White paper

Controlled double emulsification process for encapsulation

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

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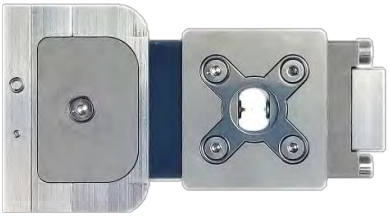
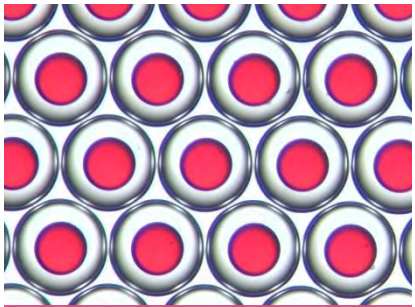
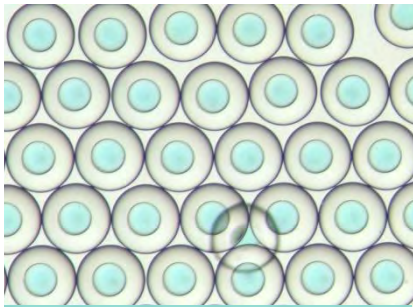
Executive summary

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

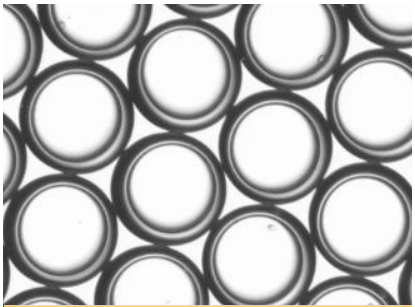




Chitosan



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



Polymethacrylate resin

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Terminology

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

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

Microcapsule and microsphere: made 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 process in industry 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 processes are 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 to obtain monodisperse emulsions and to improve the quality of the product, with notably reduced APIs losses thanks to a higher encapsulation efficiency as opposed to batch processes.

In this context, Secoya has developed a device named Raydrop® which is a microfluidic droplet generator that facilitates the production of emulsions. The Raydrop® technology aims for a more robust production with less wear as 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, this device and the multiple possibilities that it offers concerning the production of double emulsions are presented.

Furthermore, the Raydrop® is now part of a platform that make the utilization of microfluidic easier. This platform already contains all elements needed to produce a reproducible and high-quality emulsion, such as the fluidic elements, the mechanic compounds and optical material. The capacities of this platform are underlined in this white paper as well as a performance illustration using different application cases.

Equipment

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 to generate controlled double emulsions and to produce highly monodispersed and perfectly 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.

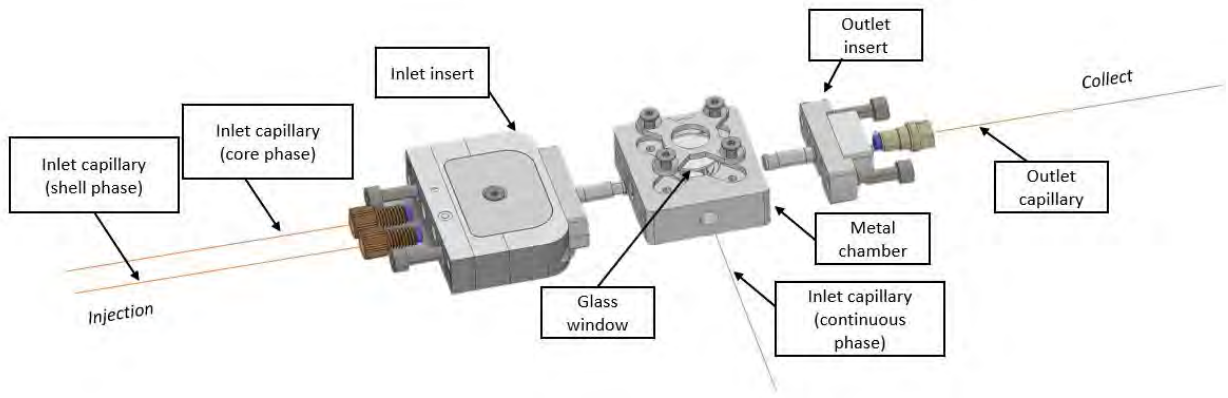

Figure 1: Exploded view of the Raydrop®, non-embedded capillary device developed by Secoya Technologies

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 a last 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 to have a visibility on the formation on the double emulsion inside the device. Both inserts

can easily be disassembled from the chamber to clean the device, for instance when changing fluids.

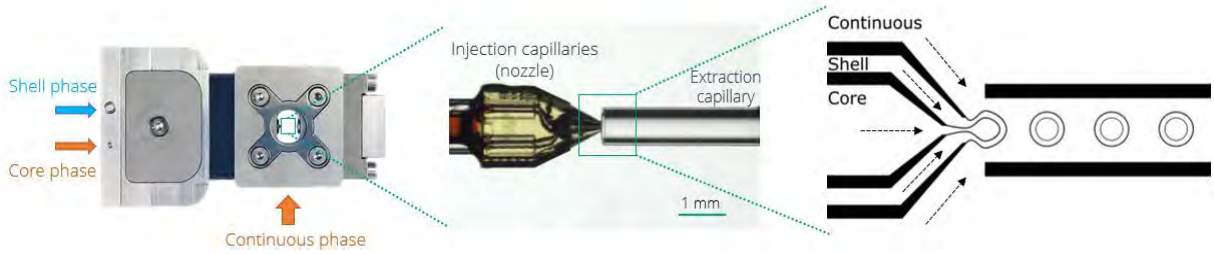

Figure 2: Internal structure of the Raydrop® [4]

The mechanism of the Raydrop® 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 come out of 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. At the outlet, the droplets can be collected.

The device is based on an axisymmetric flow focusing geometry which removes all the wettability issues, so no coating is needed. 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 as well by applying different variations in the flowrates of all fluids.

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

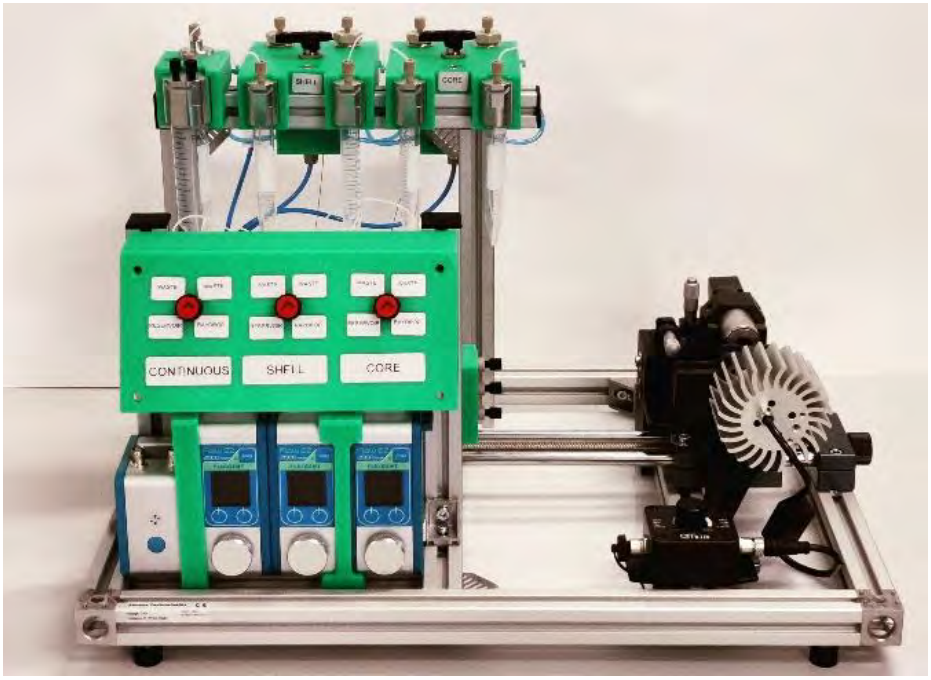

Figure 3: Raydrop® platform

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

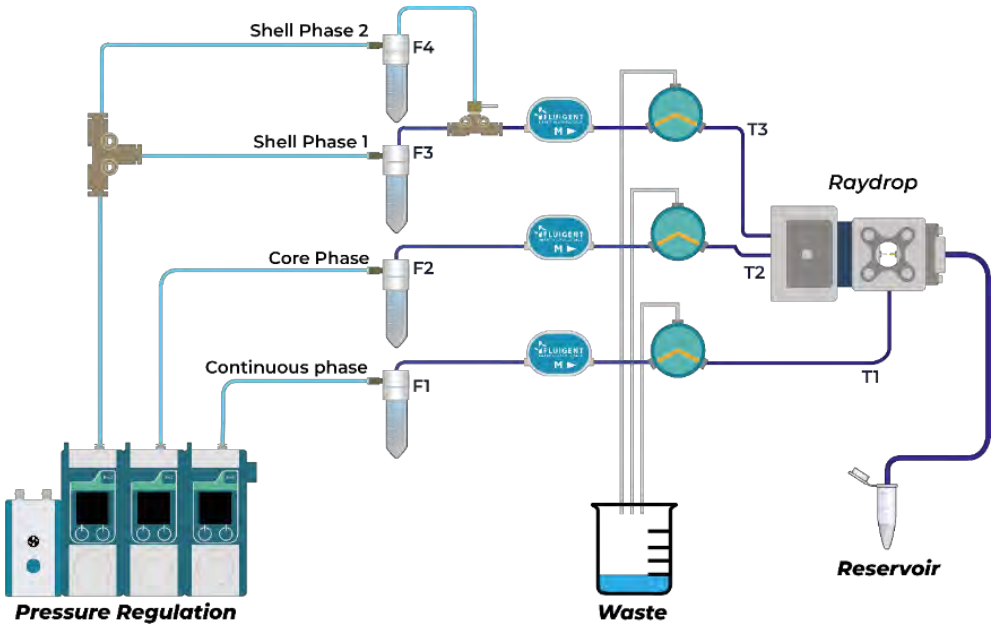

Figure 4: Fluidic system diagram corresponding to the platform. This flow scheme can be slightly different from the one corresponding to the commercialized version of the platform, where normally two reservoirs for the core phase are included.

In the following of this document, three different applications made with the microfluidic plateform containing a Raydrop® present an overview of the possibilities of this platform. These cases include three families of polymers. The first case concerns chitosan, a water-soluble polysaccharide crosslinkable ex situ in the presence of a polyaldehyde (here glutaraldehyde), for a soybean oil encapsulation. Then, another encapsulation mode is underscored to encapsulate an aqueous core containing proteins in a poly(lactic-co-glycolic acid) (PLGA) capsule formed by in situ precipitation. Finally, capsule formation by in situ UV cross-linking of a commercial polymetacrylate resin is highlighted.

Use case 1

Chitosan microcapsules

The first application of the Raydrop® is the production of 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 is interesting to contain lipophilic drugs or volatile oils. [5] Moreover, 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.

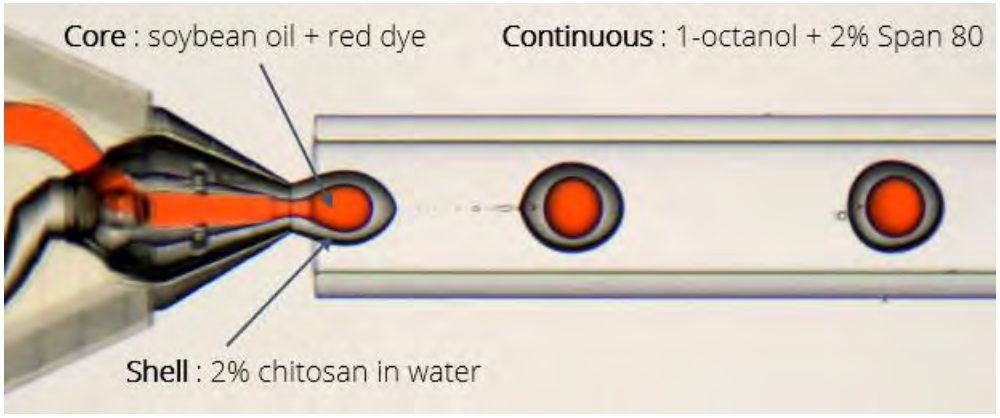

Figure 5: Formation of the chitosan double emulsion

After the formation of droplets in the Raydrop®, they are collected in a vessel. The self-induced restructuring in a single layer of ordered droplets forming the double emulsion underlines the monodispersity of the droplets.

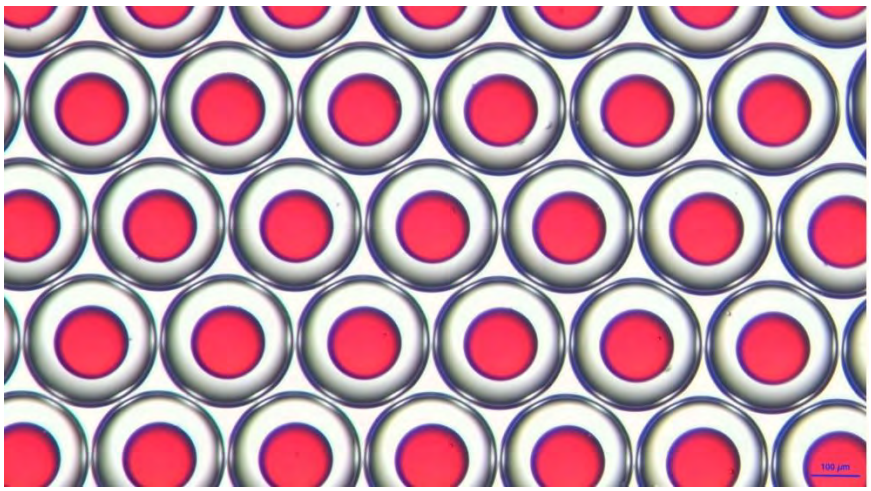

Figure 6: Oil/water/1-octanol double emulsion just after collect, observed under the microscope

Once that the double emulsion is generated, capsules are formed by the curing of the chitosan shell. In order 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.

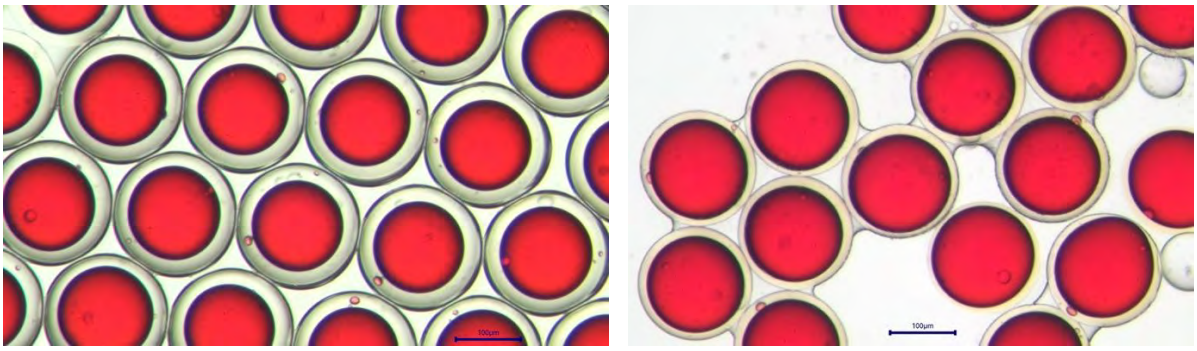

Figure 7: Glutaraldehyde cross-linked chitosan microcapsules on the cross-linking bath. On the left, after 4 minutes in the cross-linking bath. On the right, after 1h in the cross-linking bath. The shell thickness decreases and becomes progressively yellow, as a part of its water content diffuses in the continuous phase. Expelled water is clearly visible wetting the capsules.

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 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 feel free to contact us at* marie.mettler@secoya-tech.com

Use case 2

PLGA microcapsules

Another application of the Raydrop® is the production of a water in oil in water emulsion. The formation of PLGA microcapsules is very interesting due to the good biocompatibility and biodegradability of PLGA. [7] The use of microfluidic allows a

controlled production of these capsules. This control is necessary in order 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). Finally, the continuous phase is water with 1% poly(vinyl alcohol) (PVA).

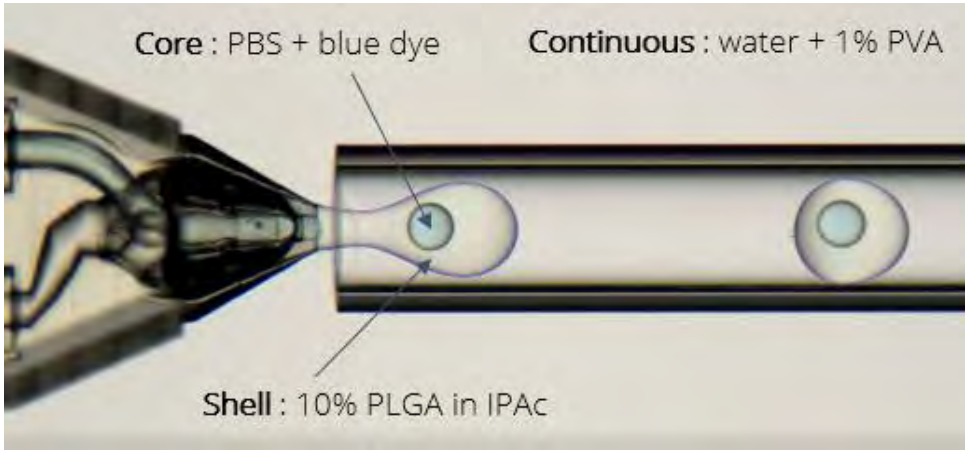

Figure 8: Formation of the PLGA double emulsion

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

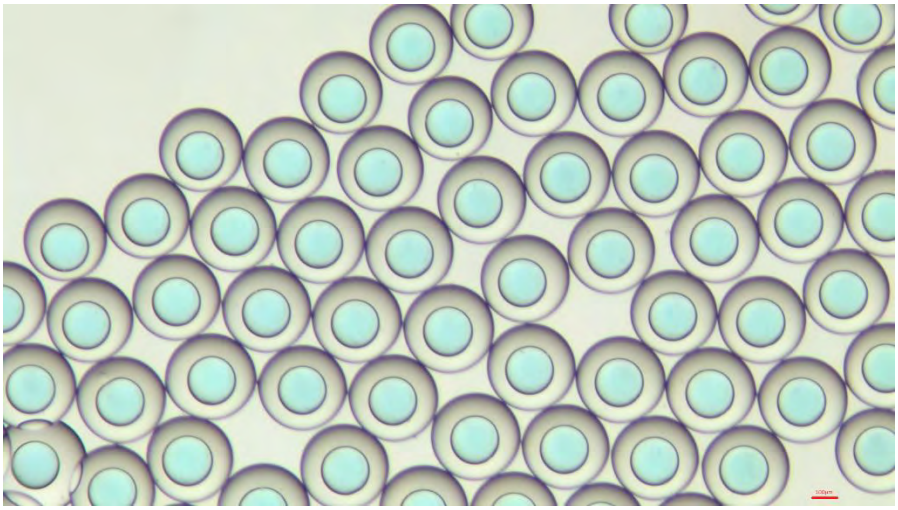

Figure 9: PBS/IPAc/water double emulsion just after collect, observed under the microscope

After being generated, 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 that the IPAc has spread enough of the shell, PLGA precipitates and the shell solidifies. Finally, the droplets become biocompatible capsules.

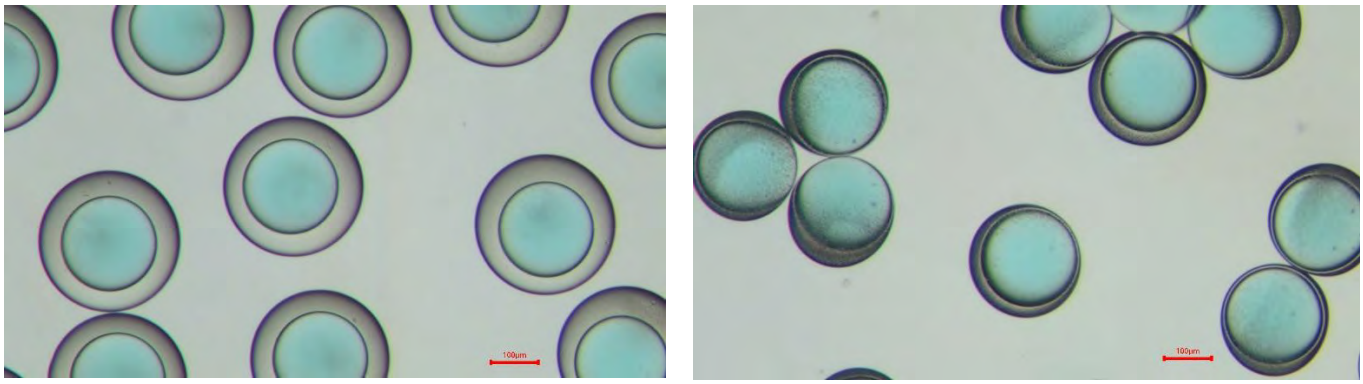

Figure 10: PLGA microcapsules in the PBS solution. On the left, 15 seconds after the creation in the Raydrop®. On the right, 200s in the PBS solution after the creation in the Raydrop®. The shell thickness decreases, as the IPAc contained in the shell phase diffuses in the continuous phase.

Here, a stable production method to generate a monodispersed double emulsion has been presented. These microparticles have a strong potential, especially for public health applications as the PLGA is biocompatible and its use is approved by the Food and Drug Administration (FDA). The PLGA is interesting for microcapsules of water-soluble compounds as on itself it is soluble in oily phases. Specific drugs can also be encapsulated and the drug release is possible by enzymatic triggers thanks 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 feel free to contact us at* marie.mettler@secoya-tech.com

Use case 3

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, an 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.

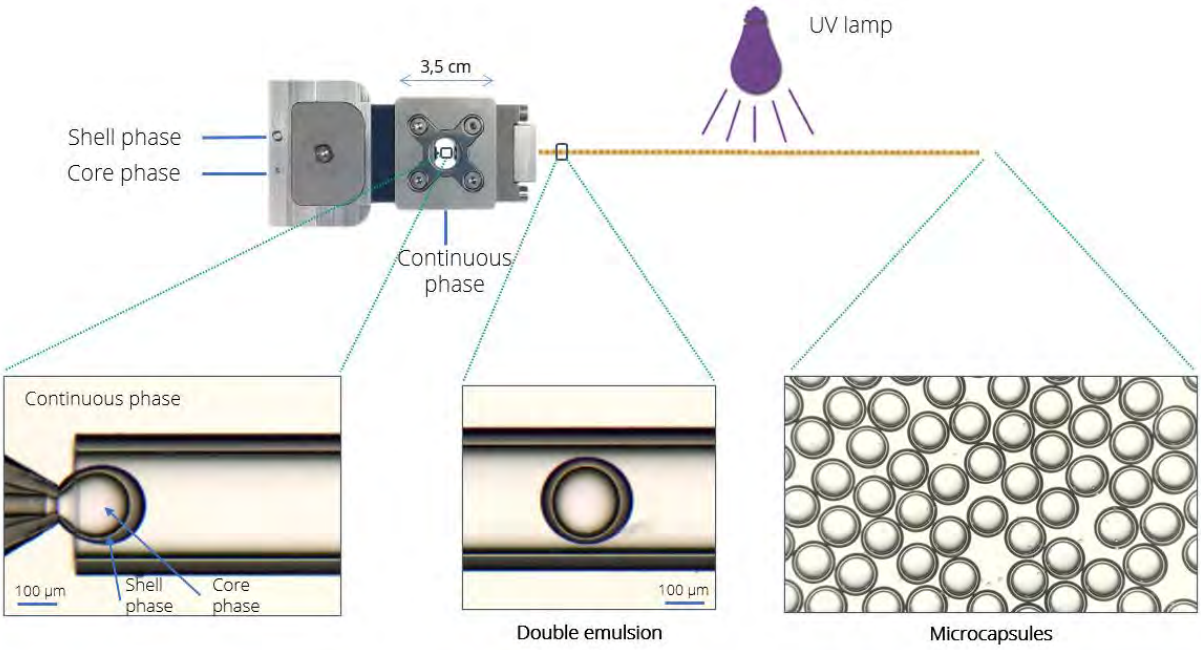

Figure 11: Setup containing the Raydrop® and a UV lamp for the formation of microcapsules in polymethacrylate resin

The solutions used to form a double emulsion in this system are the following: the continuous phase is composed by water with 2% of PVA; the shell phase is polymethacrylate resin with 20% of 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 some water-soluble compounds in the core phase to encapsulate them.

Figure 12: Formation of the polymethacrylate double emulsion. As this is a picture taken during the real experiments, the yellow color comes from a UV protection placed between the light source associated to the camera and the Raydrop®. This device allows to filter the UV light which could irradiate in the Raydrop®. Indeed, it is necessary to avoid triggering the cross-linking of the resin in the Raydrop® to avoid any clogging of this one since 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 that the cross-linking takes place. As the droplets are collected in a vessel after irradiation, monodispersed capsules have been generated.

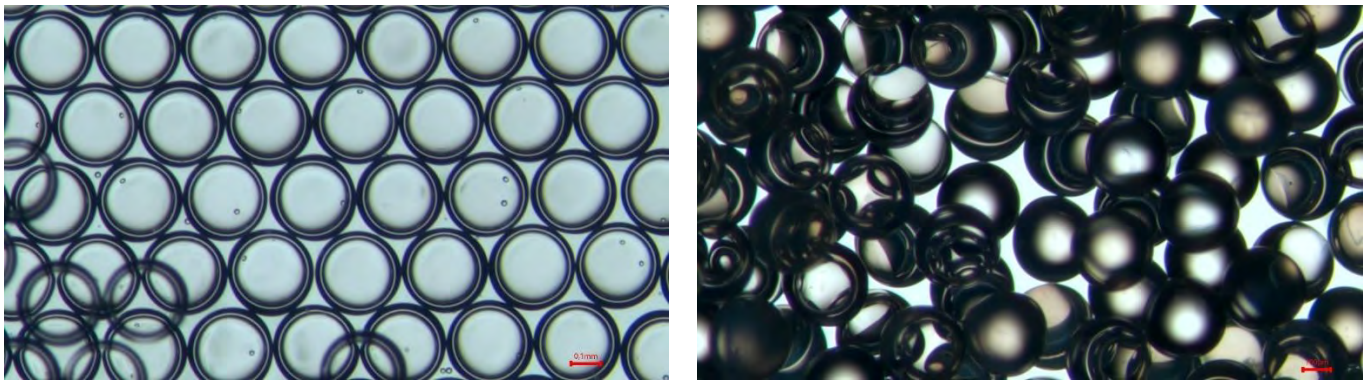

Figure 13: Observation of resin microcapsules with a microscope. On the left, the microcapsules are collected in water. On the right, an accumulation of dry capsules is shown.

If necessary, it is possible to dry the capsules in order 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® 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).

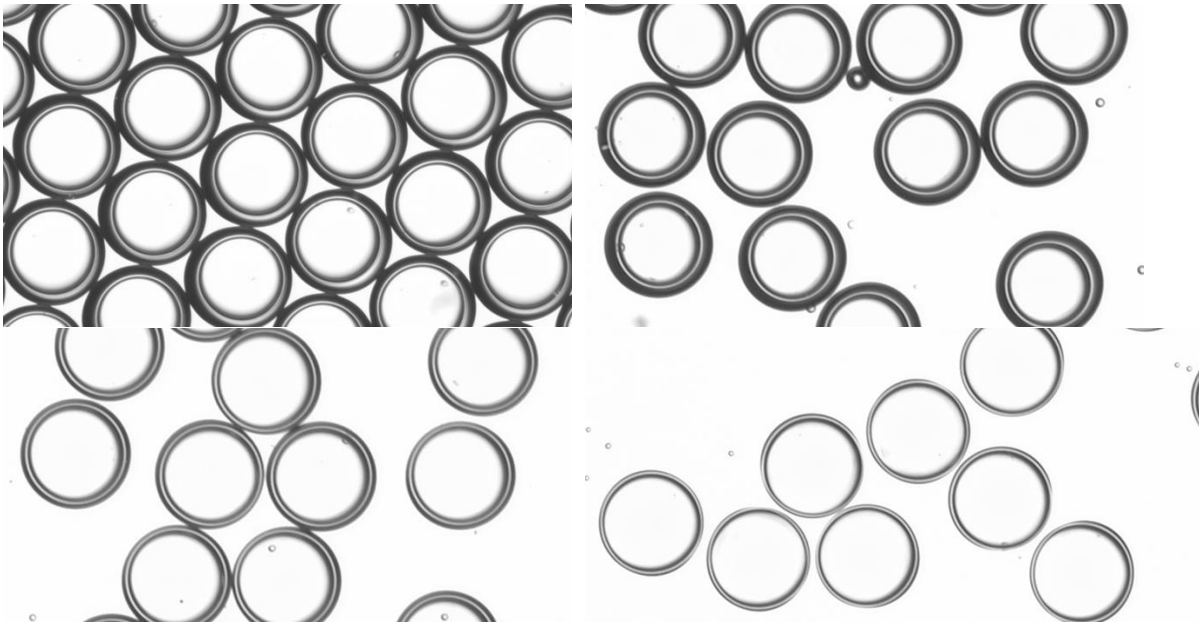

Figure 14: Microcapsules with different diameters and shell thicknesses

The addition of a UV lamp following the droplet generation process including a Raydrop® allows the production of 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 feel free to contact us at* marie.mettler@secoya-tech.com

Conclusions

The use of a microfluidic droplet generator enables the controlled production of double emulsions. On one single Raydrop® and by only a cleaning cycle of the device in between tests, numerous double emulsions have been produced, as underlined in this paper. One oil/water/oil double emulsion composed of water-soluble chitosan have been developed and using a cross-linking bath, it is also possible to produce chitosan microcapsules thanks to this double emulsion. These microcapsules allow to encapsulate 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 monodisperse with always an adjustable particle size possible.

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