Microfluidic Production of 20 to 50 µm Core-Shell PLGA Beads

Version: 1.0

Issue Date: 30/09/2019

Author: DR
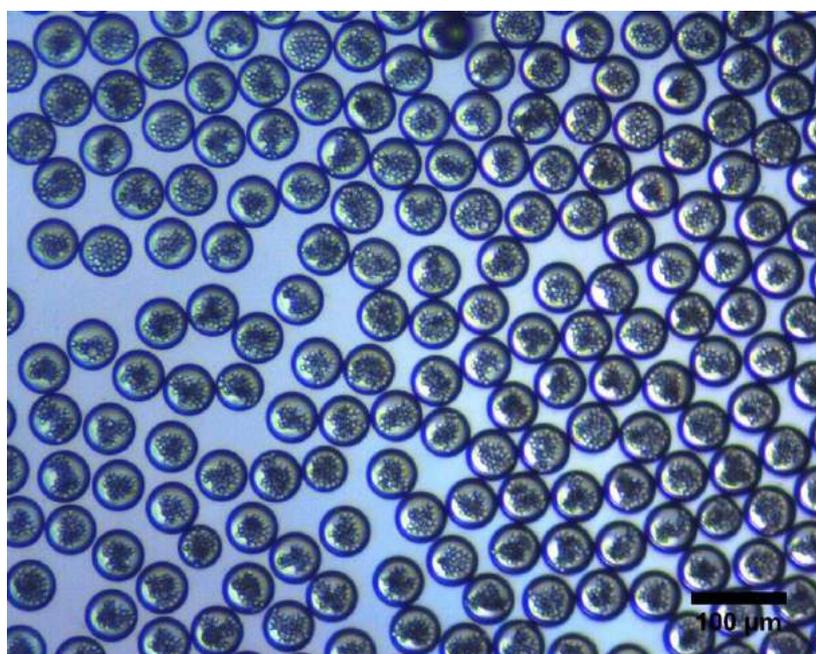
Contents

1 Introduction

This application note provides all the information necessary to make 20 to 50 um PLGA core-shell microparticles (or multiple emulsions) using the Dolomite API encapsulation system.

The encapsulation of hydrophilic materials (such as APIs, nutraceuticals, or other compounds of interest) can be challenging, as the droplet phase environment is hydrophobic during the solvent extraction process to form the PLGA particles. PLGA multiple emulsions offer a solution to this problem by introducing a number of aqueous droplets to the PLGA/DCM solution before forming the DCM emulsion in water. By controlling the relative flow rates and concentrations of the solutions, it is trivial to control the loading of the target hydrophilic material as well as the release profile of the material once encapsulated.

This note provides all the information to produce poly(lactic-co-glycolic acid) (PLGA) beads using the Dolomite API encapsulation system.

2 Experimental method

2.1 Bead formation process

The process for producing multiple emulsion microparticles first involves the emulsification of an aqueous solution in an organic continuous phase, followed by the emulsification of this primary emulsion in a second aqueous phase (containing an emulsion stabiliser). The result is a water-in-oil-in-water emulsion. This is composed by an inner phase made of deionised water, a middle phase of PLGA dissolved in dichloromethane (DCM) with 0.5 wt % CRODA Synperonic™ PE/F 68 and an outer phase made of Aqua-Phase, a Dolomite aqueous emulsion stabiliser available for purchase through https://www.dolomite-microfluidics.com/product/aqua-phase/. Once formed, this emulsion is dried to produce monodisperse PLGA microparticles containing multiple aqueous droplets.

The formation of aqueous the first emulsion droplets on a Small Droplet Chip (hydrophobic) (Dolomite part number 3200137) can be seen in Figure 1. This will produce inner droplets in PLGA/DCM solution. The size and frequency of the droplets production can be readily tuned by adjusting the flow rates of the droplet and continuous phases.
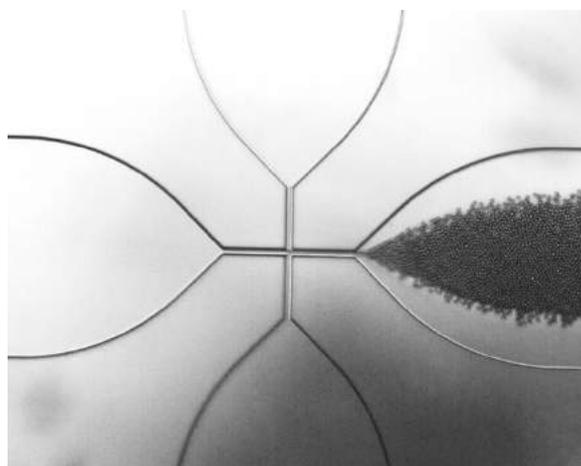

Figure 1- Image of deionised water droplets in PLGA/DCM with 0.5 wt % CRODA Synperonic™ PE/F 68

The formation of final droplets, various water droplets in a larger PLGA droplets on the second chip - 100 µm 3D pore hydrophilic chip (Dolomite part number 3200433) - can be seen in Figure 2. The size of the droplets produced can be readily tuned by adjusting the flow rates of the droplet and continuous phases.
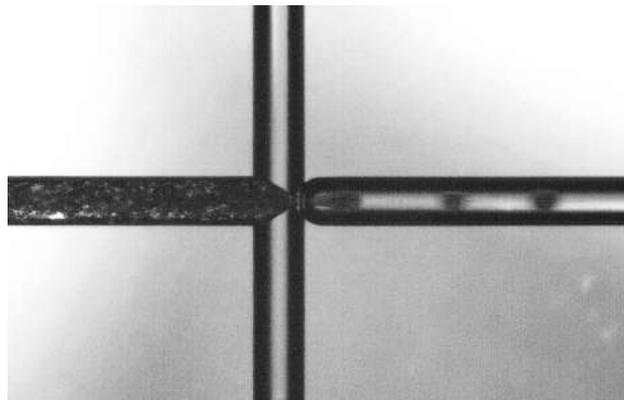

Figure 2 - Image of formation of water-in-PLGA-in-water droplets

When collected onto a glass slide for imaging (Figure 3 and Figure 4), the polymer droplets settle at the bottom of the water continuous phase on the glass slide. The evaporation process occurs indirectly, as the DCM first dissolves into the aqueous phase, and subsequently evaporates from the water-air interface. This process occurs rapidly at the thin droplet edge, where DCM evaporates fast, and is slower within the bulk (centre) of the droplet where the continuous phase forms a thicker layer and DCM evaporates slowly.

See https://www.dolomite-microfluidics.com/drug-encapsulation-in-plga-particles/ for more information about PLGA droplet shrinkage.
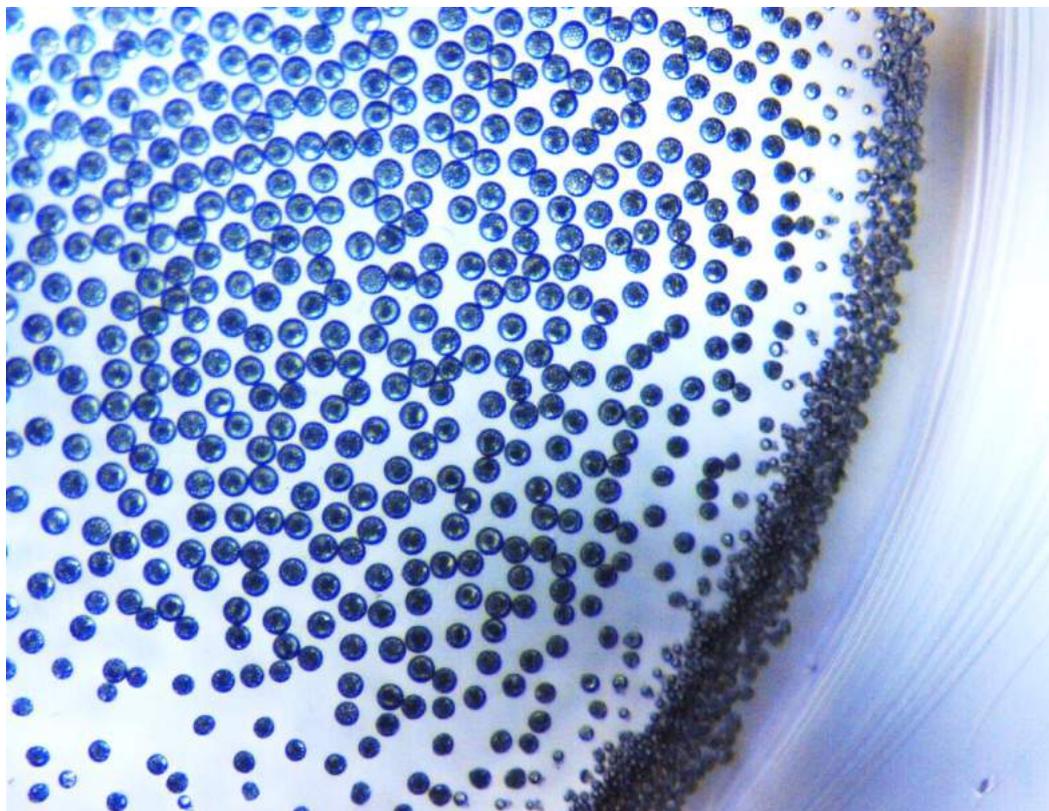

Figure 3 - Water-in-oil-in-water droplets on glass slide. DCM is slowly evaporating from the droplets starting from the droplets on the edge of the glass slide (right side of the picture)
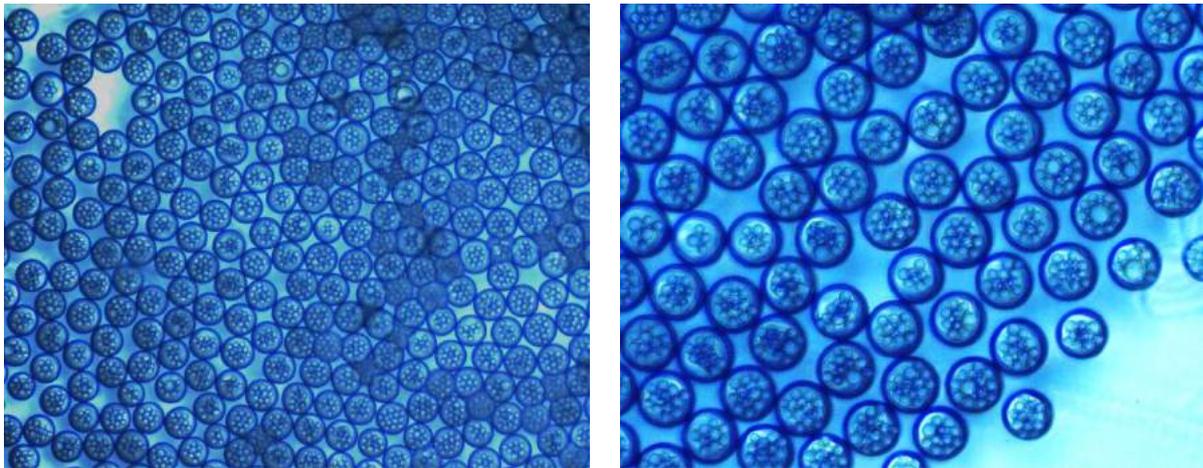
Figure 4 - Water-in-oil-in-water droplets on glass slide (bulk)

It is important to gradually evaporate the DCM from the droplets in a controlled way, to do so these can be collected in a vial containing excess continuous phase solution (Aqua-Phase), with gentle overhead stirring to ensure that inner droplets remain contained within the outer droplet. This will result in uniform gradual shrinkage of the droplets and an increase in the concentration of PLGA, until eventually all the DCM has evaporated, leaving behind a solid PLGA microparticle.

At the end of the evaporation process, the final dry beads result monodisperse as they are formed from droplets that are monodisperse initially. Once all the DCM has evaporated, the beads can be washed with fresh water to remove any Aqua-Phase stabiliser from the bead surface, and they are then ready for use in a wide range of applications. Once the process for the preparation of PLGA beads has been mastered, drug molecules and APIs can be incorporated into the beads, resulting in a functional material that can be used for drug encapsulation, controlled drug release, API solubilisation, as well as many other applications.

2.2   Different solvent

The most common production process of PLGA particles is solvent based and can involve different solvents depending on user requirements. In this work DCM is used as conventional solvent to demonstrate PLGA particle production. However, the Dolomite API encapsulation system is flexible and can be adapted for working with different solvents. E.g. ethyl acetate or other less hazardous solutions that show better biocompatibility can be alternatively used for outstanding monodisperse particle production. Contact Dolomite for more information for other solvents https://www.dolomite-microfluidics.com/contact/contact-us/.

3 Preparing reagents

There are 3 solutions to prepare for the formation of PLGA microparticles:

- Deionised Water

- 3 wt % and 6 wt % PLGA - 0.5 wt % CRODA Synperonic™ PE/F 68 in DCM (see above, ethyl acetate can be also used)

- Aqua-Phase (https://www.dolomite-microfluidics.com/product/aqua-phase/)

Simply decant 20 ml of the solutions in 20 ml scintillation vials.

4 System setup

Figure 5 shows the system setup for multiple emulsion generation. The fluids (water, PLGA/DCM/Surfactant and Aqua Phase) are stored within the reservoirs of Dolomite pressure driven P-Pumps. Each pump is coupled with a flow sensor, tubing, connector and valve to accurately control the flow rates from the pump reservoirs to the chips. The pumps are controlled using the dedicated software (FCC) provided with the system. The Dolomite high speed microscope focussed at the chip junction of the microfluidic chip enables real time droplet visualization.
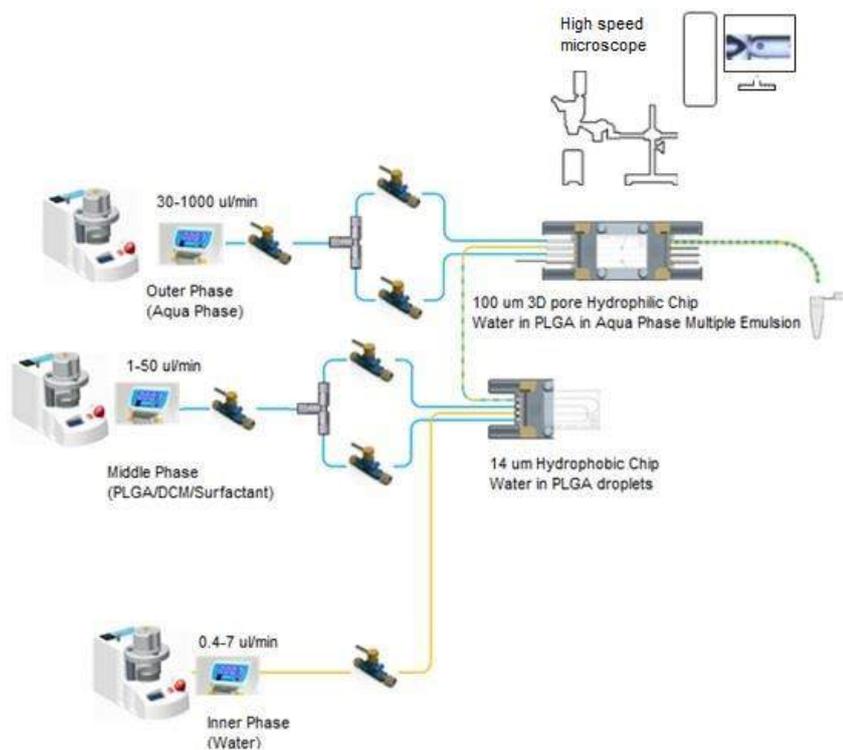

Figure 5 - Schematic of the microfluidic setup used to make multiple emulsion PLGA beads
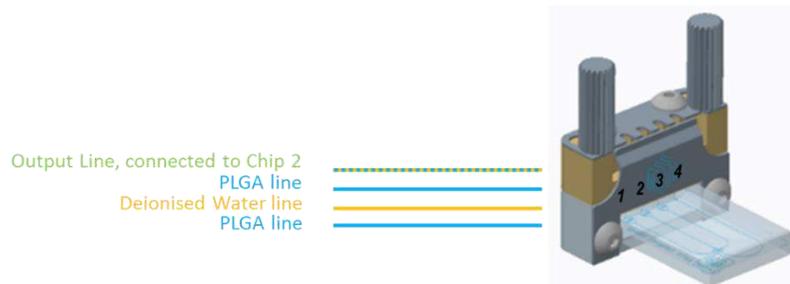
*Figure 6 - Setup of 14 um chip hydrophobic, top interface and linear connector*
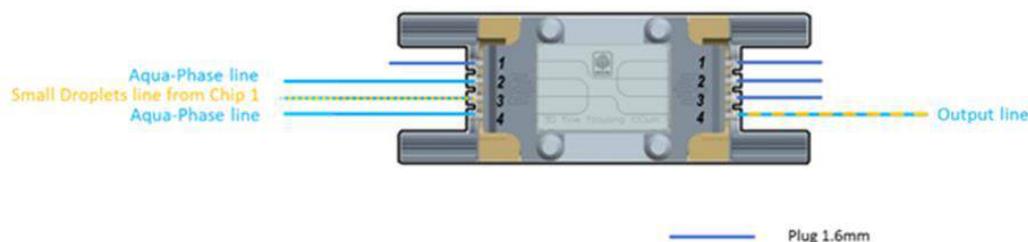
Figure 7 - Setup of 100 um 3D pore chip hydrophilic, H interface and two linear connectors

5 Experimental procedure

Sequence of operations to run the PLGA multiple emulsion:

- Prime all the tubing with fluids while keeping the chips disconnected.

- Connect the tubing to 14 um and 100 um 3D chips.

- Run the middle phase through the 14 um chip until you reach a flow rate of about 3 ul/min. Select water as reference fluid for the flow sensor on the FCC. Keep all the other valves closed.

- Set up the same pressure of the middle phase on the inner phase, open the valve and adjust the flow rate until you get droplets. Dripping regime is normally achieved when inner phase flow rate = ½ middle phase flow rate.

- Switch to flow mode.

- Run the outer phase through the 100 um 3D chip until you form the multiple emulsion. Select water as reference fluid for the flow sensor on the FCC. This is normally achieved when outer phase flow rate = 10 times middle phase flow rate.

- Switch to flow mode.

The final product is collected within a vial containing a volume of Aqua-Phase (we suggest a volume of Aqua-Phase which is half the volume of the collected product). Slow overhead stirring helps to gradually evaporate DCM and ensure that inner droplets remain contained within the outer droplet. Gentle droplet shrinkage is also achieved by DCM/Aqua-Phase partial solubility.

To confirm that PLGA droplets are being produced, a small quantity of liquid from the outlet tubing is collected onto a glass slide. The fluid on the microscope slide will contain many spherical droplets containing smaller spherical droplets.

To stop the system:

- Ensure that the outlet is collecting into a waste vial

- Set all Pumps to pressure control and close in this order: middle phase valve, followed by inner phase valve and outer phase valve. Stop Pumps

- Replace PLGA vial with pure DCM vial

- Set all Pumps to pressure control, open all valves and flush the tubing and chips

- Disconnect input/output connectors and remove the chips from the top interface and the H interface.

NOTE: a common cause of system failure during initial set-up is due to a blockage in the chip from improper cleaning or storage. A well-cared for chip can be used many times, but it is essential that they are cleaned thoroughly and stored properly

6 Factors influencing bead size and production rate

Once your system is up and running, the parameters below can be controlled to adjust the size and the production rate of PLGA core-shell beads:

6.1   Ratio of PLGA : Water : Aqua-Phase

The most effective way to alter the size of the final bead is to adjust the size of the droplets forming at the two chip junctions. Adjusting the relative flow rates of PLGA and Aqua-Phase is the easiest way to achieve this. In general, a lower flow rate of continuous phase and a high flow rate of droplet phase will result in larger droplets.

In this multiple emulsion process, a balance between the water droplet production rate and the encapsulation rate has to be found. A larger production rate of water droplets will lead to a larger amount of water droplets encapsulated in the PLGA droplet.

6.2   PLGA concentration

Changing PLGA concentration is a convenient way of fine-tuning the bead size. Increasing the concentration of PLGA means that more PLGA will be present in each droplet. This in turn means that as the droplet dries, it will form a larger PLGA microparticle.

The total amount of PLGA present is directly proportional to the volume of the resulting PLGA microparticle. Because volume is proportional to the cube of the radius, this means that an 8x increase in PLGA concentration would be required to double the diameter of the PLGA microparticle. This method therefore has a more limited effect on bead size than e.g. changing the droplet size.

In practice, highly concentrated solutions of PLGA become increasingly viscous, which can affect droplet formation. Generally, it is beneficial for the viscosities of the droplet and continuous phases to be relatively similar. Since DCM is less viscous than Aqua-Phase (which has a viscosity very similar to water), a 1-2.5 wt % solution of PLGA will improve the viscosity match, resulting in clean droplet formation and a high rate of droplet production. Increasingly concentrated solutions of PLGA result in solutions significantly more viscous than Aqua-Phase, and thus the droplet production rate reduces and there is an increased risk of the formation of satellite droplets (small droplets that form when the continuous phase does not pinch off the droplet cleanly). Using the described approach in this application note, PLGA concentrations up to 7.5 wt % perform well, however increasing PLGA concentration beyond this may result in a deterioration of the system performance. Alternatively, reducing the concentration of PLGA can be used to produce much smaller PLGA microparticles. This approach remains effective down to very small droplet sizes, though whilst the number of particles produced will be the same, the

mass yielded will be significantly smaller due to the very small amount of PLGA per droplet. A more efficient way of generating smaller microparticles is to form a larger number of smaller droplets.

6.3 Total combined flow rate

Increasing the flow rate of both the droplet and continuous phase in proportion with one another will increase the frequency of droplet formation. The droplet size will remain relatively similar, though the increased velocities will alter the fluid dynamics at the chip junction, which may result in some shift in droplet size. After increasing total flow rate, the droplet size can then be refined by adjusting the overall ratio of flow rates.

At a certain point, the continuous phase will be unable to pinch off droplets due to the rate of droplet phase flow resisting the pinching force. This will cause the droplet phase to jet through the junction, as a continuous cylinder of PLGA solution. Occasionally droplets will still form further down the outlet tubing, but these droplets are much more likely to be polydisperse compared to those formed within the confines of the chip junction.

6.4 Combinations of factors

Each of the variables discussed above acts like a slider that can be fine-tuned to achieve your desired product. However, each variable may influence, droplet size, flow stability or production rate. It is therefore important that throughput is balanced against increasing flow instability; it may be the case that a higher stable throughput may be achieved by using a higher concentration of PLGA and targeting a smaller droplet size, or that a lower rate of production with a high PLGA concentration and smaller droplets may be more stable than trying to make overly large droplets at a low PLGA concentration.

Thankfully, it is very simple to adjust these variables within Flow Control Centre (FCC); adjustments to flow rates have an immediate visible effect at the droplet junction, meaning that it is possible to rapidly screen a wide range of potential combinations of variables to find the production conditions that best suit your needs. A table, including flow rates, droplets size and frequency is available in the Appendix of this document.

Swapping to a different PLGA concentration is also simple – just switch off the P-Pump and switch out the vials, making sure to allow time for the old PLGA solution to be cleared from the tubing before collecting new samples.

7 Case study – 1: Multiple emulsion size control

It is indeed possible to produce various water-in-oil-in-water particles with many compositions of many sizes: a few small water droplets in large PLGA droplet or many large water droplets in small PLGA droplet. This following data will show you examples of particles you can achieve.

The drying step in multiple emulsions is complex and has to be done carefully. Droplets must be collected in a vial half-filled with Aqua-Phase. The resulting beads, with water droplets inside, will have to be washed once with Aqua-Phase and then dried slowly until eventually all DCM has been evaporated. For these samples, droplets have been collected in a 1mL Eppendorf with Aqua-Phase to improve droplets stability and avoid droplet coalescence. DCM must evaporate slowly, to avoid coalescence or popping out of water droplets (especially for beads with high loadings of primary emulsion droplets). The resulting beads are collected on a glass slide and then measured using ImageJ.

With a constant water flow rate of 1 µL/min, Aqua-Phase:PLGA ratio and PLGA (lactide : glycolide (75:25), mol wt 66,000-107,000) concentration have been modified, leading to various composition and size of particles. A larger PLGA flow

rate, in the small droplet chip, will enhance frequency of droplet production and droplet sizes, indeed there will be more water droplets in each larger PLGA droplet. A larger Aqua-Phase flow rate, in the 100 μm 3D flow focussing chip, will increase the final droplet sizes and the frequency of the final droplet production.

Few examples are exposed in the following table:

PLGA wt %	PLGA Flow Rate * ul/min	AP Flow Rate ul/min	Outer Droplet size um	Picture **	Final bead size um	Picture **
2	5 (11.6)	70	57.2		28.7	
	2 (2.6)		57.7		25.6	
	2 (2.6)	30	67.4		29.9	
6	5 (10.1)	70	61.6		36.9	
	2 (2.3)		58.4		36.7	
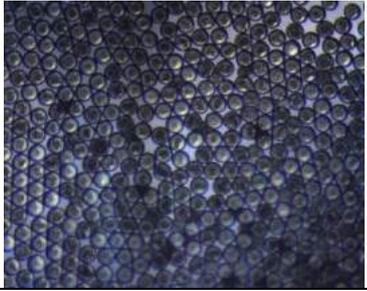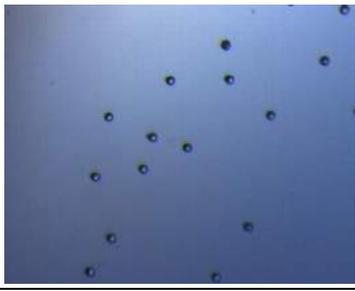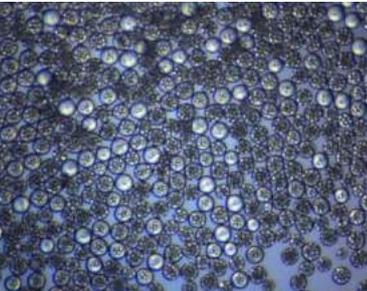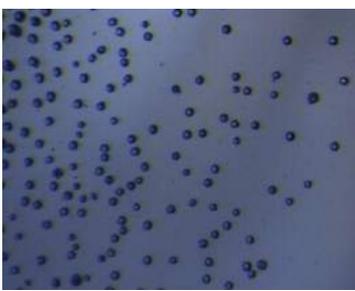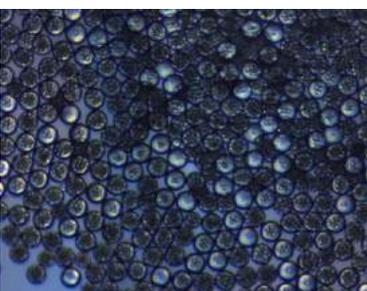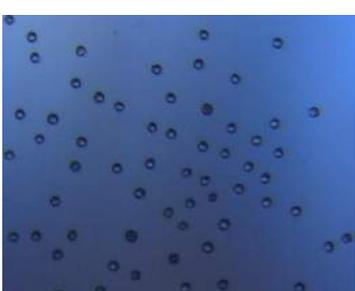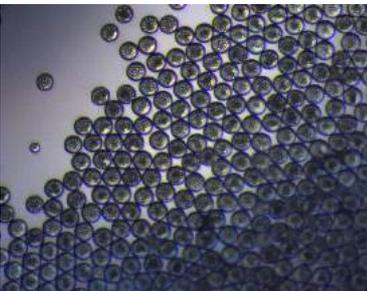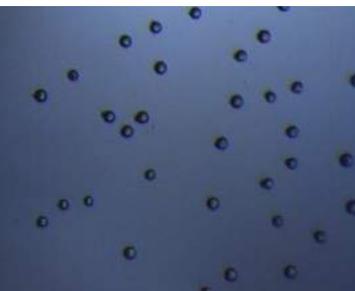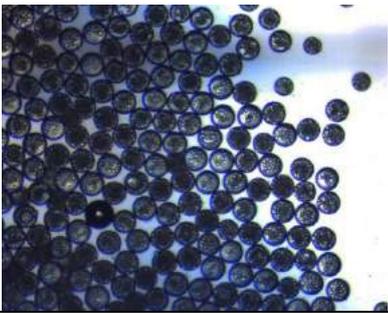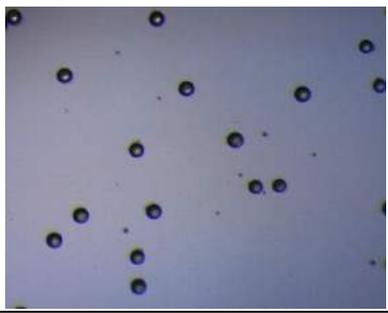
	2 (2.3)	30	74.9		45.4	
	5 (10.1)	30	72.2		45.4	
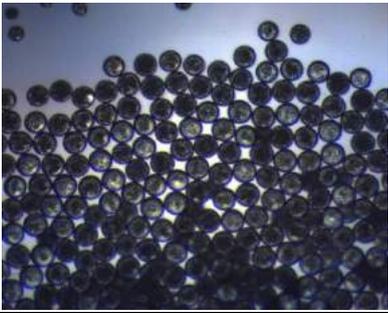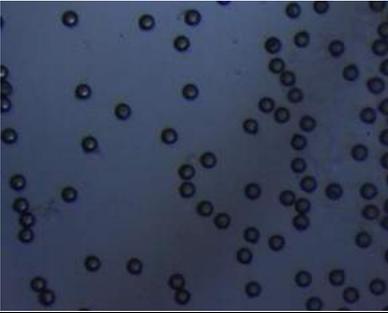
* Actual flow rates are showed in table within brackets and are calculated from set flow rate using the calibration curves reported in the Appendix.

** The core-shell PLGA droplets are collected on a microscope slide before and after shrinkage. The particles look highly monodispersed with the water droplets clearly visible within.

The inner water droplets produced using the 14 um chip are between 3 um and 14 um depending on the water:PLGA flow rate ratio (Figure 8).
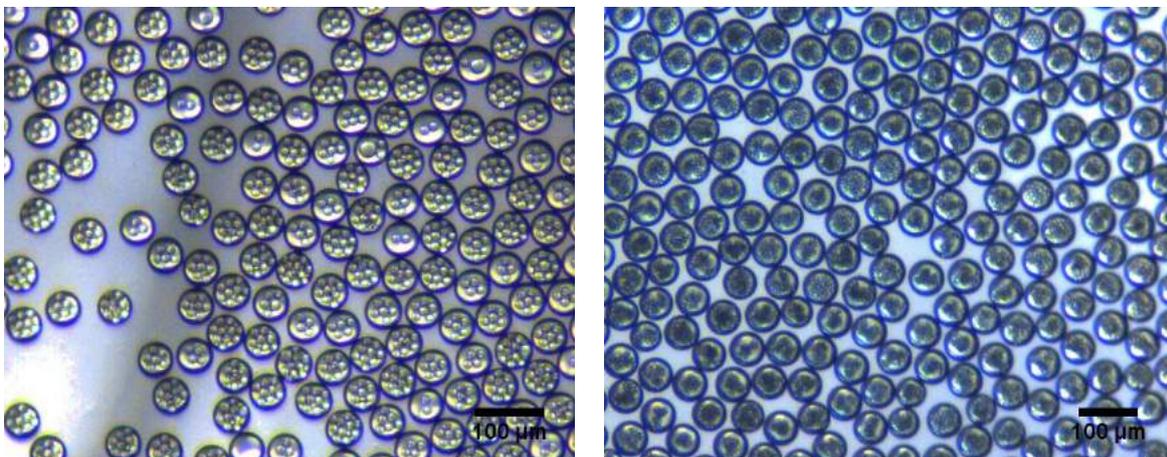

Figure 8 - 50 um PLGA beads before shrinkage with 14 um inner droplets (left) and 3 um inner droplets (right).

8 Case study – 2: Encapsulation of APIs

The Multiple Emulsion Dolomite system can produce PLGA microparticles with multiple water droplets using two single junction chips placed in series (14 um chip followed by 100 um chip) as showed in the previous sections. Using this method, we can produce multiple emulsion in a wide range of sizes depending on the dimension of the two chip junctions that are coupled together. Dolomite offers a large range of flow focusing chips https://www.dolomite-microfluidics.com/product-category/microfluidic-components/microfluidic-chips/ to target a vast range of PLGA core-shell formation (from 10 to 100 um dry beads).

Incorporating active pharmaceutical ingredients (APIs) within very tiny inner aqueous droplets (around a few micron) can be achievedusing a first small junction chip. However, in this case the throughput can be low due to the extremely small junction size. To overcome this issue, we can generate the primary emulsion (with API) using a traditional sonication method and then flow this emulsion directly through the 100 um chip to form the final monodisperse core-shell beads.

To form the primary emulsion a 2 wt % PLGA/DCM 10 v/v % water mixture is sonicated until a uniform suspension (1-3 um water droplets) is obtained.

A small quantity of fluorescein is added to the water phase to analyse the droplets using fluorescent microscopy. This has the aim of verifying that the water gets encapsulated in the PLGA beads at the end of the process.

After all DCM evaporated the beads look highly monodispersed with the water droplets clearly visible in brightfield (Figure 9). Under fluorescence excitation light water droplets appear bright green (Figure 10).
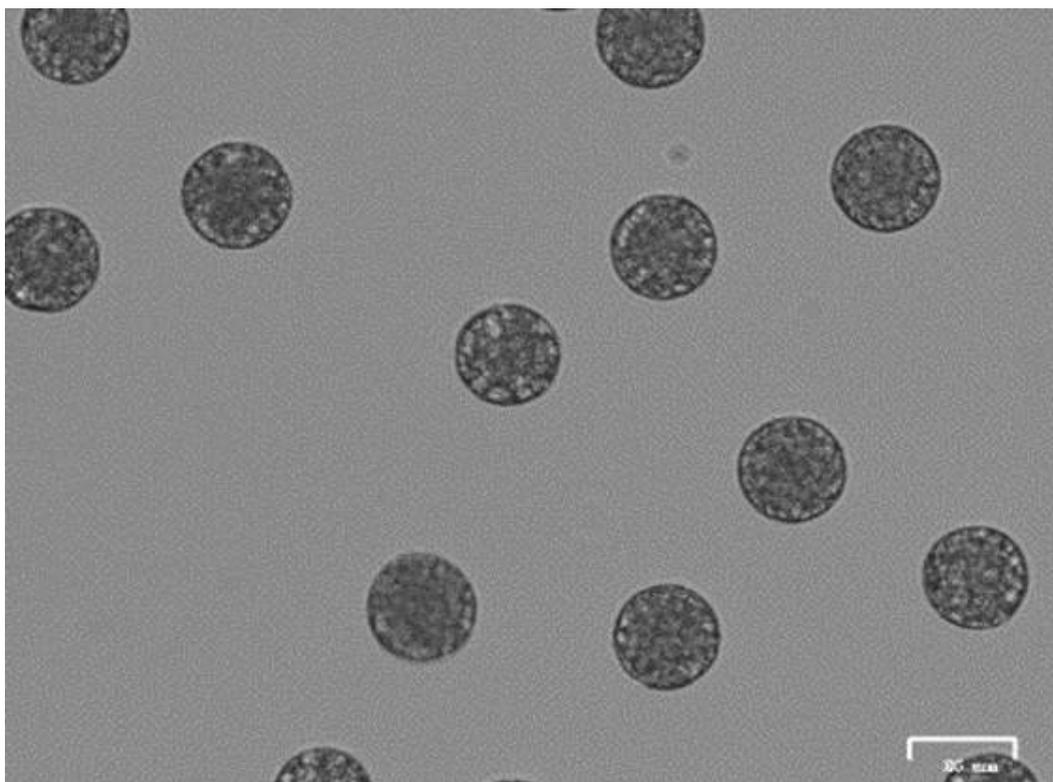

Figure 9 - 25 um PLGA dry beads containing water after DCM evaporation (bright field image). First emulsion = 5 ul/min, Aqua Phase = 50 ul/min
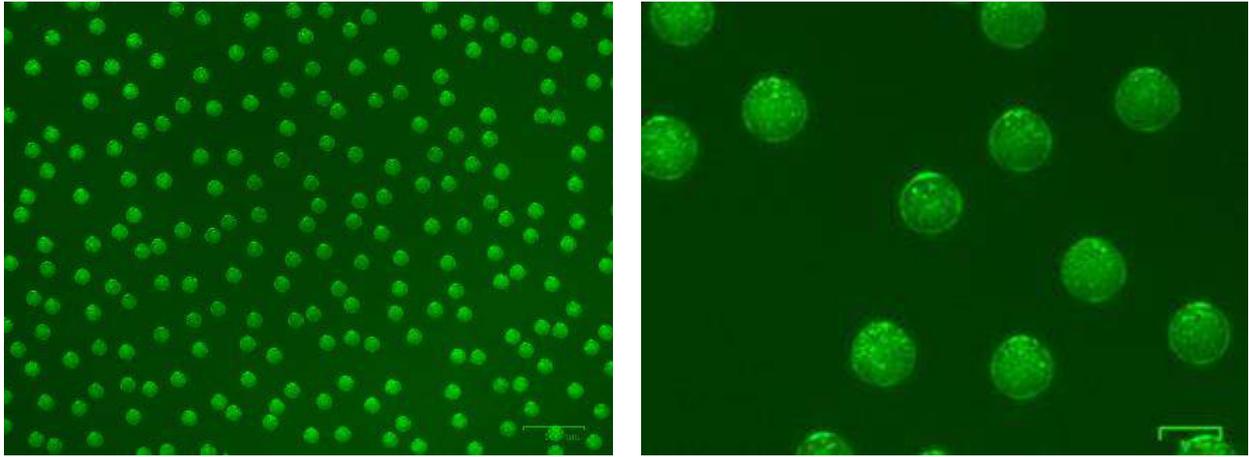
Figure 10 - 25 um PLGA dry beads containing water after DCM evaporation (fluorescent light). First emulsion = 5 ul/min, Aqua Phase = 50 ul/min

9 Scale up your production

The PLGA microparticle fabrication described in this application note is ideal for the rapid iteration of formulations and optimisation of material performance characteristics. Dolomite API encapsulation system can produce up to about 0.5 g/h of dry PLGA core-shell beads depending on flow rates and PLGA concentrations.

If you have used the manufacturing method described in this note to develop a new material formulation that you want to scale up, then you may be interested in our Telos system. This increases the number of junctions per chip to 7 and allows for expansion up to 10 chips per system. This system significantly increases the achievable quantity of material manufactured per hour (up to about 20 g/h dry beads).

To find out more, visit www.dolomite-microfluidics.com.

10 Appendix

10.1 Calibration curve

Calibration curves allow to calculate the actual flow rates of non-standard fluids from the set flow rate of common standard fluids. The calibration chart reported below is used to determine the actual DCM/PLGA flow rates at reference (set flow rate) read by the flow sensor which is set on water as working fluid on FCC.


Calibration Curve - Droplet Phase

$y = 0.903x^{1.542}$
$y = 0.8002x^{1.6441}$
$y = 0.8158x^{1.6282}$
$y = 0.8298x^{1.6388}$
$y = 0.7333x^{1.6257}$

- × DCM
- × 0.50%
- × 1.00%
- × 3.00%
- × 7.50%
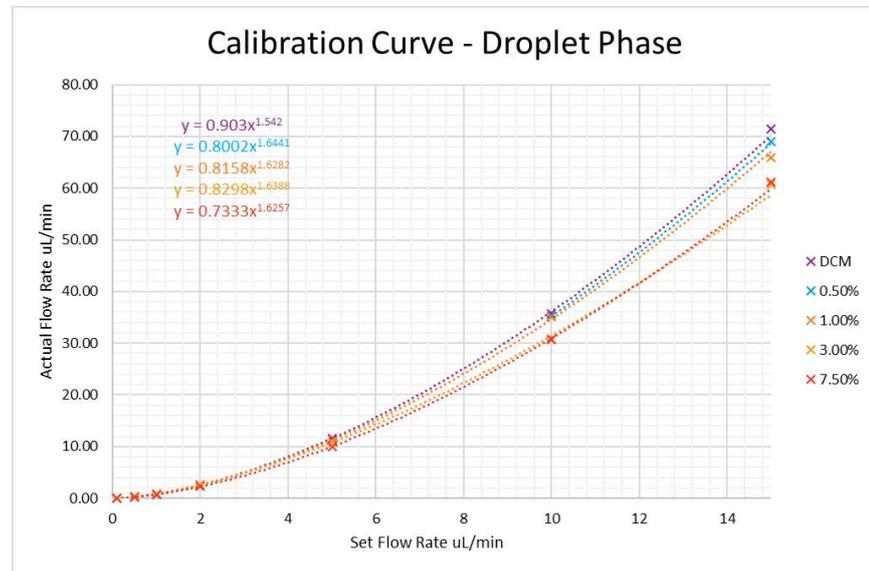
Figure 11 - Calibration curve for various concentrations (up to 7.5%wt) of PLGA/DCM solutions

10.2 System component list

PART NO.	DESCRIPTION	QUANTITY
3200732	API encapsulation system - 20um to 50um PLGA particles - Enhanced Control	1
3200433	3D Flow Focusing Chip - 100µm - Hydrophilic	3
3200734	Upgrade kit for aqueous soluble API encapsulation	1
3200117	Mitos Compressor 6bar	1
2 days On-site Installation and Training included		