Production of Highly Monodisperse Pectin Particles

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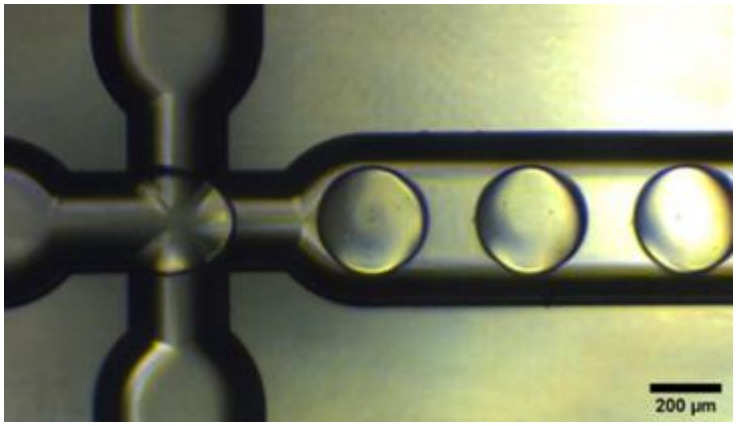

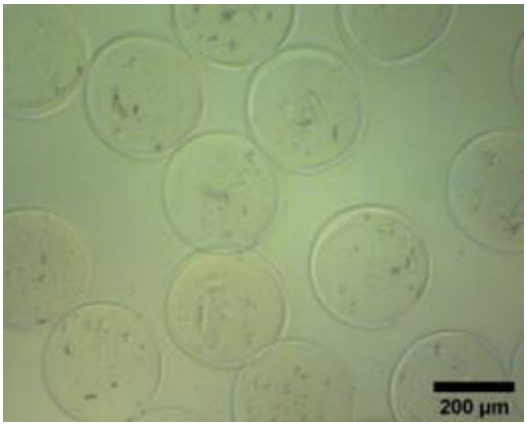

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1 Summary

In this Application Note, we demonstrate the use of the Dolomite Large Droplet System for the formation of biocompatible highly monodisperse micron particles of Pectin gel. Pectin droplets are initially produced using a standard Dolomite chip and stabilized using FluoSurf, a Dolomite emulsion stabiliser for aqueous droplets in fluorinated oil. Pectin droplets are collected into a gelation bath for curing and final washing. Different flow conditions are tested by altering the dispersed and continuous phase flow rates to achieve different droplet and bead sizes.

2 Introduction

Controlled drug delivery remains a research focus for public health to enhance patient compliance, drug efficiency and reduce the side effects of drugs. Pectin, an edible plant polysaccharide, has been shown to be useful for the construction of delivery systems for specific pigments, flavour, fragrances, nutrition supplements, microorganisms, and active pharmaceutical ingredients; for example, Pectin derivatives carrying primary amine groups are more mucoadhesive and have shown potential in nasal drug delivery and other mucosal drug delivery. Pectin derivatives with highly esterified galacturonic acid residues are more hydrophobic and able to sustain the release of incorporated fragrances for a prolonged duration. Less esterified pectin derivatives can penetrate deeper into the skin and may be useful in aromatherapy formulations pigments [1].

Several methods have been reported for producing pectin hydrogel microspheres, such as the capillary flow-based system, dripping, and the electrostatic generation system. Microfluidics offers economic feasibility, precise control of volume, efficient use of reagents, and high monodispersity. These capabilities have made microfluidics an attractive platform for studying biochemical reactions, tissue engineering, drug delivery, control of release and the encapsulation of nanocomposites. Microfluidic approaches for Pectin bead production offer many advantages over conventional batch method of production. Firstly, the shape of hydrogels in drug delivery systems can influence the release kinetics of the drug. In general, a spherical shape results in release rate uniformity, reproducible results and allows for uniform encapsulation in a single microsphere. Secondly, monodisperse cell-laden microspheres offer suitable microenvironments with spatially homogeneous distributions to observe cell-cell and cell-matrix interactions. Cell-cell distances and structural arrangements influence cellular properties and function. Thirdly, the spherical shape provides an effective delivery system as a payload for nutrients, cells, proteins, and inorganic or organic materials. Lastly, hydrogel microspheres, as a confined space system, allow for real-time observation of fast reactions in microenvironments [2].

Dolomite Microfluidics offers a microfluidic platform to enable users to produce Pectin beads with the desired size for their applications. Reusable glass chips enable the production of reproducible beads and allow real-time control to generate monodispersed droplets. Each droplet undergoes identical process conditions, preventing fusion and product waste.

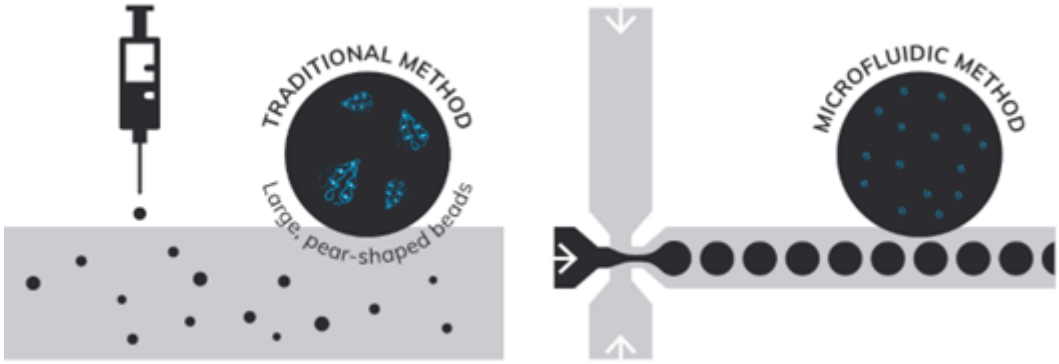


Figure 1 Dolomite's microfluidic methods of emulsification offer better control of droplet formation, as compared to traditional batch methods, which rely on chaotic emulsification processes such as stirring, ultrasonication or syringe injection.

Pectin beads are crosslinked using a similar microfluidic method employed with another biocompatible hydrogel material such as alginate (https://www.dolomite-microfluidics.com/applications/alginate-particles/).

The aim of the following work is to 1) produce different monodispersed Pectin droplets via microfluidics 2) crosslink Pectin droplets to produce monodispersed beads 3) identify parameters for high throughput.

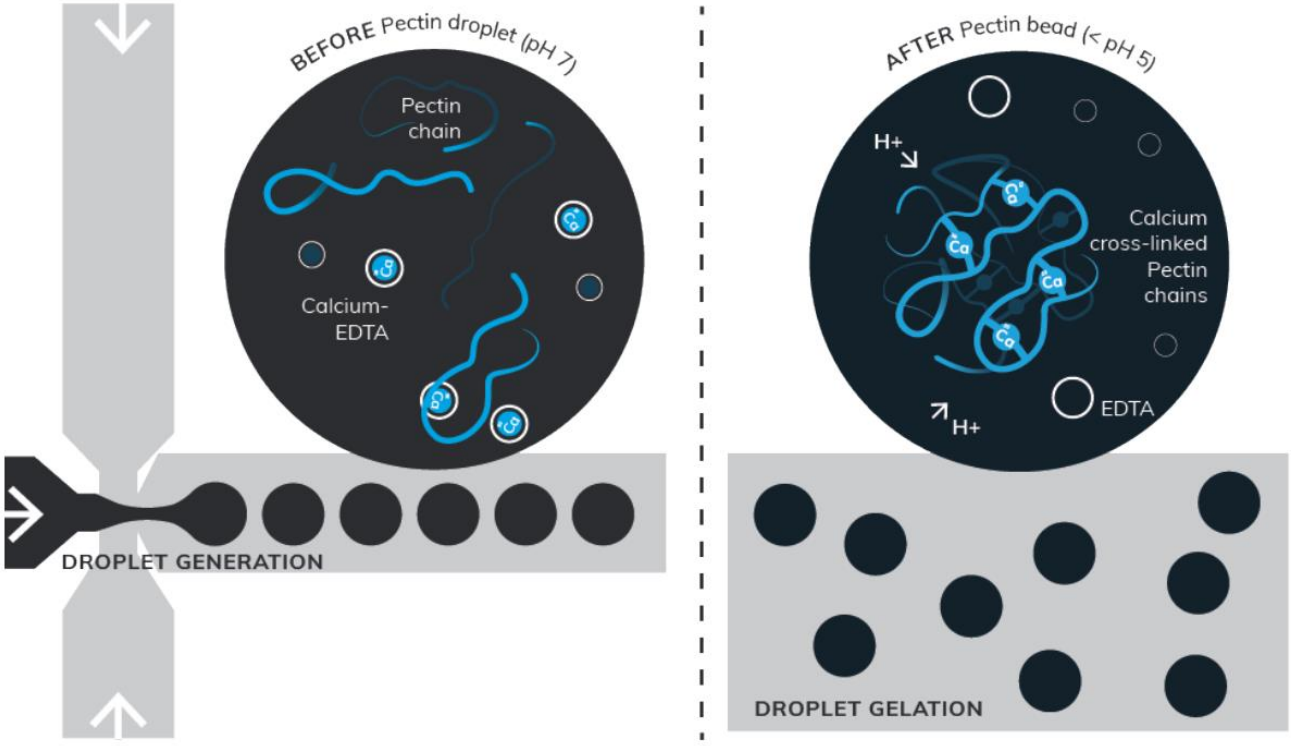
3 Methods

3.1 Materials

3 % (w/w) polygalacturonic acid sodium salt PGA or Pectin (from Sigma UK CAS 9049-37-0) is dissolved in water after 3 h stirring at 80 °C. The solution is cooled at room temperature and then mixed to 0.2 M Ca-EDTA (from Sigma UK CAS 62-33-9) to form a final 1.5 % (w/w) PGA 0.1 M Ca-EDTA solution which is used as the droplet phase. For the continuous phase, FluoSurf in HFE- 7500 (Dolomite Microfluidics, U.K.) is used. The collection phase comprised 0.1 % (v/v) acetic acid (from Sigma UK CAS 64-19-7) added to the continuous phase. Fluoro-Stop (Dolomite Microfluidics, U.K) is used to break the emulsion post pectin crosslinking. Water or Phosphate buffer saline (PBS) is used to suspend the final beads.

3.2 Forming Pectin Beads

In this section, we present the Dolomite microfluidic method which is employed to produce Pectin particles. PGA and Ca-EDTA droplets are first generated in a Large Dolomite Fluorophilic Droplet Chip using FluoSurf as the continuous phase. Droplets are then collected in a gelling bath where the crosslinking mechanism takes place (Figure 2). At low pH (acetic acid gelling solution) H^+ ions diffuse into the droplets, Calcium-EDTA complex dissociates and releases Ca^{2+} ions that crosslink Pectin chains giving a Sodium-EDTA complex.



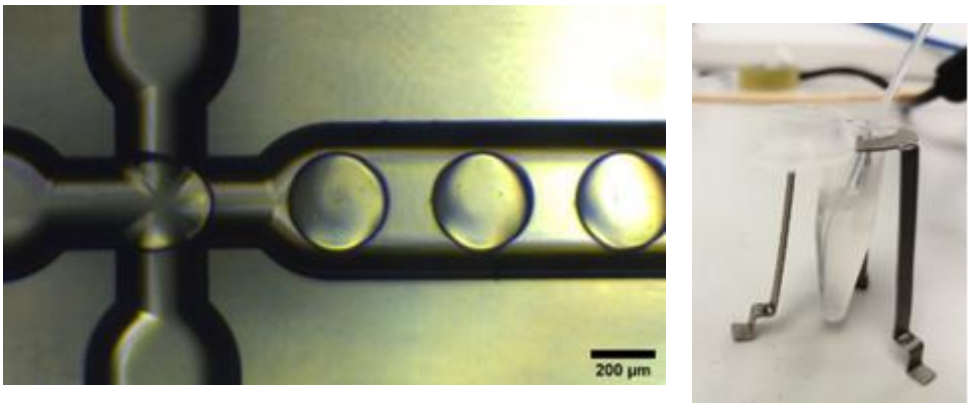
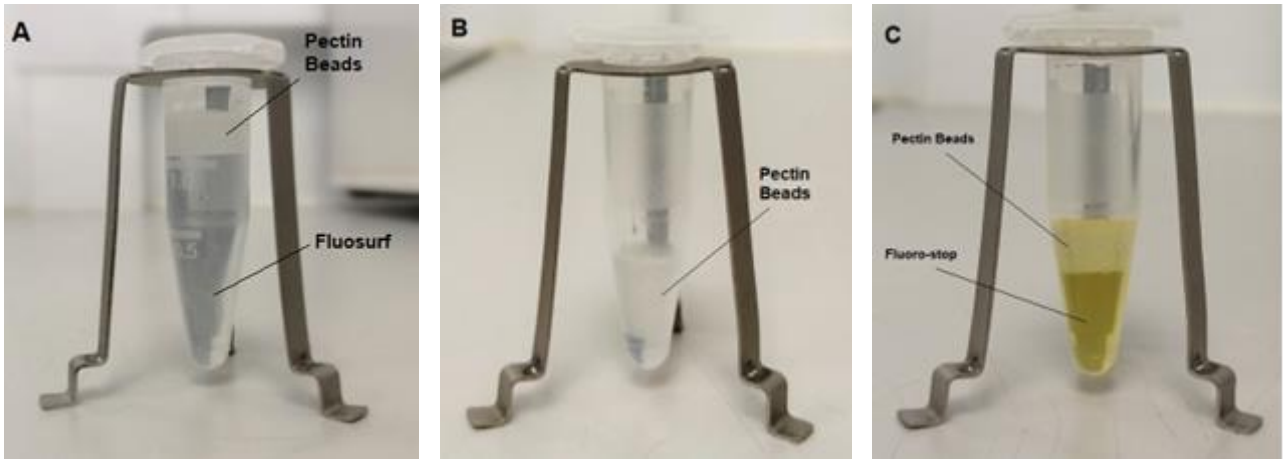


Figure 2 Schematic of Pectin production and crosslinking mechanism using 275 µm Large Dolomite Fluorophilic Chip.

Dolomite Microfluidics' approach is substantially different from the other two main microfluidic methods reported in literature by Kim, 2016 [2] and Ogończyk, 2011 [3] where the risk of chip blockage is very high. These authors demonstrated the formation of PGA beads using mineral and rapeseed oils, respectively with Ca2+ ions in solution, with the continuous phase and crosslinking mechanism occurring via diffusion as soon as the droplets were produced in the chip outlet channel. The method we propose in this application note separates the generation of PGA droplets, which occurs in the chip, from the gelling mechanism, which occurs in the gelling bath after droplet collection. In this way we minimize the risk of chip fouling.

PGA and Ca-EDTA droplets are collected in a desired volume of FluoSurf continuous phase with 0.1 % (v/v) acetic acid. The experiment runs until we reach a total continuous phase dilution of 0.05 % (v/v) acetic, which means that an equal volume of pure FluoSurf is collected in the gelling bath to reach a total volume of continuous phase; twice the original. Once the droplets are collected, 2 minutes of crosslinking time is allowed under gentle stirring.

After collection, the solution is kept stagnant for a few minutes to allow the Pectin beads and FluoSurf to separate by their difference in density: Pectin beads float to the top of the vial and FluoSurf accumulates at the bottom (Figure 3A). FluoSurf is pipetted away and the gelled Pectin beads are left in the vial (Figure 3B). An equal amount of Fluoro-Stop is added; the vial is agitated manually to perfectly mix the beads and Fluoro-stop and to break the emulsion (Figure 3C). Water or phosphate buffer saline (PBS) is added to re-suspend the final beads in the aqueous medium: Pectin beads suspended in water float at the top of the vial and Fluoro-Stop accumulates at the bottom (Figure 3D). Finally, Fluoro-Stop is pipetted away and the Pectin beads are left suspended in the aqueous medium (Figure 3E). The entire process is repeated to remove any trace of FluoSurf and the beads are imaged under the microscope (Figure 4).



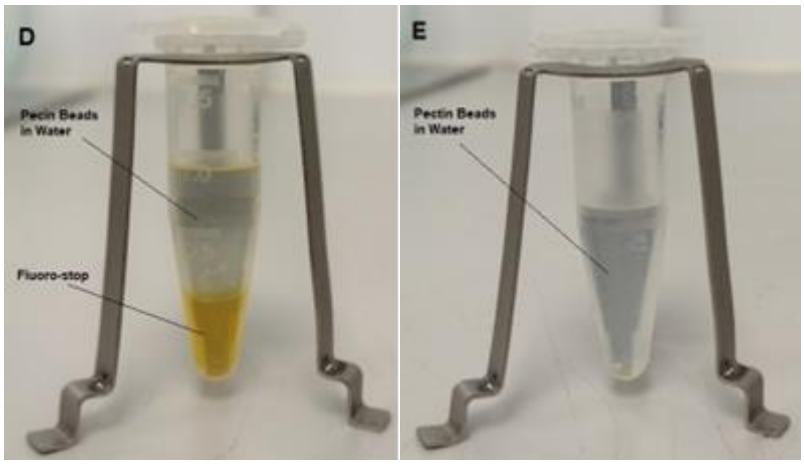
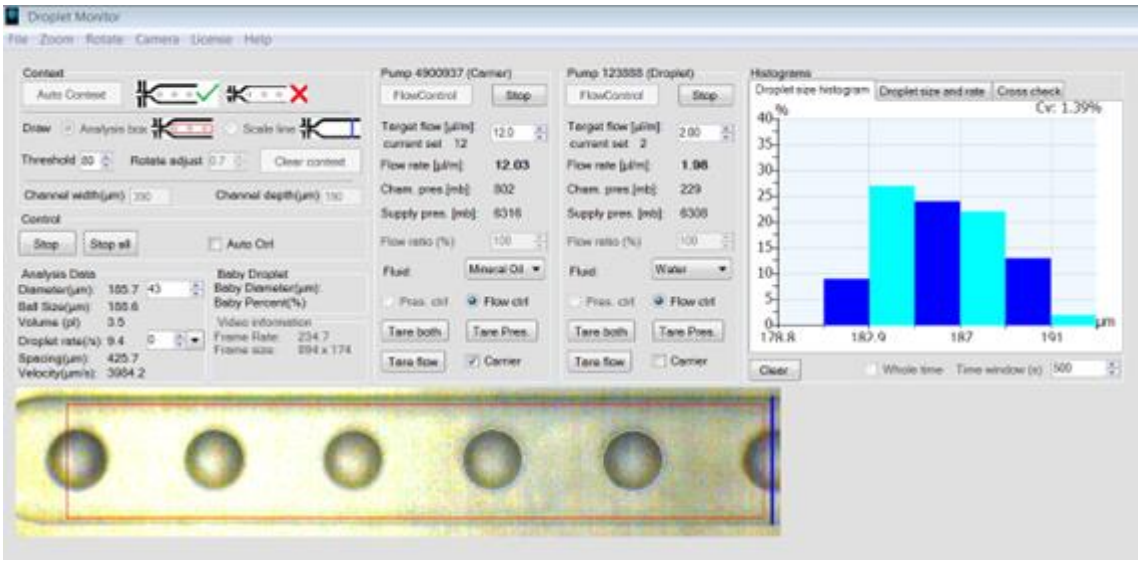


Figure 3 Pectin beads separation and washing.

The crosslinking mechanism of Pectin beads does not significantly change the size and monodispersity of the original droplets produced. In fact, the same droplet diameter and coefficient of variations (CV) are measured optically before and after curing. This is confirmed by comparing the size of the droplets as soon as these are produced in the chip (in real time) with the same droplets collected on a glass slide after the previous procedure (Figure 4). This comparison is performed using the proprietary Dolomite Droplet Monitor Software[1] for the measurement of the droplets in the chip, and by means of the commercially available ImageJ software for the measurement of the droplets collected on the glass slide.

Since the crosslink mechanism does not affect the size of the droplets produced, the analysis of the effect of flow rates on droplet size is carried out using the Droplet Monitor Software[1], simply measuring the droplet dimensions within the chip in real time.

Droplet Monitor Software for real time droplet size measurement before curing (Mean size = 186 µm, CV = 1.39 %)



[1] Contact Dolomite to request further information about the Droplet Monitor Software (https://www.dolomite-microfluidics.com/contact/contact-us/)

Droplets collected on a glass slide and measured via ImageJ software after curing (Mean size = 188 µm, CV = 1.85 %)

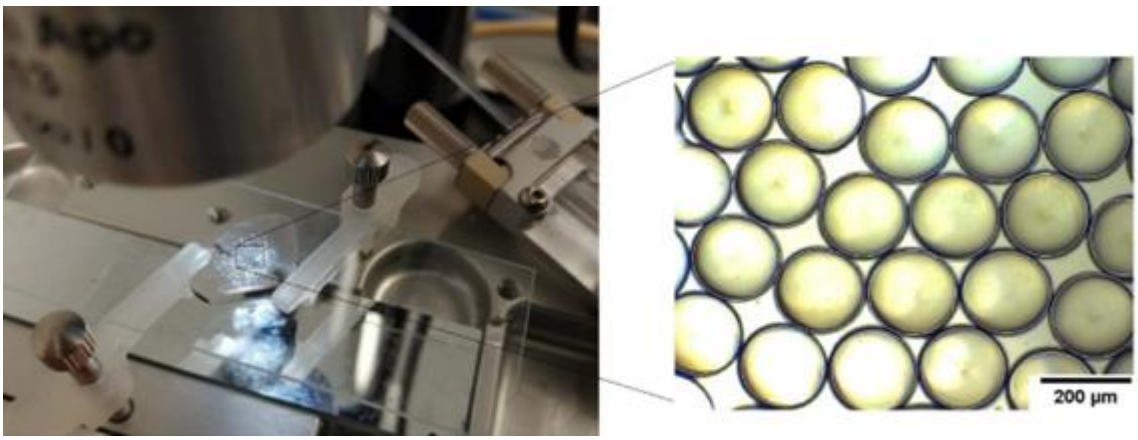


Figure 4 Highly monodisperse Pectin beads measured via Droplet Monitor Software and ImageJ software. The two optical methods of particle measurement show very similar Mean size and CV before, and after, particle curing.

3.3 Dolomite Large Droplet System

The fluids are delivered using a three Pressure Pump system connected to a 10 bar gas supply (compressor) and a droplet microfluidic chip where Pectin beads are formed. Each pump works in combination with a Sensor Display and a Flow Rate Sensor (30-1000 µl/min flow rate range). The first pump at the top of the figure (blue line) delivers the continuous phase FluoSurf, and the second Pump below (yellow line) delivers the Pectin and Ca-EDTA droplet phase. A third Pump is also required for washing the system. IPA or acetone are used to quickly wash the system in between experiments. Pectin droplets produced in the chips are collected into a final gelling bath.

All the fluidic connections between the elements in the figure are made using FEP OD 1.6 mm ID 0.25 mm tubing and specific Dolomite microfluidic fittings and connectors. 2-way in-line valves are placed on each fluid line to provide an easy-to-use solution to quickly stop flow streams. To ensure that fluids are equally split using the T-connectors, the lengths of the tubes on each branch of the two T-connectors must be the same.

The fluids are delivered from the pumps to a droplet microfluidic chip of various size depending on the pectin bead we want to produce. The chips are assembled with the H-Interface and two Linear Connectors 4-way.

Visualization is achieved using a High-Speed Digital Microscope.

Pumps and microscope are controlled remotely via the dedicated Dolomite Flow Control Centre Software (FCC).

See Appendix for a list of all components and spare consumables included in the Dolomite Large Droplet System.

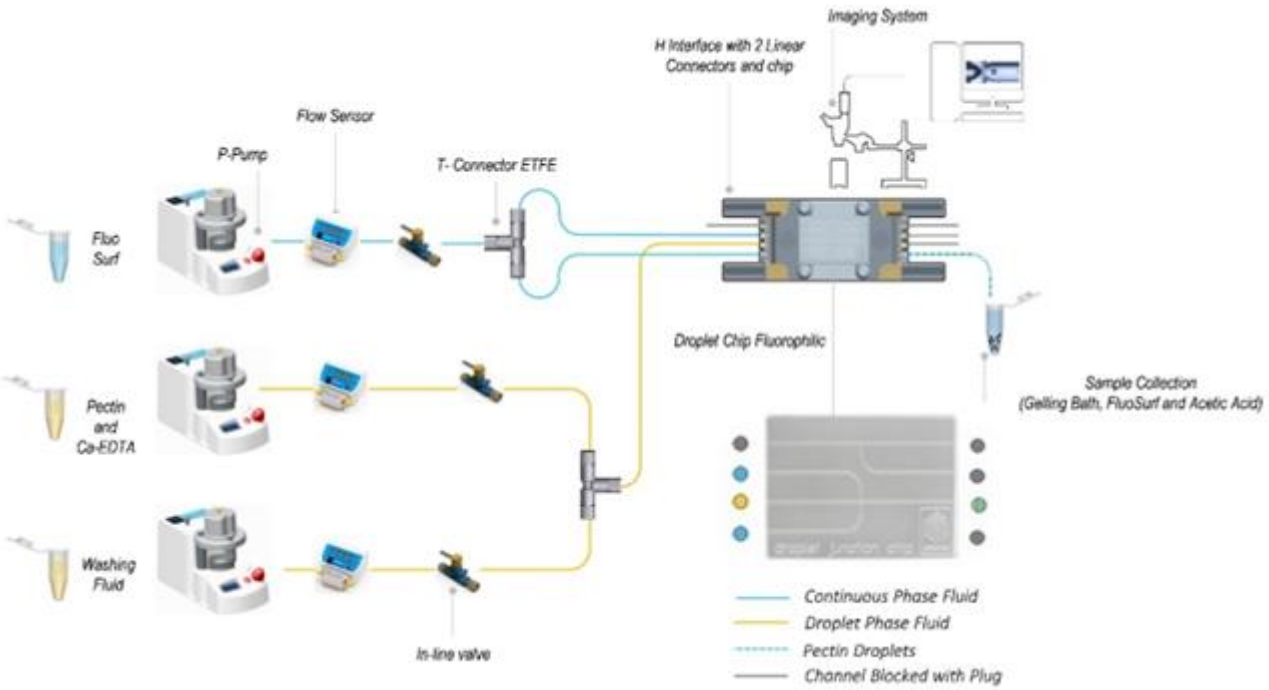


Figure 5 Schematic of the Dolomite Large Droplet System to produce Pectin beads in FluoSurf.

Figure 6 Dolomite Large Droplet System for Pectin bead production.

All fluids used (FluoSurf, Pectin Ca-EDTA, water and IPA or acetone) are pre-filtered using a 0.45 µm syringe filter (MF-Millipore Sigma-Aldrich) and placed in 20 ml scintillation vials prior to being loaded onto the system. The system is primed by running FluoSurf and Ca-EDTA fluids at 1 bar until liquid appears at the linear connector (note: the tubing is not yet connected to the chip; FCC operates in pressure mode). Once this occurs (within seconds), the valves are closed and pumps stopped. The connector is then attached to the chip via the H-interface. The continuous phase is started first, then, the dispersed phase flow according to the flow rates showed in the charts in Section 4. During this step, FCC operates in flow mode.

Beads are collected in the final gelled bath, resuspended in water, and analysed as described in Section 3.2.

In between experiments, the system is washed with IPA or Acetone using the third pump. The washing fluid is flowed through the chip at 1 bar for a couple of minutes. During the washing step, both valves on the Pectin Ca-EDTA and FluoSurf lines are closed to avoid backflow. At the end, the washing fluid vial is removed from the pump and the droplet phase line is dried flowing air at 1 bar.

4 Results and Conclusions

In this section we present the results obtained with three different Dolomite droplet chips:

- Large Droplet Junction Chip 100 µm (Fluorophilic)
- Large Droplet Junction Chip 190 µm (Fluorophilic)
- Large Droplet Junction Chip 275 µm (Fluorophilic)

Pectin bead production is optimized in each chip geometry. Flow rates of both continuous and droplet phases are adjusted to maximize the throughput of droplet formation for a given droplet dimension. The maximum droplet throughput is considered the one obtained increasing both continuous and droplet phase before a jetting regime is achieved (Figure 7).

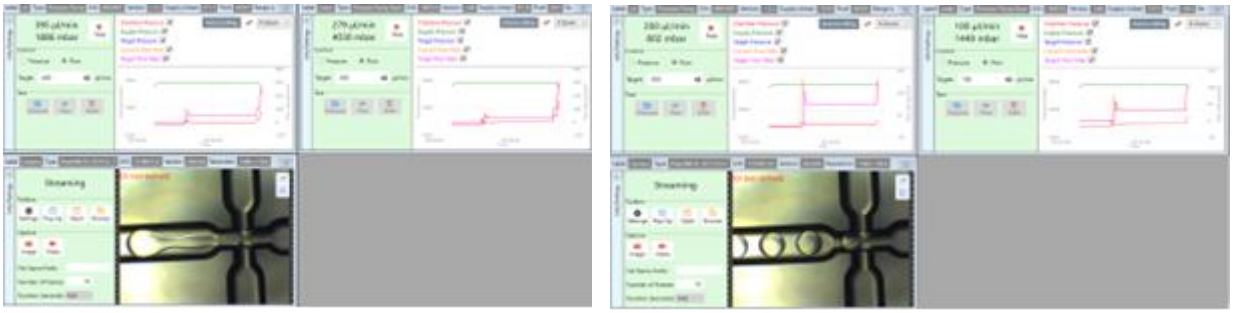


Figure 7 Example of jetting (left) and dripping (right) regimes achieved in the 275 µm chip. FCC control tabs.

The charts below respectively show the droplet size at different droplet flow rates (Figure 8), and the droplet size at different continuous to droplet flow rate ratios (Figure 9). The different Pectin bead sizes obtained in the various chip geometries before jetting are also presented in Figure 10.

Note, the droplet flow rates set on the FCC are calculated using the calibration curve reported in the Appendix. The continuous phase does not require calibration as FluoSurf is an HFE oil based, and is one of the standard fluids implemented within the FCC for real time flow rate measurement[2].

The results demonstrate that the 100 µm, 190 µm and 275 µm chip set can cover the 65 - 305 µm size range with a maximum throughput varying from 52 µl/min (65 µm) to 172 µl/min (305 µm). Smaller or larger Pectin beads can be produced using other chips available in Dolomite[3]. Higher production rates, up to 70 times higher than the previous flow rates, can be achieved using the Dolomite Telos System https://www.dolomite-microfluidics.com/microfluidic-systems/telos-high-throughput/. This would allow production of monodisperse Pectin of several litres per minute.

2 Contact Dolomite for more information on how to build a calibration curve

(https://www.dolomite-microfluidics.com/contact/contact-us/)

3 Contact Dolomite for more information on various chips available for your applications

(https://www.dolomite-microfluidics.com/contact/contact-us/)

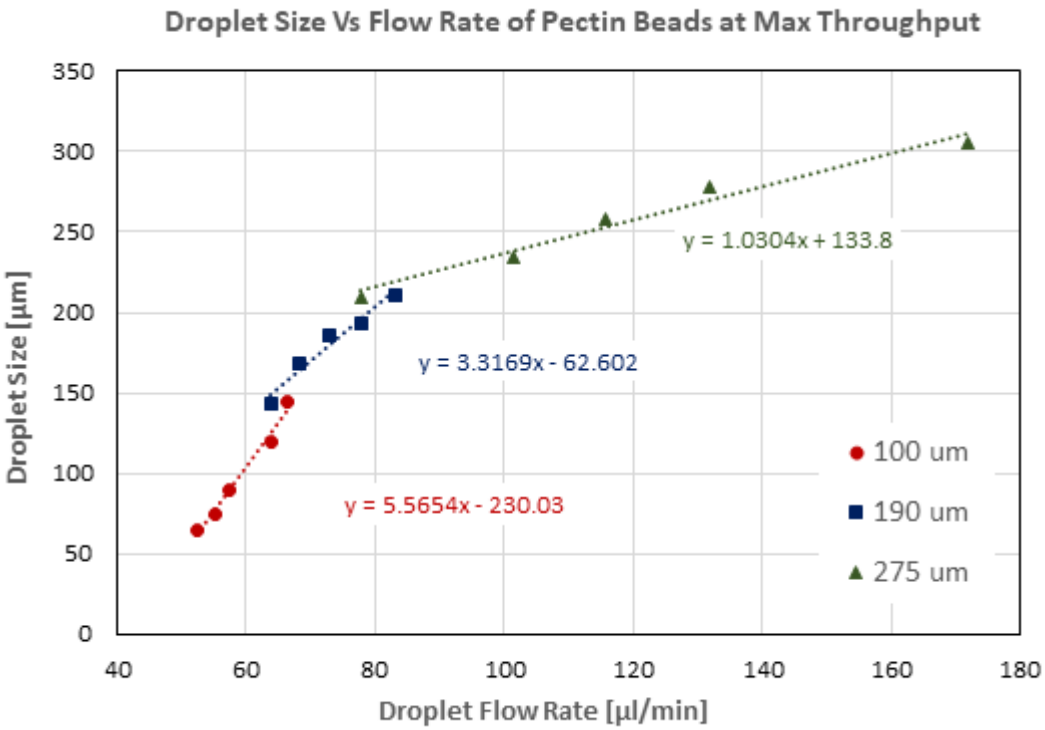


Figure 8 Pectin bead size as a function of droplet flow rate before jetting.

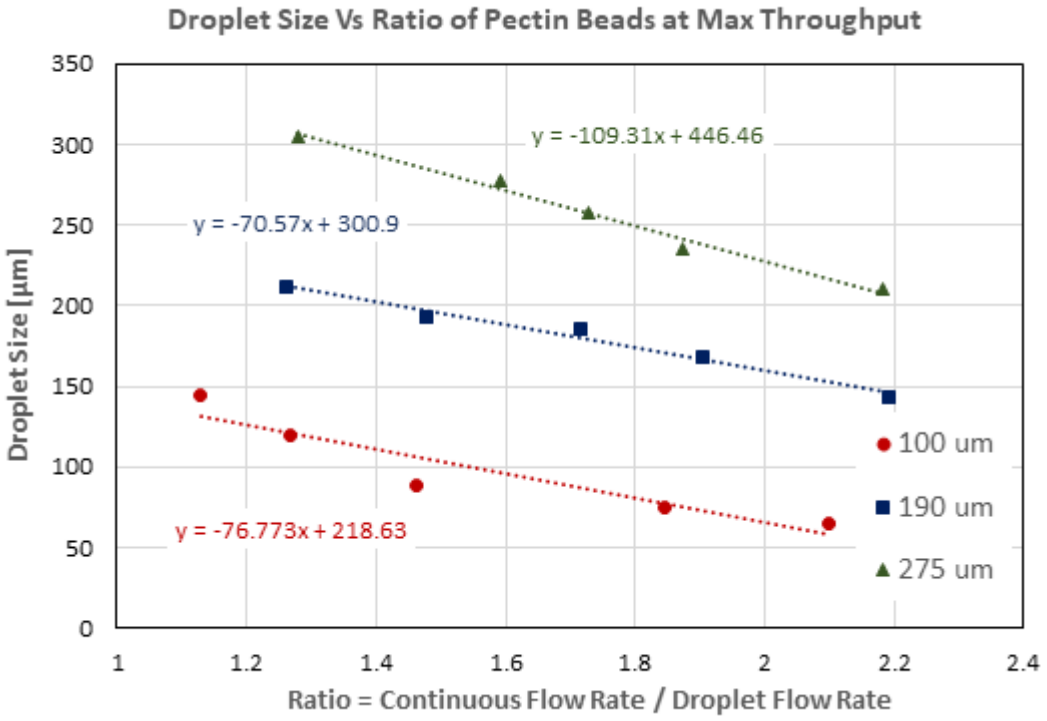


Figure 9 Pectin bead size as a function of flow rate ratio before jetting

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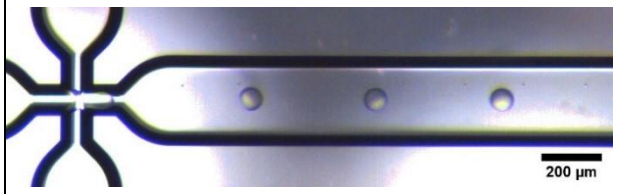
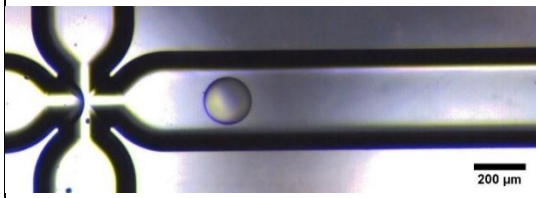
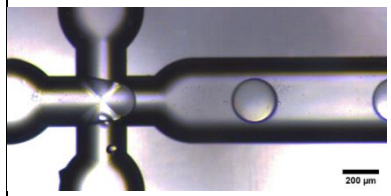
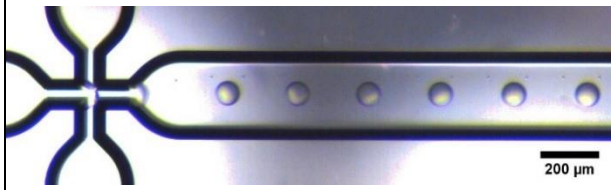
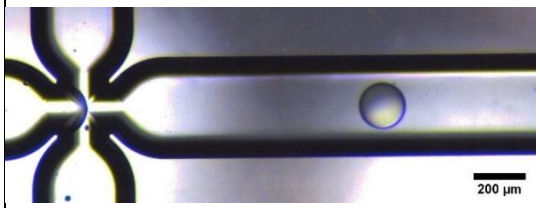
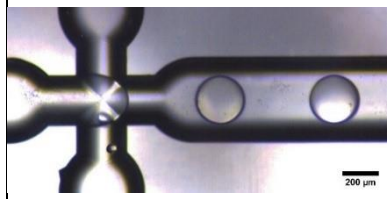
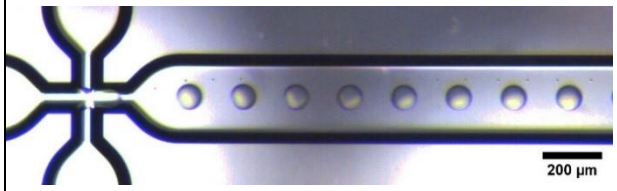
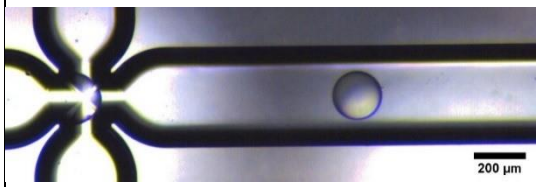
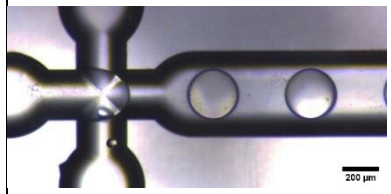
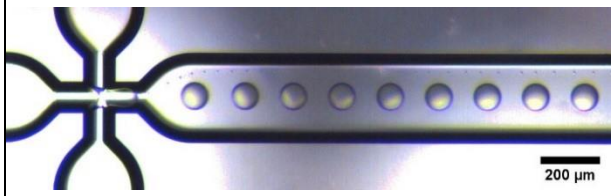
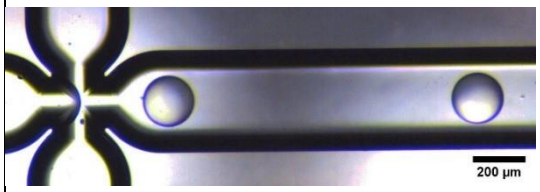
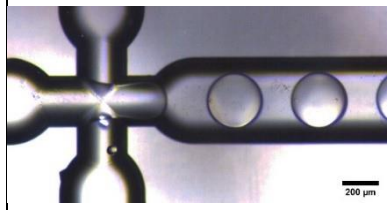
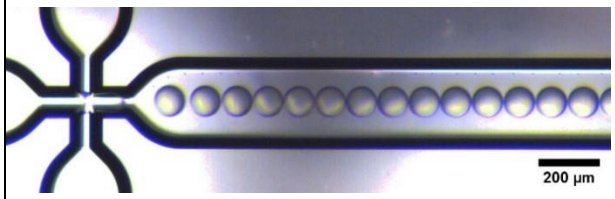
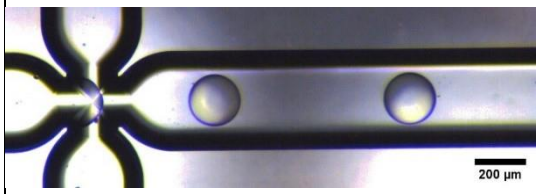
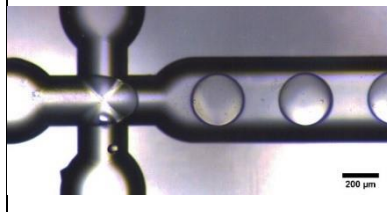
65		143		210	
75		168		235	
89		186		258	
120		193		278	
145		211		305	

Figure 10 Pectin bead size before jetting in different large droplet chips.

5 Appendix – Calibration Curve

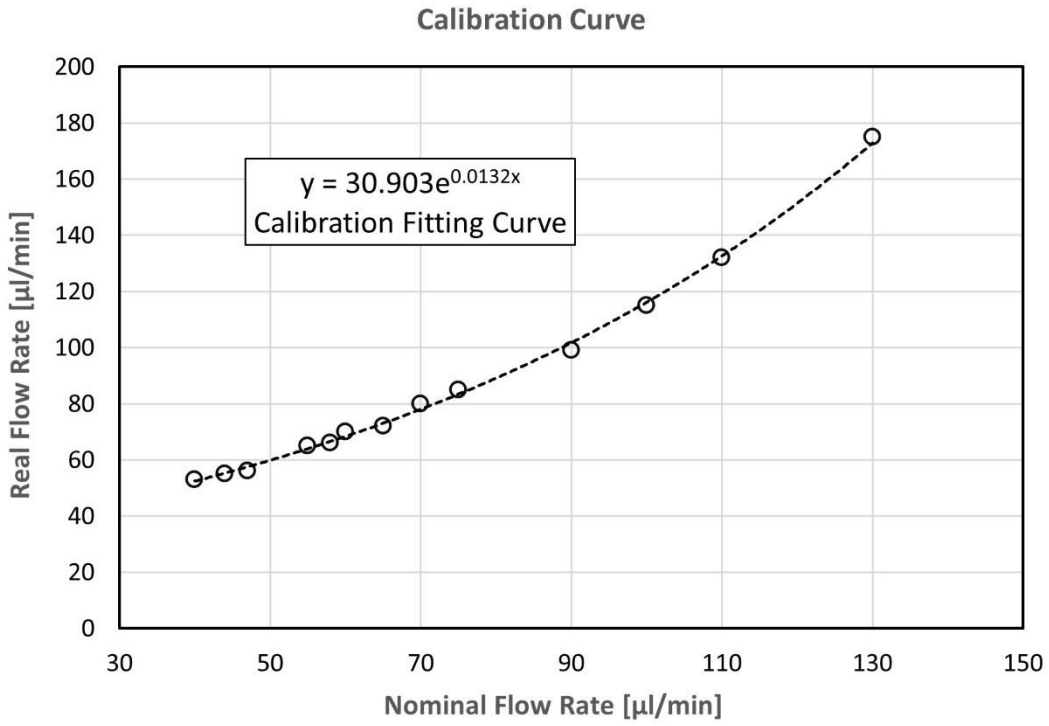


Figure 11 Pectin 1.5 % 0.1 M Ca-EDTA calibration curve.

6 Bibliography

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[2] C. Kim, K. S. Park, J. Kim, S. G. Jeong, and C. S. Lee, "Microfluidic synthesis of monodisperse pectin hydrogel microspheres based on in situ gelation and settling collection," J. Chem. Technol. Biotechnol., vol. 92, no. 1, pp. 201–209, 2017, doi: 10.1002/jctb.4991.

[3] D. Ogończyk, M. Siek, and P. Garstecki, "Microfluidic formulation of pectin microbeads for encapsulation and controlled release of nanoparticles," Biomicrofluidics, vol. 5, no. 1, 2011, doi: 10.1063/1.3569944.