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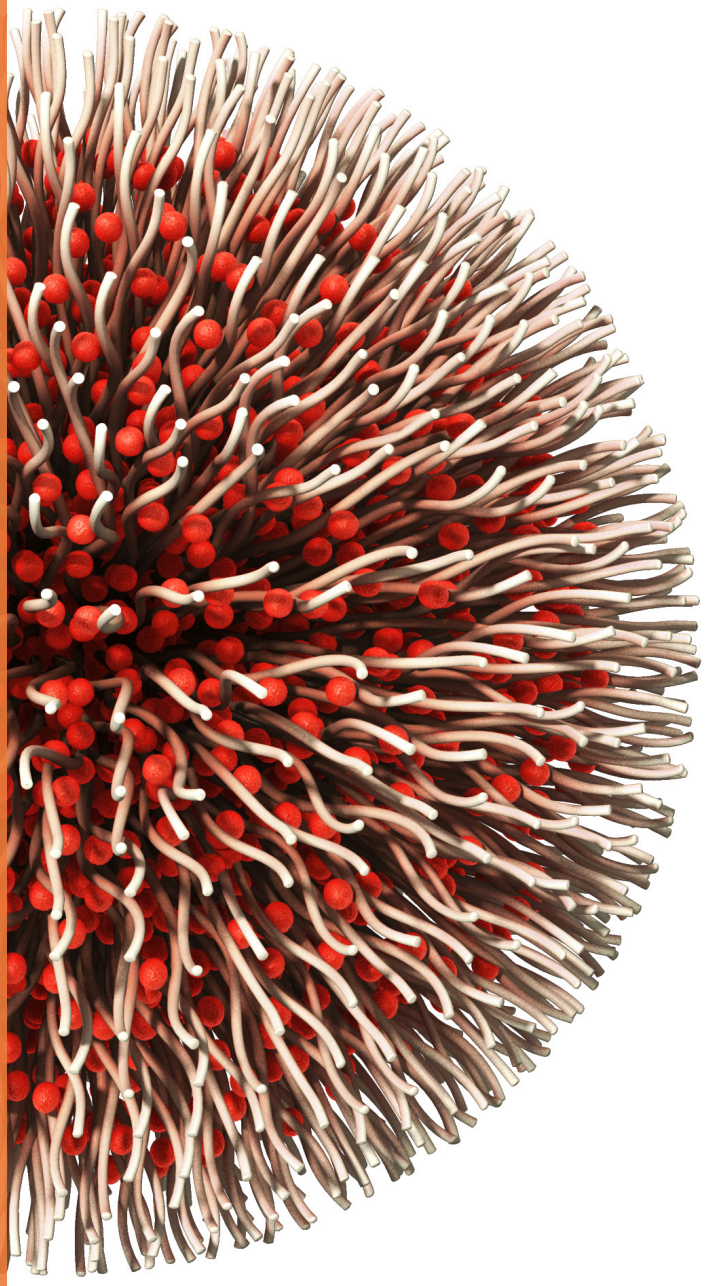
PLGA Nano-particles

Tuning Particle Size Using The NanoAssemblr® Benchtop Instrument



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Abstract

Emerging evidence from a growing number of studies about nanoparticle-based drugs indicates that the size of these drug-loaded nanoparticles (NPs) plays a critical role in drug efficacy and ultimate clinical success. It is therefore important that manufacturing processes enable precise NP size control during the production process. Current methods for producing NP drugs offer a range of achievable NP sizes but lack the ability to specifically control NP size while maintaining high drug encapsulation efficiency and low polydispersity. Here, we present a novel solution for NP production using the NanoAssemblr® Benchtop instrument, which utilizes microfluidic mixing driven by computer controlled pumps to offer a high degree of control over the solvent/antisolvent nanoprecipitation process and hence the ability to rationally optimize NP properties. Instrument and formulation parameters were systematically explored to tune NP size on the NanoAssemblr® using a representative polymeric poly(lactide-co-glycolide) (PLGA) NP system. Higher flow rates of reagents through the microfluidic mixer resulted in smaller particles, while higher aqueous-to-solvent mixing ratios increased particle size. Additionally, increasing the polymer concentration in the solvent phase led to increased particle size. Through examination of 4 parameters, particle sizes were tuned between 70 and 200 nm with PDIs < 0.2.

Introduction

With the increasing amount of research and development centered on nanoparticle (NP) based therapeutics and drug products, it is evident that NP size plays an important role in drug efficacy. NP size may impact the drug's tissue adsorption and bio-distribution,[1-3] so the ability to reproducibly tune the size of drug-loaded NPs is extremely important for clinical success. Current NP production methods lack precise size control and are difficult to scale up to large clinical production batches. The NanoAssemblr microfluidic platform addresses these shortcomings by enabling users to control the environment during NP precipitation through computer controlled parameters. This leads to precise NP size control, a high degree of particle uniformity, and batch-to-batch reproducibility. Furthermore, formulations can be scaled up by increasing the quantities of materials pumped through the system or by employing multiple microfluidic mixers in parallel. These features, significantly reduce time and cost associated with developing NP-based drug candidates.[4]

Optimization of NP size on the NanoAssemblr platform is achieved via modulation of built-in instrument parameters that control fluid mixing within the microfluidic cartridge and/or by altering the formulation parameters. The first instrument parameter is the total flow rate (TFR), which controls the speed at which the aqueous and solvent phases are mixed together within the microfluidic cartridge. The second instrument parameter is the flow rate ratio (FRR), which controls the mixing ratio of the aqueous and solvent phases. By systematically varying one or both of these instrument parameters, one can achieve a variety of reproducible nanoparticle sizes that can be optimized for a given application.

Additionally, NP size can be tuned on the NanoAssemblr by altering formulation parameters, such as the concentration of the starting materials dissolved in the aqueous and solvent phases (i.e. increase/decrease the concentration of polymers in the solvent phase and/or changing the concentration of stabilizers in the aqueous phase). Here, we present a case study on how these different parameters can be used to tune the size of a NP composed of poly (lactic-co-glycolic) acid (PLGA) core and a poly (vinyl alcohol) (PVA) coating using the NanoAssemblr microfluidic mixing platform. The tested parameters are summarized in ***Table 1.***

Instrument Parameters

Total flow rate (TFR)	2 - 12 mL/min
Flow rate ratio (FRR)	1:1 - 9:1 (aqueous:solvent)

Formulation Parameters

Polymer (PLGA) concentration	5 - 40 mg/mL
Stabilizer (PVA) concentration	0.5 - 4.0 % w/v

Table 1. *Parameters Tested on the NanoAssemblr Benchtop Instrument*

Result

PLGA NPs were manufactured on the NanoAssemblr Benchtop instrument via microfluidic mixing, illustrated in ***Figure 1.*** This approach achieves rapid and uniform mixing of the PLGA (solvent phase) with the stabilizer poly(vinyl alcohol) (PVA, aqueous phase) which drives controlled precipitation of the PLGA NP.

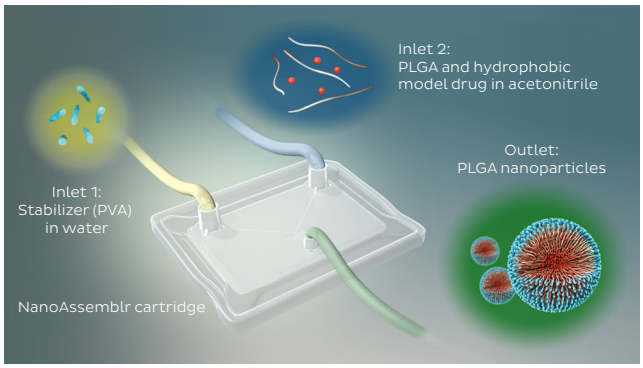
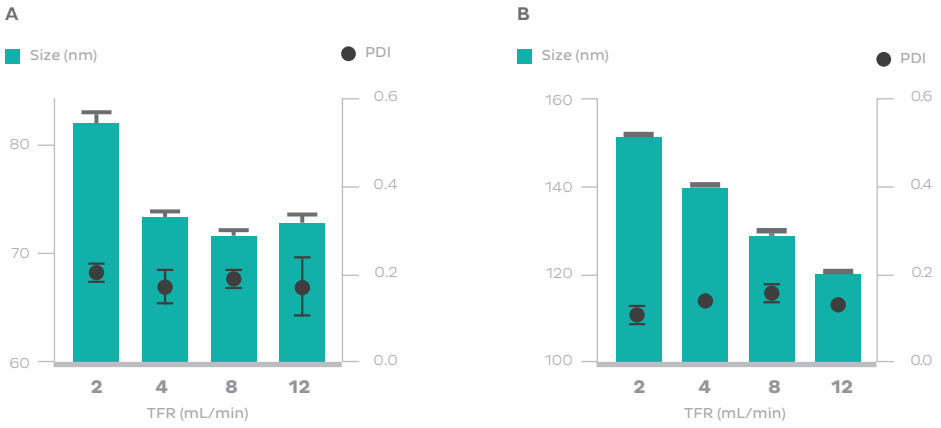


Figure 1. *Illustration showing the manufacture of PLGA nanoparticles using the NanoAssemblr Benchtop.*

EXPLOITING INSTRUMENT PARAMETERS TO TUNE THE SIZE OF PLGA NP

The effect of total flow rate (TFR) on the size of PLGA particles using a fixed concentration of PLGA and PVA is demonstrated in ***Figure 2.*** Shown in ***Figure 2A,*** NPs produced using 5 mg/mL of PLGA showed a small decrease in size, from ~82 to ~73 nm, as TFR increased. NPs produced using a higher concentration of PLGA at 20 mg/mL ***(Figure 2B)*** exhibited a larger decrease in the particle size, ranging from ~150 nm down to ~120 nm, as TFR increased from 2 to 12 mL/min. The PDI remained consistent (~0.2) regardless of PLGA concentration or TFR.



Polymer (PLGA) concentration	5 or 20 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	2 - 12 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

Figure 2. *Effect of total flow rate (TFR) on the size and polydispersity (PDI) of PLGA NP at polymer concentrations of (A) 5 mg/mL and (B) 20 mg/mL using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for three independent size/PDI measurements on three independent samples (n = 3).*

Figure 3 demonstrates the effect of flow rate ratio (FRR) on the size and PDI of PLGA NPs. The aqueous:solvent FRR is the ratio of the two phases that are mixed with each other as they are pumped through the microfluidic device; a ratio of 3:1 indicates that 3 parts aqueous phase (PVA in water) is mixed with 1 part solvent phase (PLGA in acetonitrile). As shown in ***Figure 3,*** as the FRR increased from 1:1 to 9:1, the PLGA NP size increased from ~135 to ~160 nm.

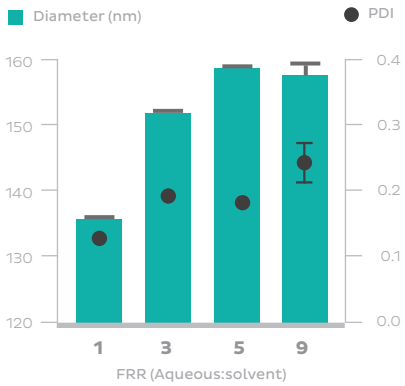


Figure 3. *Effect of aqueous:solvent flow rate ratio (FRR) on the size and PDI of PLGA nanoparticles at polymer concentrations of 20 mg/mL using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	20 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	12 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1 - 9:1

EXPLOITING FORMULATION PARAMETERS TO TUNE THE SIZE OF PLGA NP

The other approach to tune the size of the NPs is to alter the formulation parameters, such as polymer concentration, shown in ***Figure 4.*** As the concentration of PLGA increased from 5 to 40 mg/mL, NP size also increased from ~70 to ~200 nm while maintaining a PDI around 0.2.

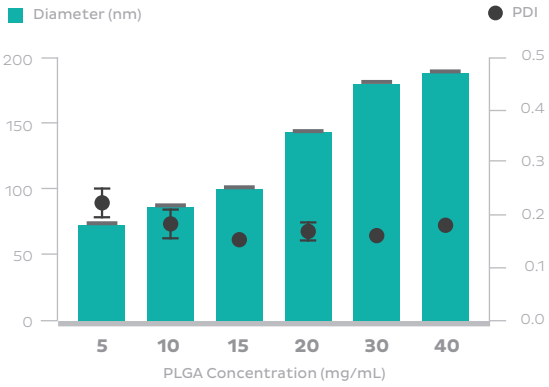


Figure 4. *Effect of PLGA polymer concentration on the size and PDI of PLGA nanoparticles using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	5 - 40 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

A PLGA concentration of 5 mg/mL may be too low for certain applications which require a final NP size below 100 nm with high polymer content. To achieve high PLGA content and NP size < 100 nm, a large batch of 5 mg/mL PLGA NPs were produced and subsequently concentrated to 25 mg/mL using centrifugal filtration, shown in ***Figure 5.*** This approach achieves a final NP size ~90 nm at the final desired PLGA concentration of 25 mg/mL, as opposed to formulating directly at 25 mg/mL which produced NP of ~170 nm as indicated in ***Figure 4.***

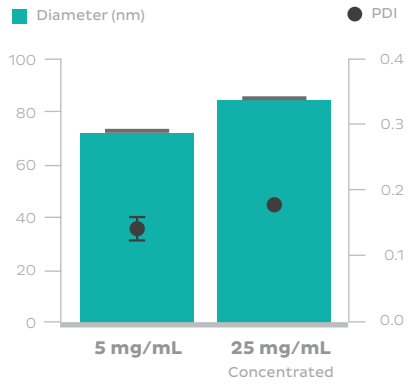


Figure 5. *Change in size of PLGA nanoparticles prepared using the NanoAssemblr Benchtop at 5 mg/mL compared to the same formulation after concentrating to 25 mg/mL. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	5 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

The concentration of the stabilizing agent, PVA, can also impact the size of the PLGA NP, which is highlighted in ***Figure 6.*** As the PVA concentration increased from 0.5 to 2% w/v, the PLGA NPs decreased in size from ~163 to ~147 nm. No further reduction in particle size was observed at PVA concentrations > 2% w/v.

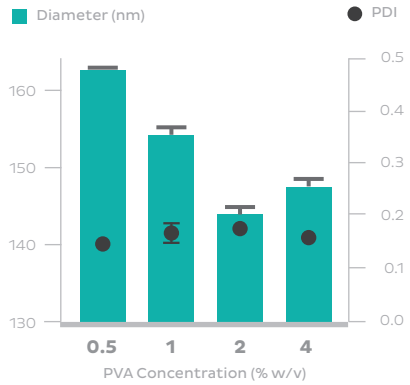


Figure 6. *Effect of PVA stabilizer concentration on the size and PDI of PLGA nanoparticles at polymer concentrations of 20 mg/mL using the NanoAssemblr Benchtop. Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

Polymer (PLGA) concentration	20 mg/mL
Stabilizer (PVA) concentration	0.5 - 2.0 % w/v
Total flow rate (TFR)	8 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

Discussion

Size plays an important role in the biodistribution, tissue penetration, drug release and drug efficacy of NP-based therapeutics,[1,2] so it is critical to maintain control over NP size during the manufacturing process. Compared to conventional methods,[5] the innovative microfluidic mixing technology employed by the NanoAssemblr platform offers exquisite control over NP size during the formulation process. This is achieved by modulating instrument parameters including the total flow rate (TFR) or flow rate ratio (FRR) and formulation parameters such as concentration. Herein, we presented examples of how these parameters can tune the size of PLGA NPs, selected as a representative biodegradable polymer that is currently approved by the FDA for drug delivery applications.[6]

The TFR in mL/min is the total combined speed at which the two fluids are being pumped into the two inlets of the microfluidic device (illustrated in ***Figure 1***). As TFR increases, faster mixing time is achieved which reduces PLGA NP size, as shown in ***Figure 2.*** As mixing time becomes much faster than the precipitation time of PLGA, NP size approaches an asymptote with increasing TFR. This asymptote is believed to be the 'limit size', which is the smallest, thermodynamically stable NP size for a given system.[7] For 5 mg/mL PLGA specifically, this limit size was ~70 nm for this particular system under these conditions, shown in ***Figure 2A.***

The relative amounts of aqueous and solvent phases being mixed at any given moment are dictated by the FRR. As solvent and antisolvent phases are rapidly mixed, there is a sudden shift in polarity that leads to a transient supersaturation of the molecule in the new solvent environment. This drives the precipitation of dissolved molecules into NPs. Here, larger PLGA particles were obtained with higher FRR, which contrasts with trends observed with amphiphilic molecules such as phospholipids (data not shown). The different trends can be understood through differences in the dynamics of particle assembly with these materials. With amphiphilic molecules which self-stabilize, greater changes in solvent polarity drive nucleation of particles which are rapidly stabilized by the hydrophilic portion of the molecule. With hydrophobic PLGA, rapid increase in polarity leads to rapid precipitation of the PLGA core, but surface passivation is limited by the kinetics of the assembly of the PVA corona. For the PVA, as the proportion of the aqueous phase to the organic phase increases, the magnitude of the polarity reduction experienced by the PVA upon mixing decreases. This lowers the driving force for the PVA to assemble on the surface of nascent PLGA particles. This delayed passivation favors growth of larger PLGA cores.

The effects of PLGA and PVA (stabilizer concentration) were also explored. Increasing the concentration of PLGA led to an increase in NP size ***(Figure 4),*** which is similar to results reported in the literature.[8] Concentrated polymer solutions are more viscous, so it is thought that this change in viscosity may decrease the speed of diffusion of the solvent phase into the aqueous phase which subsequently leads to formation of larger NPs.[8] The role of the PVA is to stabilize the particles by reducing the interfacial tension between the PLGA polymer and the aqueous phase. Changing the concentration of the stabilizer can thus have an impact on the size and PDI of PLGA nanoparticles, shown in ***Figure 6.*** These results are similar to that reported in literature and is due to the reduction in interfacial tension as concentration increases.[8] No further reduction in particle size was reported at PVA concentrations of 4% w/v which may be because concentrations of 2% w/v are enough to efficiently stabilize these nanoparticles.

It is important to note, that having a precisely controlled, reproducible process is necessary to isolate the effects of formulation parameters on particle size. Without such control, batch-to-batch variability could result in experimental errors that could obscure the effects of the formulation changes. In particular, the effect of PLGA concentration between 5-15 mg/mL on particle size is subtle. Batch-to-batch reproducibility afforded by the NanoAssemblr platform results in nearly negligible experimental error, which lowers the noise floor for these observations allowing changes in size to be both detected and attributed to changes in formulation.

Conclusion

These data demonstrate how NP size can be rationally tuned on the NanoAssemblr Benchtop by modulating the instrument parameters (TFR and FRR) and formulation parameters (polymer and stabilizer concentrations). Through a series of optimization experiments, the NanoAssemblr benchtop was able to formulate PLGA NPs in a range of sizes (70 - 200 nm) which is difficult to attain by most conventional methods of manufacture. Batch-to-batch reproducibility and the ease with which NP size can be tuned on this platform is promising for the field of nanomedicine.

Materials & Methods

MATERIALS

PLGA ester-terminated (lactide to glycolide ratio 50:50, molecular weight 45,000 - 55,000) was obtained from Akina, Inc (West Lafayette, IN). Poly(vinyl alcohol) (PVA) (molecular weight ~31,000, 87-89 mol% hydrolyzed) was purchased from Sigma-Aldrich (St Louis, MO). All other materials were reagent grade.

MICROFLUIDIC MANUFACTURE OF PLGA NANOPARTICLES.

PLGA nanoparticles were prepared using a microfluidic mixer with the NanoAssemblr Benchtop instrument (Precision NanoSystems, Inc., Vancouver, BC) by mixing appropriate volumes of PLGA solutions in acetonitrile with aqueous solutions containing PVA. Briefly, PLGA was dissolved in acetonitrile at concentrations ranging from 5 - 40 mg/mL, whereas, PVA was dissolved in deionized water at concentrations of 0.5 - 4 % w/v. For synthesis of PLGA nanoparticles, the PLGA solution in acetonitrile was injected through one inlet of the microfluidic mixer whereas, the PVA solution was injected through the other inlet of the microfluidic mixer. The NP formulations were prepared at various TFR and aqueous:solvent FRR from 2-12 mL/min and 1:1 – 9:1, respectively. The PLGA nanoparticles were collected in the sample collection tube by discarding an initial and final waste volume of 0.25 and 0.05 mL, respectively. The sample collected was then dialyzed against 1L deionized water using a dialysis bag (MWCO - 10,000) for 12 h replacing the dialysis medium twice in the first 4 h.

CHARACTERIZATION OF PLGA NANOPARTICLES.

PLGA nanoparticles prepared using the NanoAssemblr Benchtop were characterized for their size (Z-Average size) and polydispersity index (PDI) by dynamic light scattering using a Malvern Zetasizer 3000 (Malvern, UK) by diluting 50 µL of the prepared nanoparticle formulation with 300 µL of deionized water and measured at 25 ºC.

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