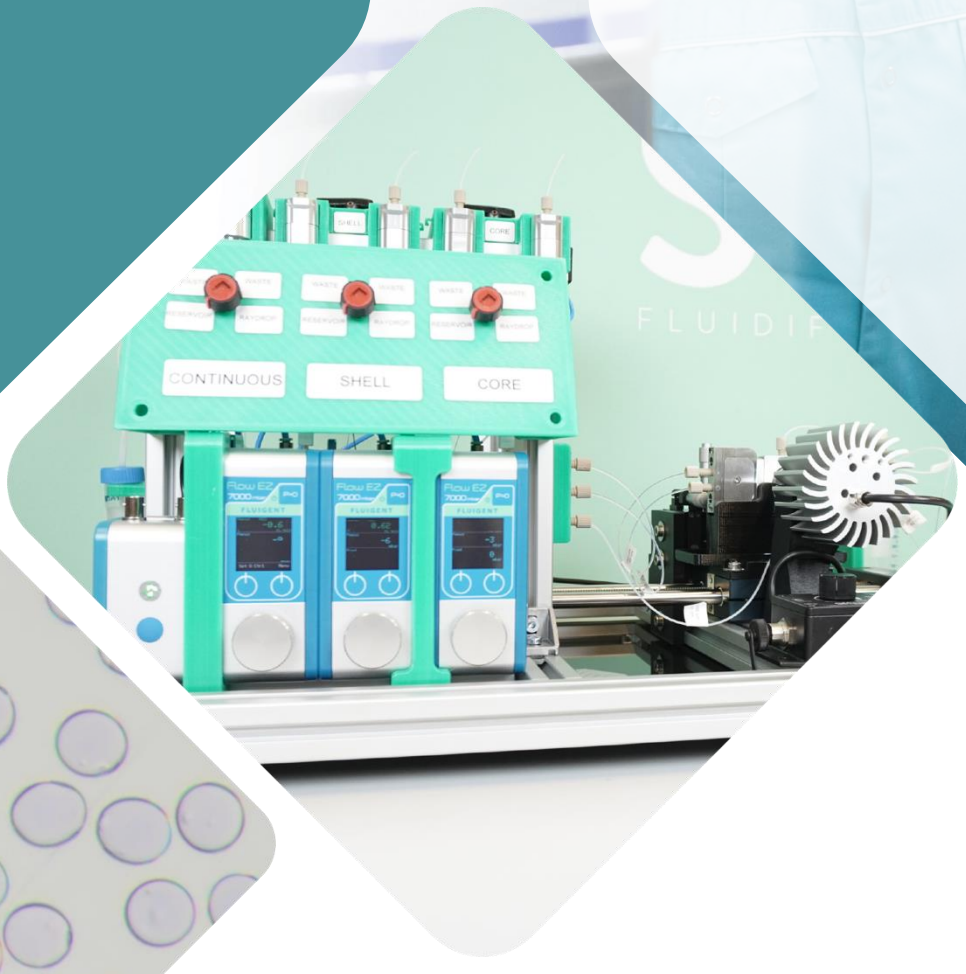
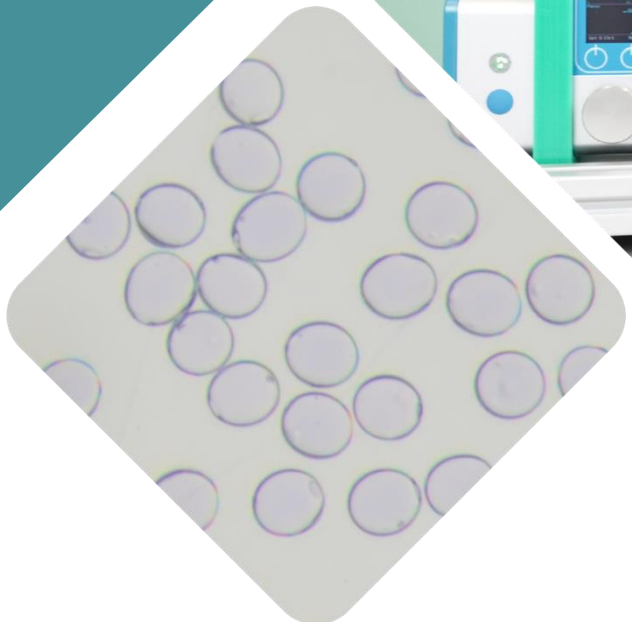
Secoya
FLUIDIFY PHARMA

QUICK START GUIDE

PLGA capsules

30 September 2022





Ref: QSP0922/1

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1 Safety precautions

Only trained personnel should be allowed to use this equipment.

The safety information in all sections of the Raydrop Platform's manual must be complied with.

The operating manual must be read and fully understood by the specialist personnel/ operators responsible prior to use.

The contents of the operating manual must be always available to the specialist personnel at the site.

The operator is responsible for ensuring compliance with all local regulations not taken into account in this operating manual.

Unattended operation of the Raydrop Platform should only be undergone after thorough risk assessment and after adequate safety and monitoring measures have been put in place.

Do not exceed maximum operating parameters.

Always wear appropriate personal protective equipment:

safety glasses

adequate gloves

lab coat

Pressure applied to the 50 mL test tube must not go above 4 bar.

When using organic solvent, the Raydrop platform must be used under a fume hood.

2 Abstract

This document is intended for trained people who want to produce microcapsules. The goal of this document is to guide you step by step from the filling of the droplet generator to the generation of microcapsules by using the Raydrop Platform. You will use the Raydrop to produce capsules of Poly(D,L-lactide-co-glycolide) (PLGA) with an aqueous core.

To follow the instructions of this guide, you will need 4 hours. However, I you just want to produce capsules and you don't need to encapsulate an API, it is possible to skip steps 5.7 and 5.8 and the whole process will take less time.

3 Overview

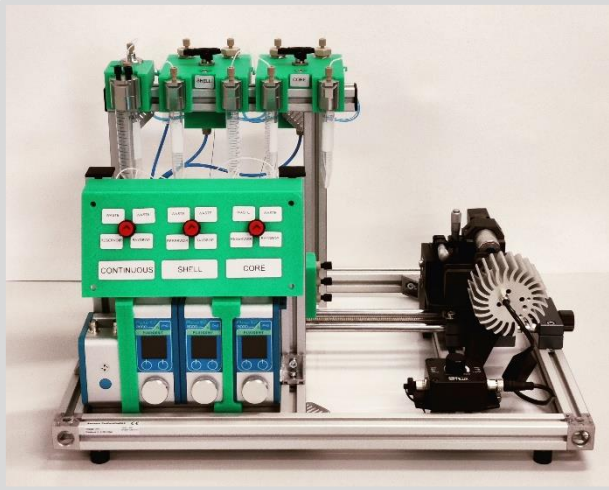


Figure 1: Front view of the Raydrop Platform

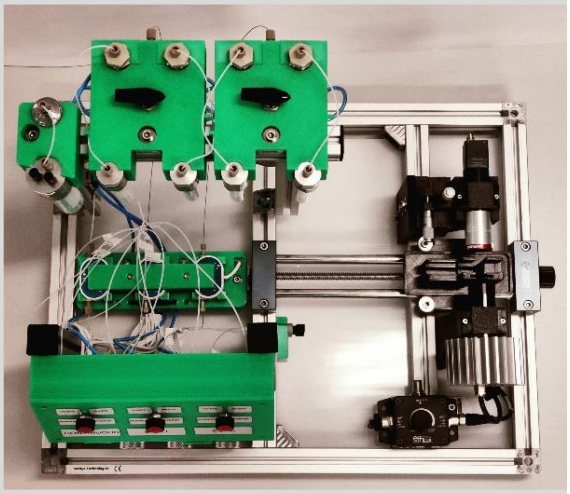


Figure 2: Top view of the Raydrop Platform

The Raydrop Platform is a fast and easy screening tool to setup double emulsion production processes using Secoya's emulsification technology: the Raydrop. It includes a comprehensive flow path with pressure controllers, filters, flowmeters, and valves to ease the start-up, shutdown and cleaning of the system in between tests. A suitable optical system guarantees the optimum visualisation of the emulsification process inside the Raydrop. The open design of the platform allows its adaptation to your needs and facilitate its maintenance. All tubing and connectors are standard, commonly used references that you can adapt to your own application. Yet, we recommend to use the references provided by Secoya.

The left-hand side of the Raydrop platform comprises the equipment needed to control the flows that will produce an emulsion. The Raydrop platform use pressure controllers that apply pressure within the tightly closed reservoirs to push the fluid into the fluidic system. It encompasses the pressure controllers, flowmeters, feeding vessels, online filters and swich valves. The complete composition of the equipment will be described later. On the right side of the Raydrop platform, the Raydrop holder is surrounded by an optical setup that will provide control on the emulsification process inside the Raydrop.

The complete flowpath of the Raydrop Platform is presented in Figure 3.

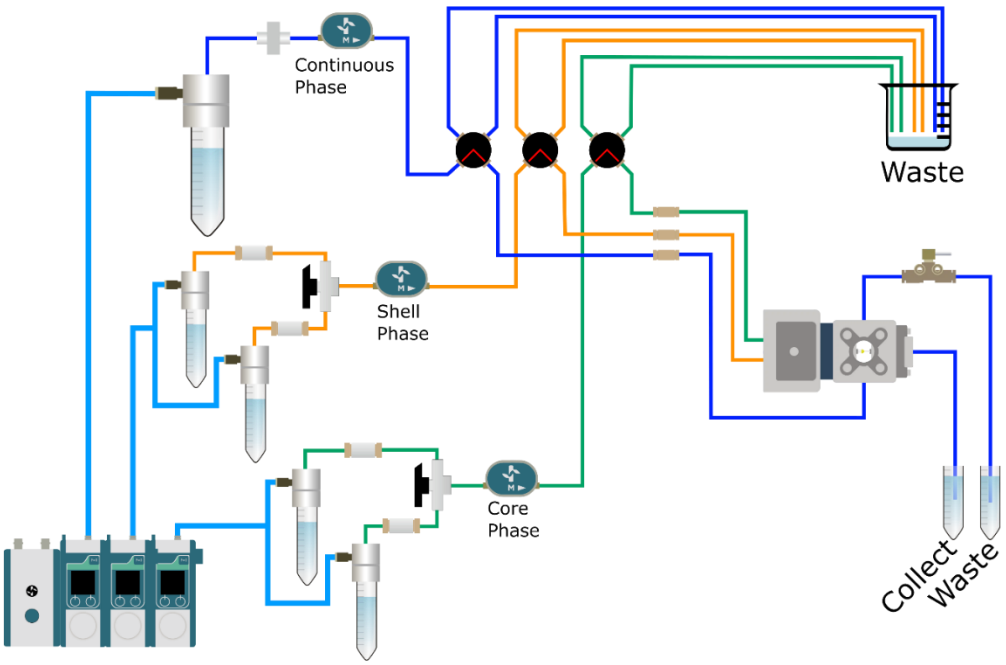
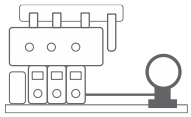
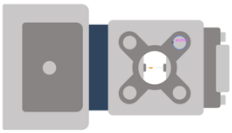


Figure 3: Flowpath of the Raydrop Platform

4 Related products

Product	Description	Reference
	An easy-to-use equipment allowing to produce emulsions using Secoya's microfluidic droplet generator: the Raydrop.	Raydrop Platform
	A droplet generator that produces simple and emulsions with a precise control of droplet size.	Double emulsion, glass, 30-100-150

5 Using the Raydrop platform to produce double emulsion

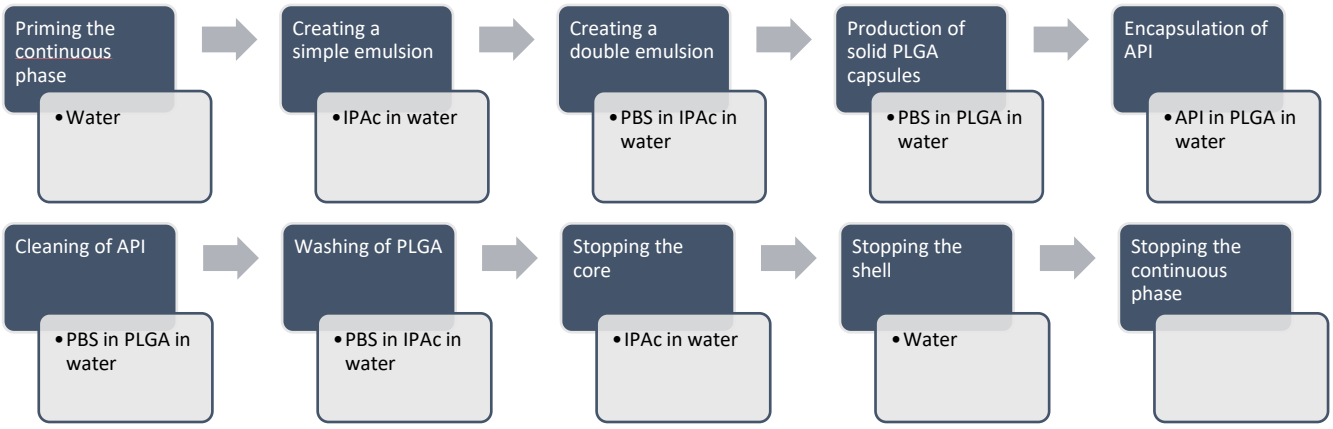
In this document, the use of the Raydrop Platform to produce Poly(D,L-lactide-co-glycolide) (PLGA) capsules is described.

The Raydrop used is the standard double emulsion version with a 30-100 glass nozzle (30µm inside diameter (ID) for the Core phase, 100µm ID for the Shell phase) and a 150µm ID collection's capillary. Other double emulsion configurations could be used, provided some parameter tuning.

The three phases used to produce the double emulsion are:

- Continuous phase: Water + 2% w/w poly(vinyl alcohol) (PVA Mw 9 000 – 10 000 80% hydrolyzed, surfactant)
- Shell priming and cleaning phase: Isopropyl acetate (IPAc)
- Shell phase: IPAc + 10% w/w Poly(D,L-lactide-co-glycolide) (PLGA Resomere RG 755 S ester terminated)
- Core priming phase: Phosphate-buffered saline (PBS, buffer solution) + 0.05% w/w bromocresol purple (dye)
- Core phase: PBS solution containing an API
- Collection bath: Phosphate-buffered saline (PBS, buffer solution)

The following diagram describes the steps to follow to produce PLGA capsules.



<u>Note:</u> The dye used in the core priming phase helps to see the formation of the emulsion. Here, bromocresol purple is used as a dye but it is also possible to use any other water soluble dye.

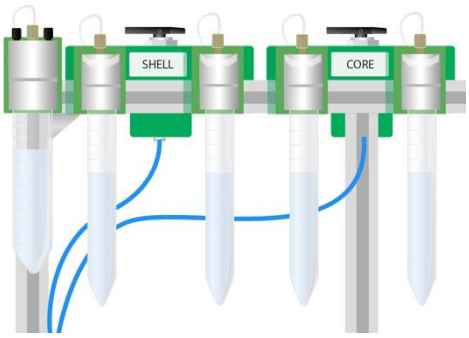
<u>Note:</u> Even if the platform integrates filters for each phase, it is a good practice to filter the solution before pouring them in the Test tube to increase the life of the online filters. Of course, the online filters are easily exchangeable for new one.

<u>Note:</u> Pressure and flowrate mentioned below are indicative. Every fluid system will need adjustment.

5.1 Filling the Raydrop

5.1.1 Fill reservoirs

F.01
Fill each test tube with the respective 0.22µm filtered solution:



- Continuous phase: 45 mL of water + 2% w/w poly(vinyl alcohol) (PVA Mw 9 000 – 10 000 80% hydrolyzed, surfactant)
- Shell priming and cleaning phase (left Test tube of the shell): 13mL of IPAc
- Shell phase (right Test tube of the shell): 13mL of IPAc + 10% w/w Poly(D,L-lactide-co-glycolide) (PLGA Resomere RG 755 S ester terminated)
- Core priming phase (left Test tube of the core): 10mL of phosphate-buffered saline (PBS, buffer solution) + 0.5% w/w bromocresol purple (dye)
- Core phase (right Test tube of the core): 10mL of API solution

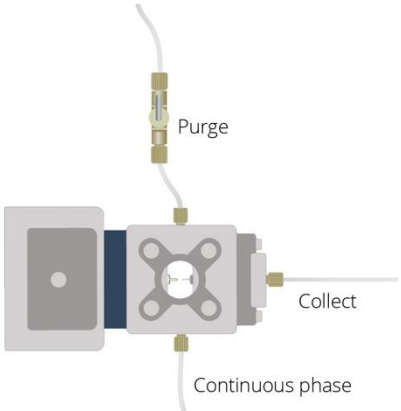
Place the test tube back in the platform.

Note: When filling the test tubes, always leave at least 1cm between the maximum level of the test tube and the liquid level. This prevents liquid from getting into the gas lines and damaging the pressure controllers.

Note: The solvent of the shell phase (here IPAc) has to be degassed before use. If this solvent is not degassed, bubbles will appear in the nozzle and the formation of the double emulsion will be difficult to stabilize. An appendix details how to degas solvents efficiently.

Note: If you do not want to encapsulate some API and you only want to produce capsules, the right test tube of the core phase can be left empty. ***However, all test tubes must be mounted on the platform (whether for the shell or the core). If a test tube is not mounted, the pressure will not increase and there will be no fluid flow.***

F.02
Connect to the Raydrop:



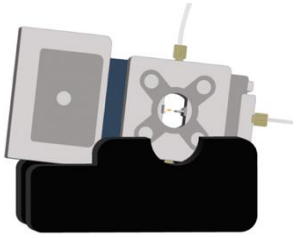
- the purge line with on/off valve
- the collect tubing: 50 cm 250µm ID, one end with the blue ferrule fitting
- Continuous phase tubing: 25 cm 250 µm ID both end with the yellow ferrule fitting.

Note: to tighten tubing to equipment, several types of fitting (nut+ferrule) exist. In the platform two types of fitting are mostly used:

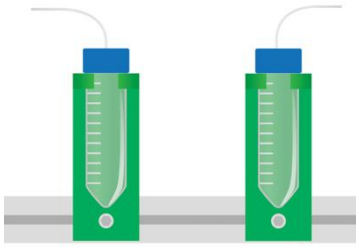
- *Flatbottom flangeless fitting (two parts, nut and blue ferrule)*
- *Flatbottom super flangeless fitting (three parts, nut, ring, and yellow ferrule).*

The super flangeless fittings offer a great benefit compared to the flangeless fittings: you don't twist the tubing while screwing the nut. However, this advantage comes with one major drawback: the tubing is not perfectly aligned with respect to the thread. As the inlet diameter of the core and shell phases tubing is only 150µm, it is mandatory to connect these two inlets with flangeless fittings (the blue one).

F.03
Place the Raydrop in its sample holder; use the groove to guide the continuous phase tubing

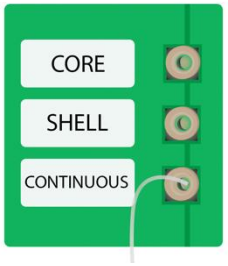


F.04
Attach to the right side of the platform both Test tube holder. Place the purge tubing in one test tube and the collect tubing in the other Test tube.



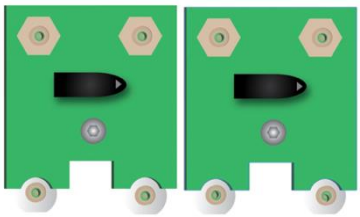
F.05
Place every waste tubing inside a waster container; here we use a GL45 bottle with the supplied GL45 pierced cap

F.06
Connect the continuous phase tubing to the platform



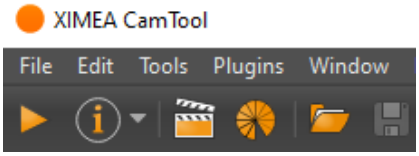
F.07
Switch all 4-way valves to the position Reservoir-Waste

F.08
Switch all black 3-way valves to the position Right Test tube



F.09
Turn on the platform lamp by turning the dimmer switch

F.10
Start the camera software xiCamTool on your computer.
Launch the live video by pressing the triangle start button.
The view of the inside of the Raydrop should appear on the screen.



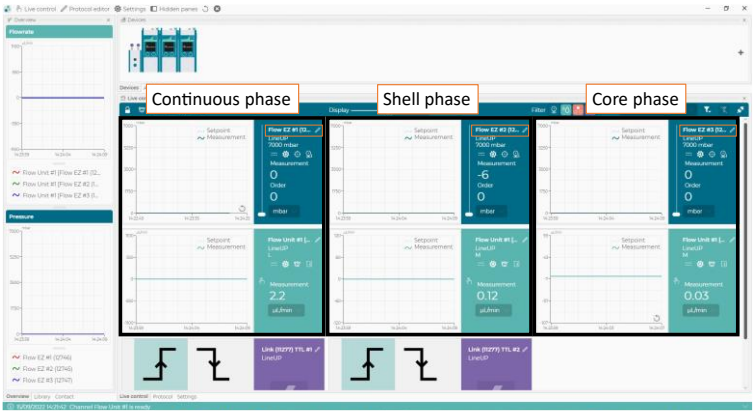
F.11
Press the blue button of pressure controllers to start them.

F.12

Start the Fluigent OxyGEN software on your computer.
This software allows to control the pressure and the liquid flowrates.

When opening the software, a landing page opens. Choose the Live control to close this window and to be able to control the flow of the fluids.

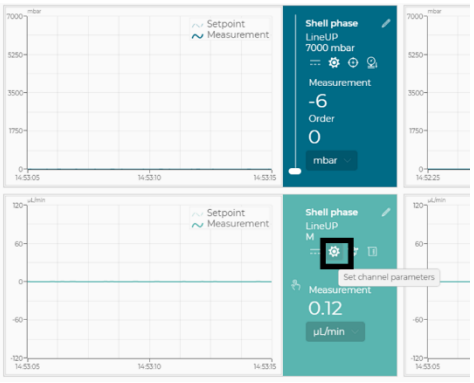
On the software, 6 small windows are available. For each phase (continuous, shell and core), there are 2 windows (one for the pressure and one for the liquid flow):
Flow #1 ⇔ Continuous phase
Flow #2 ⇔ Shell phase
Flow #3 ⇔ Core phase



Advice:
For more convenience, to easily change the flow rates and observe the inside of the Raydrop at the same time, split the computer screen in two with one part displaying the camera and a second part displaying the Fluigent software. Moreover, it is possible to rename each of the 6 small windows with the corresponding phase to clarify the use of the software.

F.13

Change the type of fluid measured by the Flow Unit for the Shell phase to Isopropanol. To do that, click on the wheel of the shell flowrate small window to set the channel parameters.

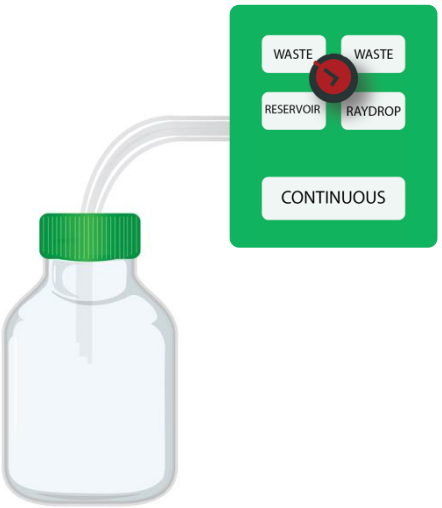


Once that the parameters of the shell are open, change the fluid of the calibration table to IPA.

For this application, the reference of the shell fluid in Fluigent software is Isopropanol (IPA).
For the continuous phase and the core phase, the fluid reference in Fluigent software is Water.

Note: Please refer to Fluigent oxygen user manual to have more information about the pressure controleur sofltware.

5.1.2 Purge the gas present in all tubing



F.14
Increase gradually the pressure for the Continuous phase up to 500 mbar on the software.

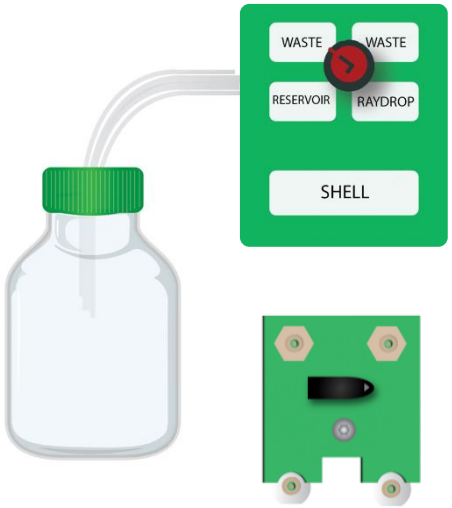
After about 1 minute, you should see a flowrates measurement. Wait until the flow measurement is stable (i.e. the value varies by maximum 5µL/min over 5 seconds).

As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Continuous pressure to 0 mbar.

F.15
Increase gradually the pressure for the Shell phase up to 500 mbar.

After 3 minutes, you should see a flowrates measurement.
Wait until the flow measurement is stable (i.e. the value varies by maximum 5µL/min over 5 seconds)

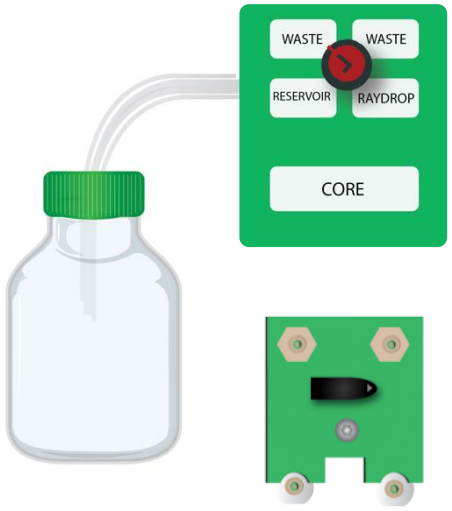
As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Shell pressure to 0 mbar.



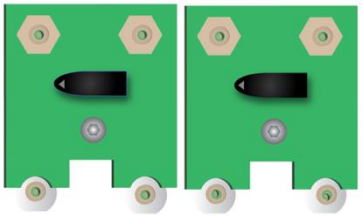
F.16
Increase gradually the pressure for the Core phase up to 500 mbar.After 3 minutes, you should see a flowrates measurement.

Wait until the flow measurement is stable (i.e. the value varies by maximum 5µL/min over 5 seconds)

As soon as the liquid flowrate is stable and once that the liquid flows out the waste tubing, you can set the Core pressure to 0 mbar

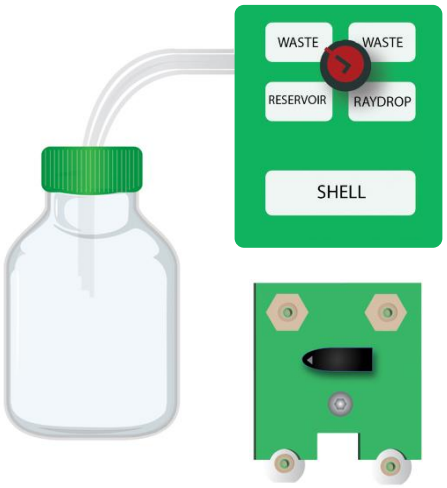


F.17
Switch all black 3-way valves to the position Left Test tube



F.18
Increase gradually the pressure for the Shell phase up to 500 mbar.
After a couple of minutes, you should see a stabilization of flowrates measurement, which mean that the flowrate value varies by maximum 5µL/min over 5 seconds.

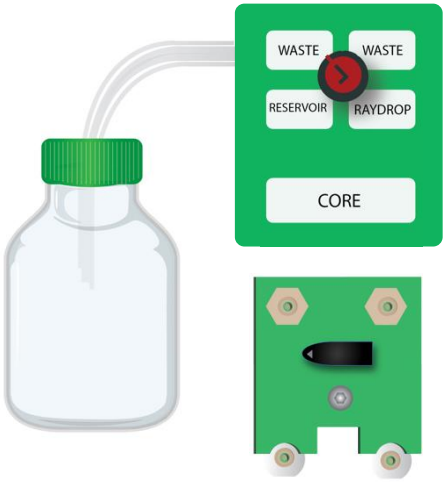
As soon as the liquid flows out the waste tubing, you can set the Shell pressure to 0 mbar



F.19
Increase gradually the pressure for the Core priming phase up to 500 mbar.

After a couple of minutes, you should see a stabilization of flowrates measurement, which mean that the flowrate value varies by maximum 5µL/min over 5 seconds.

As soon as the liquid flows out the waste tubing, you can set the Core priming pressure to 0 mbar



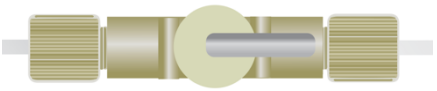
F.20
At this point, for each phase, liquids are present in the tubing from the Reservoirs to the 4-way valves and from the 4-way valves to the waste container.

To prevent liquids to flow naturally from the Test tube to the waste container, switch all the 4-way valves to the position Waste-Waste.

5.1.3 Fill the metalic chamber of the Raydrop

F.21
Switch the Continuous 4-way valve to Reservoir-Raydrop

F.22
Open the purge valve

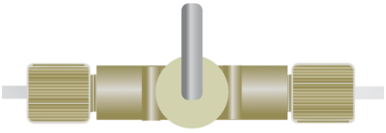


Note: the open position is where the metal bar is aligned with the tubing (see scheme on the right)

F.23
Increase gradually the pressure for the Continuous phase until you reach a flowrate of 400 µL/min.

F.24
After less than a minute, the continuous phase exits the purge tubing.

Let the continuous phase flows in the purge waste Test tube for 30 seconds.



Close the purge valve.

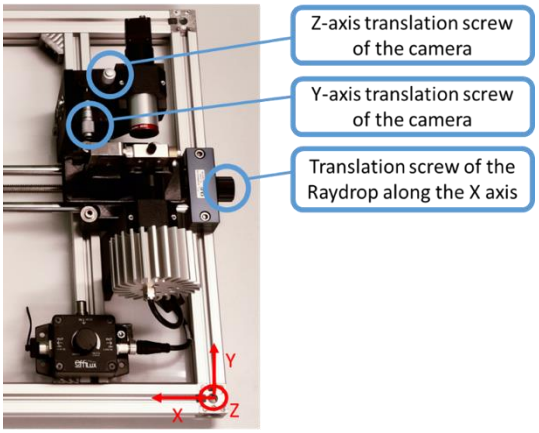
Set the pressure to 0 mbar.

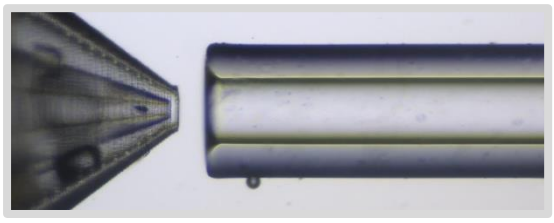
5.2 Adjusting the optical setup

O.01

Most of the time, the glass capillary and nozzle are already visible in the camera view and only a slight adjustment is needed.

- Adjust the focus of the camera by moving the camera Y-axis translation screw
- Adjust the high-low position of the nozzle by moving the camera Z-axis translation screw
- Adjust the left-right position of the nozzle by moving the Raydrop X-axis translation screw

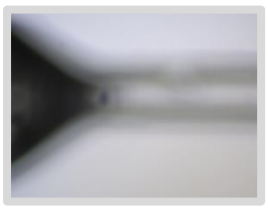




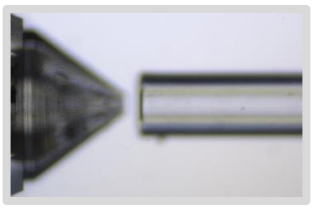
O.02

In some case, due to poor transportation condition or manutention, the focus of the Raydrop might be not good at all.

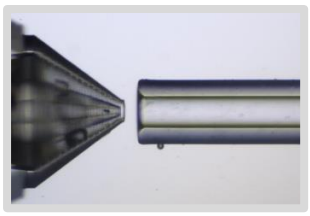
1) Check that the Raydrop is properly installed in its holder. Don't hesitate to move a bit the Raydrop in its sample holder to find the sweet spot
2) Slowly move the camera along the Z-axis to find the Nozzle and output capillary in the camera view. If the nozzle is out of focus, you will only see a shadow
3) Move the Raydrop along the X-axis to adjust the observation windows to your needs
4) Move the camera on the Y-axis to adjust the focus



Out of focus Nozzle



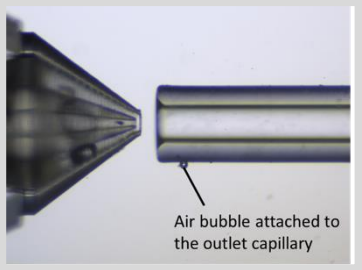
Almost in focus nozzle



In focus nozzle

O.03
Sometimes, when filling the Raydrop, a large bubble remains attached to the outlet capillary. To remove this bubble, apply a small mechanical vibration by gently tapping the Raydrop.

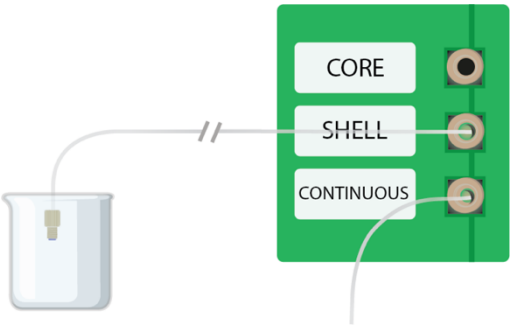
In the picture on the right, you can see a very small air bubble but it does not interfere with the formation of emulsions.



5.3 Priming the shell and the core phases

5.3.1 Connect the shell to the Raydrop

P.01
The procedure to connect the Shell and Core phases tubing is slightly different from the Continuous phase tubing as we want to avoid introducing too much air in the Raydrop.



First connect the Shell tubing (20 cm, 125µm ID, red PEEK, blue+sleeve and green fittings) to the platform. The green fitting will be connected to the platform and the blue one to the Raydrop.

Screw the green fitting to the platform and place the blue fitting in a beaker

Note: the tubing is modified from the initial configuration. The aim is to adjust the pressure drop into the ideal operating range. More details are given in Appendix 1.

Note: When using 1/16" OD fittings with smaller tubing, an element called sleeve must be added to ensure tight fitting. Appendix X specifies in which cases sleeves must be used.

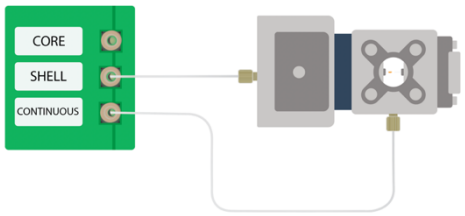
P.02
Switch the Shell 4-way valve to Reservoir-Raydrop

Increase gradually the pressure for the Shell phase up to 500 mbar
After a couple of minutes, you should see the liquid flowing out to the beaker. As soon as the liquid flows out the tubing, set the Shell phase pressure to 0 mbar

P.03
Switch the Shell 4-way valve to Waste-Waste

P.04
Connect the Shell phase tubing to the Raydrop:

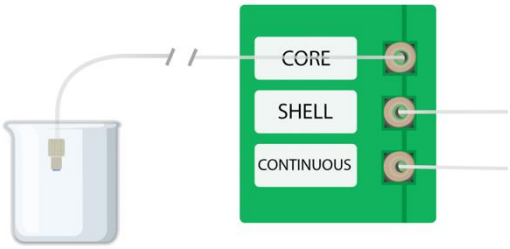
- To avoid any twisting of the tubing, loosen the Shell fitting on the platform side
- Rinse the blue fitting with acetone to prevent dust from entering the Raydrop when screwing
- Screw and tighten the blue fitting to the Shell inlet of the Raydrop. The Shell inlet is the one with the large circle mark
- Tighten the yellow fitting on the platform side



5.3.2 Connect the core to the Raydrop

P.05
Connect the Core tubing (30 cm, 125µm ID, red PEEK, blue+sleeve and green fittings) to the platform. The green fitting will be connected to the platform and the blue one to the Raydrop.

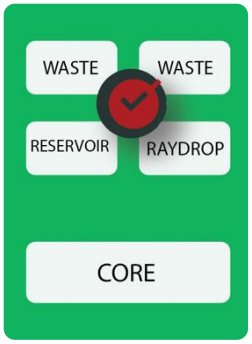
Screw the green fitting to the platform and place the blue fitting in a beaker.



P.06
Switch the Core 4-way valve to Reservoir-Raydrop.

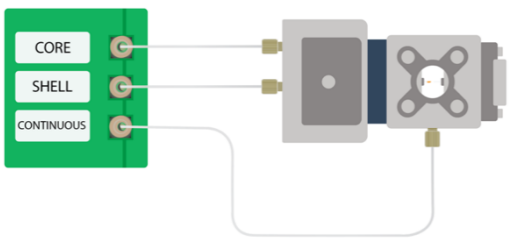
Increase gradually the pressure for the Core priming phase up to 500 mbar.
After a couple of minutes, you should see the liquid flowing out to the beaker. As soon as the liquid flows out the tubing, you can set the Core phase pressure to 0 mbar.

P.07
Switch the Core 4-way valve to Waste-Waste.



P.08
Connect the Core phase tubing to the Raydrop:

- To avoid any twisting of the tubing, loosen the Shell fitting on the platform
- rinse the blue fitting with acetone to prevent dust from entering the Raydrop when screwing
- Screw and tighten the blue fitting to the Core inlet of the Raydrop, the one with a dot
- Tighten the green fitting on the platform side

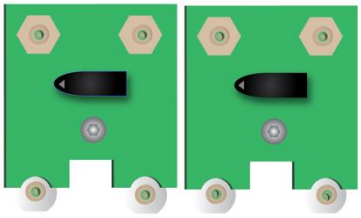


5.4 Creating a simple emulsion of shell phase

It is best to work in pressure regulation mode with the pressure controller instead of flowrate regulation mode when starting the Raydrop system. The pressure controllers' algorithm is not fast enough to compensate the large variation in pressure and flowrate. However, once the system is stable, you can switch to flowrate regulation mode as it is more convenient to use.

SE.01
Set the Continuous 4-way valve to Reservoir-Raydrop and both Shell and Core 4-way valves to Reservoir-Waste

SE.02
Check that all black 3-way valves are on the position left Test tube

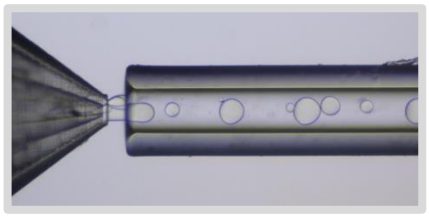


SE.03
Increase gradually the pressure in the continuous phase to obtain a flowrate of 200 µL/min

SE.04
Increase the pressure of the Shell phase to obtain a flowrate of 100 µL/min

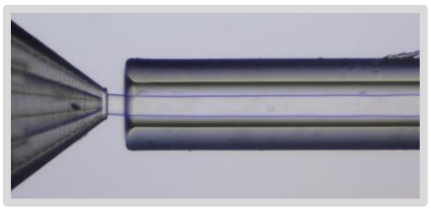
SE.05
Switch the Shell 4-way valve to Reservoir-Raydrop. Due to the difference in pressure drop between the two flow paths, this will result in a significant diminution of the flowrate.

SE.06
Adjust the Shell pressure to obtain a 35 µL/min Shell flowrate. This will help flushing the nozzle from the remaining water and air.
First, a mixture of water, IPAc and air flows through the nozzle.

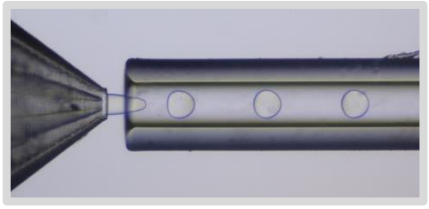


Note: the Shell flowrate might become negative when switching the valve. It is due to the pressure balance between Continuous and Shell phases. Simply increase the Shell pressure until having a positive flowrate.

SE.07
After approximatively 30 seconds, a clean jet of Shell priming and cleaning phase flows out of the nozzle.
If drops are formed instead of a jet, increase the flow rate slightly until a jet is obtained.



SE.08
When the jet is stable and regular, decrease the Shell pressure to reach a Shell flowrate of ~25 µL/min.
Shell phase droplet should now be dripping from the Raydrop.



Congratulation, you have formed your first simple emulsion with the Raydrop!

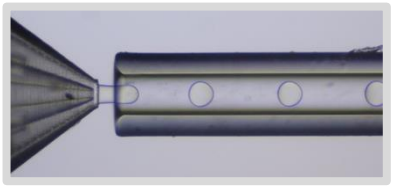
Note: dripping and jetting regimes are described in the glossary

SE.09
Here, you can play with the system to understand its basic principle:

- When you increase the Continuous flowrate (by increasing the Continuous pressure) while keeping a constant Shell flowrate: the droplet size decreases.
- When you decrease the Continuous flowrate (by decreasing the Continuous pressure) while keeping a constant Shell flowrate: the droplet size increases.
- The minimum accessible droplet diameter will depend on the fluid system (viscosities, surface tensions, etc.) and the geometry (nozzle and capillary sizes).
- When the Shell flowrate is too large compared to the Continuous phase flowrate, a jetting regime will be obtained; in such cases, decreasing the Shell flowrate will revert to a dripping regime

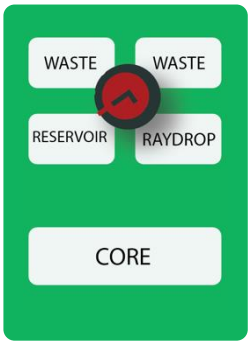
5.5 Creating a double emulsion

DE.01
Adjust again the Continuous pressure to reach a Continuous flowrate of ~150 µL/min and the Shell pressure to obtain a Shell flowrate of ~8 µL/min



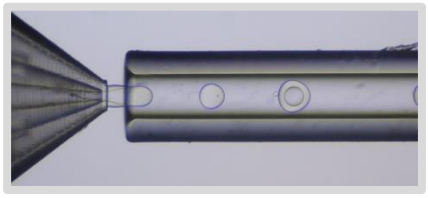
DE.02
Increase the pressure of the Core phase to obtain a flowrate of 7 µL/min

DE.03
Switch the Core 4-way valve to Reservoir-Raydrop. If the indicated Core flow rate is negative, simply increase the pressure of the core phase until it becomes positive again and drops come out of the core.

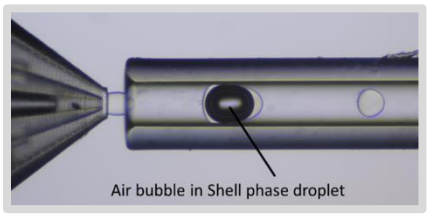


DE.04

Adjust the Core pressure to obtain a 7 µL/min Core priming flowrate. First, the remaining mixture of air/continuous phase/shell phase must be flowed out of the Raydrop.
Depending on flowrates, an emulsion with one core in every two droplets could be obtained. This can be fixed by increasing the core flowrate.



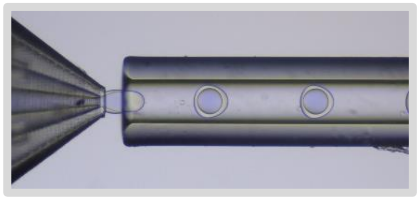
Note: the Core flowrate might become negative when switching the valve. It is due to the pressure balance between the three phases. Simply increase the Core pressure until having a positive flowrate.



Note: if an air bubble, coming from the shell or the core, breaks the double emulsion generation, stop the Core flowrate by switching the Core 4-way valve to Raydrop-Waste and repeat steps DE3 and DE4.

DE.05

Quickly after that, the double emulsion formed with your mixtures appears, as seen on the right with the violet dye.



Congratulation, you have formed your first double emulsion with the Raydrop!

DE.06

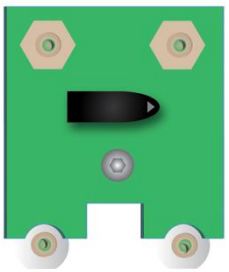
Here, you can play with the system to understand its basic principle:

- When you increase the Continuous flowrate (by increasing the Continuous pressure) while keeping constant Shell and Core flowrates: the droplet size decreases.
- When you decrease the Continuous flowrate (by decreasing the Continuous pressure) while keeping constant Shell and Core flowrates: the droplet size increases.

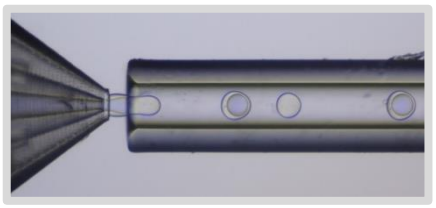
- When you increase the Shell flowrate (by increasing the Shell pressure) while keeping constant Continuous and Core flowrates: the shell thickness of the double emulsion increases.
- When you decrease the Shell flowrate while keeping constant Continuous and Core flowrates: the shell thickness of the double emulsion decreases.
- When you increase the Core flowrate (by increasing the Core pressure) while keeping constant Continuous and Shell flowrates: the core of the double emulsion increases and so the size of droplets increases.
- When you decrease the Core flowrate while keeping constant Continuous and Shell flowrates: the core of the double emulsion decreases and so the size of droplets decreases.

5.6 From a double emulsion to solid PLGA capsules production

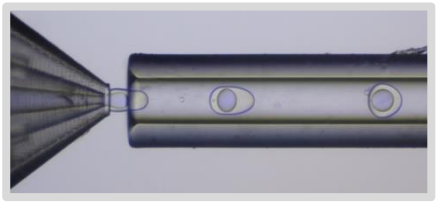
CAPS.01
Once that a stable double emulsion is generated, switch the black 3-way valve of the shell on the position right Test tube.



CAPS.02
After the switch, wait 6 minutes so that the PLGA solution substitutes the pure IPAc. The change of fluid (and so of viscosities) has an influence on the shell flowrate. Once that the shell flowrate stabilized, the shell phase flowing is IPAc with PLGA.



CAPS.03
Readjust the pressure of the shell and core phase to have a flowrate respectively of 24 µL/min and 8 µL/min to form a stable double emulsion with one core in every droplet.



CAPS.04

Now, adjust the size of the core and the shell thickness by modifying flowrates:

- When you increase the Continuous flowrate (by increasing the Continuous pressure) while keeping a constant Shell and Core flowrate: droplet size decreases.

Increase of Continuous phase flowrate

- When you decrease the Continuous flowrate (by decreasing the Continuous pressure) while keeping a constant Shell and Core flowrate: droplet size increases.

Decrease of Continuous phase flowrate

When the Shell flowrate is too large compared to the Continuous phase flowrate, a jetting regime will be obtained; in such a case, decreasing the Shell flowrate will revert to a dripping regime

Note: details about the influence of flowrates on the double emulsion and on the regime are presented in annex XX

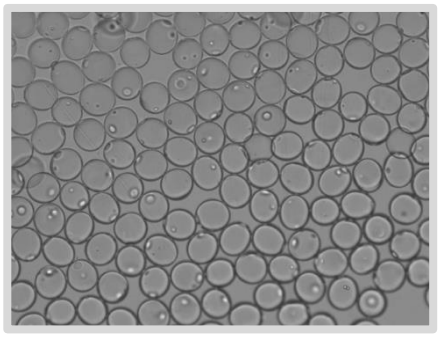
CAPS.05

Fix the continuous flowrate at 150 µL/min, the shell flowrate at 24 µL/min and the core flowrate at 8 µL/min to have a stable double emulsion with a shell that is not too thin.

Collect the droplets at the outlet of the Raydrop in a vessel of 15mL half filled with PBS buffer. The PBS buffer is used here to match the osmolarity of inner and outer aqueous phases.

CAPS.06

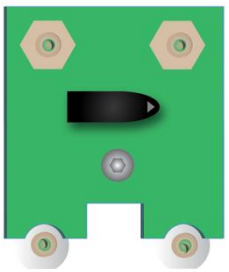
Upon solidification, the shell thickness of the collected droplets decreases. It is normal and is due to the diffusion of IPAc into the PBS collection bath so that the PLGA precipitates.
When the droplets fall to the bottom of the collection container, they became capsules that are totally solid. The solidification of PLGA is a solvent extraction process. IPAc is extracted from the shell, which leads to an increase of PLGA concentration in the shell until it precipitates



5.7 From encapsulation of PBS to encapsulation of API

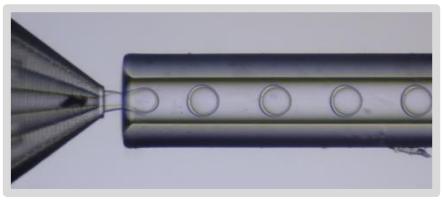
ENCAPS.01

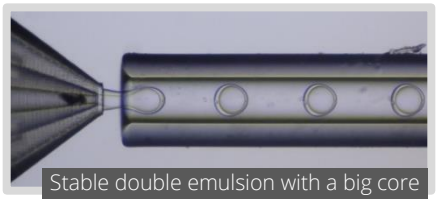
Once that a stable double emulsion with PLGA is generated, switch the black 3-way valve of the core on the position right Test tube



ENCAPS.02

After the switch, wait 5 minutes so that the API solution substitutes the pure PBS. The change of fluid can have a small influence on the core flowrate, depending on the viscosity difference between the two fluids that are used as cores. Once that the core flowrate stabilized, the core phase flowing is the API solution.



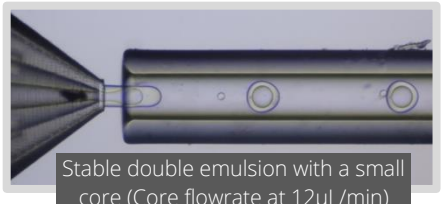


Stable double emulsion with a big core (Core flowrate at 40µL/min)

ENCAPS.03

Readjust the pressure of the core phase to form a stable double emulsion with one core in every droplet.

Note: it is of course possible to use another flowrate for the core phase to adapt the volume of API to encapsulate.



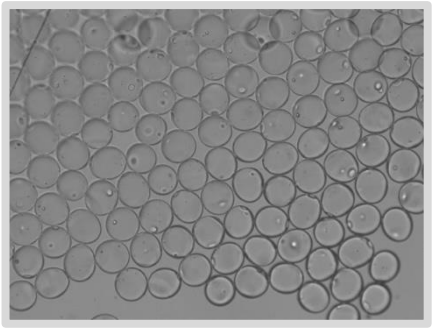
Stable double emulsion with a small core (Core flowrate at 12µL/min)

ENCAPS.04

Collect the droplets at the outlet of the Raydrop in a vessel of 15mL half filled with PBS. The solution should be used to match the osmolarity of inner and outer aqueous phases.

ENCAPS.05
The shell thickness of the collected droplets decreases. It is normal and is due to the diffusion of IPAc into the PBS collection bath so the PLGA precipitates.

When the droplets fall to the bottom of the collection container, droplets became capsules that are totally solid.

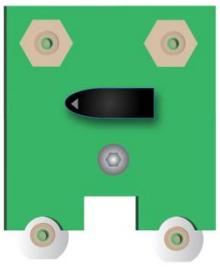


ENCAPS.06
The API is now encapsulated.

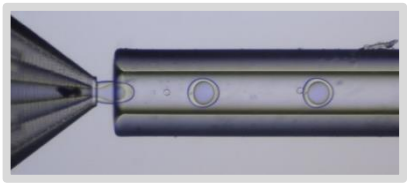
5.8 Cleaning of API

Once that all samples have been collected, the core has to be washed before shutting down to avoid any clogging of the system.

C.01
Switch the black 3-way valve of the core back to the position left Test tube-Filter.



C.02
After the switch, wait 5 minutes so that pure PBS substitutes the API.

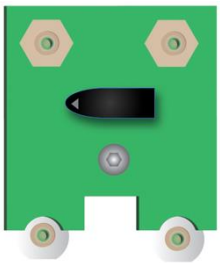


C.03
The core phase tubing is now clean and the flowrate can be reduced to 5 µL/min.

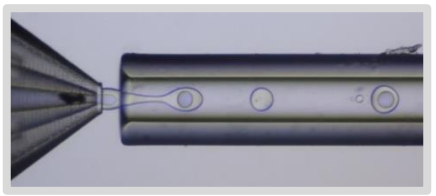
5.9 Washing of PLGA

Before stopping the experiment, it is important to flush the shell phase and the nozzle of the Raydrop with IPAc. This priming but also cleaning solution contains pure IPAc, which will dissolve and evacuate the PLGA. This way, tubing stay clean and clogging is avoided.

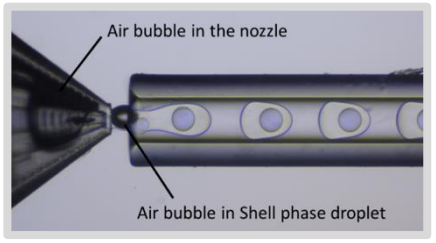
W.01
Switch the black 3-way valve of the shell back to the position left Test tube-Filter.



W.02
After the switch, wait 5 minutes so that pure IPAc substitutes the PLGA solution. The shell flowrate can be increased to 60 µL/min to accelerate the cleaning process.

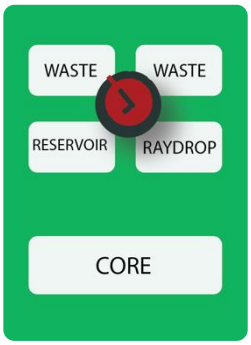


W.03
It is possible that the IPAc forms bubbles in the nozzle. This is why degassing organic solvents like IPAc is necessary. The formation of bubbles in the nozzle destabilizes the double emulsion process. However, the formation of bubbles is not a problem for the washing.

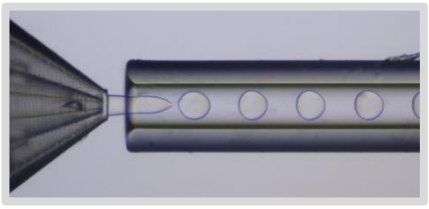


5.10 Stopping the core

SC.01
First, switch the Core 4-way valve to Reservoir-Waste and set the Core pressure to 0 mbar.



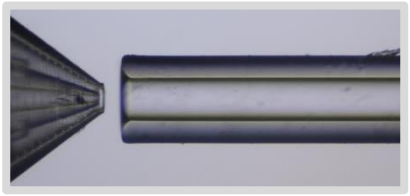
SC.02
A simple emulsion is produced.



5.11 Stopping the shell and the continuous phase

SSC.01
Switch the Shell 4-way valve to Reservoir-Waste and set the Shell pressure to 0 mbar.

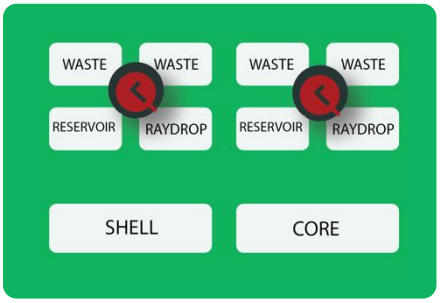
SSC.02
Now, only the Continuous phase is flowing.



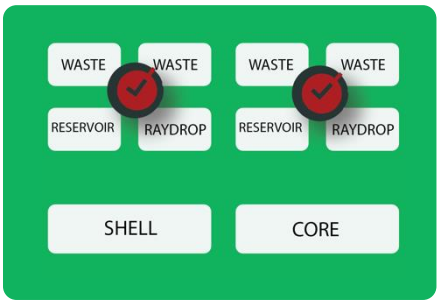
SSC.03
Reduce slowly the Continuous pressure to reach around 50 µL/min for the Continuous phase.

SSC.04
To avoid any pollution of the chamber from the Shell or Core phase, it is a good practice to let the pressure inside the system decreased for a couple of minutes

SSC.05
Switch the Shell and Core 4-way valves to Raydrop-Waste to completely decrease the pressure inside the Core and Shell tubing.



SSC.06
After approximately 15 seconds, switch the Core and Shell 4-way valves to the position Waste-Waste to completely stop the flow for these two phases.



SSC.07
Switch the Continuous phase 4-way valve to Reservoir-Waste and set the Continuous pressure to 0 mbar.

SSC.08
Switch the Continuous phase 4-way valve to Waste-Waste to completely stop the flow for the Continuous phase. The system is properly stopped.

5.12 Putting the platform in standby mode

SM.01
Turn off the platform lamp by turning the dimmer switch

SM.02
Exit software used for the camera and the pressure controllers

SM.03
Press the blue button of pressure controllers for 4 seconds to turn them off

The solutions used for the continuous, shell and core phases can be used up to 1 week after preparation. If the platform is not used for more than one week, it is necessary to clean and empty the Raydrop and all tubing on the platform.

6 Glossary

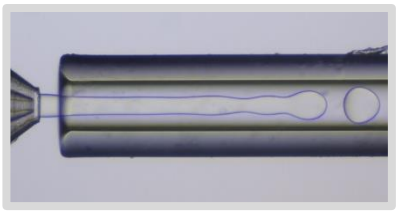
Test tube
plastic tube for centrifugal process that are used, in combination of a PCap, as a pressurised reservoir. The PCap does not accept every commercially test tubes. We recommend the use of Falcon test tubes ref. 352097 (15 mL) and 352070 (50 mL)

PCap
air-tight metal cap that allows to pressurize a Test tube

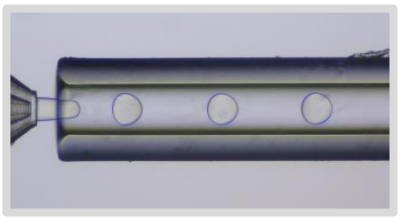
Fitting (Flatbottom Super flangeless)
ensemble of nut, ring and ferrule

Nut
hard material screwed to apply a pressure on the ferrule

Ferrule (yellow part) with ring (metallic piece)
soft material used to seal the tubing thanks to the ring action



Jetting regime
Droplets are generated through the destabilization of a jet. This regime, while giving access to higher flow rates, is also less stable than the dripping regime.



Dripping regime
droplets are generated at the outlet of the nozzle, in a stable manner.

7 Appendix