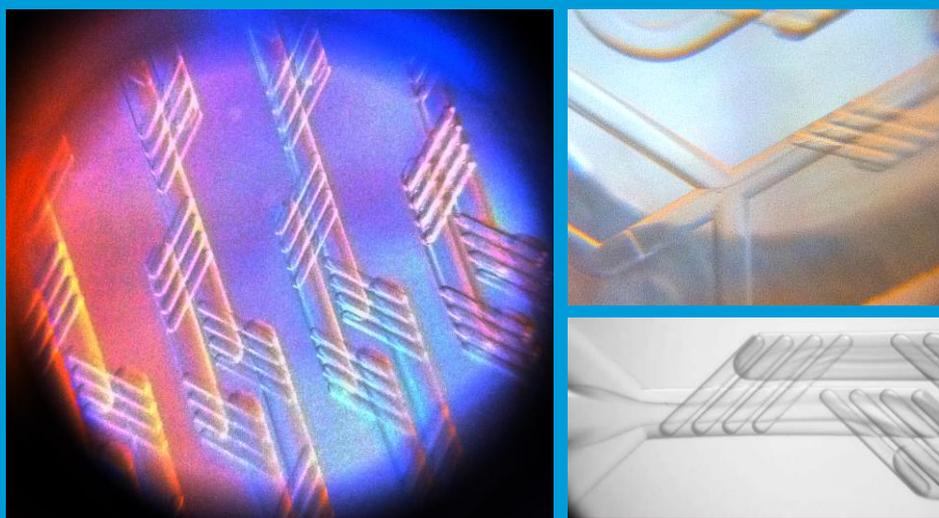


Continuous Microfluidic Synthesis of PLGA Nanoparticles

Dolomite's Micromixer System



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Summary

Robust, high-throughput methods of particle production in the size range of 50 to 500 nanometers 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 the particle fabrication process using batch techniques typically results in a reduction of control over the synthesis process, leading to wide particles 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 with satisfying criteria such as: quantity of residual solvent present, 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. Two distinct formulations are used differing in the physico-chemical process of nanoparticle formation and growth modes. Dynamic Light Scattering (DLS) is used to characterize the final product. This note demonstrates the use of Dolomite's micromixer system for the production of PLGA nanoparticles.

In this work acetone and dichloromethane were used as PLGA solvents. The two solvents differ greatly in their solubility with water. Water is used as the "antisolvent" (w.r.t PLGA) to trigger particle formation, growth and precipitation of the solid polymer content as nanoparticles. In the case where acetone is used as the PLGA solvent, rapid mixing with the aqueous phase leads to the formation of an azeotrope (two liquid mixture). By contrast, in the case of dichloromethane rapid mixing results in the formation of a microemulsion. Subsequently displacement of solvent from the polymer matrix to the surrounding aqueous phase occurs resulting in hardened particles. The table below presents particle sizes obtained by DLS from the two tests.

Solvent	Average size (nm)	PDI width	Count Rate kcps
Acetone	42.26 nm	0.075	395.3
Dichloromethane	94.03 nm	0.226	141.5

Fabrication of the PLGA nanoparticles using the Dolomite micromixer chip was shown to result in substantial improvements in nanoparticle size distributions when compared to conventional methods. This use of continuous flow techniques provides a simple, scalable methodology for high-yield, high-quality fabrication of PLGA nanoparticles for pharmaceutical applications.

Polymer Nanoparticles

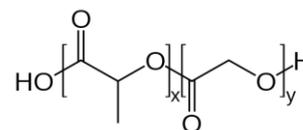
PLGA – A Biodegradable Polymer

PLGA or poly(lactic-co-glycolic acid) is a copolymer which is used in a host of Food and Drug Administration (FDA) approved therapeutic devices, 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. 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 the polymer molecular weight, ratio of lactide to glycolide, drug concentration and the particle size

Current methods of particle synthesis rely on batch type homogenizers* that typically result in wide particle size distribution. 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.

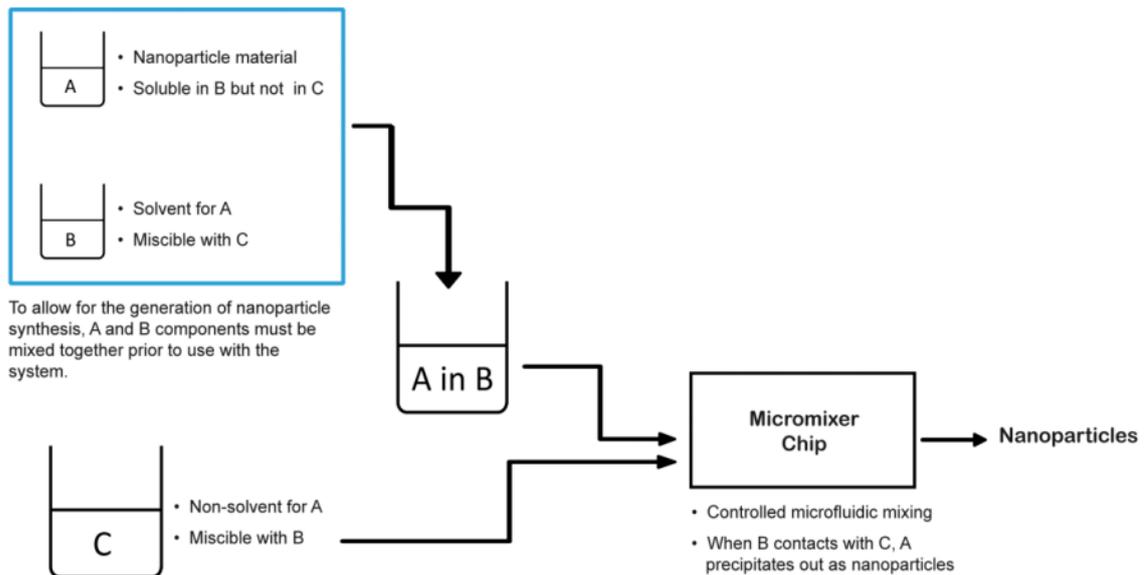


PLGA. x = number of units of lactic acid; y = number of units of glycolic acid.

* Ki Chang Song, Ho Seok Lee, Il Yeop Choung, Kyung In Cho, Yangkyu Ahn, Eun Jung Choi. The effect of type of organic phase solvents on the particle size of poly(*d,l*-lactide-co-glycolide) nanoparticles. **Colloids and Surfaces A: Physicochem. Eng. Aspects** 276 (2006) 162–167.

Microfluidic Synthetic Method

Representative production rates using rapid micromixing 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 sieve 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.

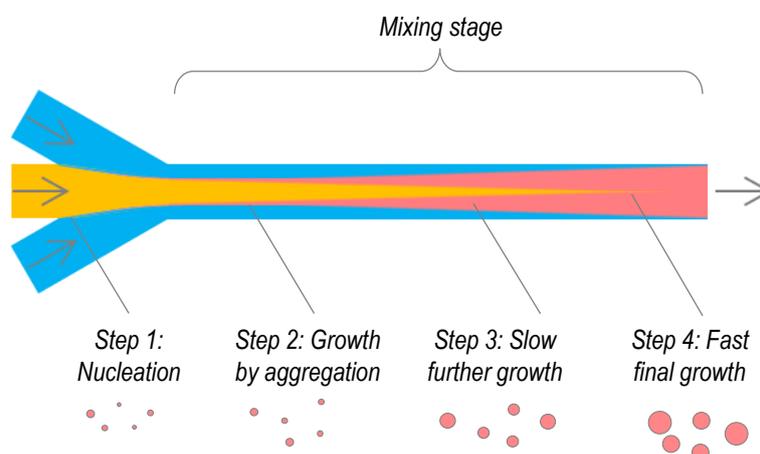


Schematic showing a strategy for synthesizing nanoparticles.

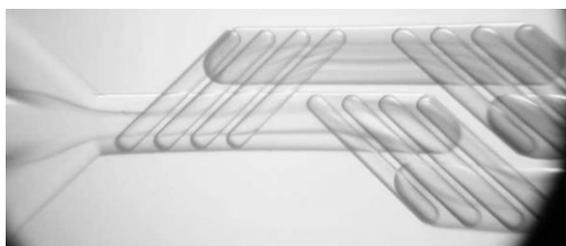
Mechanism – Micromixing – Solvent diffusion method

We propose a bottom-up mechanism of nanoparticle growth[†]. Individual polymer molecules start aggregating ultimately growing into a nanoparticle.

In the formation of PLGA nanoparticles by the Micromixing – 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 randomly 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. Effective mixing ensures that the polymer is evenly distributed throughout the solution and that the particle growth histories are close to identical. This results in the formation of highly monodisperse particles.



Schematic showing a micromixing mediated nanoparticle production strategy.  - Polymer dissolved in organic solvent with high aqueous miscibility,  DI water with 1% PVA (surfactant),  Polymer nanoparticles as solid content dispersed in water with residual solvent. The organic solvent is displaced from the polymer stream and diffuses into the aqueous stream leaving behind a hardened colloidal suspension of nanoparticles. Colors and sizes are illustrative only and not to scale.



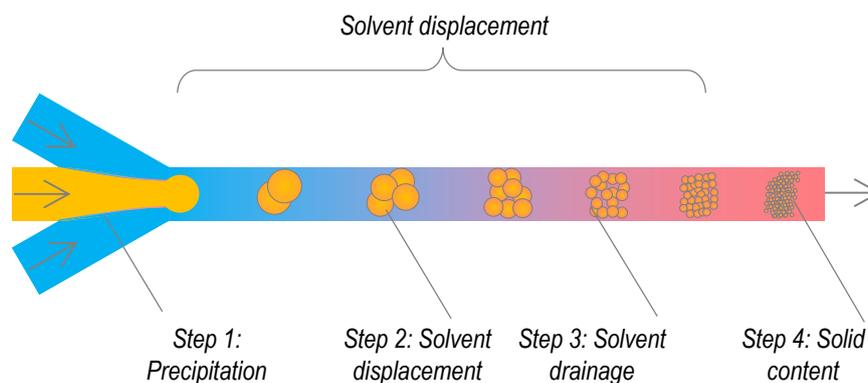
Representative image of droplet formation in a micromixer chip. The miscibility of the fluids causes quick displacement of the solvent into the antisolvent, leaving behind a trail of nucleation growth spots.

[†] *Microfluidic technologies for accelerating the clinical translation of nanoparticles.* Pedro M. Valencia, Omid C. Farokhzad, Rohit Karnik & Robert Langer. *Nature Nanotechnology* 7, 623–629 (2012) doi:10.1038/nnano.2012.168.

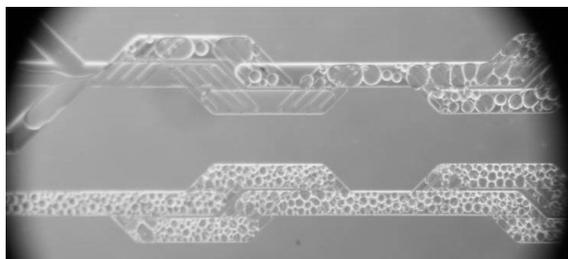
Mechanisms – Emulsification – Solvent diffusion method

We propose a Top-down mechanism of nanoparticle formation[‡]. A droplet of solvent + polymer shrinks into a small polymer nanoparticle.

By contrast the formation of PLGA particles using water-immiscible solvents such as DCM occurs via emulsification. The addition of water to the PLGA-containing solvent and the agitation of the mixture results in the formation of a micro-emulsion. The initially coarse emulsion is sequentially broken down by the successive mixing stages of the micromixer chip into a fine emulsion. Simultaneously the organic solvent diffuses into the surrounding aqueous phase (dichloromethane has a 2% solubility in water at room temperature) resulting in particle hardening and further reduction in diameter.



Schematic showing an emulsification mediated nanoparticle production strategy.  - Polymer dissolved in organic solvent with low aqueous miscibility,  DI water with 1% PVA (surfactant),  Polymer nanoparticles as solid content dispersed in water with residual solvent. The organic solvent is displaced from the organic polymer droplets and diffuses into the aqueous stream leaving behind a hardened colloidal suspension of nanoparticles. Colors and sizes are illustrative only and not to scale.



Representative image of droplet formation in a micromixer chip. The immiscibility of the fluids creates a slug flow. The mixing stages sequentially split the slug into smaller droplets – the total flow rate is expected to significantly affect the final characteristic size of the emulsion.

[‡] *Microfluidics-Nano-Integration for Synthesis and Sensing*. Simona Badilescu and Muthukumaran Packirisamy. *Polymers* 2012, 4, 1278-1310; doi:10.3390/polym4021278.



Dolomite Micromixer System used to make Nanoparticles

In another [application note](#)[§], Dolomite's micromixer system was shown to be effective at mixing fluids at a wide range of flow rates (50 - 1000 μ l/min), and at various viscosities. The mixing strategy used here exploits chaotic advection. The flow field is sufficiently three-dimensional (with secondary flows stretching and folding the fluid) to greatly increase the interfacial area across which diffusive exchange occurs.

The implementation of the micromixer chip in PLGA particle synthesis enables rapid mixing of reaction components (solvent and anti-solvent) thereby ensuring that the growth histories of all the particles in the mixture are close to identical. The outcome is a narrow particle size distributions and reduced reagent consumption,

Dolomite's micromixer system, when used for particle synthesis, brings benefits such as: wide operating pressure range 0 - 6bar, options to control the reaction temperature from room temperature up to 300°C, and improved control over reaction times and temperatures. Furthermore, the wetted parts of the micromixer system are inert and ensure that the reaction is well controlled and confined exclusively to the reactor portion of the system.

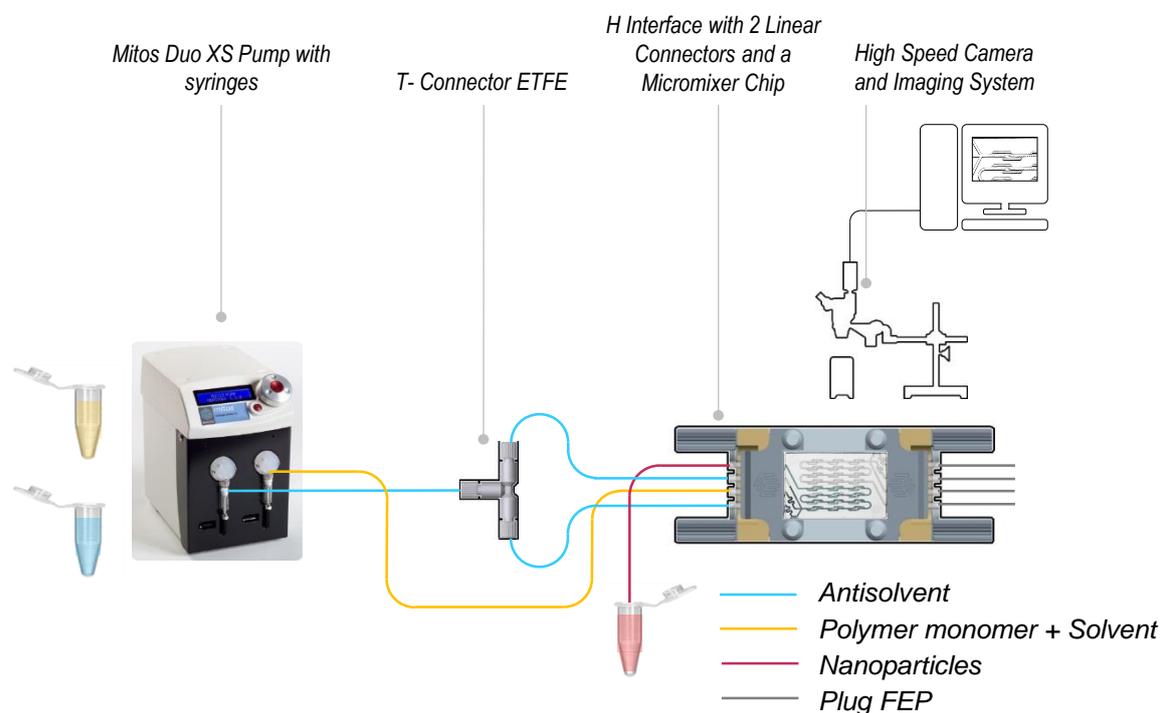
	MICROMIXING METHOD Acetone	EMULSIFICATION METHOD Dichloromethane
Water	Very soluble and forms azeotrope on contact (infinitely at 25 °C)	Sparingly soluble and forms emulsion on contact (2% (v/v) at 25 °C)
PLGA	Good solvent	Excellent solvent

Solubility of organic phase solvents in water or PLGA. .

In this note, uniform sized nanoparticles were prepared by two facile methods. The methods differ only in the reagents used; the system hardware remains identical in both cases.

[§] *Quantifying μ -Scale Mixing Time Using Sodium Hydroxide and Phenolphthalein using Dolomite's Micromixer System. **Application note published on Dolomite's Applications section.***

Test Setup



*Schematic showing representative** setup of a nanoparticle production system. The samples are loaded into the XS-Pump using the multi-port valve block especially designed for this purpose.*

The system setup is shown in the figure above. Fluidic connections between the XS-Pump (Part No. 3200057) are made using FEP tubing of OD 1.8 mm and ID 0.25 mm (Part No. 3200302). The Micromixer Chip (Part Number: 3200401) is a lamination-based compact glass microfluidic device that allows rapid mixing of two or three fluid streams. It is assembled with the H Interface (Part No. 3000155) and two Linear Connectors 4-way (Part No. 3000024). Plug FEP (Part No. 3000056) is used to block off unused ports on the linear connector. End Fittings and Ferrules for 1.6mm Tubing (pack of 10) (Part No. 3000477) are used to ensure leak proof connections between the tubing and the components. Visualization was achieved using a High Speed Camera and Microscope System (Part Number: 3200050). The polymer + solvent flow rate is set to 20 $\mu\text{L}/\text{min}$, and the antisolvent flow rate is set to 100 $\mu\text{L}/\text{min}$.

	Polymer mix(yellow) OD(mm), ID(mm); L(mm)	Antisolvent (blue) OD(mm), ID(mm); L(mm)
Pump to T-connector	1.60, 0.25, 500	1.60, 0.25, 300
T-connector to Chip (x2)		1.60, 0.25, 200
Chip to Collection	1.60, 0.25, 500	

Tubing connections between components.

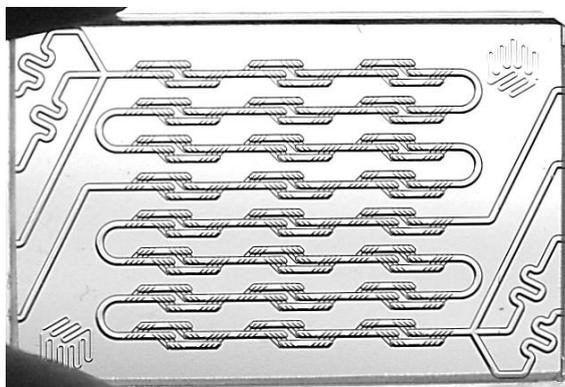
*** It is possible to pump larger volumes of fluid without refill interruption (from 20 ml up to 10 L) using Dolomite's P-Pumps.*

Reagent Preparation

Biodegradable PLGA (75:25 lactide:glycolide, MW 75,000–120,000) was purchased from Sigma Chemicals. The organic solvents acetone (ACE), and dichloromethane (DCM) were HPLC grade purchased from Aldrich Chemicals. The stabilizer poly(vinylalcohol) (PVA, MW 9000–10,000, 80% hydrolyzed) was also purchased from Aldrich Chemicals.

Method	Polymer	Solvent	Method	Antisolvent (with Surfactant)
Emulsification	2% (w/v) poly(lactic-co-glycolic acid)	Dichloromethane (DCM)	Solvent evaporation	1% (w/v) Polyvinyl acetate (PVA) in water
Micromixing	2% (w/v) poly(lactic-co-glycolic acid)	Acetone (ACE)	Solvent displacement	1% (w/v) Polyvinyl acetate (PVA) in water

Reagents are freshly prepared before the tests and loaded into the syringe pump using the multi-port valve.



Magnified view of the micromixer chip. Three fluid inlets merge and mix. The mixture is ‘churned’ by the sequential splitting and combining caused by the especially designed features.

Where possible, all liquid handling was performed in a fume hood to eliminate exposure hazards associated with the reagents used.

This product was stirred overnight to completely evaporate organic solvent. The obtained PLGA nanoparticles were washed three times and collected. Subsequent DLS analysis demonstrated particle sizes of an order of 100 nm in the case of both micromixing and emulsion mediated mechanisms.



Results

Micromixing – Solvent diffusion method

In this application note, micromixing-solvent diffusion method was successfully implemented to generate PLGA nanoparticles with an average particle size of 42.26 nm and size distribution characterised by PDI width of 0.075. Superior control over synthesis parameters and consequently the nanoparticle sizes distribution and other properties were achieved by use of a split and recombine Micromixer Chip. Based on a flow-through scheme, and being pressure driven, the fluid reservoir volume is decoupled from on-chip nanoprecipitation^{††}, thereby enabling scaling-up to large volumes of fluid.

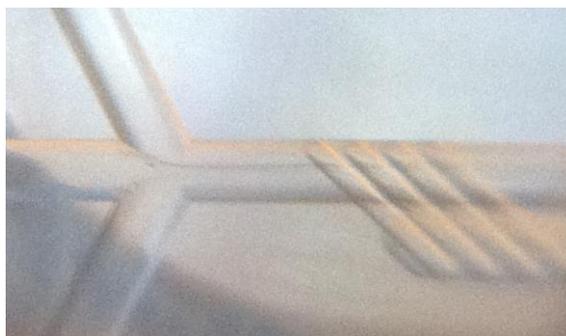
Emulsification – Solvent diffusion method

Emulsion – Solvent diffusion method was successfully implemented to generate PLGA nanoparticles with an average particle size of 94.03 nm and size distribution characterised by PDI width of 0.226. The emulsification – solvent diffusion method leads to larger particles compared to micromixing – solvent diffusion method. This is most likely a consequence of the intrinsic differences between bottom-up particle formation process that occurs during the micromixing – solvent diffusion synthesis procedure and the top-down mechanism that occurs during the emulsification – solvent diffusion process.

^{††} PLGA nanoparticles prepared by nanoprecipitation: drug loading and release studies of a water soluble drug. Thirumala Govender, Snjezana Stolnik, Martin C. Garnett, Lisbeth Illum, Stanley S. Davis. *Journal of Controlled Release*. Volume 57, Issue 2, 1 February 1999, Pages 171–185.

Bright Field Imaging of Mixing Junction

A bright field image of the mixing junction is recorded. The density difference between the fluids causes a refractive index mismatch, showing a distinct interface. This is a soft interface which disappears downstream of the junction. Visual monitoring of the junction is useful to ensure consistency of mixing.



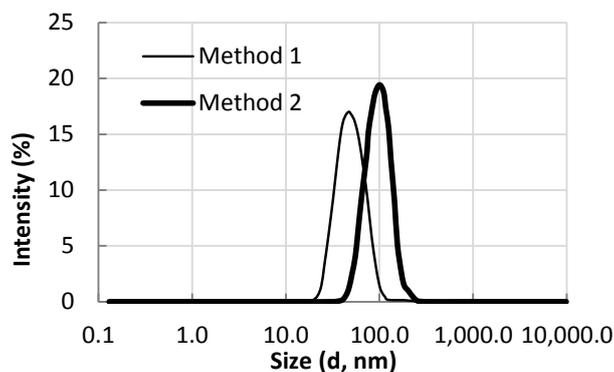
Microscope image of mixing junction.

The output from the chip is routed via the connector to the fluid tubing and finally to a collection vial. The collection vial is pre-loaded with 10 ml of deionised water with surfactant. The collection reservoir is open top so that the organic solvent dissolved in the water effuses off, and is carried away by a ventilation system in a fume hood.

After collecting 5 ml of reaction product, the system is stopped. The polymer in the syringe pump is purged out, and refilled with pure organic reagent. The setup is then run again to clear out any deposited polymer from the flow path.

DLS Characterization

The collected sample is then left on a hot plate at 45 °C with magnetic stirring for one hour. Small volumes are then taken for further analysis. The mean particle size and particle size distribution of the nanoparticles were assessed by dynamic light scattering (DLS).



Method 1: Micromixing. Method 2: Emulsification.

The particle size distributions shown above are summarized below.

	Average size (nm)	PDI width	Count Rate kcps
Micromixing	42.26 nm	0.075	395.3
Emulsification	94.03 nm	0.226	141.5

The PDI or polydispersity index is an indication of how narrow a Gaussian size distribution would be that could represent the fitted DLS data. For DLS the best PDI is 0.0 (infinitely thin delta function) and the worst PDI is 1.0 (a distribution where the mean is the same as the width). A width of 0.3 is often considered the border between mono and polydisperse.



Conclusion

PLGA nanoparticle synthesis is demonstrated using Dolomite's micromixer system. PLGA as an FDA approved material for use is attractive for drug delivery applications.

Previously, PLGA nanoparticles were batch synthesized; now the trend is changing to flow synthesis with small fluid volumes used. The benefit is production of narrow size distribution nanoparticles. This results in fuller utilization of materials as none of the product size falls outside the allowable size limits for in-vivo use.

Two different mechanisms of nanoparticle production are proposed. The difference arises from the physical properties of the reagents. Both tests use the same hardware and flow rates.

- In the first instance, high miscibility reagents are used leading to a micromixing method of fluid interaction. Acetone with a high solubility in water is rapidly displaced, leaving behind a wake of nanoparticles.
- In the second instance, dichloromethane being of lower solubility in water creates initially an emulsion. The gradual diffusion and sequential splitting of the emulsion leads to volume shrinkage based nanoparticle production.

Comparing the two methods, the size distribution from the micromixing method of production leads to smaller nanoparticles with a narrower size distribution.

The ability to synthesis PLGA nanoparticles 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 micromixer 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 A: System Component List

Part No.	Part Description	#
3200057	Mitos Duo XS Pump	1
3000252	Syringe for Mitos Duo XS-Pump (1ml) ‡	2
3000155	H Interface	1
3000024	Linear Connector 4-way	2
3200401	Micromixer Chip	1
3200050	High Speed Imaging System	1
3000397	T- Connector ETFE	1
3200063	FEP Tubing, 1/16" x 0.25mm, 10 metres	1
3000477	End Fittings and Ferrules for 1.6mm Tubing (pack of 10)	1
3000056	Plug FEP (pack of 10)	1
3800073	PTFE Tube Cutter	1

Optional Extras

Part No.	Part Description	#
3200037	Sample Injection Valve	1
3000222/ 3000223	Hotplate 110 OR Hotplate 230 (depending upon country specific voltage requirement)	1
3200111	Hotplate Adaptor	1

‡ Syringes are available in volumes of 50, 100, 250, 500, 1000, 2500 and 5000 µL.

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