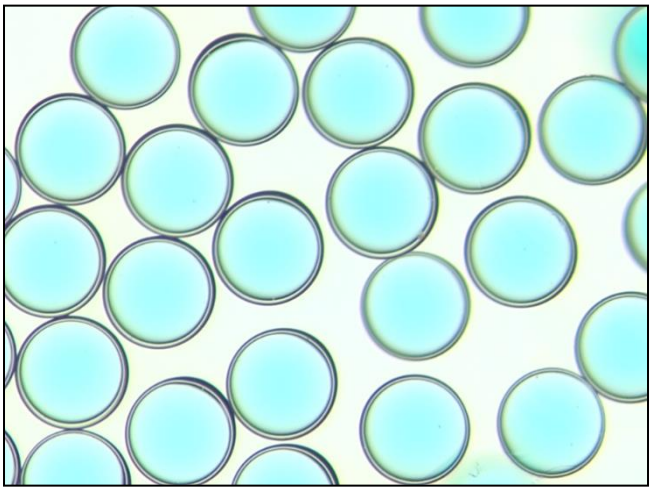
PLGA MICROCAPSULES CREATION



Application note

Marie Mettler

www.secoya-tech.com

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 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 [1].

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 [2,3]. 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, PLGA shell/aqueous core microcapsules are obtained using the Raydrop® Double emulsion device, a capillary based microfluidic device equipped with a 3D printed injection nozzle making the generation of double emulsion easy, in combination with pressure-based flow controllers. The influence of the fluidic parameters on the microcapsule size and the release from the oil across the shell are explored in this application note.

II) Materials and methods

1) Materials

Core phase:

- Phosphate Buffered Saline buffer (PBS, pH=7,28 Sigma-Aldrich) containing blue food dye (shiny blue FCF liquid by Vahiné)

Shell phase:

- Ethyl acetate (EtOAc, Merck) containing 10% Poly(D,L-lactide-co-glycolide) (PLGA, Resomer® RG 7555S ester terminated, Sigma-Aldrich)

OR

- Isopropyl acetate (IPAc, Sigma-Aldrich) containing 10% Poly(D,L-lactide-co-glycolide) (PLGA, Resomer® RG 7555S ester terminated, Sigma-Aldrich)

This application note presents all results with IPAc as shell phase. However, experiences have also been made with EtOAc and the results are similar to those presented in this paper.

Continuous phase:

- Water containing 1% Poly(vinyl alcohol) (PVA, Sigma-Aldrich)

The continuous phase requires a surfactant to stabilize the double emulsion. The PVA is used as in the publication of LEE and al. [1]

Priming and cleaning phase:

- Ethyl acetate (EtOAc, Merck)

OR

- Isopropyl acetate (IPAc, Sigma-Aldrich)

<u>Note:</u> the experiments were conducted with two different solvents: ethyl acetate and isopropyl acetate. It is therefore possible to choose one of these two solvents to repeat the experiments. However, it is not necessary to mix these two solvents.

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/ .

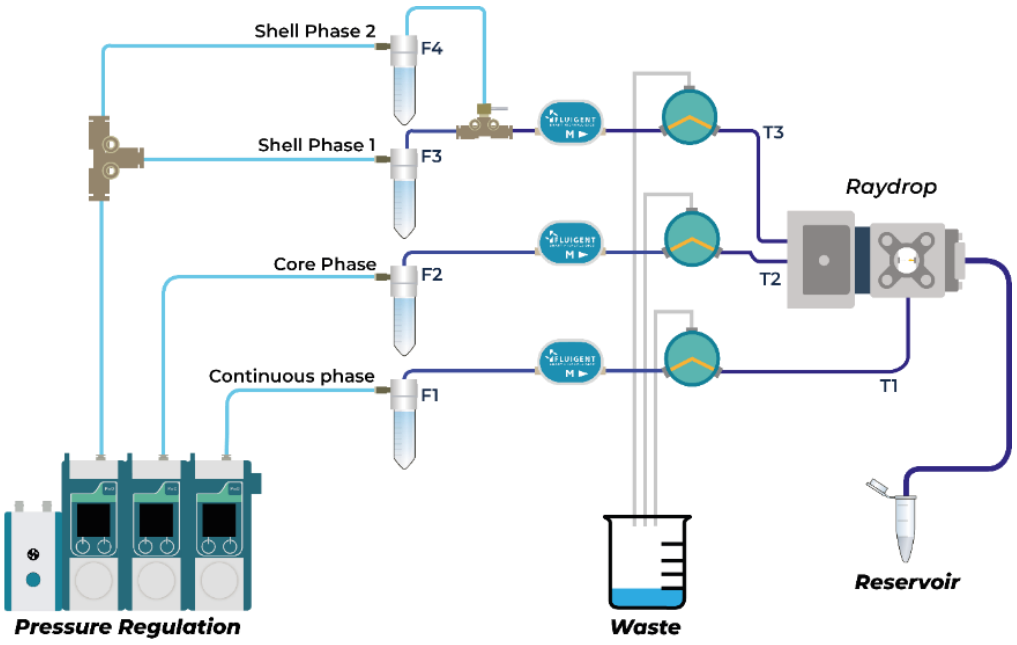


Figure 1: Experimental set-up to produce double emulsion. This flow scheme can be slightly different from the one corresponding to the commercialized version of the platform, where normally two reservoirs for the core phase are included.

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 + 1% PVA	PBS pH = 7,28 + dye	IPAc	IPAc

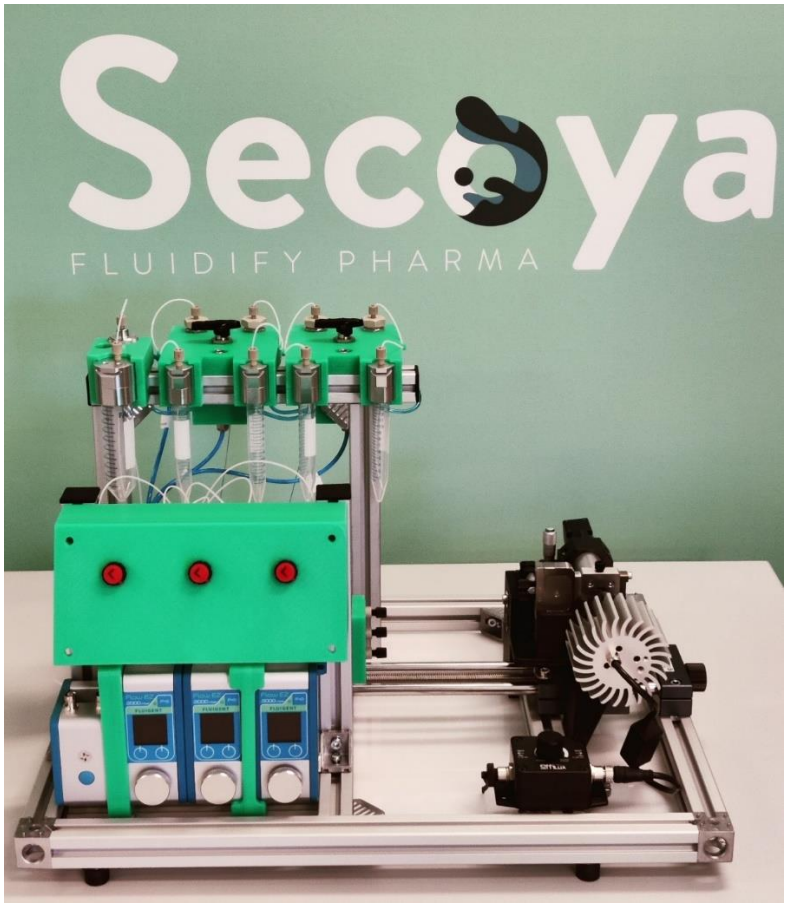


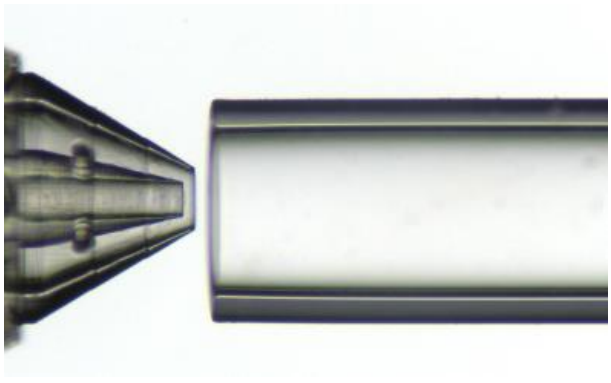
Figure 2: Raydrop® Platform

[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.*

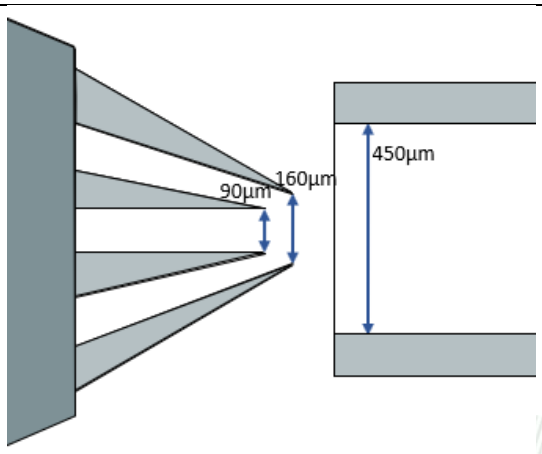
- **Mechanics:** The mechanical part includes x-y-z displacement plates that allow to adjust the focus and the observation window in the Raydrop®.
- **Fluidics:** The fluidic part consists of flowrate controllers along with the required tubing and valves, allowing for automated fluidic injection. A pressure 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

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

4) Emulsion generation

To generate droplets easily, the system must first be initiated with pure solvent in the shell phase (here pure IPAc). Then, once the droplet formation is stabilized using the priming liquid, the shell phase is switched to the solution containing PLGA. This avoids clogging problems during the transient phase:

1. Set the valve on the Falcon F3 which contain the priming solution
2. Fill the Raydrop® with the continuous phase
3. Set the continuous phase to the desired flow rate
4. Set the shell phase to the desired flow rate to establish a co-flow of water and IPAc
5. Set the core phase to the desired flow rate to generate a double emulsion
6. Once the double emulsion is stabilized, switch the valve to Falcon F4
7. Wait[2] until the PLGA solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a PLGA solution shell and a PBS buffer core in the aqueous continuous phase

[2] *This can take 5 to 10 minutes, depending on the flow rate of the PLGA phase and the diameters and length of the tubing.*

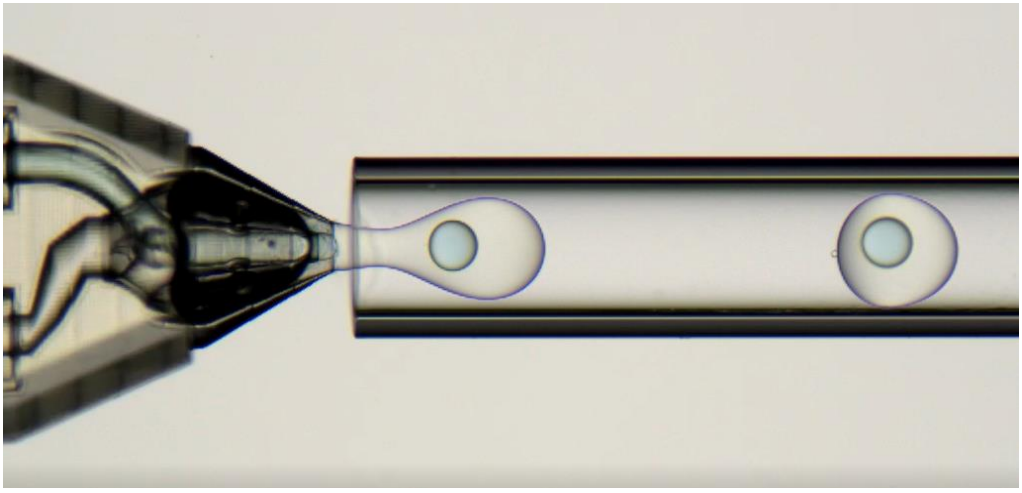


Figure 4: Generation of droplets in the Raydrop®

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® in a bit of the core solution to match the osmolarity of inner and outer aqueous phases.

Here, the core solution is PBS so you have to collect the droplets in PBS so that the capsules do not collapse and the shell has time to solidify. If you want to encapsulate an API dissolved in PBS, you just have to collect the droplets in PBS but you do not need to put some API in the collection bath.

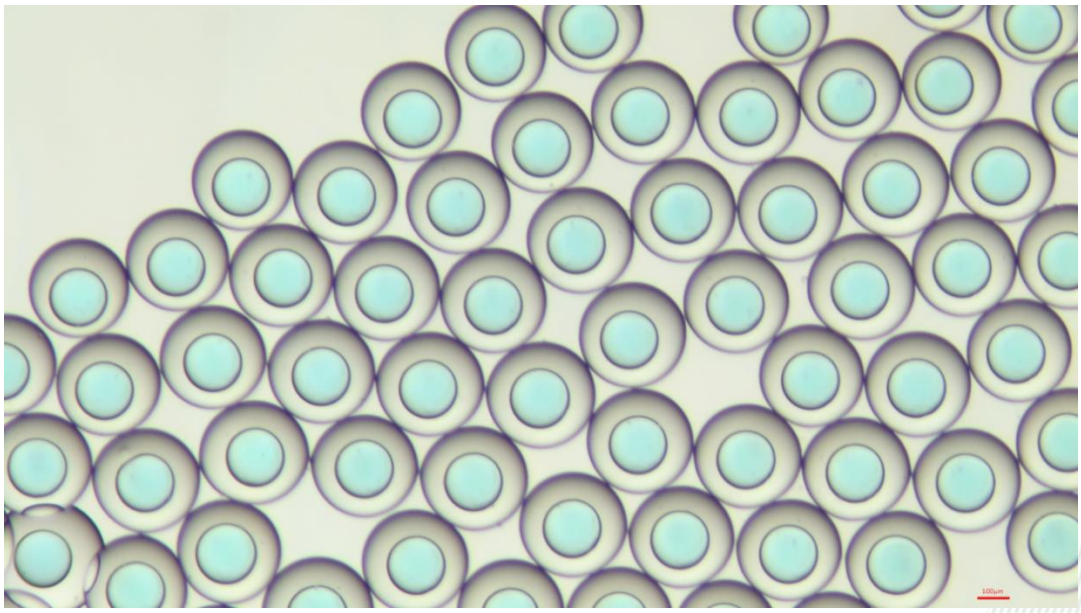


Figure 5: Double emulsion PBS/IPAc/water obtained at the output, observed under the microscope

Before stopping the experiment, it is important to flush the shell tubing (T3) and the nozzle of the Raydrop® with the solution contained in the Falcon F3. This priming and cleaning liquid will dissolve and evacuate any remaining PLGA inside the tubing or the nozzle of the Raydrop®. This way, the tubing stays clean and clogging is avoided.

11. To flush the PLGA out of tubing and Raydrop®, switch the valve on the Falcon F3 which contains the cleaning solution
12. Wait[3] until the cleaning solution crosses the tubing and reaches the Raydrop® to form a double emulsion with a PLGA solution shell and a PBS buffer core in the aqueous 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

5) Capsule formation

After being generated, the droplets are collected in a glass Petri dish containing a little of PBS solution. Then, IPAc contained in the shell phase diffuses into the PBS so the PLGA precipitates. As a result, droplets are solidified and become PLGA capsules.

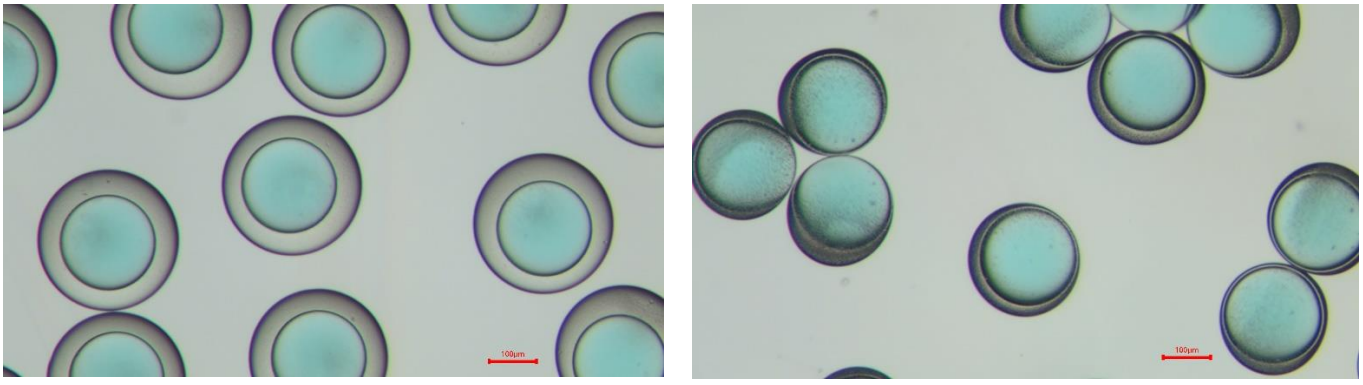


Figure 6: PLGA microcapsules in the PBS solution. On the left, 15 seconds after the creation in the Raydrop®. On the right, 200s in the PBS solution after the creation in the Raydrop®. The shell thickness decreases, as the IPAc contained in the shell phase diffuses in the continuous phase.

[3] Please refer to the footnote 2

III) Results

Evolution of the droplet diameter during the precipitation process

Once formed, the droplets are collected in the same solution that have been used in the core of droplets -so PBS buffer- to match the osmolarity of inner and outer aqueous phases. 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. The evolution of the diameter is highlighted in Table 1 and the operating conditions are shown in Figure 7.

	Continuous phase	Shell	Core
Composition	Water + 1% PVA	IPAc + 10% PLGA	PBS pH = 7,28 + dye
Pressure (mbar)	214	2404	104
Flowrate (µL/min)	109	16,5	9,1

Table 1: Operating conditions for the evaluation of the diameter of capsules

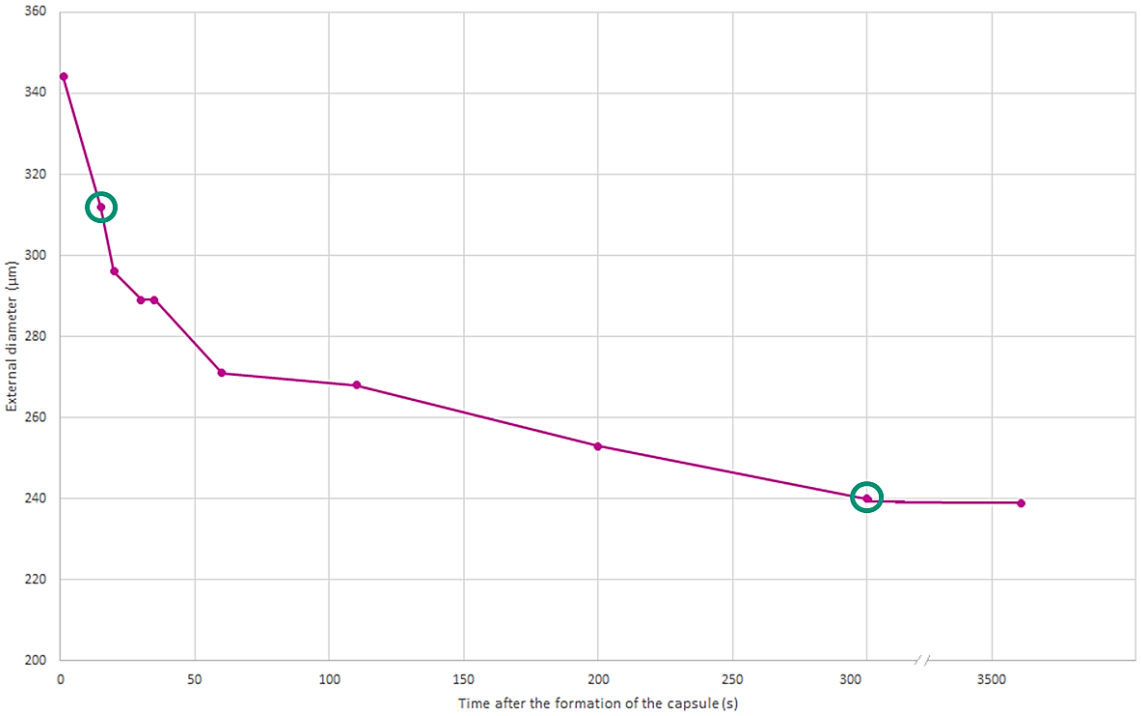


Figure 7: Size of PLGA capsules as a function of time

The capsules have a diameter ranging from 345 µm to 240 µm. Moreover, we observe that during the precipitation process, the diameter of the capsules decreases. Indeed, a diameter of 312 µm is obtained 15 seconds after droplet formation, while a diameter of 240 µm is obtained 300 seconds after droplet formation.

IV) Conclusion

The production of stable monodispersed microcapsules with a solid PLGA shell and an aqueous core using a microfluidic system consisting of pressure-based flow controllers and the Raydrop® microfluidic tool has been successfully achieved. Microcapsules with a PLGA shell and aqueous core has been widely studied because PLGA microcapsules appear as one of the most successful new drug delivery systems (DDS) in labs and clinics. Owing to the good biocompatibility and biodegradability of PLGA, microcapsules can be used in various application such as long-term drug release systems, vaccine adjuvant, and tissue engineering [4].

The microfluidic platform allows to produce capsules in PLGA, which is very interesting as it is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, owing to its biodegradability and biocompatibility (ref publi). These microcapsules can be used in a wide range of applications, like the encapsulation of active ingredients such as specific drugs, which will be delivered according to the pH acidity [2].

V) References

[1] LEE, Myung Han, HRIBAR, Kolin C., BRUGAROLAS, Teresa, KAMAT, Neha P., BURDICK, Jason A. and LEE, Daeyeon, 2012. Harnessing Interfacial Phenomena to Program the Release Properties of Hollow Microcapsules. Advanced Functional Materials. 11 January 2012. Vol. 22, no. 1, p. 131–138. DOI 10.1002/adfm.201101303.

[2] MONTAZERI, Leila, BONAKDAR, Shahin, TAGHIPOUR, Mojtaba, RENAUD, Philippe and BAHARVAND, Hossein, 2016. Modification of PDMS to fabricate PLGA microparticles by a double emulsion method in a single microfluidic device. Lab on a Chip. 2016. Vol. 16, no. 14, p. 2596–2600. DOI 10.1039/C6LC00437G.

[3] TU, Fuquan and LEE, Daeyeon, 2012. Controlling the Stability and Size of Double-Emulsion-Templated Poly(lactic- co -glycolic) Acid Microcapsules. Langmuir. 3 July 2012. Vol. 28, no. 26, p. 9944–9952. DOI 10.1021/la301498f.

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