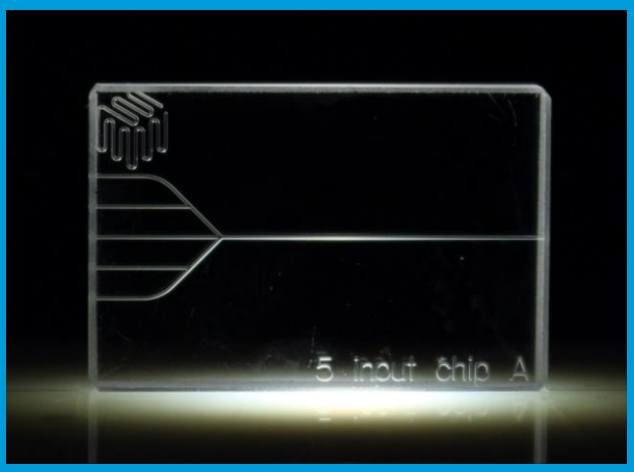
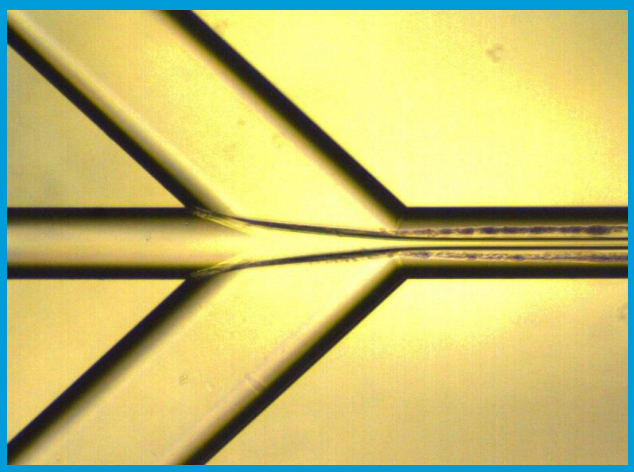
Continuous Microfluidic Synthesis of PLGA Nanoparticles by Hydrodynamic Flow Focusing

Dolomite's API encapsulation system for PLGA 50 nm to 30 µm particle synthesis





dolomite

Aim & Objectives

In this application note we demonstrate the use of the Dolomite API encapsulation system for the controllable production of PLGA 50 nm to 30 µm nanoparticles. The system is based on continuous microfluidic synthesis of PLGA nanoparticles using hydrodynamic flow focusing techniques by means of the Dolomite's 5 Input Chip.

Introduction

Robust, high-throughput methods of particle production in the size range of 50 to 500 nm have received significant interest within the scientific and pharmaceutical communities due to a wide variety of emerging applications in the fields of targeted drug delivery and controlled drug release. Scale-up of particle fabrication process using batch techniques typically results in a reduction of control over the synthesis process, leading to wide particle size distributions and, in some cases, to uncontrolled particle aggregation.

By contrast, the use of microfluidic devices for nanoparticle synthesis brings advantages such as: enhanced control over each stage of particle fabrication process, greater particle yields, and ease of scale-up. This continuous flow methodology can be applied to produce high grade PLGA nanoparticles satisfying criteria such as: low residual solvent content, presence of processing aids and high degree of batch-to-batch consistency.

This application note reports on the production of biocompatible PLGA polymer nanoparticles (NP) for pharmaceutical applications. This note demonstrates the use of Dolomite's 5 Input Chip and hydrodynamic flow focusing microfluidic techniques for the synthesis of PLGA nanoparticles. In this work Acetone is used as PLGA solvent and Water is used as the "antisolvent" to trigger particle nucleation, growth and precipitation of the solid polymer content as nanoparticles. When Acetone is used as the PLGA solvent, rapid mixing with the aqueous phase leads to the formation of an azeotrope (two liquid mixture). Subsequently displacement of solvent from the polymer matrix to the surrounding aqueous phase occurs, resulting in hardened particles.

Fabrication of the PLGA nanoparticles using the Dolomite's 5 Input Chip results in substantial improvements in nanoparticle size distribution when compared to conventional batch methods. This use of continuous flow techniques provides a simple, scalable methodology for high-yield, high-quality fabrication of PLGA nanoparticles for pharmaceutical applications.

Dynamic Light Scattering (DLS) is used to characterize the final product.

Polymer Nanoparticles

PLGA or poly(lactic-co-glycolic acid) (Figure 1) is a copolymer which is used as a host of Food and Drug Administration (FDA) approved therapeutic drugs, owing to its predictable biodegradability and biocompatibility. It has found numerous applications in controlled release and targeted delivery of Active Pharmaceutical Ingredients (APIs) including the treatment of listeriosis, prostate cancer, and prophylactic delivery of vancomycin.

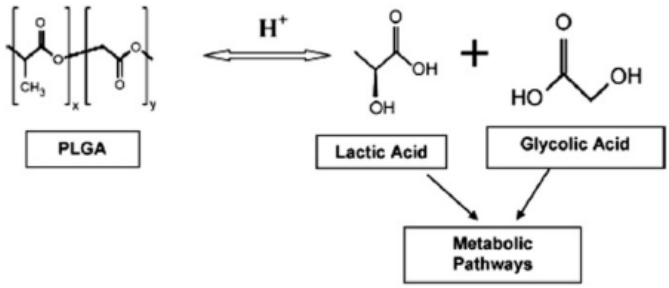


Figure 1. PLGA molecular structure.

In controlled drug release applications, predictable degradation of PLGA is employed for sustained release, at desirable doses, by non-surgical implantation. In the case of targeted drug delivery, the particles accumulate in specific tissues using the Enhanced Permeability and Retention (EPR) effect, or as a result of particle surface functionalization by targeting species such as anti-bodies. It is possible to tune the release profiles from the polymer-drug matrix by controlling polymer molecular weight, ratio of lactide to glycolide, drug concentration and particle size.

Microfluidic Synthetic Method of PLGA Nanoparticles

Microfluidic VS Batch Methods

Current methods of particle synthesis rely largely on batch stirred homogenizers. However, major challenges persist in these systems with regard to process controllability and reproducibility, owing to the rapidity of the involved processes of mixing, nucleation, growth and agglomeration and their complex interactions when they take place concurrently. Often nucleation is rapid compared with mixing and starts when the components are still not homogeneously distributed, resulting in broad crystal size distributions and substantial batch-to-batch product variability.

Pharmaceutical applications, however, require narrow particles size distributions necessitating additional particle size selection processes to be implemented leading to low particle yields and loss of a large portion of the seeded API. Continuous flow microfluidic processes offer a technology with the potential to form particles with excellent homogeneity and control the critical process parameters in a way that is difficult to achieve in batch

methods. Owing to the continuous nature of the process and better controlled fluid dynamic conditions, microfluidic systems offer improved controllability and reproducibility and therefore a precise control over the particle size, shape and crystallinity [1]. Among the numerous polymeric materials used in microfluidics for the production of drug delivery, PLGA is widely employed in biomedical applications, and has been increasingly used in several microfluidic methods to produce particles in the micro- and nano- size range [2]. This application note focuses on the production of PLGA nanoparticles by antisolvent precipitation using hydrodynamic flow focusing microfluidic techniques [3] [4].

Dolomite Microfluidic Method	Batch Method
~100% encapsulation efficiency	~30% Encapsulation efficiency
Monodisperse (CV <5%)	Polydisperse (CV >>20%)
Scale to kg/day with no waste	~50% waste
Consistent from run to run	Poor batch to batch consistency
Uniform API distribution	Uneven API distribution
Precise particle size control	Poor size control
Wide range of particle size	Limited size range without filtering

Table 1. Microfluidic VS Batch Synthesis.

Microfluidic Synthetic Method - Solvent Diffusion

Representative production rates using rapid microfluidic methodology are multiple grams of PLGA particles per day per chip. While greater throughput can be achieved using batch techniques, microfluidic methods have the advantage of producing particles with narrow size distributions, do not require the use of seize selection methods and, as a result, lead to a minimal loss of API during the encapsulation process. To date, the proof-of-concept studies found in literature have not sufficiently addressed the engineering challenges necessary to reach production volumes relevant for clinical translation.

In the microfluidic solvent diffusion method, PLGA nanoparticles are synthesized in a microchannel by rapidly mixing PLGA-Acetone solution and Water using a hydrodynamic flow focusing strategy (Figure 2 and Figure 3). In this approach, a stable laminar focussed stream is created along the central channel meeting two adjacent streams flowing at higher flow rates. Flow focusing squeezes the PLGA in Acetone stream between two anti-solvent Water streams, resulting in rapid solvent exchange via diffusion and PLGA nanoparticles precipitation. In the formation of PLGA nanoparticles by flow focusing technique and solvent diffusion method the PLGA containing solvent (Acetone) and the antisolvent (Water) form an azeotropic mixture. The PLGA particle formation takes place spontaneously at the nucleation spots that are distributed through the mixture. Particle growth then occurs by addition of PLGA to the surface of the newly formed particles. Particle hardening occurs

throughout and post the growth stage by diffusion of the solvent from the polymer matrix into the surrounding mixture (Figure 3).

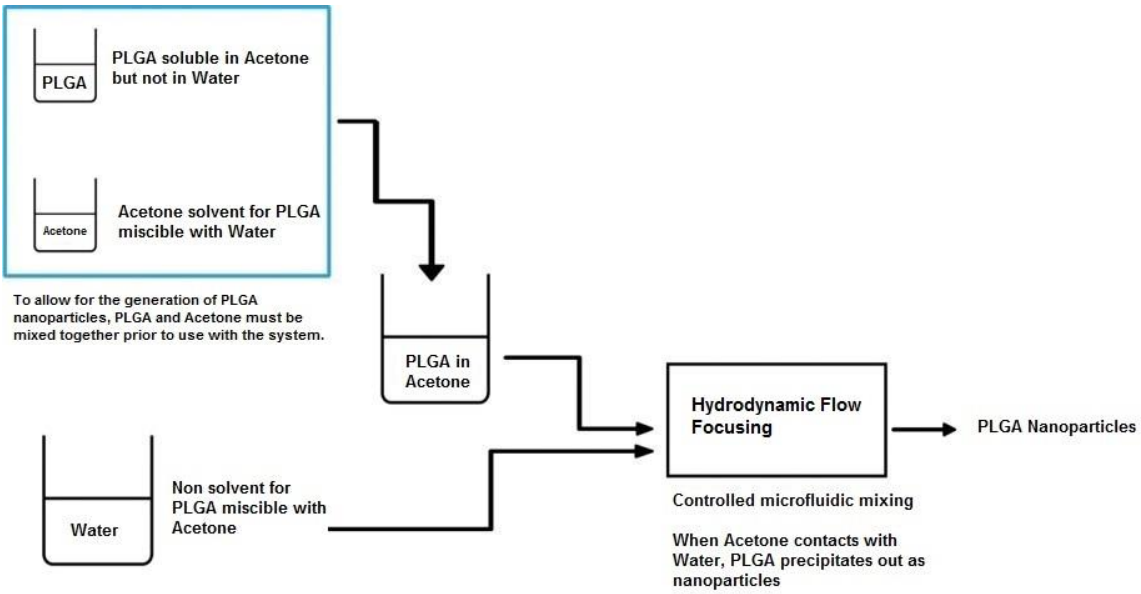


Figure 2. Schematic showing a strategy for synthesizing nanoparticles.

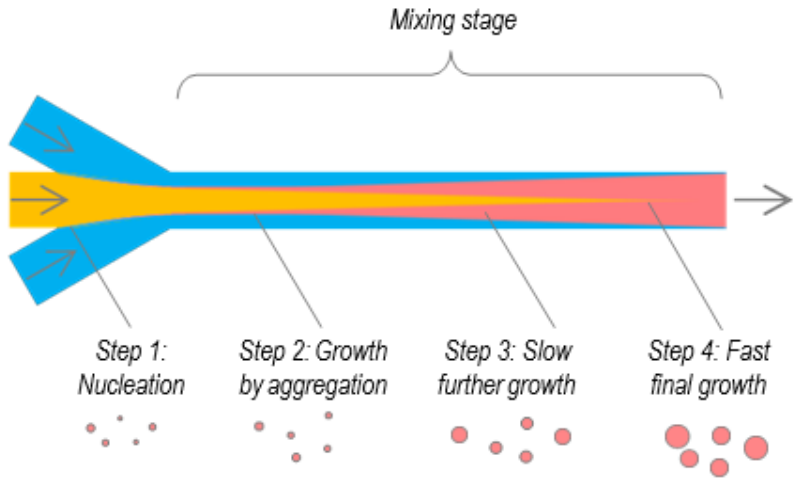


Figure 3. Schematic showing a hydrodynamic flow focusing mediated nanoparticle production strategy. Acetone is displaced from the PLGA in Acetone polymer stream and diffuses into the aqueous stream leaving behind a hardened suspension of colloidal nanoparticles.

The predictable flow pattern achieved in the microfluidic hydrodynamic flow focusing allows controlled precipitation of PLGA particles. In fact, the final mean particle size and size distribution can be tuned by changing the mixing timescale (τ_{mix}) across the focussed stream. This is true if τ_{mix} is larger than τ_{agg} which is the characteristic timescale for polymer chains to nucleate, grow and aggregate. $\tau_{agg} \sim 10^1 - 10^2\ ms$, depending on the molecular weight of the polymer chain and solvent. This quantity is difficult to estimate and is generally unknown [1]. τ_{mix} can be calculated as $\tau_{mix} \sim W^2/4D$, where W is the width of the focussed central stream and D the diffusivity of the solvent ($D \sim 10^{-9}\ {}^{m^2}/_{s}$) [4]. W can

be varied by changing the flow ratio $R = F_A/F_w$ between the PLGA/Acetone stream (F_A) and Water stream (F_A). In conclusion, provided that $\tau_{mix} > \tau_{agg}$, we can then control τ_{mix} changing R. According to the model of self-assembly of di-block copolymers during nanoprecipitation, we expect a decrease in nanoparticle size with a decrease of mixing time. This is because if mixing is not completed nanoparticles begin to assemble in an heterogenous environment forming larger particles [5].

In the opposite condition where $\tau_{mix} < \tau_{agg}$, particle self-assembly occurs primarily when the solvent change is complete. This homogenous solvent environment for nanoparticle assembly allows the hydrophilic portion of the molecule to stabilize the nanoparticles more effectively, and this yields smaller nanoparticles with uniform size. In this case nanoparticle size is expected to become independent of mixing time and polymer concentration [5].

All the previous considerations are correct as long as the average residence time of the fluid $\tau > (\tau_{mix}\ ;\ \tau_{agg})$. In other words, the particles will need to spend sufficient time within the channel to experience mixing and agglomeration phenomena under the same fluid dynamic conditions. This hypothesis will be verified and commented in the following sections.

Experimental Setup

System Description

The system setup is shown in the Figure 4. The fluids are delivered using three Pressure Pumps (Part No. 3200016). The first Pump P_1 delivers the Acetone flushing fluid (dark blue line) and works in combination with the Sensor Displays (Part No. 3200095) and the Flow Rate Sensors 1-50 µl/min (Part No. 3200098). The second Pump P_2 delivers the Water antisolvent solution (light blue line) and works in combination with the Sensor Displays (Part No. 3200095) and the Flow Rate Sensors 1-50 µl/min (Part No. 3200098). The third Pump P_3 delivers the PLGA/Acetone solution (yellow line) and works in combination with the Sensor Displays (Part No. 3200095) and the 0.4-7 µl/min the Flow Rate Sensor (Part No. 3200099).

Fluidic connections between the three P-Pumps and the two T-connectors (Part No. 3000397) are made using FEP tubing of OD 1.6 mm and ID 0.25 mm (Part No. 3200063). All the other connections are made using FEP tubing OD 0.8 mm and ID 0.25 mm (Part No. 3200302). 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, 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 5 Input Chip (Part No. 3200711) as shown in (Figure 6Figure 7). The 5 Input Chip is a hydrophilic flow focusing glass microfluidic device that allows the formation of a stable laminar stream confined by two lateral streams. It is assembled with the H Interface 7-way (Part No. 3200297) and two Linear Connectors 7-way (Part No. 3200148) (Figure 7).

Visualization is achieved using a High-Speed Digital Microscope (Part No. 3200531).

System Start-up and Shut-down

Open valve V_2 and start Pump P_2 setting the desired flow rate of the antisolvent Water solution. Then, open valve V_3 and start pump P_3. Increase gently the flow of the PLGA solution until a PLGA in Acetone stable stream is created within the outlet channel. During this stage keep the Pump P_1 and the valve V_1 closed. The thickness of the laminar PLGA in Acetone stream can be controlled by changing the ratio R between the PLGA in Acetone stream and the Water stream. To shut down the system close first V_3 and then V_2. Finally open the valve V_1 and start the pump P_1 to flush the system and clean the channel surfaces from any particles deposited.

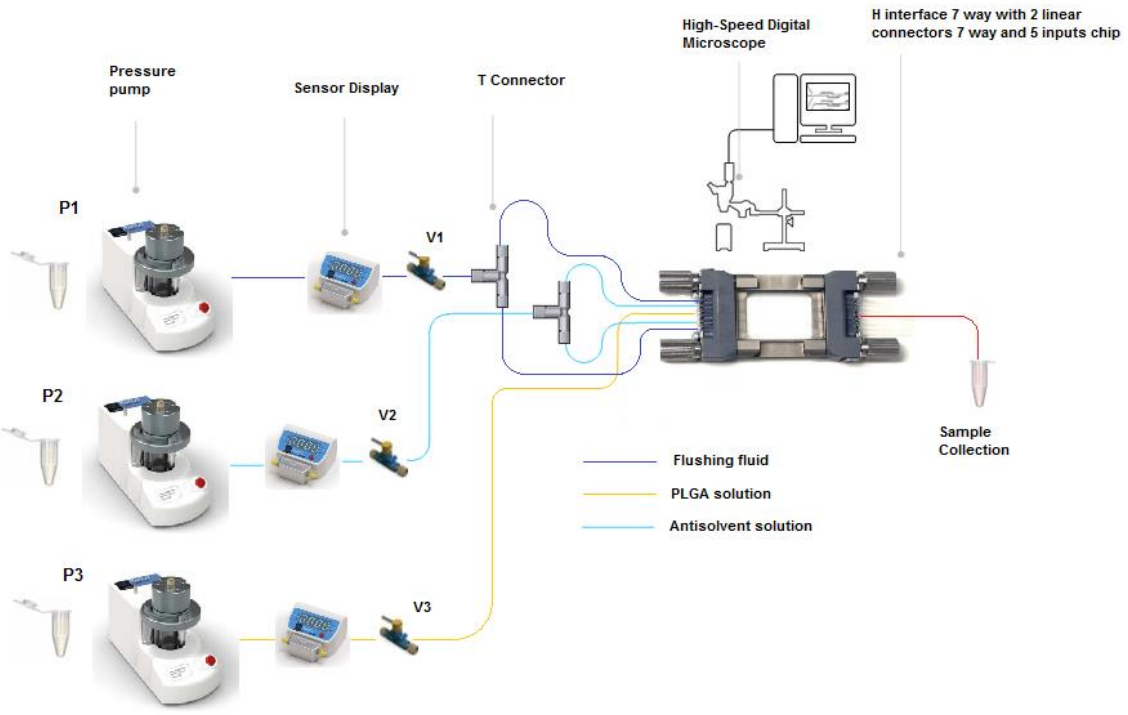


Figure 4. Schematic showing representative setup of a API encapsulation system for PLGA nanoparticle production.

Figure 5. API encapsulation system for PLGA nanoparticle production.

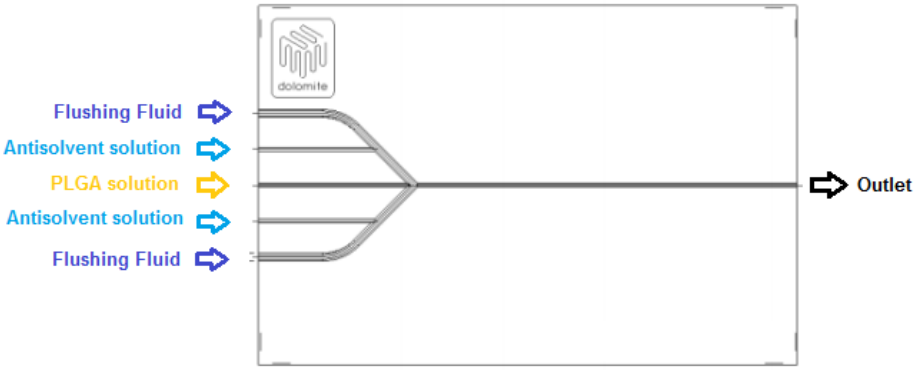


Figure 6. Fluids dispensed to the 5 Input Chip.

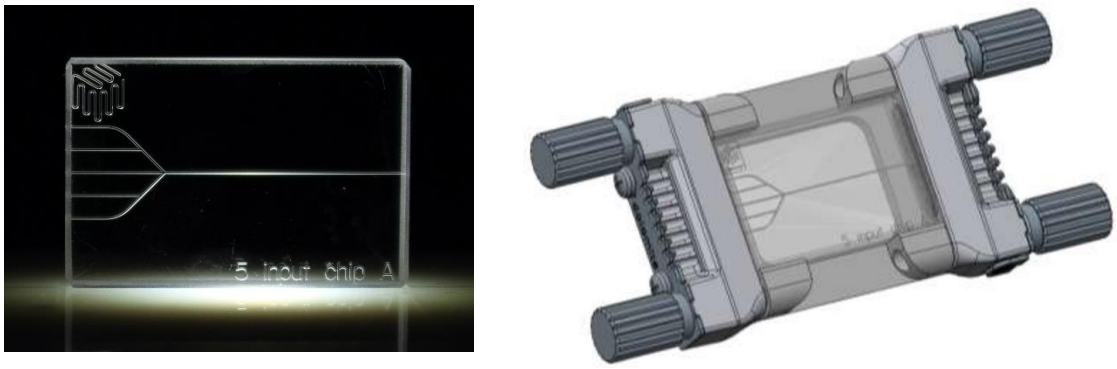


Figure 7. 5 Input Chip (left). 5 Input Chips together with H Interface 7-way and two Linear Connectors 7-way.

Experiments are performed using a 50/50 PLGA Poly(D,L-lactide-co-glycolide) 300000-60000 mol wt. 99.5 % Acetone and Deionized Water purchased from Sigma-Aldrich.

Experiments are carried out using both 1 % (w/v) and 2 % (w/v) PLGA in Acetone solutions. These are typical polymer concentrations employed when PLGA nanoparticles are generated by bulk precipitation using microfluidic devices. Higher concentrations might lead

to a particle-to-particle aggregation as well as heterogeneous nucleation on channel walls with consequent channel clogging.

The effect of flow ratio R on the PLGA particle distribution is investigated using the flows and flow ratios reported in Table 2. The average residence τ time reported in Table 2 are calculated as $\tau = A \cdot L/(F_W + F_A)$, with $L = 16\ mm$ is the length of the straight outlet channel and $A = \pi d^2/4$ the cross-sectional area of the outlet channel of average diameter $\mathrm{d} = 0.155\ mm$.

F_W $[\mu l/min]$	F_A $[\mu l/min]$	$F_W + F_A$ $[\mu l/min]$	$R = F_A/F_w$ $[-]$	τ $[ms]$
30	10	40	0.333	452
40	10	50	0.250	362
50	10	60	0.200	302
60	10	70	0.167	259
70	10	80	0.143	226

Table 2. Flows, flow ratios and average residence times used with the 5 Input Chip.

The flow rates F_A and F_W are set quite low so that the average residence time τ is likely to be larger than both τ_{mix} and τ_{agg}.

Each experiment is conducted three time and the particle number distributions and peak intensities are determined by Dynamic Light Scattering (DLS) from Malvern. The particle distributions obtained are plot of the relative intensity of light scattered by particles in various size classes and is therefore known as an intensity number distribution. Particles are collected at the outlet and analysed with DLS without further dilution.

Results & Discussion

Effect of Flow Ratio on PLGA number distribution

Figure 8 reports the DLS number distributions obtained at different flow ratios R for 1 % and 2 % PLGA in Acetone solutions. The corresponding peak intensities (I) are plotted in Figure 9 with relative error bars showing the standard deviations calculated for the set of three experiments for each R.

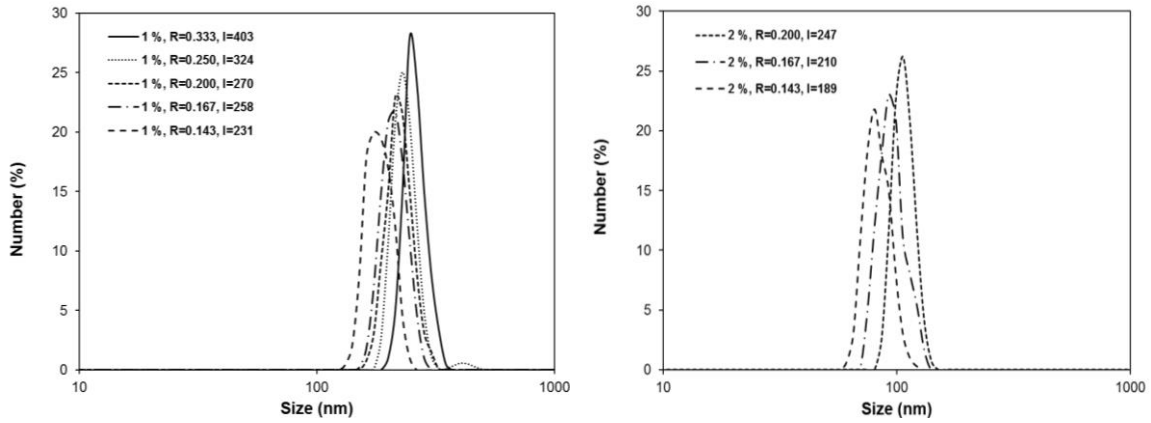


Figure 8. Number distributions for 1 % (left) and 2 % (right) PLGA in Acetone for different R.

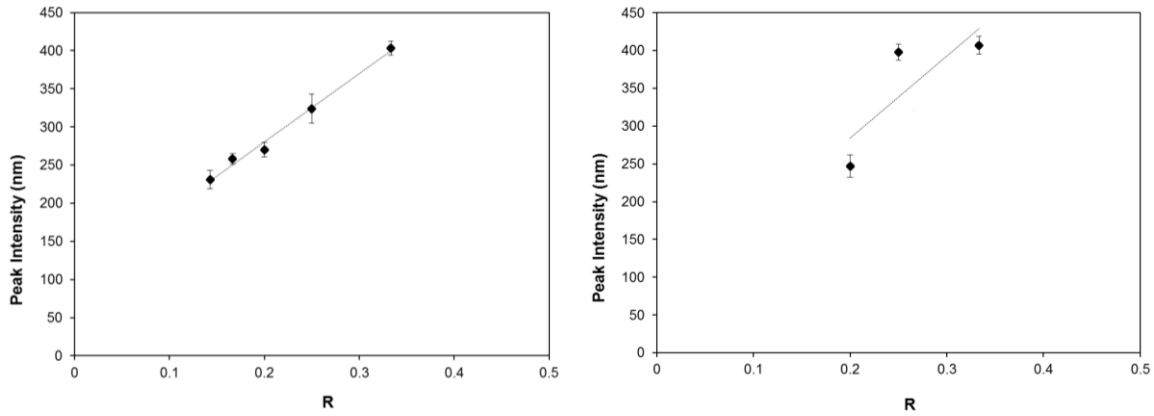


Figure 9. Peak intensities for 1 % (left) and 2 % (right) PLGA in Acetone for different R.

The results show that PLGA nanoparticles are produced in the range between ~ 400 nm and ~ 200 nm. Particle mean size (peak intensity) can be tuned by controlling the flow ratio R. A decrease of the flow ratio R leads to a decrease of the nanoparticle size with a precise control that cannot be achieved with traditional batch synthesis. As reported in the previous sections, this condition is in agreement with the theoretical model of rapid self-assembly of block copolymer nanoparticles proposed by B. K. Johnson et al. (2003) for $\tau > \tau_{mix} > \tau_{agg}$ [5]. This condition is verified in the table below for all the flow rates adopted.

F_W [µl/min]	F_A [µl/min]	$F_W + F_A$ [µl/min]	$R = F_A/F_w$ [−]	W [mm]	τ [ms]	τ_{mix} [ms]	τ_{agg} [ms]
30	10	40	0.333	0.042	452	441	
40	10	50	0.250	0.037	362	342	
50	10	60	0.200	0.034	302	289	10 – 100 ref. [1]
60	10	70	0.167	0.025	259	156	
70	10	80	0.143	0.02	226	100	

Table 3. Estimation of τ_{mix} for different flow rate ratios R.

In Table 3 τ_{mix} is calculated as $\tau_{mix} = W^2/4D$, where the width of the focussed laminar stream W is measured optically from pictures (Figure 11) and the solvent diffusivity $D = 10^{-9}\, m^2/_S$.

The distributions obtained for 2 % PLGA in Acetone at the highest ratios ($R = 0.333$ and $R = 0.250$) reported in Figure 10 show bimodal distributions. These are characteristic distributions generally caused by particle-to-particle aggregation which leads to particles of larger size. This phenomenon occurs at the highest concentration and residence times where a consistent number of particles reside in the channel for long time under low shear flow and have therefore more chance to aggregate.

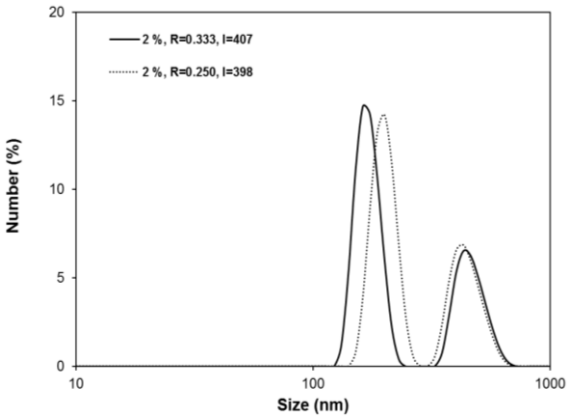


Figure 10. Number distributions for 2 % PLGA in Acetone for $R = 0.333$ and $R = 0.250$.

Figure 11 shows that PLGA precipitation is triggered at the interface between the two phases and becomes more evident while moving towards the channel outlet where mixing is improved and superasturation becomes uniform across the channel section. Particle precipitation is more significant at higher PLGA concentration (2 %) and tends to occur further away the mixing point of the two phases with the decrease of R.

Flow ratios R smaller than 0.143 gives laminar streams that are too thin and tend to break up into droplets due to fluid fluctuation and turbulence. This condition promotes uncontrolled particle precipitation along the channel length and possible channel blockage (Figure 12).

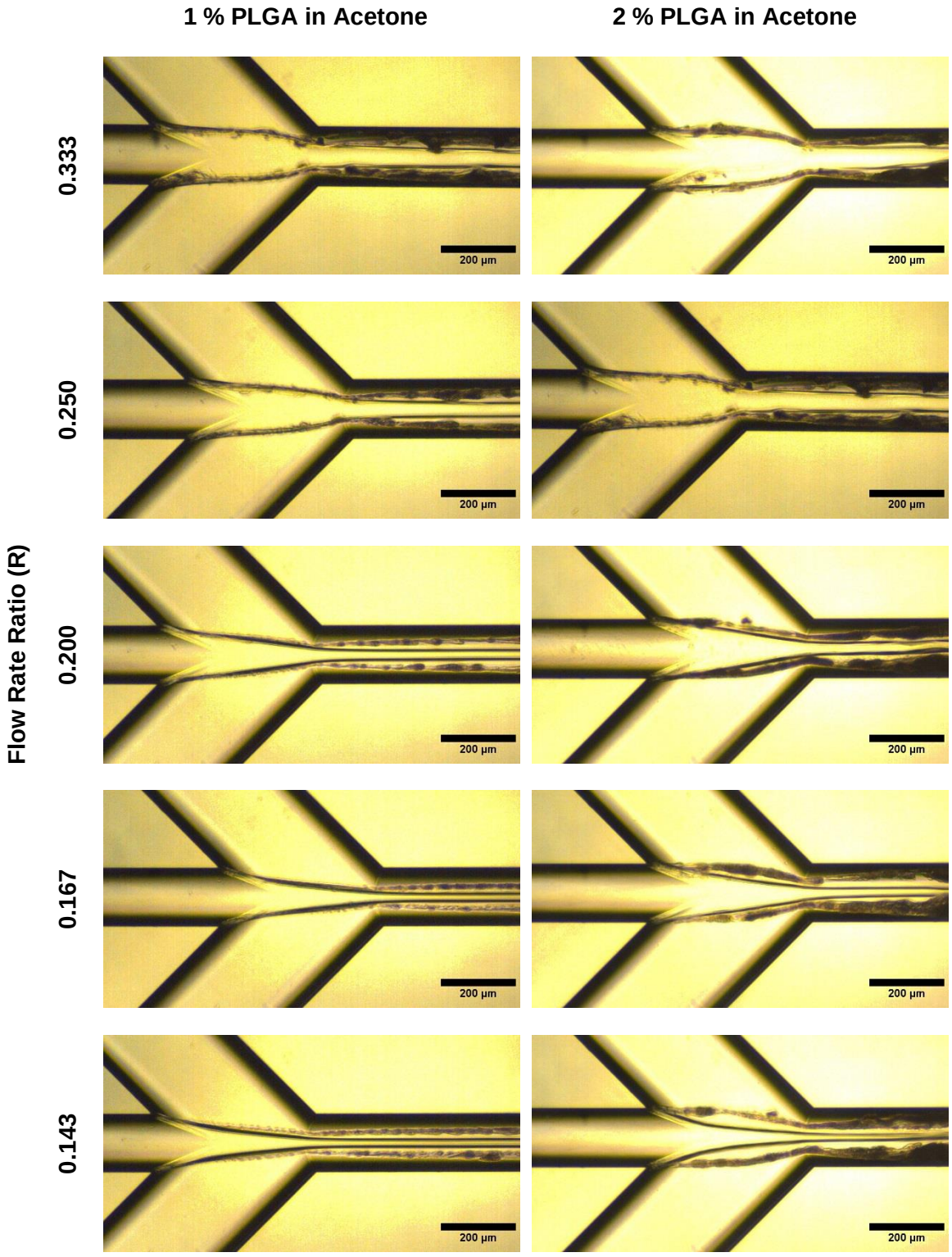


Figure 11. Hydrodynamic flow focusing streams of 1 % and 2 % PLGA in Acetone for different flow rate ratios R. PLGA particles precipitating at the interface between the two phases.

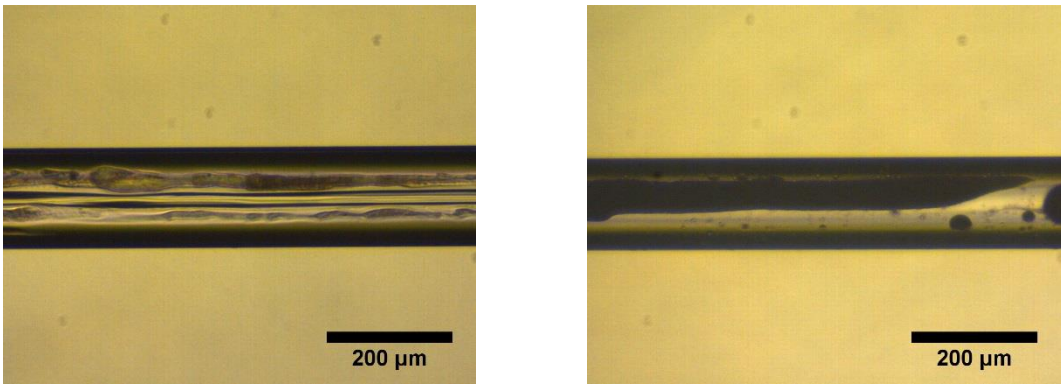


Figure 12. Stable focussed stream produced along the channel outlet $R = 0.143$ (left). Unstable focussed stream produced along the channel outlet $R < 0.143$ (right). 1 % PLGA in Acetone.

For the same batch of PLGA polymer, the peak intensity of nanoparticle number distributions was typically reproducible within about 15 nm between different experiments. This demonstrates the robustness of on-chip synthesis for the selected PLGA polymer.

Nanoparticle distributions result stable over 2 weeks within about 50 nm of the peak intensity measured by DLS. Particles stability can be improved adding surfactant to the Water phase. Typically, 1 % of polyvinyl alcohol is used in combination with PLGA polymers.

Assuming a PLGA density $\rho = 1.34\ \frac{g}{cm^3}$ and considering that most of the PLGA polymer initially dissolved will precipitate assembling in polymer nanoparticles, we can roughly estimate that at the highest concentration of 2 % we can produce up to $0.02 \cdot 10\ \frac{\mu l}{min} \cdot 1.34 \frac{g}{cm^3} = 16 \frac{mg}{h}$. This production rate can be scaled up to ~ $1 \frac{g}{h}$ using the Dolomite Telos System for high throughput. This System offers the possibility to work with 70 hydrodynamic flow focusing junctions arranged in parallel configuration and working simultaneously.

The API encapsulation system 50 nm to 30 µm PLGA particles presented in this application note is provided with an additional 5 Inputs Chip of longer channel outlet $L = 82\ mm$ (Part No. 3200712) which allows users to work with longer residence times τ and optimise particle growth and agglomeration. The system is also provided with a 3D Flow Focusing Chip - 14µm - Hydrophilic (Part No. 3200437) for larger PLGA particle synthesis (from 1 µm to 30 µm) based on droplet production method and droplet shrinkage. See the Continuous Microfluidic Synthesis of PLGA Microparticles by Droplet Method application note for more information.

Conclusions

PLGA nanoparticle synthesis is demonstrated using Dolomite's API encapsulation system 50 nm to 30 µm.

PLGA nanoparticles traditionally synthesized by batch methods are generally not a uniform and reproducible product. Consequently, these particles do not represent a very attractive solution for pharmaceutical industry; particularly in the field of smart drug delivery where narrow distributions, small particle size and controllable synthesis are desired. Nowadays, the trend is changing. New microfluidic continuous flow technologies offer an attractive solution for the synthesis of small and narrow size distribution nanoparticles. This results in more utilization of materials as none of the product size falls outside the allowable size limits for in-vivo use.

In this application PLGA polymer nanoparticles in the range between 200 nm and 400 nm were produced using the hydrodynamic flow focusing microfluidic strategy. Particle size can be controlled by changing the ratio of the phases mixed with a production rate up to about $1\frac{g}{h}$. The ability to synthesis PLGA nanoparticles in a more controllable and reproducible way opens up possibilities for custom tuning surface properties. This is achievable by adding surfactants or API to the polymer mix, or by adding downstream processes. As the entire chemistry is user controlled, Dolomite's API encapsulation system enables users to manipulate the entire synthetic route in-house with control on purity standards.

With the rapid development of microfluidic manipulation methods, new nanoparticle synthetic methods with better control and design of nanoparticle properties are expected in the coming years.

Appendix: System Component List

Part No.	Part Description	#
3200730	API encapsulation system - 50 nm to 30 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
3200711	5 Input Chip (short channel), hydrophilic	3
3200712	5 Input Chip (long channel), hydrophilic	3
3200437	3D Flow Focusing Chip - 14µm - Hydrophilic	3
	Installation and Training	-

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