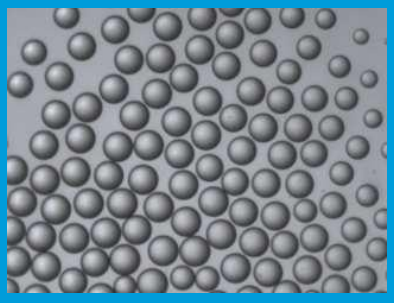
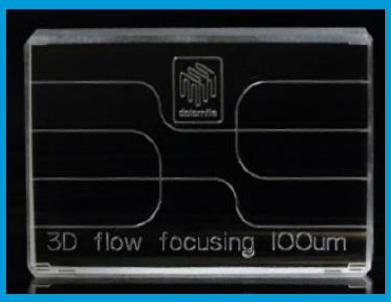
Continuous Microfluidic Synthesis of PLGA Microparticles by Droplet Method

Dolomite's API encapsulation system for PLGA 20 µm to 50 µm particle synthesis





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Summary

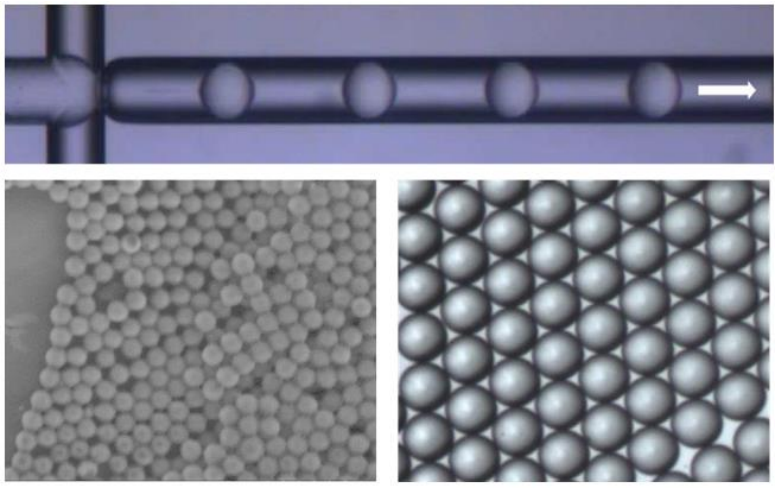
This application note describes methodology for fabrication of highly monodisperse poly (lactic- co-glycolic acid) (PLGA) beads with sizes ranging from 10 to 45 µm using Dolomite's API encapsulation system. The system is based on continuous microfluidic synthesis of PLGA microparticles with the Dolomite's 3D flow focussing droplet chip.

The methodology of bead fabrication described herein relies on dissolution of the polymer in a solvent followed by emulsification using a droplet microfluidic chip. The key advantages of this approach are the high monodispersity and batch-to-batch consistency of the resulting beads. The wide size range achievable on one system without hardware changes is a strong benefit as well.

A production setup is recommended with a priming and production protocol. This is operated over a range of test conditions where relative flow conditions are varied between the droplet fluid and the carrier fluid. The resulting droplet sizes and droplet rates are documented. A physical mechanism is illustrated describing the process of collection, and conversion of the droplet from liquid phase to solid phase.

Key parameters achieved during the tests are:

- Droplet flow rate range: 0.5 – 30 µL/min
- Carrier flow rate range: 10 – 125 µL/min
- Droplet size range: 35 – 101 µm
- Particle size range: 12 – 44 µm (2 µm size particles produced in separate tests)
- Maximum droplet rate achieved: 14 kHz
- Polymer concentration used: 1, 2, 10, & 20 % (w/v)
- Highly monodisperse particles (less than 5% cv)

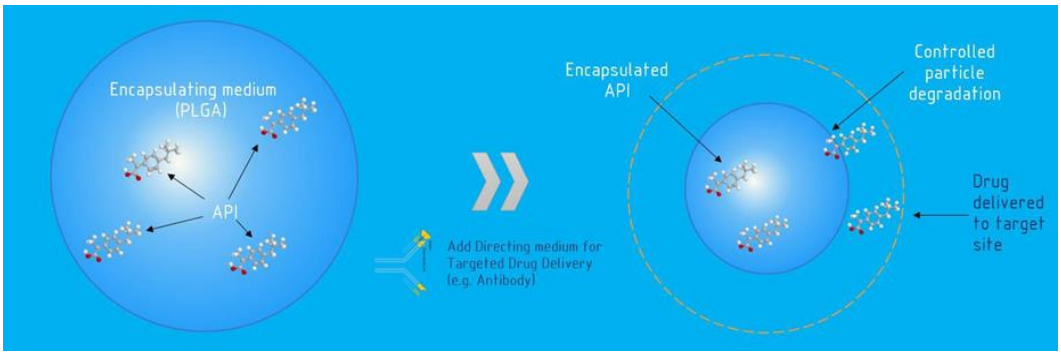


PLGA particles by droplet method.

Introduction

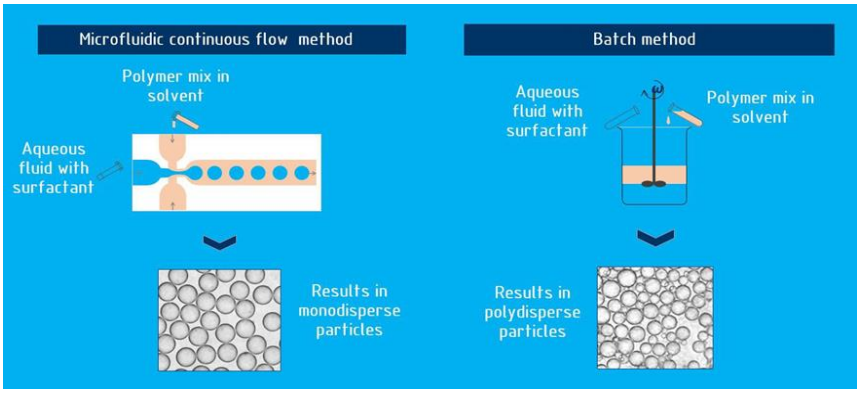
Poly(lactic-co-glycolic acid) or PLGA is a polymer that has broad utility as vehicles for drug delivery and form the basis of several therapies approved by the US Food and Drug Administration, owing to its biodegradability and biocompatibility.

There is a recognized need to for fabrication of highly monodisperse beads for applications such as controlled drug release and targeted drug delivery. In these applications precision control over the bead size distribution is particularly important as it has a significant effect on preferential segregation within the body (Enhanced Permeability and Retention Effect). Further, bead size determines the degradation rate and consequently the rate of drug release. Bead size has a dramatic effect on the surface area to volume ratio and consequently affects the quantity of the functional coating groups. In targeted drug delivery applications, this in turn determines the preference the beads have for navigation to specific site.



API is released constantly over time thanks to the controlled degradation of the PLGA bead.

Conventional emulsion-based methods of manufacturing PLGA particles produce beads with a wide range of diameters (and thus properties) in each batch. There is limited degree of size selection by controlling the shear energy input. The droplet based microfluidic method demonstrated here yield highly monodisperse particles in a single step, thereby increasing the yield of the process.



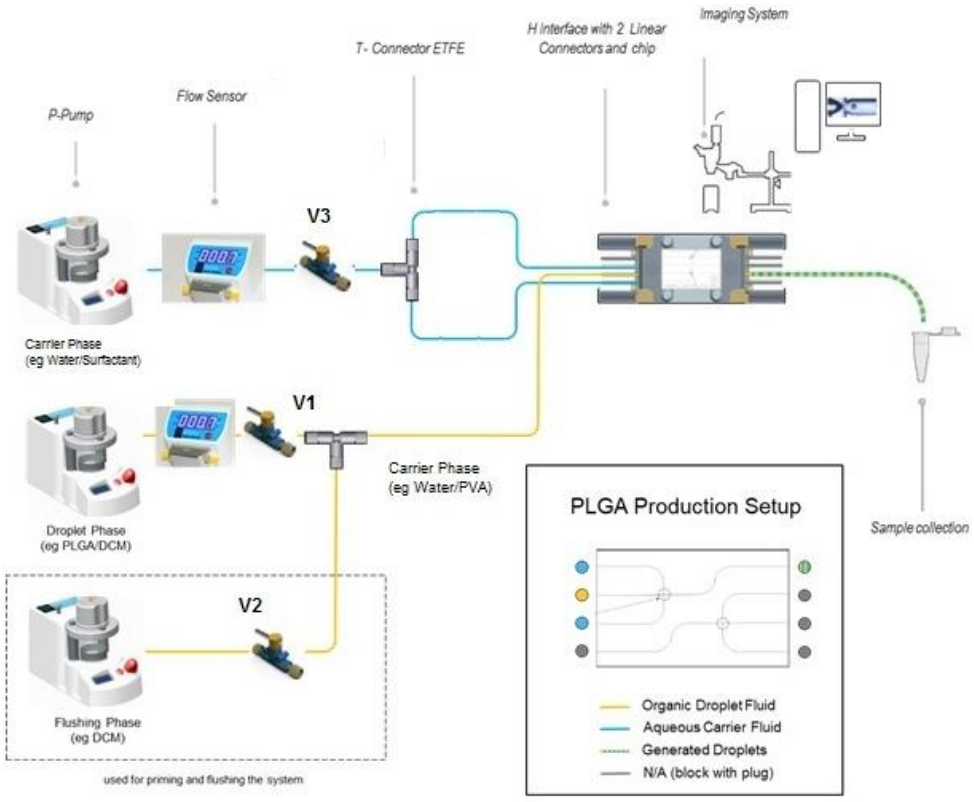
PLGA synthesis. Batch VS Microfluidic method.

Materials & Methods

System Description

PLGA beads production using the microfluidic droplet method is achieved by formation of droplets followed by solvent extraction. Two partially miscible solvents dichloromethane (DCM) and water are used. PLGA is dissolved in DCM and forms the droplets fluid. Aqueous surfactant blend forms the continuous phase fluid. The surfactant adsorbs to the fluid interface between the droplet and carrier, and stabilizes the emulsion.

The system is set-up with 3 Mitos P-Pumps (Part No. 3200016) and in-line Mitos Flow Rate Sensors to monitor the flow rates. A flow rate sensor 30 - 1000 µl/min (Part No. 3200097) is placed on the organic line and the flow rate sensor 1 - 50 µl/min (Part No. 3200098) is placed on the aqueous line. A calibration is carried out to give accurate flow rate readings for the droplet phase (see Appendix A). A helium gas supply up to 10 bar is connected to the P-Pumps. This along with the flow resistance offered by the system prevented outgassing of the DCM - PLGA solution. Outgassing leads to uncontrolled precipitation of polymer in the system which creates flow instabilities (and blockages in some extreme cases).

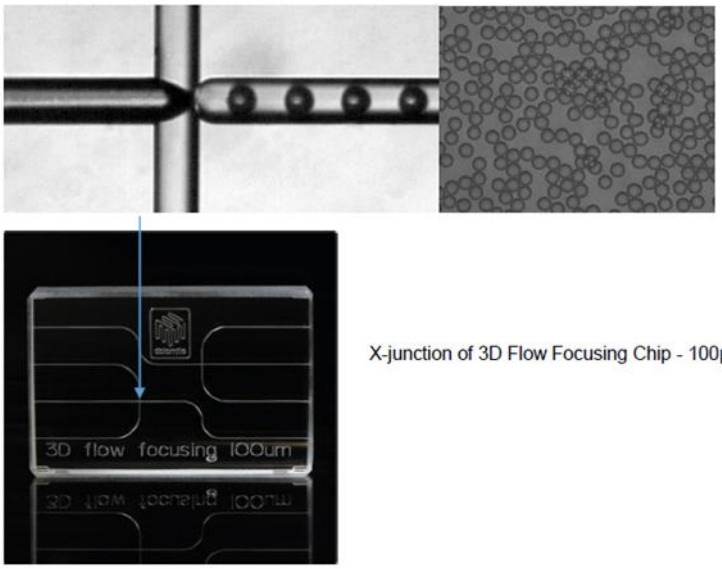


Schematic of the microfluidic setup used to make PLGA beads. The organic flow line sensor is connected to the pump containing the polymer mix to enable flow control mode of operation during production. The priming is done in pressure-control mode.

System for API Encapsulation in PLGA particles 20µm to 50µm diameter.

Fluidic connections are made using FEP tubing of OD 1.6 mm and ID 0.25 mm (Part No. 3200063). 2-way in-line valves (Part No. 3200087) are placed on each fluid line to provide an easy-to-use solution to quickly stop flow streams. To ensure that fluids are equally divided using the T-connectors (Part No. 3000397), the lengths of the tubes on each branch of the two T-connectors have to be the same.

The fluids are delivered from the pumps to the 3D flow focussing Droplet Chip 100µm hydrophilic (Part No. 3200433) which is mounted in a Chip Interface H (Part No. 3000155). Two 4-Way Linear Connectors (Part No. 3000024) help create a leak proof seal between tubing and the chip. Visualization is achieved using a High-Speed Digital Microscope (Part No. 3200531). The 3D flow focussing Droplet Chip is used to create droplets. The 3D flow focussing design minimises fouling of the channel walls after the junction and enable multi-hours operation of the chip without risk of blockage. This is due to the particular ‘pore’ structure on the outlet side of the droplet forming junction which creates a 3D sheath flow in the droplet pinch-off region. This flow pattern is especially useful when handling droplet fluids which are liable to foul the junction surface



X-junction of 3D Flow Focusing Chip - 100µm

3D Pore Chip.

There are three valves on the pump lines. The use of three valves allows priming the system (see Appendix B), and then bringing in the droplet fluid without fluidic interruption on-chip. This also enables the continuous phase flow to be started first before opening the valve to start the droplet flow.

Reagents

The three pumps are loaded with droplet fluid, priming fluid, and carrier fluid.

- Priming fluid: Dichloromethane (DCM) CHROMASOLV® for HPLC, ≥ 99.8% is passed through a 0.2 µm pore filter and used without further modification.
- Droplet fluid preparation: Poly(D,L-lactide-co-glycolide) (PLGA) - ester terminated, lactide : glycolide 75 : 25, Mw 76,000 - 115,000PLGA is dissolved in DCM at room temperature by stirring over the course of an hour. 10 ml volumes of 4 concentrations are prepared at 1, 2, 10, and 20 % (w/v).
- Carrier fluid: Water and 2 % (w/v) Poly(vinyl alcohol) (PVA) surfactant is passed through a 0.2 µm pore filter and used without further modification.

Results & Discussion

PLGA particle formation

After droplet formation, DCM begins to diffuse into the surrounding aqueous phase (DCM has a 2% (v/v) solubility in water at room temperature) thereby depleting the droplets of solvent. The solvent depletion from the droplet increases PLGA concentration until supersaturation is reached and therefore PLGA particles precipitate. This diffusive release of solvent continues as there is sufficient fresh water available close to each droplet. Eventually, as most of the solvent is removed from the droplet solid content remains. At this stage, the droplet is converted to a PLGA bead. The removal of the solvent causes a proportional reduction in volume, and therefore size. Thus, the droplets visibly shrink in size.

To demonstrate the workflow of estimating size changes, a sample is collected using test conditions in the below table.

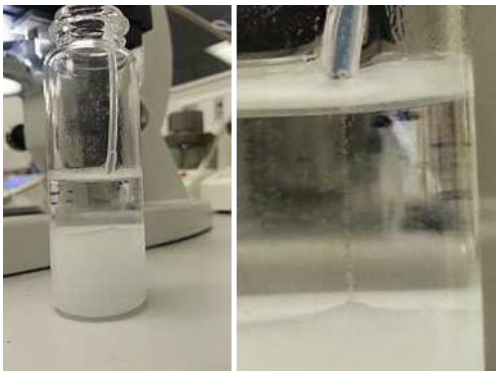
Carrier		Droplet		Junction Image	Droplet Diameter	Droplet Rate
Pressure (mbar)	Flow Rate (µl/min)	Pressure (mbar)	Flow Rate (µl/min)		µm	Hz
270	10	202	1.35		101	50

The size of the droplet is measured by using a pixel analysis software. The reference dimension is taken as the channel width of 100 µm. Videos of droplet production are recorded at a frame rate of 1 kHz for a pixel size of 592 px × 144 px using the high-speed imaging system.

Where the droplet production rate is higher than the video capture rate, a still image is recorded. This is found to sufficient to measure the droplet size. Droplet rate is then estimated by

$$\textit{Droplet rate (Hz) = Droplet phase flow rate (µL/s) / Droplet volume (µL)}$$

The droplets made on chip are collected in a vial. The vial is pre-filled with 100 µL of carrier fluid (Water + 2 % PVA).



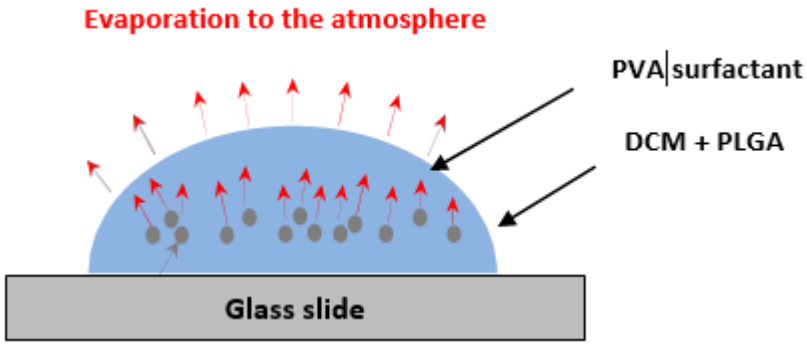
Left: Outlet tubing from chip dips into the collection fluid. Right: Droplets exiting tubing, falling through the aqueous phase, and collecting at the bottom of the vial.

Solvent removal is the diffusive movement of dichloromethane from the droplet, across the droplet/carrier fluid interface, and into the carrier. The dichloromethane is progressively removed from the collected sample, and effused to the atmosphere. Left behind are solidifying PLGA beads suspended in carrier fluid.

Conceptual molecular schematic of solvent removal.

In the presence of abundant aqueous fluid, the solvent continuously diffuses out of the droplet. The remnant hydrophobic polymer forms a microparticle of smaller size than the initial droplet. The surfactant molecules (not illustrated above) self-assemble at the fluid interface between the organic droplet and aqueous carrier - the hydrophobic part of the surfactant molecule intrudes into the droplet while the hydrophilic part extends outwards.

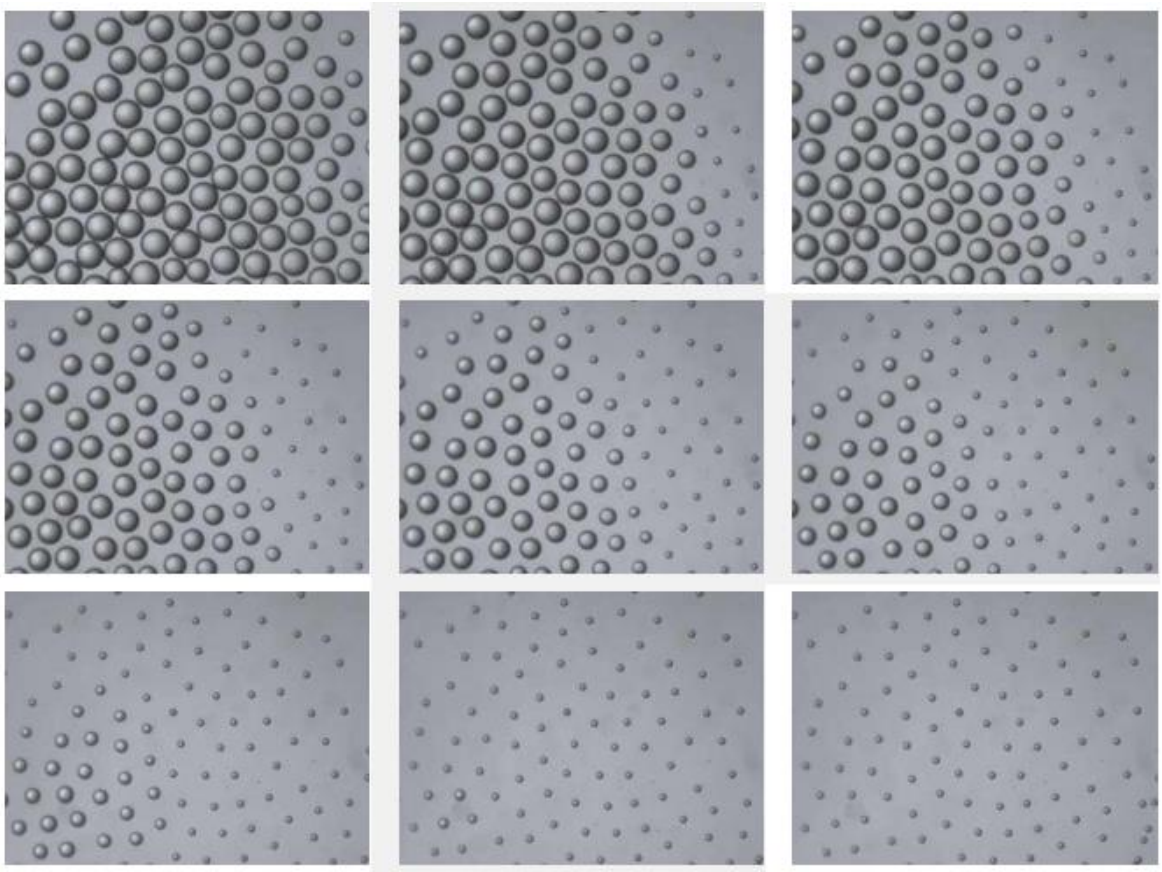
To collect and analyse a small sample, the outlet tubing is removed from the collection vial, and the sample is instead collected on a small glass cover slide. The cover slide is inspected under a microscope, and images are recorded over fixed time intervals.



Arrows indicate transfer route of DCM from the droplets to the water.

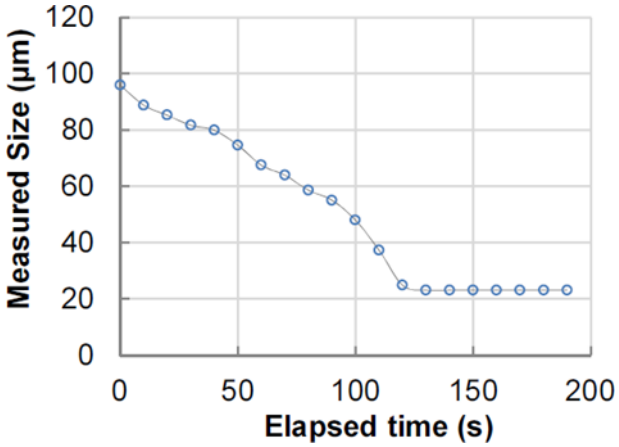
The polymer droplets settle at the bottom of the water droplet on the glass cover slip. The cover slip is uncoated, and hence hydrophilic, the same surface as the droplet chip. There is sufficient exposed surface area for the DCM to transfer from the polymer droplets to the surrounding water, and eventually diffuse out to the atmosphere.

The image sequence below shows the size evolution of the collected sample.



Sequence of images 30 seconds apart. Pixel resolution for size estimation is 1.77 µm/px. The variability of solvent removal is apparent from the differences between droplets. However, being monodisperse, all droplets have identical initial size and final size.

One droplet/particle is selected, and tracked during the solvent removal process. The sizes are recorded. The particles continue to be surrounded by the carrier phase fluid, and hence in a fluid suspension.

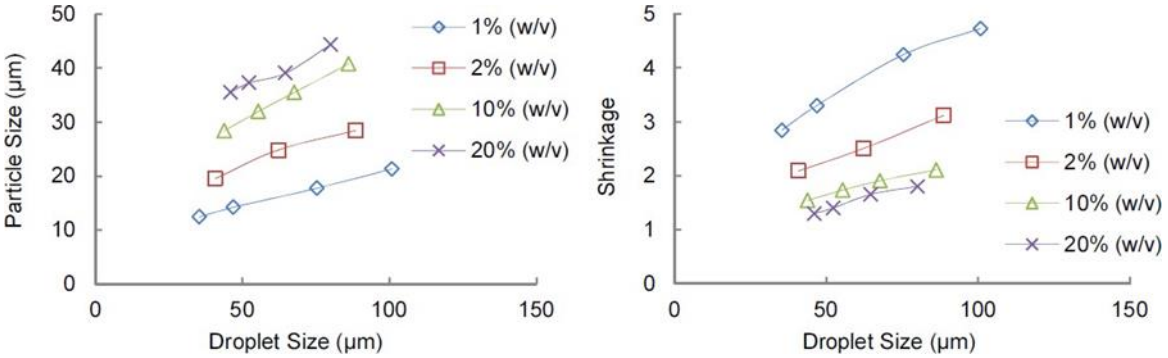


Shrinkage of a tracked droplet.

The shrinkage starts as soon as the droplet is formed on the chip. For this reason, by the time the size is tracked under a microscope, the size has reduced from 101 µm to 95 µm. The graph shows the terminal size of the droplet to be 23 µm.

Shrinkage = (Size of droplet) / (Size of particle)

In the above test case, shrinkage is 101 µm / 23 µm = 4.36. At the terminal size, the droplet is said to have lost most of the DCM content, leaving behind a semi-gel like PLGA micro particle.



Left: Relationship showing the final particles sizes achieved with starting droplet size for various polymer concentrations. Right: Shrinkage versus starting droplet size for various polymer concentrations.

Droplet sizes and bead sizes

The rate of removal of the solvent is strongly tied with the strategy of removal. On a cover slip, there is large exposed surface area for evaporation, and therefore solvent removal progresses to completion in a few minutes. In column method, solvent exchange, the solvent removal may take longer yet be more controlled.

The droplet sizes vary as a function of the flow ratio (flow rate of droplet fluid/flow rate of carrier fluid). Full list of results can be found in Appendix C. The pinch off of the droplets depends on various physical parameters such as flow ratio, interfacial surface tension, and fluid viscosities. The droplets are produced on-chip, and transport off-chip for conversion from liquid phase to solid phase. The surfactant adequately stabilized the emulsion during this conversion phase.

	Droplet size/Bead size (smallest)		Droplet size/Bead size (largest)	
Polymer concentration	Droplet size	Bead size	Droplet size	Bead size
1% (w/v)	35 µm	12 µm	101 µm	23 µm
2% (w/v)	26 µm	16 µm	88 µm	28 µm
10% (w/v)	44 µm	28 µm	86 µm	40 µm
20% (w/v)	46 µm	35 µm	80 µm	44 µm

Droplet production rate

Polymer concentration	Maximum droplet rate
1% (w/v)	14,007 Hz
2% (w/v)	10,996 Hz
10% (w/v)	2,832 Hz
20% (w/v)	471 Hz

Conclusions

Droplet microfluidics has been demonstrated to be a very effective tool for production of PLGA beads with high monodispersity and size homogeneity.

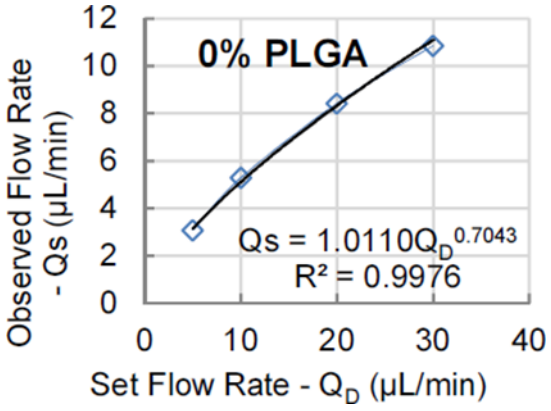
Microfluidics allow seamless control of the size of the beads by varying the flow rates of carrier and droplet phases, as well as changing the concentration of the PLGA in the droplet phase. In this work, bead sizes ranging from 12 µm to 44 µm have been obtained on the same microfluidic system with no change in hardware.

Optical microscopy of samples obtained within this study has demonstrated extremely high bead uniformity, which in turn results in a homogenous degradation and drug release.

Options for scaling-up the production of PLGA beads and increase the throughput are available, notable Dolomite Telos multi-channel platform.

Appendix A: Flow Rate Calibration for Dichloromethane

A calibrated positive displacement pump is used to pump the DCM solution at reference flow rate Q_D. The observed and uncalibrated flow rate read by the flow sensor (set to water as the working fluid) is Q_S. Plotting the two results in the calibration curve.



Calibration curve for 0% PLGA solution.

Inverting the trendline equation gives:

$$Q_D = (Q_S/1.011)^{(1/0.7043)}$$

During tests, the P-Pumps are run in flow control mode, and the calibration fluid set to water. The actual flow rates are determined by the calibration done *a priori*.

Flow rate on P-Pump via FCC (Q_S)	Actual flow rate through chip (Q_D)
1.00 µL/min	0.98 µL/min
5.00 µL/min	9.67 µL/min
10.00 µL/min	25.88 µL/min

Appendix B: Priming Method

We start the priming with valve V1 closed and valve V2 open. In pressure control mode, DCM droplets are established in the carrier fluid. This is a safe start considering that a backflow, jetting, or chaotic flow regime do not cause any irreversible effects (polymer would precipitate and create blockages). The objective is also to purge the fluid pathways of gases, and to condition the chip surface with the surfactant. This is a check to ensure chemical compatibility with all wetted parts in the system. It is advisable to do the priming at reasonably high flow pressure (2-3 bar).

It should be kept in mind that valves should be closed only when pumps are in pressure control mode. When priming fluid droplet production is well established, the valves may be switched so that priming valve V1 is open, and droplet valve V2 is closed. The pump with the droplet fluid is kept at the same pressure as the priming pump. This valve switch will cause the droplet fluid to arrive at the chip. There will be very little visible change when observing droplets via the imaging system. After a few minutes, the polymer mix arrives at the chip, and starts producing polymer particles.

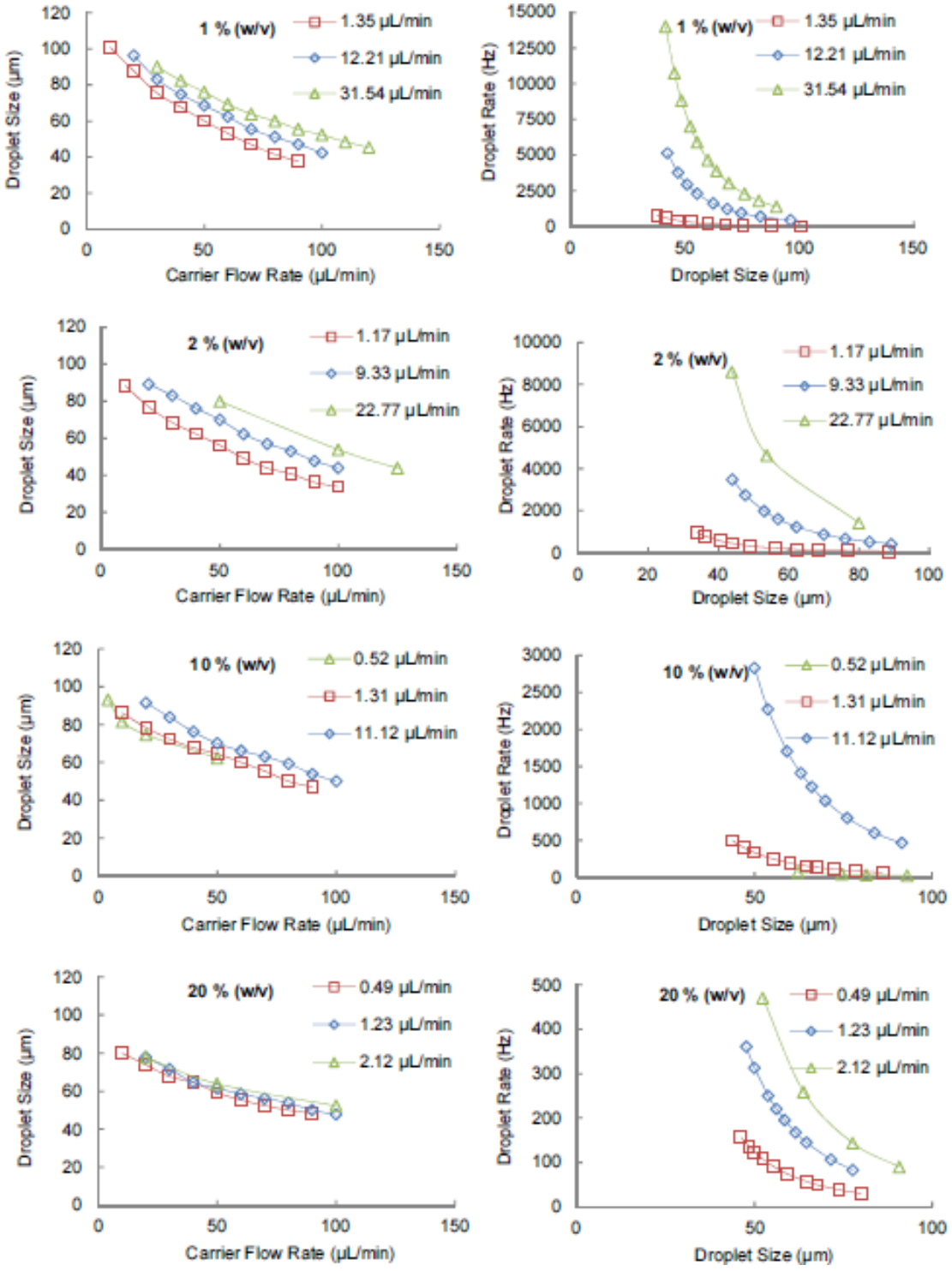
After a few minutes more, both pumps are then switched to flow control mode.

Shutdown at the end of the production run follows the reverse routine followed for priming. The pumps are switched to pressure-control mode. The valves are switched to change the flow from droplet to priming fluid. The system is allowed to cleanse out for about 10-15 minutes.

- When operating in pressure control mode, the temperature of the fluid is important in maintaining constant flow rate. For long duration running, it may be beneficial to use hotplates to control the temperature of the input liquids, as changes in temperature lead to a change in viscosity causing a drift in flow rate.
- Flow control mode is helpful for long duration running in making small adjustments to the pressure to maintain constant flow rate. However, significant blockages in the system cannot be compensated using this method.

Appendix C: Droplet Diameters and Rates

The following charts show droplet size and rates for many different test conditions. Each test condition differs in the set flow rate on the two fluid lines.



Left: Droplet size variation with carrier flow for 3 droplet flow rates.

Right: Size and droplet rate dependence for the same for 3 droplet flow rates.

Appendix D: System Component List

Part No.	Part Description	#
3200732	API encapsulation system - 20 µm to 50 µm PLGA particles - Enhanced Control The system includes:	-
	Mitos P-Pumps	3
	Sensor Displays	3
	Flow Rate Sensors	3
	High-Speed Digital Microscope	1
	Valves, Chip Interfaces, Fittings and Tubing	-
	Mitos Compressor 6bar	1
3200433	3D Flow Focusing Chip - 100µm - Hydrophilic	3
	Installation and Training	-