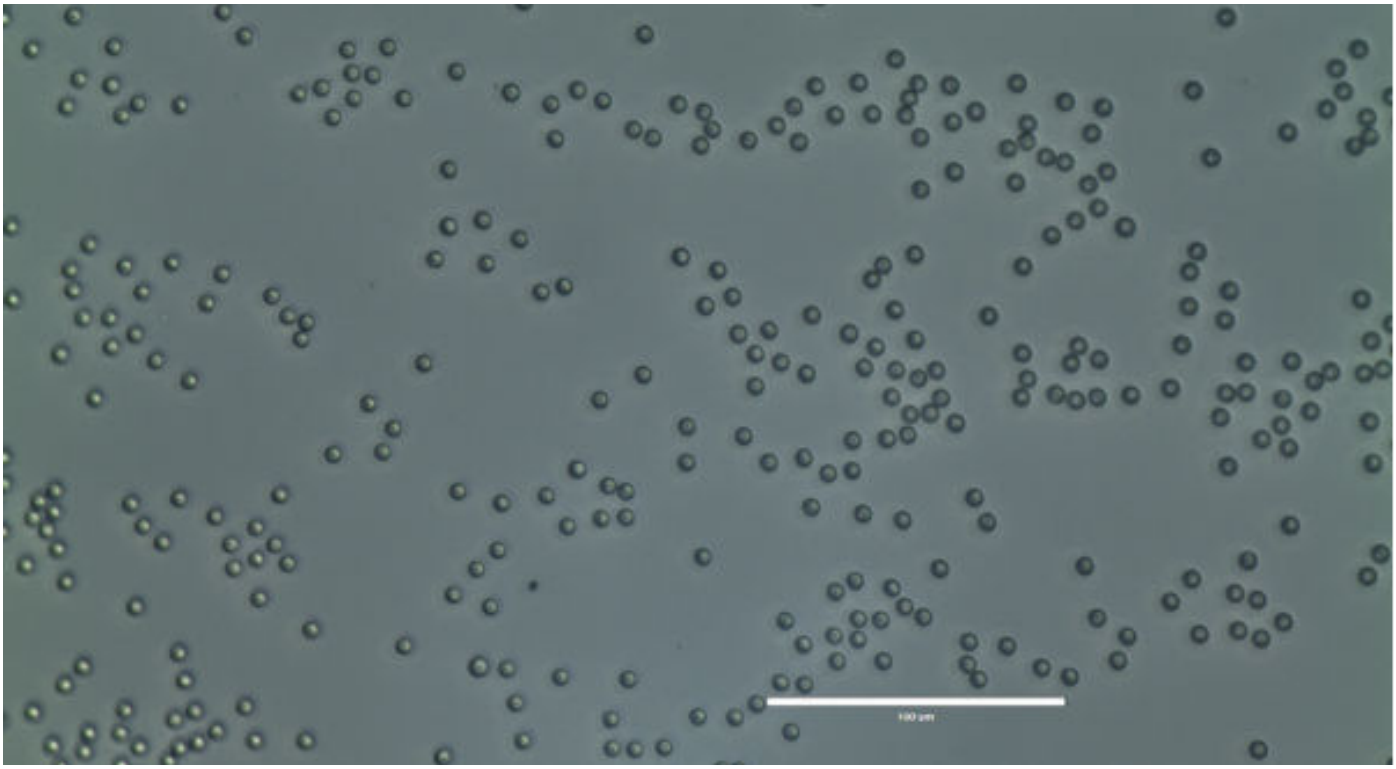
FLUIGENT

1-10 µM PLGA MICROSPHERE PRODUCTION USING THE RAYDROP



The RayDrop, developed and manufactured by Secoya Technologies, is used in this application note.

Secoya
FLUIDIFY PHARMA

INTRODUCTION

The pharmaceutical field's progress in effective disease treatment relies on the precise delivery of drugs, vaccines, and biomolecules to specific sites while ensuring stability and safety. Polymeric microparticle systems, particularly Poly (lactic-co-glycolic acid) or PLGA microspheres, are gaining attention for controlled drug release due to biodegradability, biocompatibility, and customizable drug release profiles. PLGA microparticles find applications in various fields, including cancer, cardiovascular diseases, and COVID-19 research.[1,2]

Different techniques for PLGA microparticle preparation exist, with emulsification–solvent evaporation being simple but having drawbacks like low encapsulation efficiency. Spray drying and electrospray present alternative methods but have challenges such as adhesion and control over particle size.

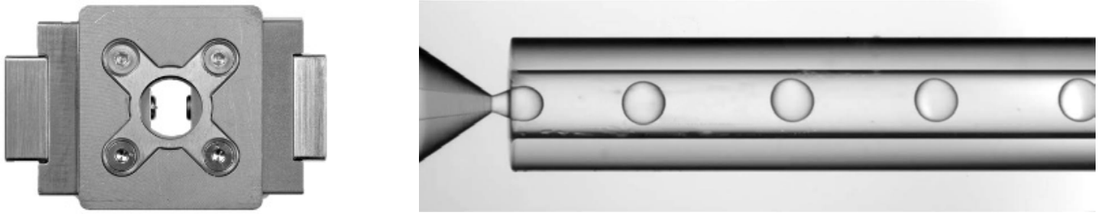
Microfluidic technologies emerge as a powerful tool for PLGA microsphere production, offering precise control over size and structure. However, classical microfluidic chips face challenges in prolonged multi-hour operations. Secoya Technology's RayDrop addresses these limitations with its cylindrical capillaries, preventing contact with walls and ensuring high-quality microfluidics for prolonged operations.[3,4]

METHOD	ADVANTAGES	DRAWBACKS
Emulsification solvent – evaporation	• Easy scaling-up • Certain ability to control particle size	• Biomacromolecule instability • Batch-to-Batch variance • Polydispersityof particle size
Spray-drying	• Fast and convenient • Suitable for industrial scaling up • Less harsh conditions for proteins	• Adhesion of the microspheres to the inner walls of the spray dryer • Difficulty in control of size
Microfluidics	• Precise of processing parameters • Monodispersity • Ease of fabricating double, triple, and even higher-order emulsions	• Instrument dependent • Relatively low yield

Advantages and drawback of microparticule production techniques.[5]

Our previous application note demonstrated the capability of the RayDrop to produce PLGA microparticles with diameters between 15 and 50 µm. It offered enhanced reproducibility and increased monodispersity compared to other technologies, enabling uninterrupted, long-term production for investigations. However, the minimum microparticle diameter was 15 µm due to the available RayDrop geometry (30-150) at the time of redaction.[6,7]

With new RayDrop geometries capable of generating smaller droplets, the current application note showcases the RayDrop's ability to generate PLGA microparticles in the range of 1 to 10 µm and demonstrates that any formulation developed for one specific RayDrop configuration can directly be used with another RayDrop configuration to achieve different particle sizes.



Ethyl Acetate - PLGA droplet production with the RayDrop.

MATERIALS & METHODS

1. Products

The reagents used for the PLGA microsphere production are: deionized water, PLGA Resomer RG 755 s, Poly (vinyl alcohol) (MW13000-23000, 87-89% hydrolyzed), and Ethyl Acetate (purity 99.7%). All reagents were purchased from Sigma-Aldrich.

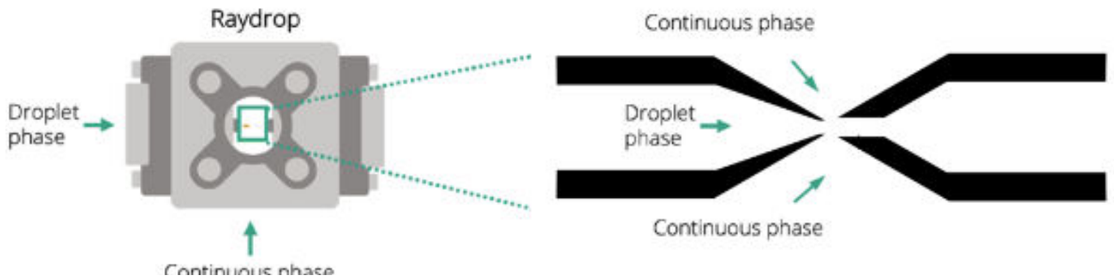
The composition of the different phases used are:

CONTINUOUS PHASE	DROPLET PHASE PRIMING & CLEANING	DROPLET PHASE PRODUCTION
WATER+1% PVA	ETHYL ACETATE	ETHYL ACETATE+2% PLGA

2. Microfluidic Setup

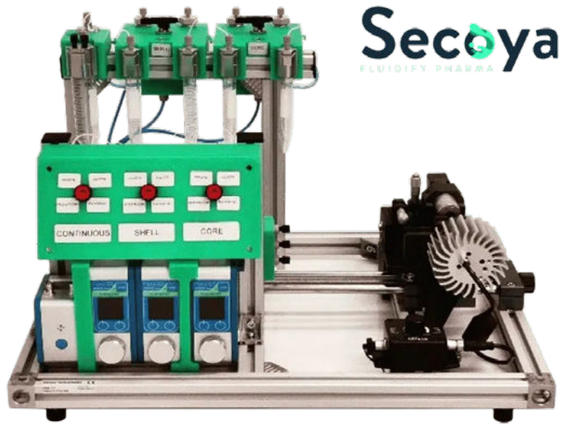
A RayDrop with a 30 µm inlet nozzle and a 45 µm counter nozzle is used to perform the PLGA microsphere generation. Its functionality is based on aligning two capillaries within a pressurized chamber containing the outer phase. The inner phase exits through a 3D-printed nozzle positioned in front of a second capillary, where it becomes enveloped by the outer phase.

This coflow-focusing approach deviates from the conventional embedded approach and facilitates the creation of a hydrodynamically focused 3D stream. This unique design eliminates wettability issues observed in classical microfluidic chips.

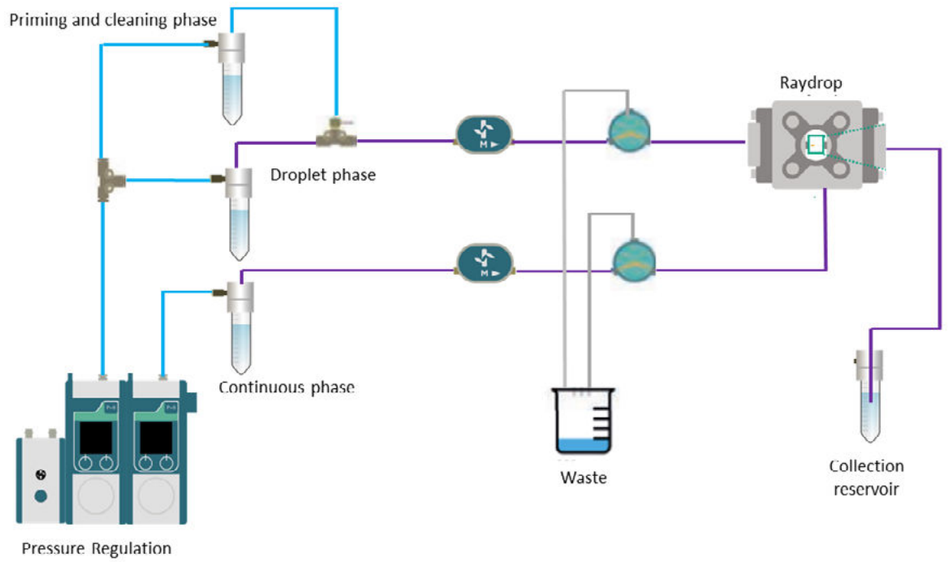


Simplified structure of the RayDrop

The RayDrop (30-45) was installed in the Complex Emulsion Production Platform for easy fluids and droplet flow, control, and imaging. The platform is divided into three parts: mechanical, fluidic, and optical.



The complex emulsion production platform



Simplified microfluidic circuit of the Complex Emulsion Production Platform for PLGA microparticle synthesis.

The mechanical assembly includes different displacement plates. These allow one to adjust the camera by moving it in x, y, and z directions. It is also possible to position the RayDrop to optimize the visibility of the nozzles on the screen.

The fluidic part contains all the pressure-driven controllers, tubing, and valves necessary for the circulation of fluids. Fluigent's FlowEZ Flow controller and Flow Units enable real-time control and measurement of the flow rates, and transitioning from pressure control to flow rate control becomes feasible. Our pressure-driven controllers permit high flow stability and fast flow change response. This capability **enables the continuous production of highly uniform droplets** over an extended duration. Also, it is possible to easily set a different pressure or flow rate for each phase.

The optical section contains an LED light source and a color USB 3.0 camera. This camera is connected to a computer to observe droplet formation in real time, as well as to control the stability of the emulsion and measure the size of the generated droplets.

Some modifications were done to the platform to adapt to the production of 10 µm PLGA microsphere as described below:

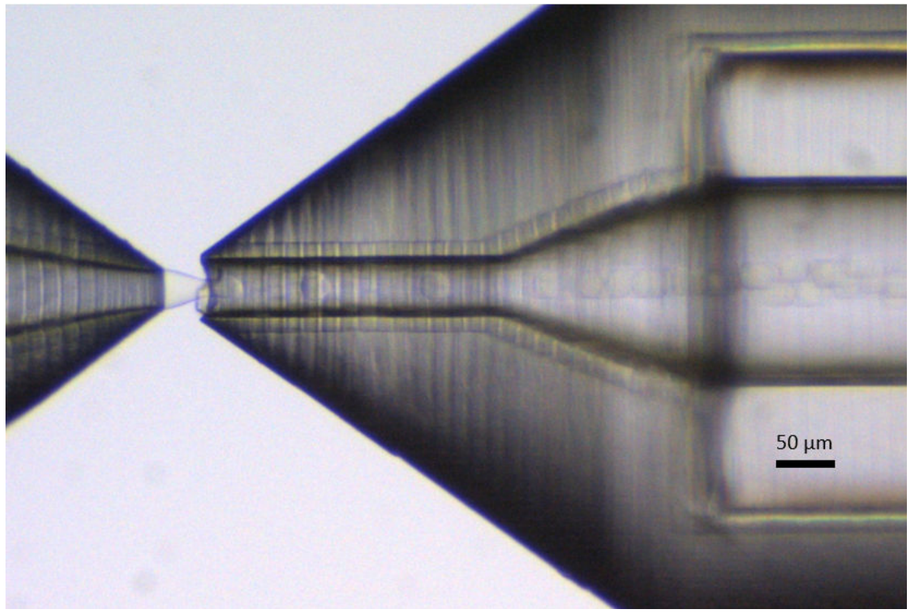
- Use of the 10x optical module
- Addition of 15 cm of PEEK tubing with 125µm ID on the continuous phase
- Addition of 20 cm of PEEK tubing with 125 µm ID on the droplet phase
- Addition of 30 cm of PEEK tubing with 125 µm ID for the pure Ethyl Acetate phase
- Use of a peak tubing with 180 µm ID at the outlet of the RayDrop

3. PLGA Microsphere Production Methods

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

Droplet Generation Steps:

1. Fill the RayDrop with the continuous phase (refer to the user guide for detailed instructions).
2. Set the continuous phase to the desired flow rate (35 µL/min as an example).
3. Set the droplet phase to the desired flow rate to establish a co-flow of water and Ethyl Acetate (1.5 µL/min as an example).
4. After stabilization, switch the valve to the reservoir containing the PLGA solution.
5. Wait until the PLGA solution traverses the tubing and reaches the RayDrop, forming a single emulsion with PLGA droplets in the aqueous continuous phase.
6. Optimize the emulsion properties by adjusting flow rates, through Fluigent's software Oxygen, to achieve the desired droplet diameter.
7. Collect the PLGA-Ethyl Acetate droplets by immersing the tip of the outlet tubing in a small bath of deionized water.



PLGA-Ethyl Acetate droplets in the counter nozzle.

Cleaning and Priming:

Before concluding the experiment, flush the droplet phase tubing and the RayDrop nozzle with Ethyl Acetate to dissolve and remove the PLGA, ensuring tubing cleanliness and avoiding clogging issues.

To flush the PLGA out of the tubing and RayDrop:

- Switch the manual valve to the reservoir containing the cleaning solution (Ethyl Acetate).
- Wait until the cleaning solution traverses the tubing and reaches the RayDrop,

Stop the flow of the droplet phase followed by the continuous phase.

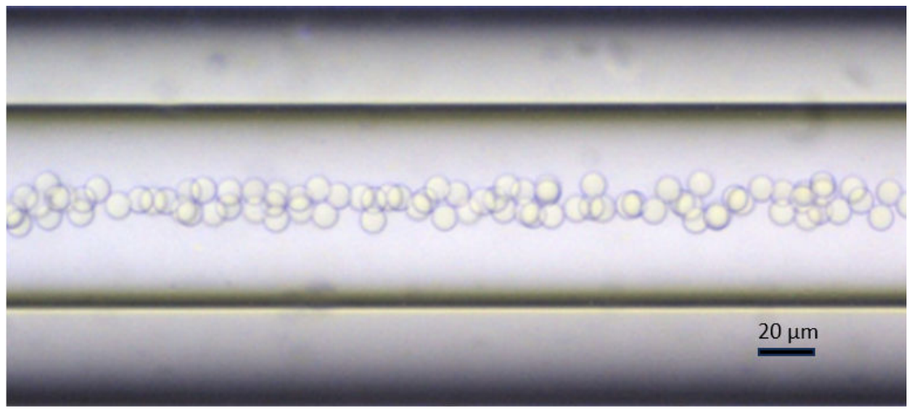
Finally, the PLGA-Ethyl Acetate droplets were left for 5 minutes to complete the polymerization process. and were observed with the EvosXL microscope and a 40x magnification.

RESULTS

Table 2 summarizes the flow rates used to generate the PLGA-Ethyl Acetate droplets and the size of the droplet generated. As usual, the RayDrop produces highly monodisperse droplets (21.3 µm mean diameter) with a CV<2%.

Table 2: PLGA-Ethyl Acetate droplet production and PLGA microparticle production.

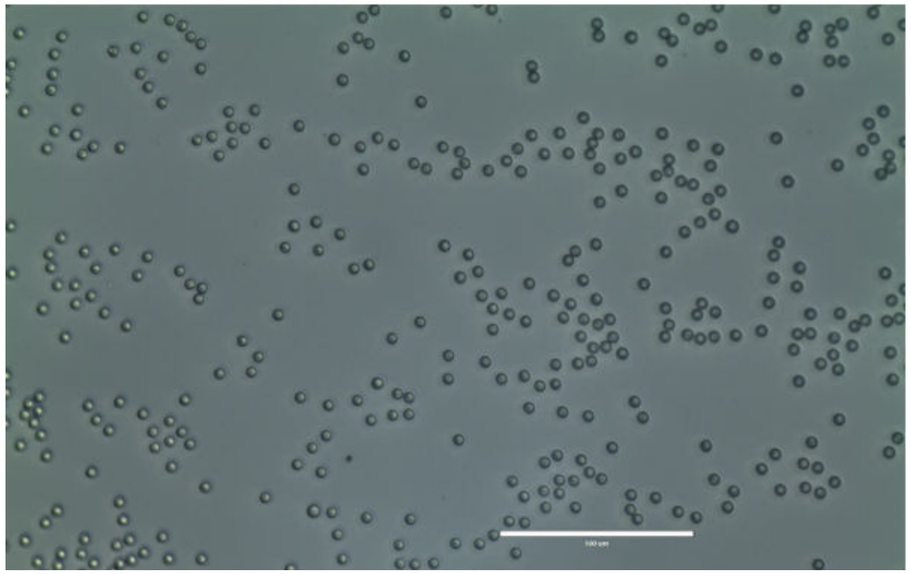
	PRESSURE (MBAR)	**FLOWRATE (µL/MIN)**
Continuous phase (Water+1% PVA)	400	Ethyl Acetate+2% PLGA
Droplet phase (EthylAcetate+2% PLGA)	80	1.8
	Mean diameter (µm)	**CV (%)**
Ethyl Acetate+2% PLGA Droplet size	21.3	1.5
PLGA microparticle size	6.9	2.4



PLGA-Ethyl Acetate droplets in the counter nozzle.

After collection, Ethyl Acetate starts to gradually diffuse into the surrounding aqueous solution. Thus, PLGA undergoes precipitation, transforming the droplet into a solid bead. The solvent removal process leads to a reduction in droplet volume, resulting in the formation of a solid bead with a final fixed size.

The figure below shows the produced PLGA microparticles. The PLGA microspheres present a mean diameter of 6.9 µm with a high monodispersity (CV=2.4%).



PLGA microparticles observed with an EvosXL and 40x magnification objective. The size bar measures 100 µm.

CONCLUSION

The present proof of concept demonstrates the capabilities of the RayDrop single emulsion device to produce PLGA microparticles in the range 1-10 µm with microparticles having a mean diameter of 6.9 µm. As expected, the combination of a known formulation1,2 with the correct RayDrop allows for the production of the desired microparticle size.

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