nanoComposix
A FORTIS LIFE SCIENCES COMPANY

PLGA Nanoparticles – Bridging the Gap from R&D to GMP

White Paper

1. Introduction

Over recent decades, drug delivery vehicles and other biomedical technologies based on **poly(lactic-co-glycolic acid) (PLGA)** have been developed and approved, taking advantage of the excellent biocompatibility, tunable degradation and release characteristics, and high versatility of the polymer. While the bulk polymer is approved by both the Food and Drug Administration (FDA) and European Medicine Agency (EMA) and is listed in the US Pharmacopoeia (USP) as a pharmaceutical excipient, there are few examples taking advantage of the unique polymer properties for use in nanomedicine applications.

Recent research and development efforts have focused on changing this, however, using PLGA not just as an inactive material but as an integral component of nanoparticle formulations that enable a wide range of nanomedicine applications including drug targeting and delivery, imaging, immunoassays, and medical devices.

One past barrier in translating laboratory discoveries to commercial application has been the lack of robust manufacturing techniques that allows production of PLGA nanoparticles to be prepared at commercially relevant scales. This white paper provides a comprehensive description of available technologies for PLGA nanoparticle scale-up, along with characterization and quality control methods, that can be used for initial feasibility and development studies, through to process development, and regulated manufacturing. The combination of robust fabrication methods, characterization, and manufacturing controls are critical for a successful translation from R&D through to clinical use.

2. Initial Development of PLGA Nanoparticle Formulations

One of the first steps involved with development of PLGA nanoparticle formulations involves selection of the PLGA composition and molecular weight, along with selection of other integral components of the particles. PLGA can be synthesized in a wide range of molecular weights (MW) to control the degradation rate of the synthesized materials. Depending on molecular weight, this degradation can occur over a few weeks (low MW) to several months (high MW). In addition, the relative ratio of the two polymers (lactic and glycolic acid, LA and GA, respectively) also has a significant impact on the degradation rate of the particles. **Figure 1** shows a schematic overview over the chemical structure of PLGA and options for modifying PLGA nanoparticle formulations for biomedical applications, and an electron microscope image of typical PLGA nanoparticles is shown in **Figure 2.**

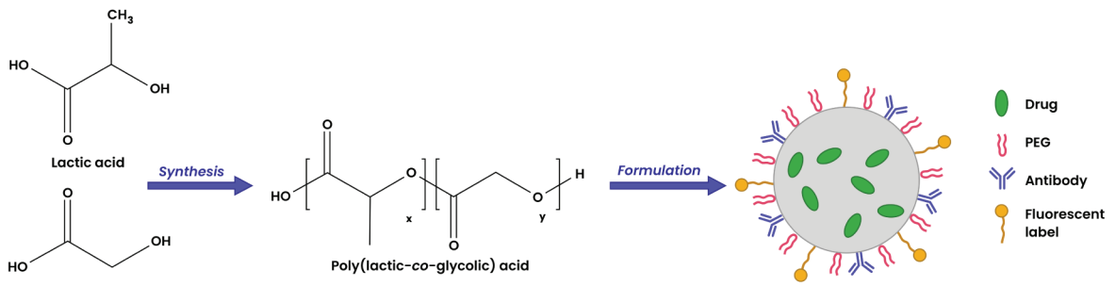


Figure 1. Chemical structure of the PLGA polymer and formulations of PLGA particles for drug delivery and therapeutics.[1]

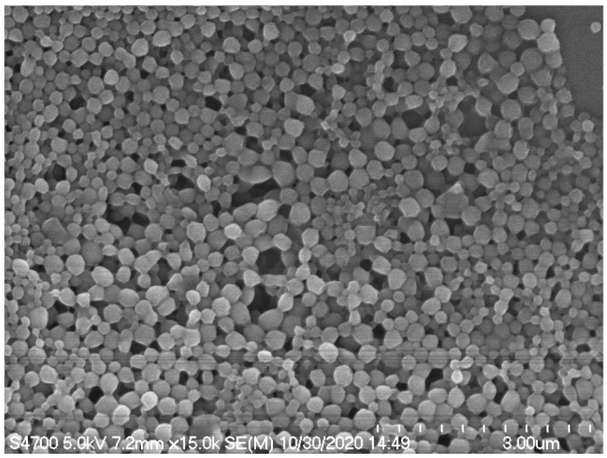


Figure 2: Scanning Electron Microscope (SEM) image of PLGA nanoparticles prepared at nanoComposix

Often, initial particle development work is performed to produce demonstration batches at the laboratory scale for proof-of-principle testing and refinement of nanoparticle properties. Several technologies and devices can be used for the production of PLGA nanoparticles at this scale, commonly via emulsion or nanoprecipitation.

EMULSION FABRICATION

Emulsion fabrication is the most common preparation method at small bench scales and allows encapsulation of both hydrophobic and hydrophilic drugs in PLGA nanoparticles or microparticles. Briefly, PLGA is dissolved into a volatile organic solvent (oil) that is emulsified with a surfactant or stabilizer in an immiscible phase (usually water). If drug loading is to be performed, hydrophobic drugs are added directly to the oil phase, whereas hydrophilic drugs may be first emulsified with the polymer solution prior to particle formation. High intensity sonication bursts can be employed to facilitate formation of small polymer droplets. The resulting emulsion is added to a larger aqueous phase and stirred for several hours, allowing the solvent to evaporate. The polymer precipitates as the solvent is removed, and the final nanoparticles are collected and washed by centrifugation prior to lyophilization and long-term storage. The method is illustrated in Figure 3.[2,3,4]

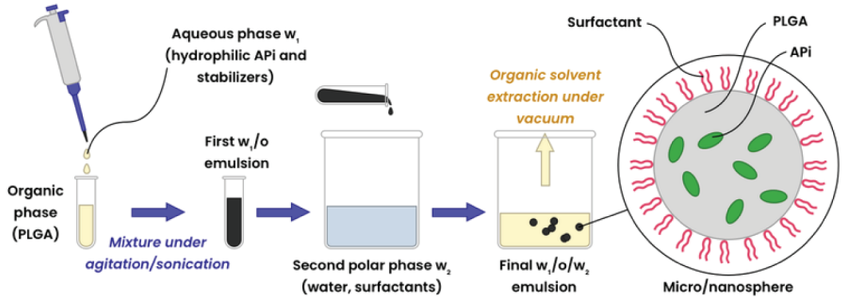


Figure 3: Overview of the double emulsion procedure (water/oil/water, w1/o/w2) to obtain PLGA micro-/nanoparticles.

Although this is one of the most well know methods to produce PLGA particles it utilizes techniques – vortexing, evaporation, centrifugation, and sonication – that are sensitive to the production scale and difficult to translate to larger production volumes and may limit the reproducibility of drug encapsulation efficiency and particle size from batch to batch. The evaporation step requires use of heat and vacuum, which results in varied particle formation kinetics at different reaction scales, introducing additional variability in the process during scale-up. To avoid these issues, an alternative nanoprecipitation approach is often used.

NANOPRECIPITATION

Nanoprecipitation is a simple, one-step process with high reproducibility and low energy input. Also known as interfacial deposition, nanoprecipitation uses two solvents that are miscible with each other. First, the polymer and drug are dissolved in an organic solvent, such as acetone or DMSO, and then controllably introduced into an aqueous solution under agitation. The agitation causes interfacial turbulence, rapid polymer dissolution, and precipitation of the polymer to form nanoparticles.[5]

To enhance particle stability, the introduction of a surfactant or polymer (such as polyvinyl alcohol, PVA) stabilizer can be valuable. After removal of the organic solvent the nanoparticles are rinsed with water to remove excess surfactant and collected as a pellet using centrifugation. A schematic of the process is shown in **Figure 4**. The size and polydispersity index of the resulting nanoparticles can be tailored to specific applications by varying the solvent, polymer, surfactant, their concentration, solvent/non-solvent ratio, and mixing speed.[6]

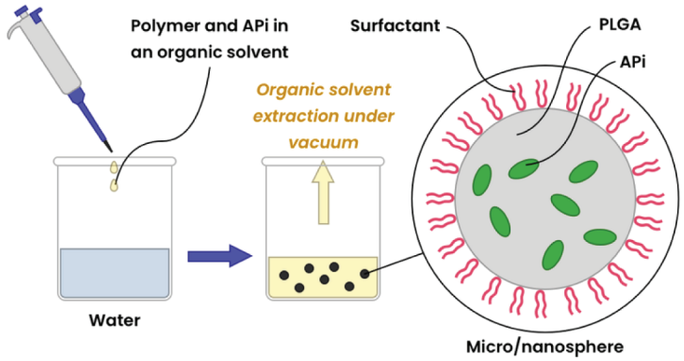


Figure 4: Overview of the nanoprecipitation formulation method

At nanoComposix, we have developed nanoprecipitation methods for formulating PLGA nanoparticles loaded with both hydrophobic and hydrophilic cargo, and with molecular weights from <0.5 kDa up to 500 kDa. molecular weight. The particle size can be tuned between ~50 to ~300 nm. Some examples of PLGA nanoparticles fabricated with different cargo are show in **Table 1**, demonstrating that this method yields stable particles with a low polydispersity index and high encapsulation yield.

Table 1: PLGA nanoparticles prepared by nanoprecipitation encapsulating hydrophobic (disulfiram, fluorescein) and hydrophilic (IgG) cargos.

Cargo	Hydropathic character	Hydrodynamic diameter (nm)	PDI	Encapsulation efficiency (%)	Loading (%)
None (PLGA only)	-	150	0.02	-	-
Disulfiram	Hydrophobic	140	0.20	39	4
Fluorescein	Hydrophobic	200	0.05	18	2
IgG antibody	Hydrophilic	220	0.14	95	2

Although it does not offer the flexibility in reaction conditions provided by other fabrication methods, nanoprecipitation is still a robust and scalable method that can be transitioned from benchtop fabrication to industrial-scale production. As an example, PLGA nanoparticle reactions were carried out at a standard bench scale and run under similar conditions by scaling the reaction by a factor of 10×. The results shown in **Table 2** demonstrate consistency in particle size, polydispersity index, and zeta potential compared to the original reaction scale. At nanoComposix, we have the capability to bulk manufacture these nanoparticles in reactors ranging from 2 to 50 liters.

Table 2: : Scale up of PLGA nanoparticles fabrication to 10× scale.

Cargo	Reaction Scale	Hydrodynamic diameter (nm)	PDI	Zeta potential (mV)
None (PLGA only)	1x	160	0.05	-8
None (PLGA only)	10x	160	0.06	-8
IgG antibody	1x	210	0.04	-5
IgG antibody	10x	230	0.09	-5

3. Scale-up and Reproducibility of PLGA Formulations

Although nanoprecipitation does offer a route to scaling the fabrication of PLGA nanoparticles, other fabrication techniques provide greater control over process parameters and an easier route for scale-up. Below we discuss two methods of flash nanoprecipitation and their advantages with moving toward a robust, scaled fabrication process.

FLASH NANOPRECIPITATION

Flash nanoprecipitation (FNP) is a new technique that uses rapid mixing to generate polymeric nanoparticles encapsulating a wide variety of substances inside the core. Though often used to encapsulate hydrophobic cargo, at nanoComposix we have successfully loaded PLGA particles with hydrophilic molecules including nucleic acids, proteins, peptides, and antibodies. This method involves the rapid combination and mixing of a water-miscible organic solvent containing polymers and cargo with an aqueous anti-solvent in a specially designed turbulent mixing chamber. This leads to a kinetic process that includes two steps: (1) nucleation and growth of the cargo under high supersaturation conditions, and (2) simultaneous stabilization by the precipitation of the polymer. Compared to traditional nanoprecipitation, the FNP process generates supersaturation more rapidly through turbulent micro-mixing, making it more robust and scalable.

Recently, systems that can manipulate fluids in millimeter-scale channels have been shown to be suitable for large scale production of PLGA nanoparticles. The process uses either confined impingement jet mixer (CIJM) or multi-inlet vortex mixer (MIVM).[7] Using rapid micromixing that takes place on the order of milliseconds, homogeneous supersaturation conditions and controlled precipitation occur in the mixing chamber. At nanoComposix we are equipped with both mixers and typically recommend the CIJM for rapid formulation screening and the use of the MIVM in the scale-up process. Both techniques may be employed to prepare PLGA nanoparticles with sizes ranging between 60 and 300 nm. The flexibility of these equipment types is important, as the type of cargo, solvent and anti-solvent composition, addition of stabilizers, and reaction temperatures also have a significant influence on the formation of nanoparticles and this equipment allows greater tuning of the particle formation process to achieve a desired particle size and formulation. The continuous, controllable, and repeatable production of uniform nanoparticles by microfluidic mixer devices makes it easy to scale from laboratory to industrial scale, while maintaining high loading capacity and encapsulation efficiency. Both types of mixers are described in more detail below.

Confined Impingement Jet Mixer (CIJM)

A CIJ mixer is the simplest mixer design for FNP, permitting mixing of two streams in a scalable and continuous fashion, shown schematically below in Figure 5. While CIJM is an excellent tool for developing PLGA formulations, it has broader applicability and has also been used in the preparation of drug polymeric micelles[8], other polymeric nanoparticles,[9] and solid lipid nanoparticles.[10]

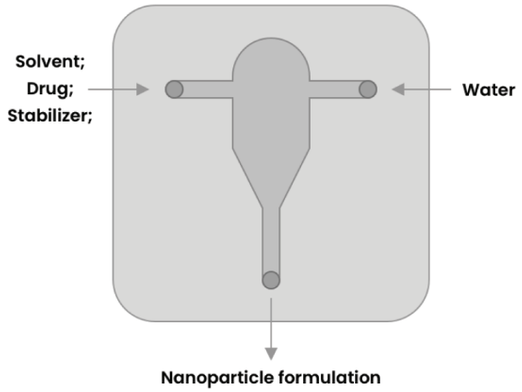


Figure 5: Detailed configuration of the Confined Impingement Jet Mixer (CIJM) mixing chamber, components flow and nanoparticles formation.

CIJM consists of two streams driven by a syringe pump that jet into the mixing chamber in opposing directions. The CIJM design is compact making it an ideal option for rapid screening and developing formulations during early development phases. The high mixing efficiency and uniformity via CIJM creates high supersaturation and high nucleation rates, generating small and uniform nanoparticles with high drug encapsulation and drug loading capacity. CIJM is a continuous process, and scale-up is accomplished simply by increasing the run time. In CIJM, the flow rate of the liquid jets is a critical parameter to control the nanoparticle precipitation and drug encapsulation. An increase in flow rate contributes to a higher supersaturation level, resulting in a higher nucleation rate, leading to smaller and more uniform particles. The main limitation of this type of mixer is that the maximum of two inlet streams limit the diversity of formulation.

An example of the ability to tune PLGA nanoparticle size using the CIJM by varying the flow rates of water and solvent (keeping the ratio between the two fixed at a one-to-one volume ratio) and varying the stabilizer concentration is shown below in **Figure 6.** Keeping the preparation conditions fixed, using 0.1% PVA, and varying the total flow rate allows the particle size to be tuned from approximately 150 nm down to 90 nm in size. Increasing the PVA concentration increases the particle size, and additional tuning of the formulation composition and mixing parameters allows further rational adjustment of the particle size.

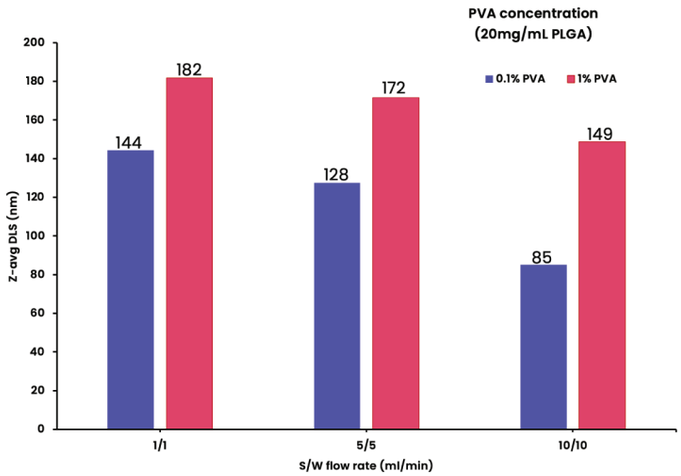


Figure 6: The relationship between the flow rate of Solvent/Water and stabilizer (PVA) concentration on the PLGA nanoparticle size, measured by DLS.

As a separate example, the CIJM was used to formulate PLGA nanoparticles encapsulating a hydrophobic anticancer drug, disulfiram, and characterization of the particles indicated successful loading of disulfiram inside PLGA nanoparticle core. The CIJM-produced formulation achieved a drug loading of 3-4% (w/w) at a targeted particle size of 290 nm and 170 nm by adjusting the flow rate of S and W. The results are presented in **Table 4.**

Table 4: PLGA nanoparticles prepared by flash nanoprecipitation using the CIJM encapsulating hydrophobic (disulfiram) cargos. Mixer S/W ratio (v/v) is 1:1.

Disulfiram concentration	Flow rate (mL/min)	Hydrodynamic diameter (nm)	PDI	Encapsulation yield (%)	Loading yield (%)
10%	1/1	290	0.17	30	3
10%	5/5	170	0.09	41	4

Multi-Inlet Vortex Mixer (MIVM)

The MIVM, also commonly used for flash nanoprecipitation, has similar rapid mixing capabilities as the CIJM and similar ease of operation, but offers additional flexibility in formulation parameters and further scaling capabilities. A schematic of a MIVM mixer is shown in **Figure 7.**

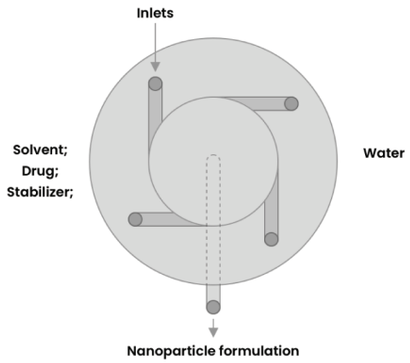


Figure 7: Detailed configuration of the Multi-Inlet Vortex Mixer (MIVM) mixing chamber, components flow and nanoparticles formation.

This device features four inlets and liquid streams at fixed angle that are propelled at high velocity by syringe pump or pulse-free continuous flow pump, producing rapid mixing in a specially designed chamber. Like the CIJM, the flow rate is an important parameter for MIVM. In addition to the flow rate, MIVM has a higher flexibility for stream arrangement and flow due to multiple inlets, allowing the use of different solvent and water flow rates for each inlet. Because of the flexibility to adjust the solvent ratios and materials by varying the content and flow velocity of incoming streams, MIVM has wider application than CIJM in the preparation of drug nanoparticles. The MIVM can produce stable and small drug nanoparticles without further dilution; in addition to PLGA nanoparticle formulations, the MIVM can also be used for the preparation of drug polymeric micelles[15], polymeric nanoparticles[16], polyelectrolyte complexes[17], nanocrystal drugs[18], and solid lipid nanoparticles[19].

Using the MIVM we formulated PLGA nanoparticles using ester-terminated PLGA or carboxyl-terminated PLGA. The size of the nanoparticles prepared from the PLGA-ester polymer is around 140 nm and from the PLGA-COOH polymer is 120 nm, with some tuning of particle size again possible through adjustment of the overall solvent ratios as shown in **Figure 8**, and both type of nanoparticles had PDI values below 0.2, indicating good uniformity.

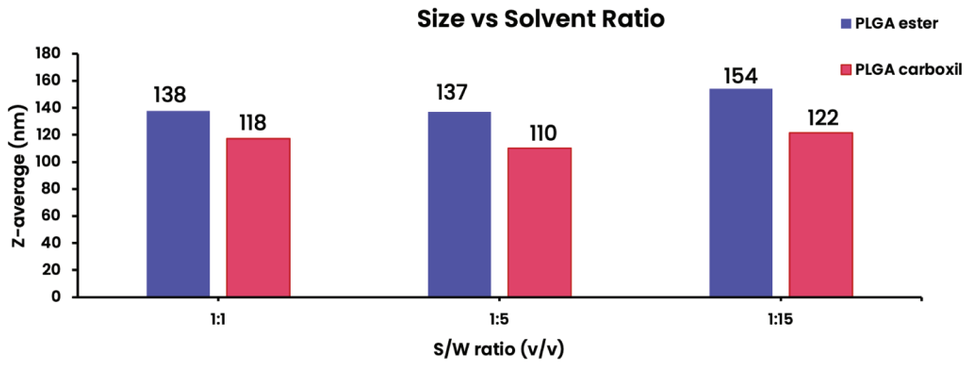


Figure 8: noparticle size produced using either ester-terminated or carboxyl-terminated PLGA using the MIVM.

The PLGA-ester polymer was further used to encapsulate ovalbumin (OVA) or doxorubicin HCl (DOX), and the PLGA-COOH polymer to encapsulate disulfiram. The inclusion of cargo in the PLGA nanoparticles had some small effects on particle size **(Table 5)**, which could be tuned by further optimization of the system parameters. Overall, even without additional optimization, nanoparticles formulated using this method maintained similar size and the PDI remained small, again indicating good uniformity. The loading encapsulation ranged from 2% for the PLGA-ester(DOX) to 6% PLGA-ester(OVA), comparable to typical loading efficiencies of around 5% reported in literature.

Table 5: PLGA-ester and PLGA-COOH terminated nanoparticles prepared by flash nanoprecipitation using the MIVM encapsulating hydrophilic (ovalbumin and doxorubicin HCl) and hydrophobic (disulfiram) cargos. Mixer configuration is S/S/W/W.

Cargo	Final S/W (v/v)	Hydrodynamic diameter (nm)	PDI	Loading efficiency (%)
Ester-terminated PLGA				
None (PLGA only)	1/5	140	0.05	-
Ovalbumin (10%)	1/5	110	0.12	6
Doxorubicin (10%)	1/19	220	0.19	2
Carboxyl-terminated PLGA				
None (PLGA only)	1/5	100	0.14	-
Disulfiram (10%)	1/5	130	0.26	4

Both CIJM and MIVM mixer produces very uniform particles in the size range of 100-300 nm with PDI values below 0.3. Moreover, the MIVM offers more flexibility by using different configurations of the solvent to water ratio together with the ability to scale-up the process while still being able to control the size and polydispersity.

GENERAL STRATEGY FOR BUILDING A PLGA NANOPLATFORM

When building a PLGA nanoplatform, the overall size of the particles is one of the critical parameters. Nanoparticle size can influence factors such as drug release, biodistribution, and other critical drug delivery parameters. Ideal formulation size is disease indication and administration route dependent, so this varies by application. Nanoparticle size can be tuned by carefully choosing the solvent or simply adjusting the method of preparation. For example, as can be seen in **Figure 10**, within one synthetic method, changing the solvent from DMSO to acetonitrile can have a significant impact on the final particle size. In addition, if the application requires the use of a specific solvent, due to drug solubility or better removal of the solvent, it is possible to generate particles with different sizes simply by changing the method from MIVM to nanoprecipitation. On the other hand, this impact of the method on the final dimensions needs to be considered when planning the scalability of the reaction. For this reason, the use of CIJM for small scale optimization is an appropriate option if the goal is to use MIVM at larger scale due to the minimum variation between the two techniques but choosing the nanoprecipitation during the small scale optimization might not be appropriate if the final scale requires the MIVM.

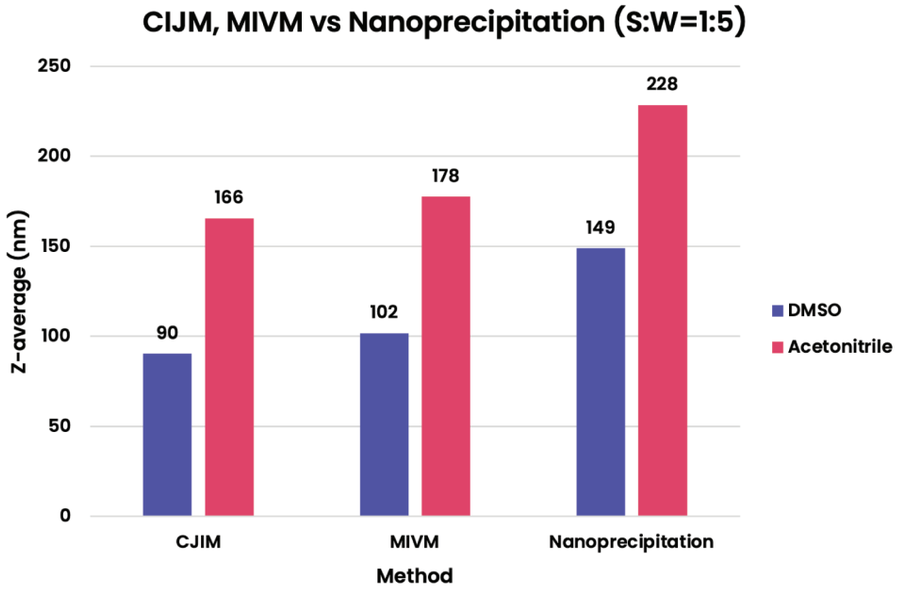
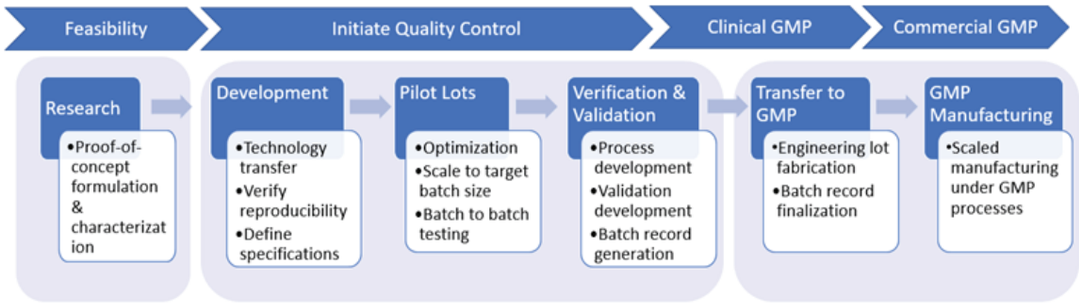


Figure 10: Impact of solvent and/or method of synthesis on overall dimension of PLGA particles prepared at a solvent:water ration of 1:5

4. Development and GMP Manufacturing

Usually, a complex project that is aimed for a Phase I clinical trials is run in phases. A Phase I starts with a Technology Transfer and/or development and optimization of the process, if needed. Usually, this phase will run for about 1-3 months depending on the complexity of the project. Next, in the Phase II the scaling up of the process will start to be developed based on the findings from Phase I. Towards the end of a Phase II engineering batches are going to be run. The length of this phase will be determined based on the findings from Phase I. The last phase, is the actual GMP manufacturing of the product of interest. The flow of the process is described in the figure below.



nanoComposix offers services to produce PLGA particles at scale to supply material for pre-clinical and Phase I/Phase II clinical trials with GMP and ISO 13485 compliance. This includes the following capabilities:

Purification: The foremost exploited technologies for nanoparticle purification are membrane separation techniques(tangential flow filtration (TFF), also called cross-flow filtration) and continuous flow centrifugation. At nanoComposix, we are experts in purifying nanoparticles by tangential flow filtration in order to wash and remove remaining solvent and unreacted precursors. TFF is a type of filtration that can be run continuously and the fluid flows along the surface of a filter membrane rather than passing through it. The main advantage is that the filter cake does not settle on and block the filter during washing, but rather is flushed away during the process, increasing the length of time a filter unit can remain operational.

Storage: In addition to the degree of purity, the physicochemical integrity of nanomedicine must be preserved over time throughout its shelf-life. It has been shown that hydrolytic degradation of PLGA particles in aqueous media can take place as early as 15 days depending on the PLGA molecular weight, lactide: glycolide ratio and particle morphology[20]. Therefore, an efficient removal of water content is particularly needed to improve the nanomedicine stability, as well as to facilitate handling, reduce storage space and transportation costs. Fortis Life Sciences lyophilizes the colloidal suspensions to facilitate particle stability and storage. The freeze drying/lyophilization process is performed in the presence of sugars as a cryoprotectant to avoid particle aggregation.

Sterilization: Lastly, for systems intended for parenteral administration, finding an appropriate sterilization method is a crucial final step in manufacturing. The most common method used at nanoComposix is the sterile filtration of the final nanoparticle product. Nevertheless, this process only works for particles with a size distribution that falls almost entirely below 0.22 µm, to allow for terminal sterilization via filtration. Other sterilization methods utilize E-beam or gamma radiation, but this can be detrimental to sensitive APIs, cause PLGA chain alteration, and affect the overall characteristics of the nanoformulation itself. Finally, endotoxin levels are tested as routine characterization procedure. Another option is to totally isolate the production from

the surrounding environment and operators by implementing and aseptic manufacturing. However, this process can be costly and difficult to implement. Another possible route towards bioburden reduction is pasteurization.

At this phase, batch records and analytical methods will be developed, qualified, and validated in a phase appropriate manner. One or more engineering runs will be performed using materials from an Approved Supplier List. Engineering runs may be performed in the nanoComposix cleanroom and a demonstration batch under cGMP will be performed. Additional lots for process qualification or validation will be included as required. Milestones will be developed in partnership with nanoComposix prior to beginning this stage.

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Table comparing fabrication methods

Emulsion	Nanoprecipitation	Flash nanoprecipitation (CIJ and MIVM)
ADVANTAGES		
Single emulsion for hydrophobic agents Multiple emulsions for hydrophilic agents High and industrial scale production Control over the particle size Different particle size achievable from nm to µm Easy and fast process Different probe size available to afford different batch sizes Continuous processing possible High loading and encapsulation efficiency	Scalability Low energy mixing Reproducibility Single step High simplicity Fast and reproductible Simple and inexpensive set-up Low sample and reagents consummation Narrow size distribution Moderate conditions suitable to process sensitive APIs Continuous processing possible Reduced energy usage	Fast processing Simple equipment Scalable Moderate conditions suitable to process sensitive APIs Continuous processing possible Fast and reproductible Simple and inexpensive set-up Narrow size distribution Versatile instruments, easy to use, simple to set up and cost effective

DISADVANTAGES		
Needs heat or vacuum for evaporation Instability of w/o/w emulsion Solvent or stabilizer residuals Negative effect of sonication and organic solvent on APIs integrity Batch to batch variation Only for small batch preparation Change of formulation properties upon scaling up the production Direct sample contact with probe Cooling jacket necessary during the sonication process Complicated and specific instruments	Low encapsulation efficiency of hydrophilic APs Particle size distribution can be affected by mixing Negative effect of organic solvent on hydrophilic APIs integrity Size range around 200 nm only	APIs and solvents limited Channel clogging and fouling Size range around 200 nm only