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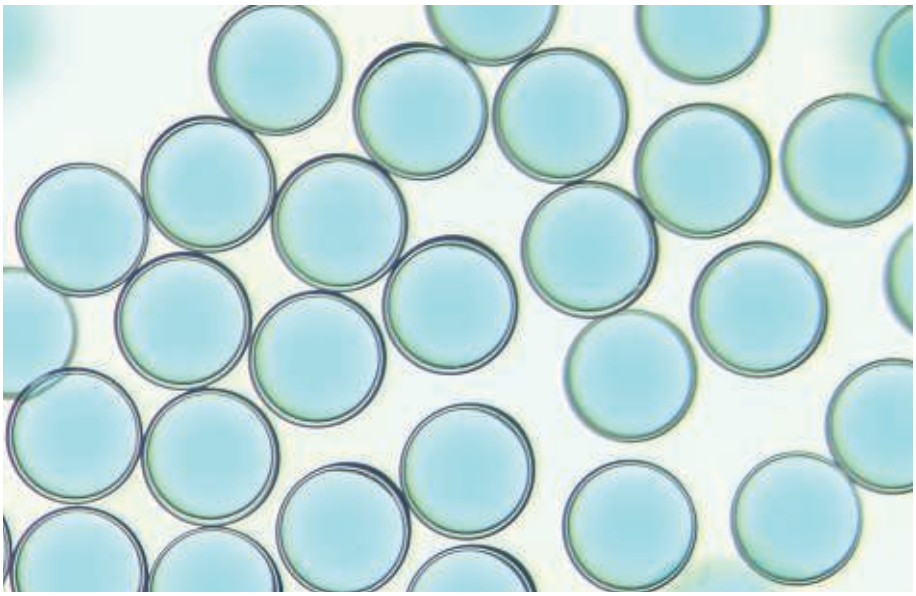
PLGA MICROCAPSULE SYNTHESIS

APPLICATION NOTE



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INTRODUCTION

Encapsulation of Active Pharmaceutical Compounds in core-shell microcapsules is of great interest for several purposes including: taste and odor masking as well as controlled release of drugs. In pharmaceutics the possibility to encapsulate drugs, nutrients, and living cells that can be protected by a solid biocompatible shell can be used to target a specific site for therapy. [1]

In this context, microcapsules with a PLGA shell and aqueous core have been widely studied because PLGA microcapsules appear to be successful new drug delivery systems (DDS). Due to the good biocompatibility and biodegradability of PLGA, microcapsules can be used in various applications such as long-term drug release systems, vaccine adjuvants, and in tissue engineering [2].

Classical methods of microencapsulation, like coacervation, spray drying, solvent evaporation, etc, require complex process and equipment and make it difficult to control the size and load of the microcapsules.

In contrast, microfluidics allows for the production of monodisperse double emulsions which lead to monodispersed microcapsules with a high control over both the size and the structure [3] [4].

In this Application Note, PLGA shell/aqueous core microcapsules are obtained using the Raydrop Double emulsion, a capillary based microfluidic device equipped with a 3D printed injection nozzle simplifying the generation of double emulsion when used in combination with pressure based flow controllers. The influence of the fluidic parameters on the microcapsule size and release from the oil across the shell are explored in this application note.

MATERIALS

1. Products

Core phase (and collect phase):

- Phosphate Buffered Saline buffer (PBS, pH=7,28 Sigma-Aldrich) containing blue food dye

Shell phase 1: Priming and cleaning phase

- Ethyl acetate (EtOAc, Merck)

OR

- Isopropyl acetate (IPAc, Sigma-Aldrich)

Shell phase 2:

- 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)

Continuous phase:

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

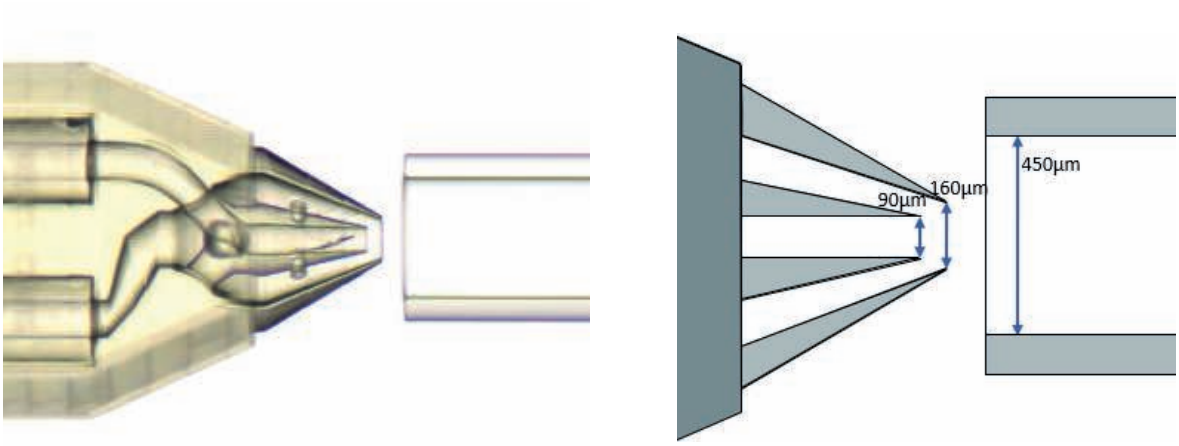
Note: Experiments were conducted with two different solvents: ethyl acetate and isopropyl acetate. Either of these two solvents may be used to repeat these experiments. It is not necessary to mix these two solvents.

2. Materials

Microfluidic system:

- Pressure controller: LineUp™ Flow EZ 7 bar[1]*
- Flow sensor: Fluigent Flow Unit M (x2) and L (x1)
- Microfluidic droplet generator: Raydrop double emulsion*

**Raydrop™ is a registered trademark of Secoya Technologies*



Picture of the Raydrop **Scheme of the Raydrop**

Figure 1: *Microfluidic droplet generator*

Nozzle information

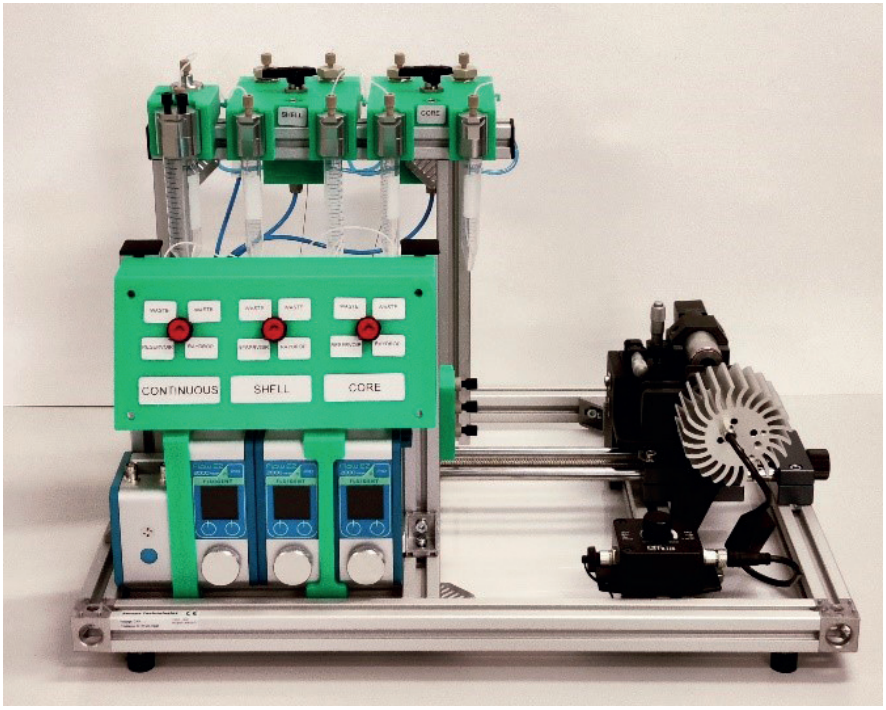
Part	Core nozzle	Size-shell nozzle	Size-extraction capillary
Inside diameter (µm)	90	160	450

The RayDrop is developed and manufactured by Secoya

[1] The pressure controllers used are 7 bar full scale. The maximum pressure used for the generation of double emulsions with large shells is 2440 mbar (corresponding to a shell phase flow rate of 24.3 µL/min). Except from this scenario, maximum working pressure is 1650 mbar. Priming and cleaning steps can require a pressure higher than 2 bar.

3. Set-up

The production of droplets has been realized with the Complex emulsion production platform. This includes a microfluidic device designed by Secoya Technologies, as well asthe needed components needed to produce simple and double emulsion with the Raydrop device. This platform is divided into three parts: mechanical, fluidic, and optical. More information about this can be found on the platform webpage available at https://www.fluigent.com/research/instruments/packages/complex-emulsion-production-platform/



The mechanical assembly includes different displacement plates. These allow one to adjust the camera by moving it in x, y and z directions. It is also possible to position the Raydrop to optimize the visibillityof the nozzles on the screen.
The fluidic part contains all the tubing and valves necessary for the circulation of fluids in the system plus the pressure controllers. It is possible to set a different pressure or fow rate for each phase. It also includes the Falcon fluid reservoirs and the Raydrop, which is the main component for formation of the double emulsion.
The optical section contains an LED light source and a color USB 3.0 camera. This camera is connected to a computer to observe droplet formation in real-time, as well as to control the stability of the emulsion and measure the size of interest (core, shell).

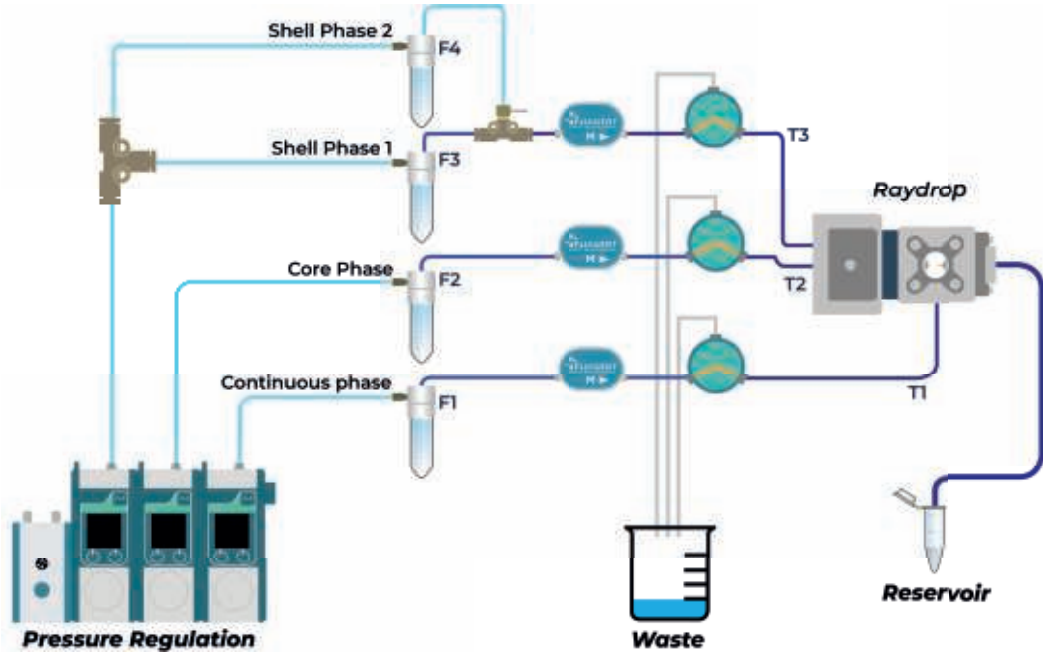


Figure 3: *Experimental set-up to produce double-emulsion*

Fluid reservoirs

Falcon identification	F1	F2	F3	F4
Volume (mL)	50	15	15	15
Phase*	Continuous	Core	Shell (priming and cleaning)	Shell
Composition	Water + 1% PVA	PBS pH = 7,28 + dye	EtOAc OR IPAc	EtOAc + 10% PLGA OR IPAc + 10% PLGA

**Each phase must be filtered in order to avoid clogging the tubing or the nozzle of the Raydrop. There is a filter after each 50 mL and 15 mL reservoir. In this case, the filter at F1 has a 10 µm filter pore size and the filters F2 and F3 have a 2 µm filter pore size. The reservoir F4 has no filter as the solution contained in it is filtered before use.*

METHOD: SYNTHESIS OF PLGA CAPSULES

Monodisperse PLGA particle synthesis is performed in 2 main steps:

- Generation of monodisperse double emulsion in the Raydrop
- Capsule formation by precipiatiof the PLGA shell

1. Double emulsion generation

To generate droplets easily, the system must first be started with pure solvent in the shell phase (here IPAC). Once droplet formation is stabilized, the shell phase is switched to the solution containing the PLGA. This avoids possible clogging issues during the transient phase.

- Set the manual valve on reservoir F3 which contain the priming solution (Shell phase 1) solution
- Fill the Raydrop with the continuous phase (refer to the user guide for more precisions)
- Set the continuous phase to the desired flow rate
- Set the shell phase to the desired flow rate to establish a co-flow of water and IPAC
- Set the core phase to the desired flow rate to generate a double emulsion
- Once the double emulsion is stabilized, switch the valve to reservoir F4 (shell phase 2)
- Wait** 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.

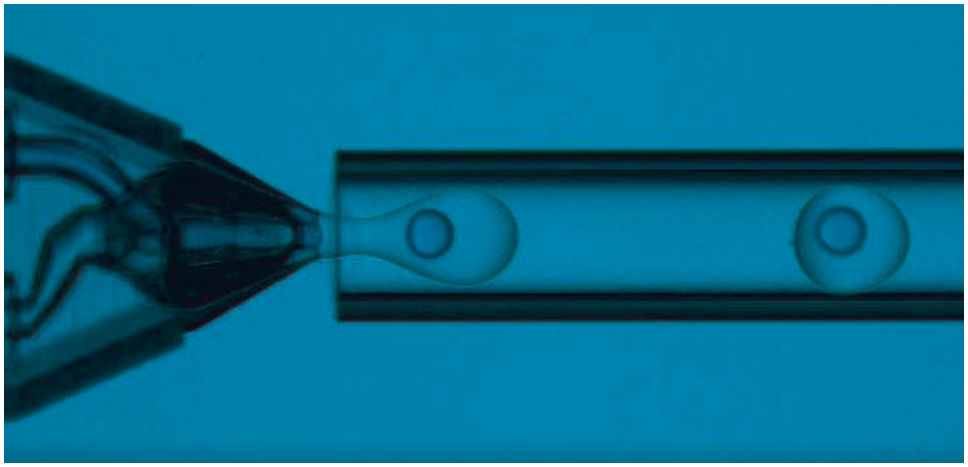


Figure 4: *Generation of double emulsion in the Raydrop*

- Optimize the double emulsion properties by varying the flow rates to obtain the desired droplet diameter and shell thickness
- 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.

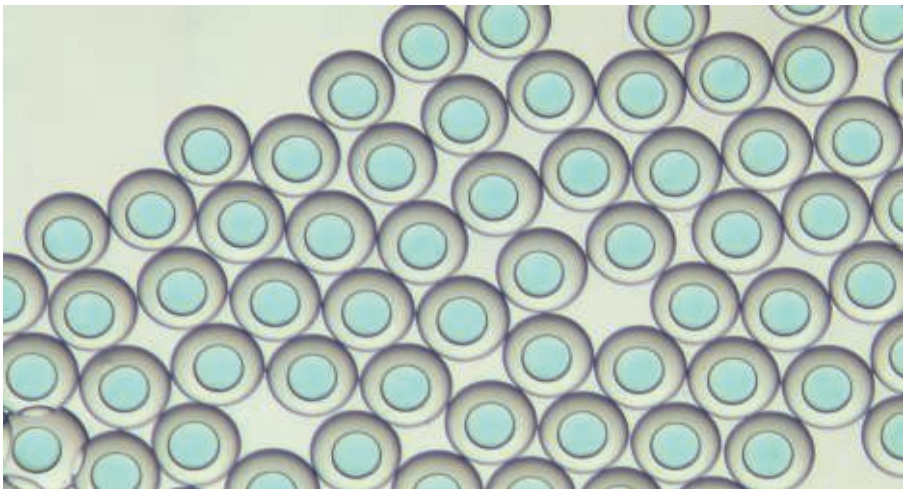


Figure 5: *Double emulsion water/IPAC/PBS obtained at the output, observed under the microscope*

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 F3 reservoir. This priming but also cleaning solution only contains ethyl acetate, which will dissolve and remove the PLGA. The tubing stays clean and clogging issues are avoided.

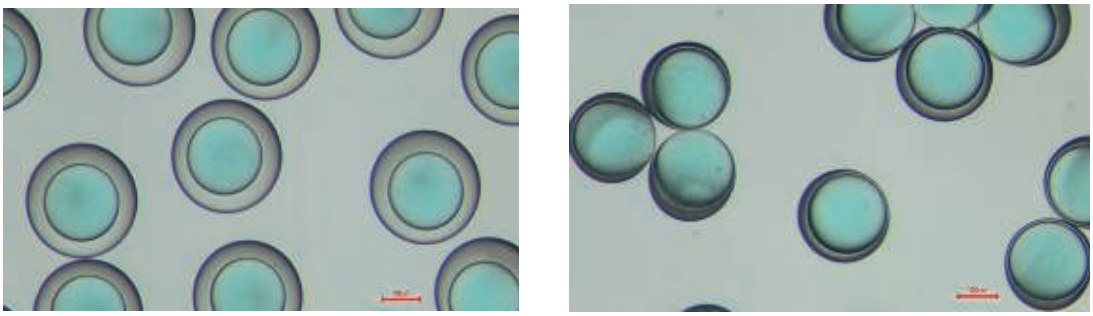
- To flush the PLGA out of tubing and Raydrop, switch the manual valve at F3 which contain the cleaning solution (shell phase 1)
- Wait** until the cleaning solution crosses the tubing and reaches the Raydrop to form a double emulsion with an ethyl acetate shell and a PBS buffer core in the aqueous continuous phase
- Stop the flow of the core phase
- Then, stop the flow of the shell phase
- Finally, stop the flow of the continuous phase

*** This can take 5 to 10 minutes, depending on the flowrate of the PLGA phase and the diameters and length of the tubing.*

[2] *DCA-MAM-019-video1-frame-rate-4.75*

2. Microcapsule formation

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



15s after formation

200s after formation

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.*

RESULTS

In this Application Note, different parameters have been studied. Firstly, the evolution of the droplets over time have been observed. Then, the release rate of the capsules was measured.

1. Evolution of the droplet diameter during the precipitation process

Once formed, the droplets are collected in the same solution used in the core of droplets to match the osmolarity of inner and outer aqueous phases. An analysis of the size of the capsules is performed using a microscope and 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 Figure 7 and the operating conditions are shown in Table 1.

[2] *DCA-MAM-019-video1-frame-rate-4.75*

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

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

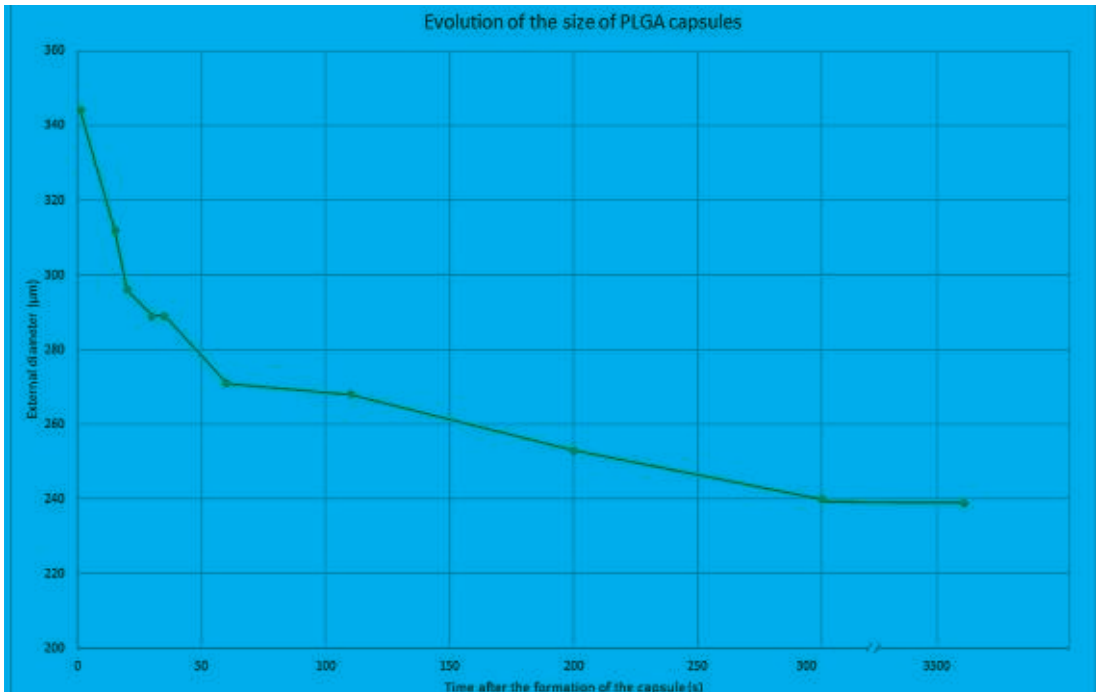


Figure 7: *Size of chitosan capsules as a function of time*

The capsules have a diameter ranging from 312 µm to 230 µm. Moreover, we observe that during the precipitation process, the diameter of the capsules decreases. Indeed, a diameter of 312 µm is obtained 20 seconds after droplet formation, while a diameter of 230 µm is obtained 300 seconds after droplet formation. After 300s we observed that microcapsules are reaching a steady state and do not decrease further.

2. Conclusion

The production of stable monodispersed microcapsules with a solid PLGA shell and an aqueous core using a microfluidic has been successfully achieved. The microfluidic platform allows one to optimize not only the core diameter but also vary the shell thickness by adjusting the flow rates of the different fluids. 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 [3].

3. 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] Qi, F., Wu, J., Li, H., & Ma, G. (2018). Recent research and development of PLGA / PLA microspheres / nanoparticles: A review in scientific and industrial aspects

[3] 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.

[4] 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.