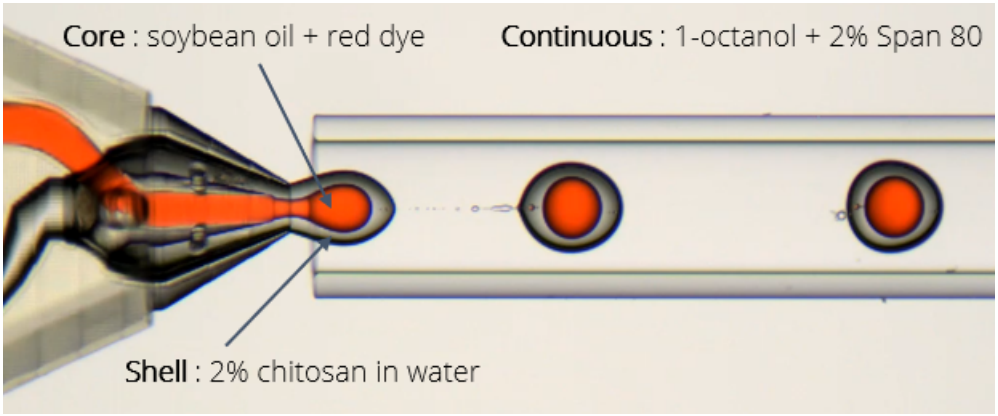


Figure 6: Formation of the chitosan double emulsion.

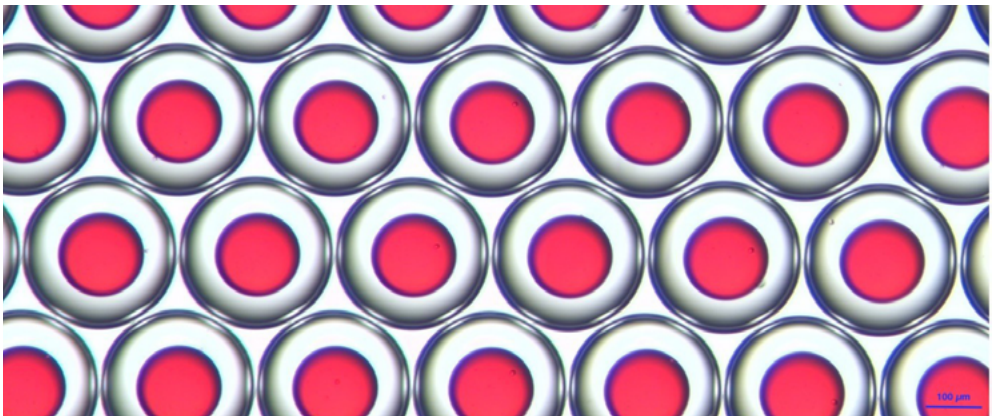


Figure 7: Oil/water/1-octanol double emulsion just after collection, observed under the microscope.

Once the double emulsion is generated, capsules are formed by the curing of the chitosan shell. To solidify the shell, droplets are collected in a cross-linking bath containing 0.3% glutaraldehyde in hexane.
As a result, chitosan reacts with glutaraldehyde by solvent extraction and chemical cross-linking based on the Schiff base reaction. The droplets are solidified and become glutaraldehyde cross-linked chitosan microcapsules.

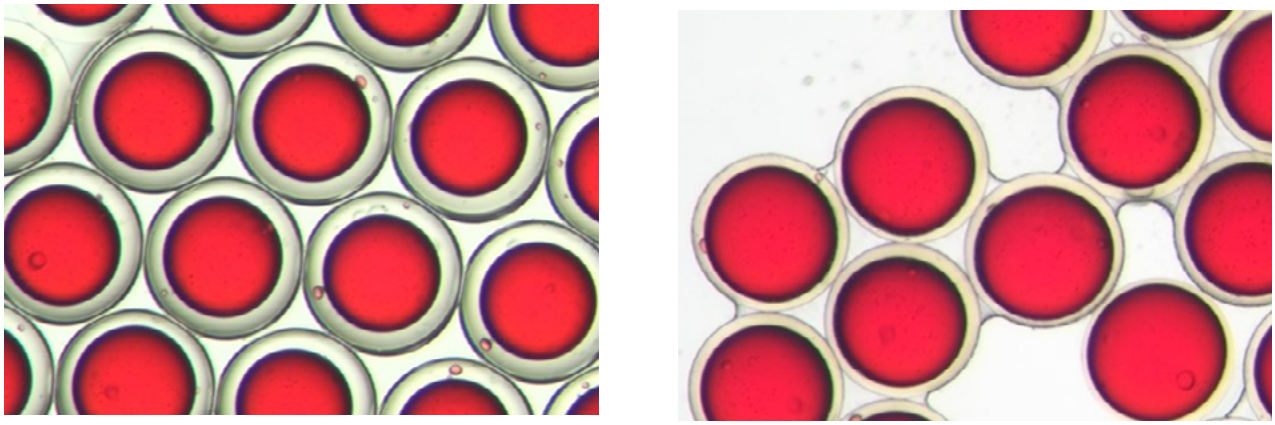


Figure 8: Glutaraldehyde cross-linked chitosan microcapsules in the cross-linking bath. On the left, after 4 minutes in the cross-linking bath. On the right, after 1h in the cross-linking bath. The shell thickness decreases and becomes progressively yellow, as a part of its water content diffuses in the continuous phase. Expelled water is clearly visible wetting the capsules.

The production of stable monodispersed microcapsules with a solid chitosan shell and a liquid oily core using a microfluidic system allows the encapsulation of lipophile compounds but also enables to encapsulate volatile products.
The microfluidic platform allows users to choose not only the core diameter but also the shell thickness adjusting the flow rates of the different fluids.
Thanks to excellent oil encapsulation properties and a very limited leakage over time (less than 5% after 24h), these microcapsules can be used in a wide range of applications, like the encapsulation of volatile products like mint oil [5] as well as specific drugs, which will be delivered according to the pH acidity [6].

For more information about the production of chitosan capsules, please refer to our application note on our website https://secoya-tech.com/ or contact us at marie.mettler@secoya-tech.com.

USE CASE 2

Agarose microcapsules

Another o/w/o microcapsule type uses agarose as the shell phase. The use of this hydrogel to generate emulsions has recently gained popularity due to its biocompatibility, chemical inertness, and common use for numerous applications such as protein immobilization and detection.

Instead of a cross-linking reaction, the solidification of agarose is temperature-dependant: under a certain temperature, the molecules constituting the agarose form double helix structures that assemble to form a solid gel. This gelation process is reversible: when heated up to a certain temperature, the structure disassembles and returns to a liquid state.

In this application, the core phase consisting of medium-chain triglycerides (MCT) oil. The shell phase is distilled water with 1% Tween 80 and 1%, 2% or 3% of agarose with a specific purple dye (to have better visualisation and contrast after capsule production) and the continuous phase is made of MCT oil with 2% w/w Polyglycerol polyricinoleate (PGPR) as a surfactant.

The concentration of agarose depends on the need: the more concentrated, the more viscous and difficult to handle it will be, but the more solid, the more obtained the microcapsules will be. To enable the easy and reproducible generation of monodisperse capsules, the Raydrop®, tubing, and shell phase must be heated to reach a liquid state, and then maintained above the gelation temperature (ideally at 50°C).

After production, the microcapsules are collected in a tube and the agarose cooling induces gelation, forming a solid capsule. Monodispersed double emulsions of various sizes could be made, and a fine tuning of the shell thickness was enabled by playing with the flowrates. In addition, the production of the emulsions was found to be stable, without clogging, for at least 30 minutes.

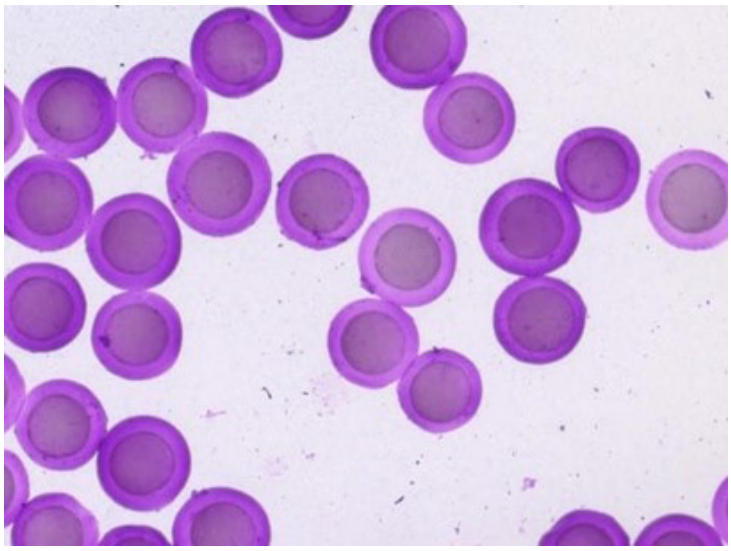


Figure 9 – Oil/Agarose/Oil microcapsules observed under the microscope.

USE CASE 3

Alginate microcapsules

Similarly to agarose, the use of alginate continues to expand, as its low viscosity, biocompatibility, and ability to mimic tissues make it suitable for experiments that require hydrogels. Here, the gelation occurs by cross-linking the alginate with a bivalent cation, most commonly Ca2+ and Ba2+.

In droplet microfluidics experiments, we use CaEDTA and acetic acid to allow the in-droplet gelation of alginate without gelation in the chip, which would cause clogging, resulting in an unstable droplet production.

Alginate and CaEDTA are encapsulated together in the dispersed phase and cannot crosslink in this state. Acetic acid, diluted in the continuous oil phase, will break the CaEDTA chelate and release bivalent calcium ions Ca2+ causing the alginate to gelify.

The core phase for alginate microcapsules generation is, as for agarose, MCT oil, the shell phase is made of distilled water with 1% tween 80, 1%, 2% or 3% w/w alginate depending on the experiment's needs, and a 2:1 ratio of CaEDTA. Finally, the continuous phase for this generation is MCT oil with 2% w/w PGPR and 5% acetic acid.

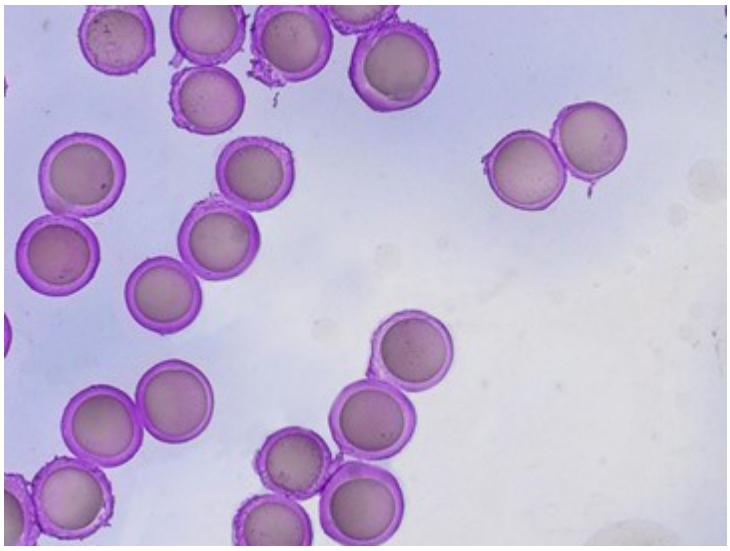


Figure 10 - Oil/Alginate/Oil microcapsules observed under the microscope.

After production, the microcapsules are collected in a tube and left for 3h in the continuous phase to allow crosslinking to occur and form polymerized alginate chains, resulting in a solid shell phase. After this, the beads were flushed with water and resuspended in MCT oil. The generation of microcapsules was found to be stable for at least 30 minutes with no chip clogging.

USE CASE 4

PLGA microcapsules

Another application of the Raydrop® is the production of a water in oil in water emulsion. The formation of PLGA microcapsules is a viable option due to its biocompatibility and biodegradability of PLGA. [7] The use of microfluidics allows for a controlled production of these capsules. This control is necessary to make the process stable and to have a suitable encapsulation efficiency. [8]

The production of PLGA double emulsion requires different solutions. In this case, the core phase is a Phosphate Buffered Saline buffer (PBS, pH = 7.4) with blue dye. The shell phase is isopropyl acetate (IPAc) containing 10% PLGA but it is also possible to use ethyl acetate (EtOAc). The continuous phase is water with 1% poly (vinyl alcohol) (PVA).

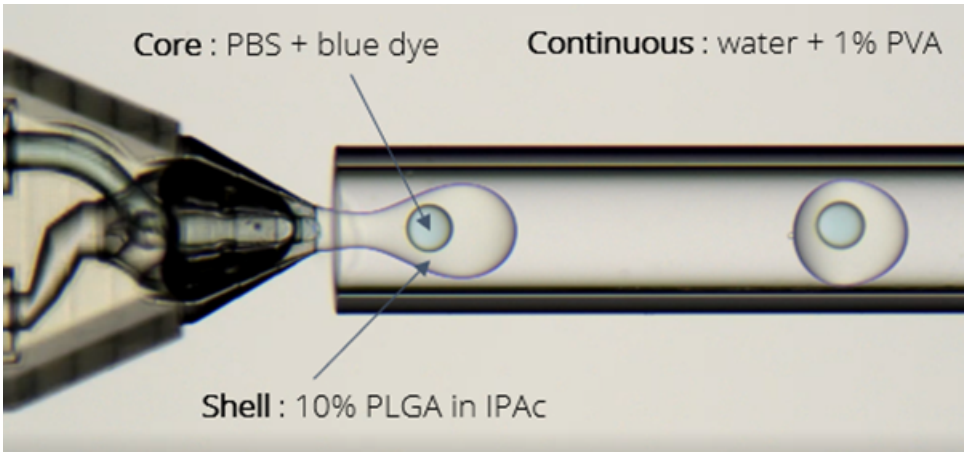


Figure 11: Formation of the PLGA double emulsion.

Once the double emulsion is formed, it is collected in a recipient. The obtained droplets are monodispersed at a diameter of 240 µm. However, the diameter of particles can be adjusted by varying the flowrates of the different phases.

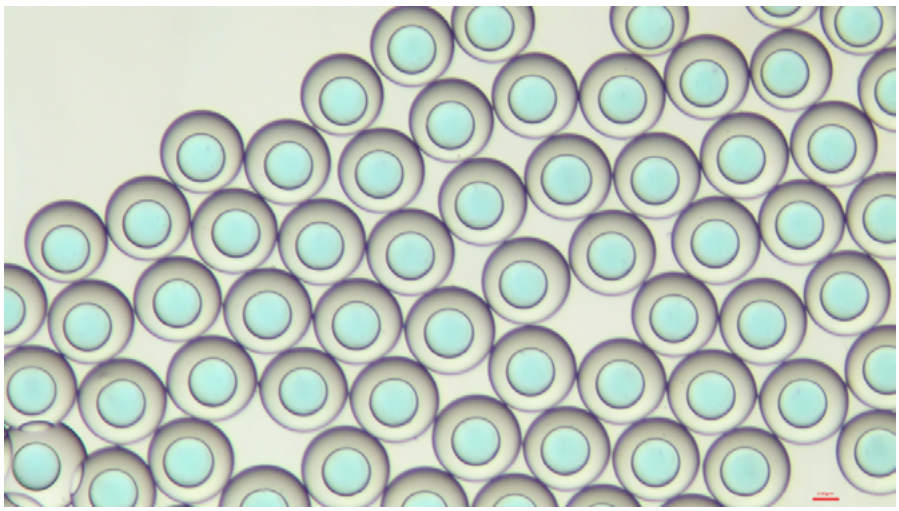


Figure 12: PBS/IPAc/water double emulsion just after collection, observed under the microscope.

After generation, the droplets are collected in a PBS solution so that the IPAc contained in the shell diffuses into the PBS solution of the vessel. Once the IPAc has spread to enough of the shell, PLGA precipitates, and the shell solidifies. The droplets then become biocompatible capsules.

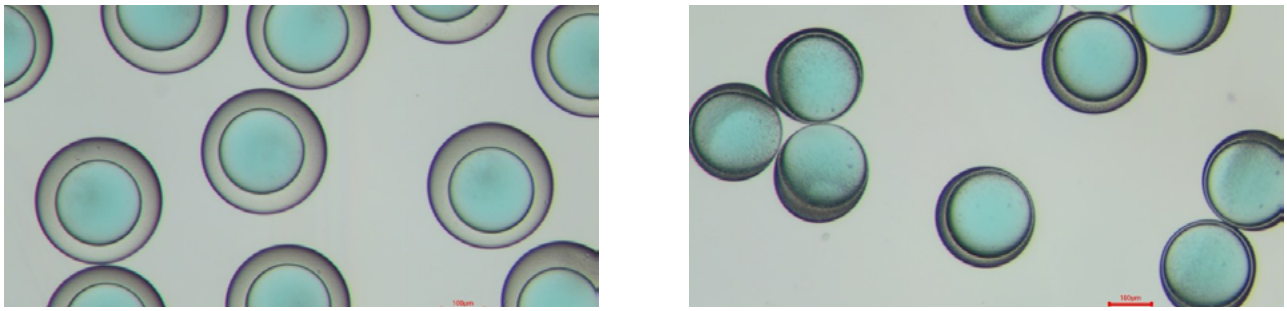


Figure 13: PLGA microcapsules in the PBS solution. On the left, 15 seconds after creation in the Raydrop®. On the right, 200s in the PBS solution after creation in the Raydrop®. The shell thickness decreases, as the IPAc contained in the shell phase diffuses in the continuous phase.

Here, a stable production method to generate a monodispersed double emulsion is presented. These microparticles have a strong potential for public health applications, as the PLGA is biocompatible, and its use is approved by the Food and Drug Administration (FDA).
The PLGA is a viable option for microcapsules of water-soluble compounds it is soluble in oily phases. Specific drugs can also be encapsulated, and the drug release is possible via enzymatic triggers due to the capabilities of PLGA molecules. [9]

For more information about the production of PLGA capsules, please refer to our application note on our website https://secoya-tech.com/ or contact us at marie.mettler@secoya-tech.com.

USE CASE 5

Polymethacrylate resin microcapsules

In the third set-up, the microfluidic platform has been used with a polymethacrylate resin to form capsules by UV cross-linking. For this purpose, a UV lamp has been added to the platform to irradiate the droplets.
This method of capsule formation is an in-situ cross-linking [10], which mean that the droplets are exposed to UV-light when they are still in the production system. Thanks to this operation, capsules formed by a solid shell and a liquid core are obtained.

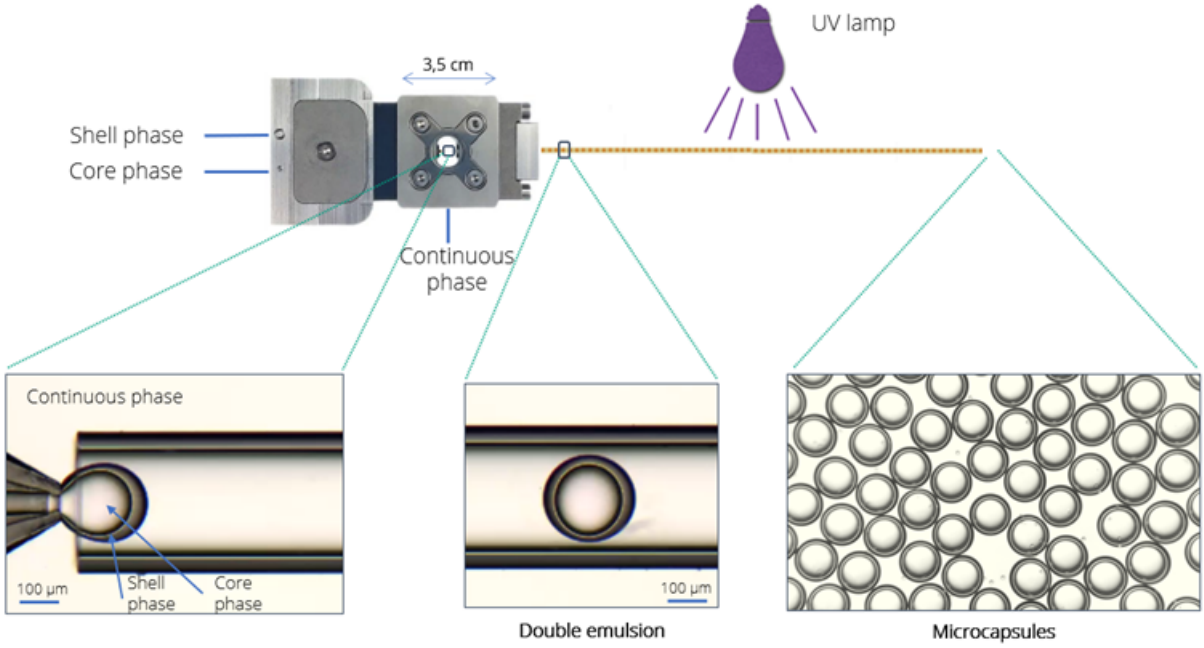


Figure 14: Setup containing the Raydrop® and a UV lamp for the formation of microcapsules in polymethacrylate resin.

The solutions used to form a double emulsion in this system are the following: the continuous phase is composed by water with 2% PVA; the shell phase is polymethacrylate resin with 20% EtOAc% EtOAc and 0.1% of diphenyl(2,4,6-trimethylbenzoyl) phosphine oxide (TPO); the core phase is water. With this composition, it is possible to add wateradd water-soluble compounds in the core phase to encapsulate them.

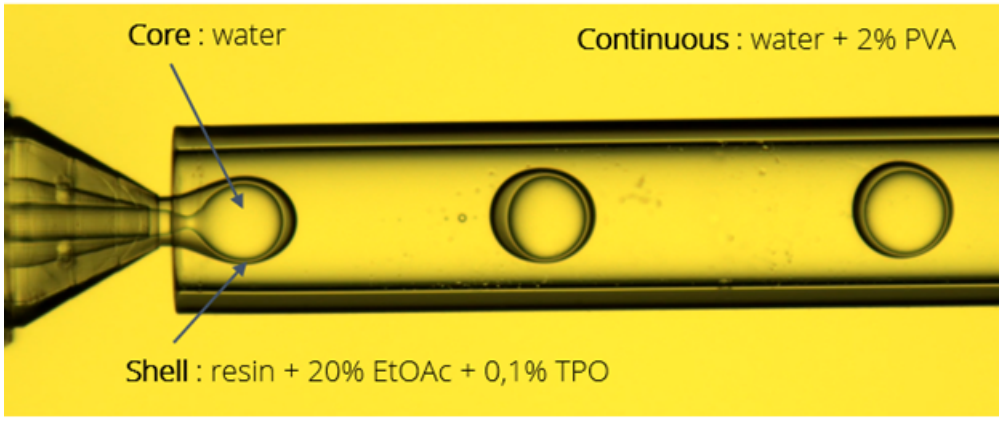


Figure 15: Formation of the polymethacrylate double emulsion. As this is a picture taken during the real experiment, the yellow color comes from UV protection placed between the light source associated to the camera and the Raydrop®. This device allows users to filter the UV light, which could irradiate in the Raydrop®. It is necessary to avoid triggering the cross-linking of the resin in the Raydrop® to avoid any clogging as the process of cross-linking occurs in a few seconds.

After the formation of droplets in the Raydrop®, the UV-lamp irradiates the outlet tubing so the cross-linking can take place. As the droplets are collected in a vessel after irradiation, monodispersed capsules have been generated.

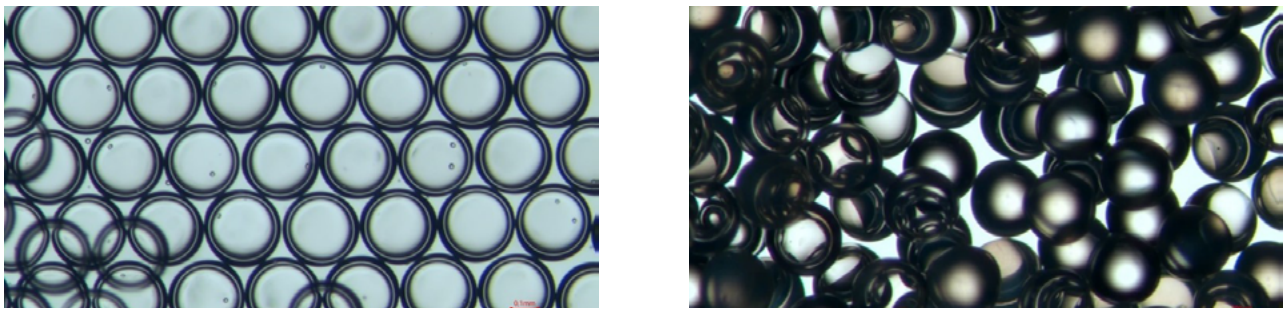


Figure 16: Observation of resin microcapsules with a microscope. On the left, the microcapsules are collected in water. On the right, an accumulation of dry capsules is shown.

If necessary, it is possible to dry the capsules to store them. In this case, the capsules were washed with water and dried using a Büchner filtration device. The liquid in which the capsules are contained ends up in a vacuum flask while the capsules remain on the filter.

By adjusting the flow rates of the different phases during the formation in the Raydrop® droplet generator, it is also possible to choose the shell size (between 10 µm and 50 µm) as well as the capsule diameter (from 200 µm to 300 µm).

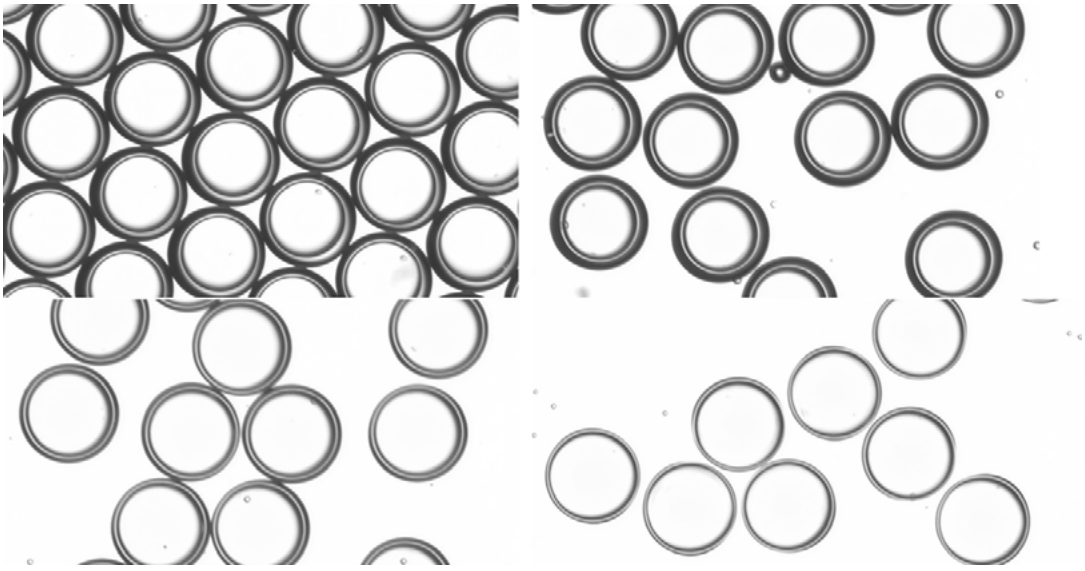


Figure 17: Microcapsules with different diameters and shell thicknesses.

The addition of a UV lamp following the droplet generation process including a Raydrop® allows users to produce monodisperse and solid capsules in polymethacrylate. By adjusting the flow rates of the different phases, it is also possible to vary the droplet diameter as well as the shell thickness. With this system, it is possible to choose a microparticle size and to encapsulate water-soluble substances.

For more information about the production of polymethacrylate resin capsules, please refer to our application note on our website https://secoya-tech.com/ or contact us at marie.mettler@secoya-tech.com.

CONCLUSION

The use of a microfluidic droplet generator enables the controlled production of double emulsions. One Raydrop® can produce numerous double emulsions with only one cleaning cycle of the device required between tests. One oil/water/oil double emulsion composed of water-soluble chitosan has been developed and can produce chitosan microcapsules when using a cross-linking bath via this double emulsion.

In addition, 2 other oil/water/oil double emulsion were made using algae-based hydrogels: agarose and alginate. These microcapsules allow for the encapsulation of oily-soluble molecules as well as volatile products with a high encapsulation yield (higher than 90%).

Moreover, the same microfluidic generator can produce a water/oil/water double emulsion. Two different applications have been underlined: one double emulsion with a shell in PLGA, and another with a shell in a polymethacrylate resin. For both cases, droplet sizes were monodispersed with an adjustable particle size.

For requests, please contact Secoya Technologies:

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FLUIDIFY PHARMA

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