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Formulating Polymer Nanoparticles with the Nova System

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Abstract

Polymer-based or polymeric nanoparticles (PNPs) have been at the forefront in the development of novel drug carriers and medical imaging agents. Despite the recent successes of their lipid nanoparticle (LNPs) counter-parts during the COVID-19 global pandemic, PNPs can bring about more efficient and targeted treatments due to their ease of tunability, control over critical quality attributes (e.g. size and polydispersity), excellent retention time, and drug-load protection. However, their lack of commercial success is tied to the difficulties in scaling-up their manufacturing and production to larger scales. We have developed a unique nanoparticle (NP) manufacturing platform—the Nova Benchtop (BT) platform—that helps address these challenges. Our platform offers versatile and efficient production of both PNPs and LNPs, greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability than existing platforms. In this article, we base our PNP formulation on previous work done by Prud'homme and coworkers (Pagels et al., Nano Letters 2018 18 (2), 1139-1144) for comparison and validation. We will also provide a detailed overview of PNP production, including small sample screening and process optimization using our novel Nova BT platform.

Introduction

Polymer nanoparticles (PNPs) have been developed over the years to increase the efficacy and specificity of poorly soluble drugs, and improve medical imaging diagnostics. Clinically relevant PNPs typically possess a particle diameter between 40 to 200 nm. They often contain a core composed of gelatin, chitosan, poly-ε-caprolactone, polystyrene (PS), etc. Drugs can either be loaded within the PNP core or conjugated onto the PNP surface post-particle formation. This procedure allows drugs to increase their bioavailability and thereby increase their retention time within the body, promoting their drug efficacy. The surface of these particles can also be tuned by conjugating small molecules, biological or synthetic, to promote cell/tissue specific targeting or other interesting properties. In these ways, PNPs are a very attractive alternative to the well-known and established lipid nanoparticles.

PNPs constitute a wide array of differing polymer bases, some notable ones include polyethylene glycol (PEG), polylactic acid (PLA), polylactic-co-glycolic acid (PLGA), polyglycolic acid (PGA), and Polystyrene-b-poly(ethylene glycol) (PS-b-PEG). The diversity of these polymers and the unique properties they can confer onto NP formulations is what has drawn considerable research attention. Most of the polymer materials used in PNP synthesis have also already been approved by the FDA due to their biocompatibility and low cytotoxic effects. Thus the question becomes: *Why aren't there more polymer-based drug carrier systems available on the market?* One major obstacle is in translating PNP synthesis from the small laboratory-scale to a large commercial process, while maintaining a consistent and high quality end product - the drug-loaded PNP.

To help address limitations in the available NP manufacturing techniques, we employed our novel NP manufacturing platform - the Nova BT Impinged Jet Mixing (IJM) platform. Our platform offers faster and more efficient production of NPs with different compositions and payloads, greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability than existing platforms. In this article, we systematically produce PS-b-PEG PNPs with PS cores, including small sample screening and process optimization, using our novel Nova Benchtop platform.

Results

Percent Core Analysis

The effect of varying PS percent core and total mass concentration (TMC) on a polymer-based formulation composed of PS and PS-b-PEG was evaluated. At a TMC of 20 mg/mL, PS percent core was varied 25%, 50% and 75% (Figure 1) and produced LNPs with low poly-dispersity (PDI) <0.20. The particle size ranged from 56.2 nm to 167 nm.

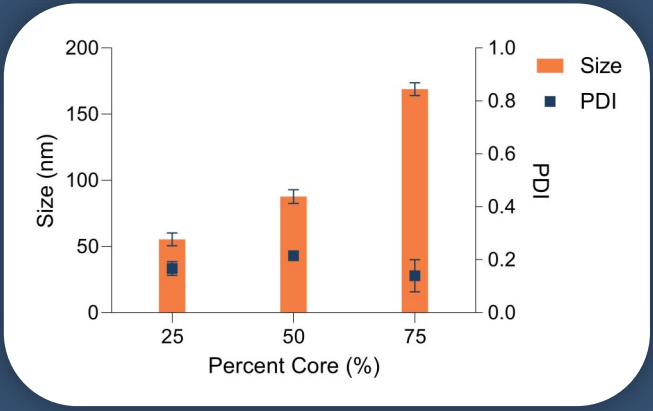


Figure 1. Size and PDI of PNPs made with the Nova BT IJM and with varying PS % core.

Formulation Parameters	
Total Mass Concentration	20 mg/mL
Polymer Type	PS-b-PEG
PS Core %	Varied, tested
Final PNP Concentration	4 mg/mL
Flow Rate Ratio	1.0
Total Flow Rate	18 mL/min

Total Mass Concentration Analysis

The effect of varying TMC on a PNP formulation was evaluated with samples formulated at TMCs of 5, 20 and 80 mg/mL and a 50% PS core. The particle size increases with TMC from 63.7 nm to 210 nm.

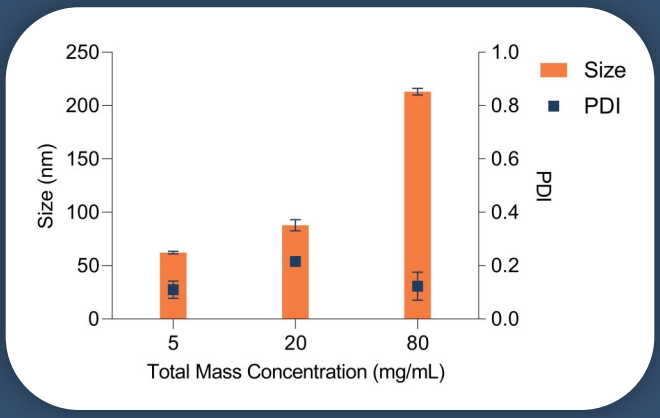


Figure 2. Size and PDI of PNPs made with the Nova BT IJM and with varying TMC.

Formulation Parameters	
Total Mass Concentration	Varied, tested
Polymer Type	PS-b-PEG
PS Core %	50%
Final PNP Concentration	Varied
Flow Rate Ratio	1.0
Total Flow Rate	18 mL/min

Results

Comparative Analysis

Comparing these data to Pagels et al., Nano Letters 2018 18 (2), 1139-1144, most of the PNP sizes fall within error between the two groups and deviations are seen only at 80 mg/mL TMC + 50% core with particles in this study being larger.

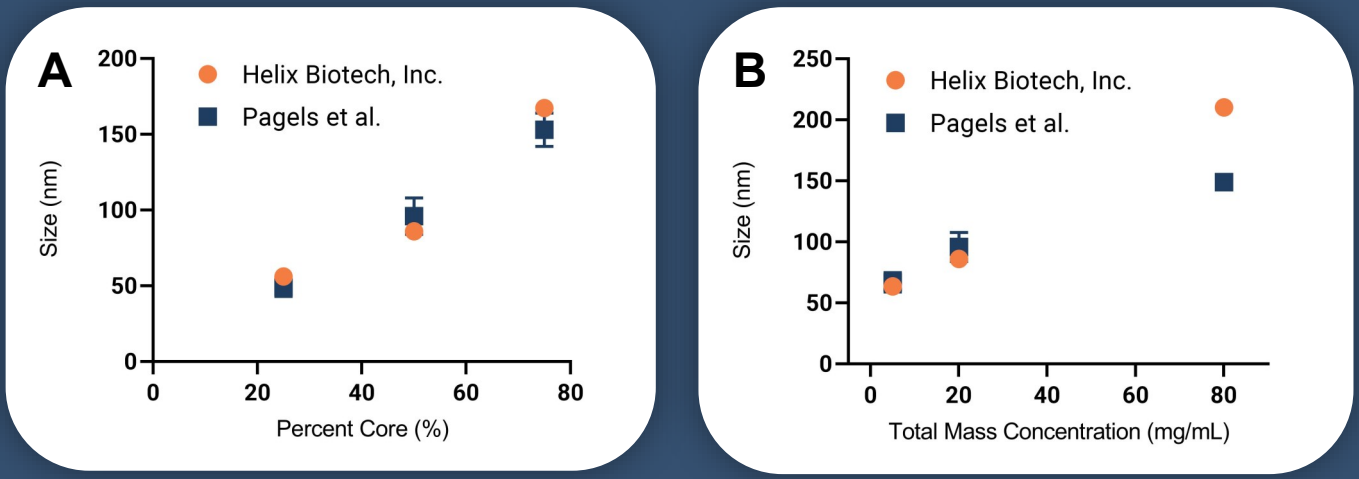
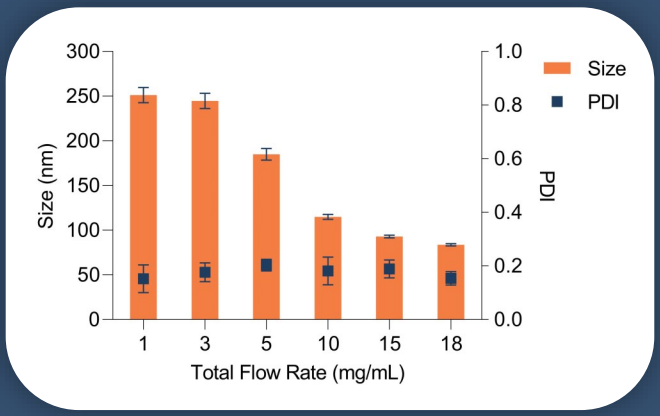


Figure 3. (A) Particle size as a function of percent PS core with a fixed TMC of 20 mg/mL. (B) Particle size as a function of TMC with a fixed percent core of 50%. Orange circles and navy blue squares represent Helix Biotech, Inc. and Pagels et al. data, respectively.

Total Flow Rate Analysis

Diverging from the Pagels et al. manuscript, the effect of total flow rate (TFR) on the polymer blend of PS and PS-b-PEG was investigated. The total flow rate was varied from 1, 3, 5, 10, 15 to 18 mg/mL at a TMC of 20 mg/mL and 50% PS core. While the PDI remains constant ~0.19, the PNP diameter size decreases from 250nm to 82nm with increasing TFR.



Formulation Parameters	
Total Mass Concentration	20 mg/mL
Polymer Type	PS-b-PEG
PS Core %	50%
Final PNP Concentration	4 mg/mL
Flow Rate Ratio	1.0
Total Flow Rate	Varied, tested

Figure 4. Size and PDI of PNPs made with the Nova BT IJM with varied TFRs.

Discussion

In the present study, we introduced our novel impinged jet mixing platform - the Nova BT - for the fabrication of polymer nanoparticles composed of PS-b-PEG block copolymers and PS as the NP core. We confirm that the Nova BT is able to control the nanoparticle size through changes in the PS percent core, the total mass concentration, and total flow rate in the solvent feed streams. Increases in the PS percent core and the TMC led to a non-linear increase in both the PNP size and PDI. Gradually increasing the TFR from 1 to 18 mg/mL led to a decrease in PNP diameter from 250 nm to 83.5 nm. Each data point found in all Figures represent triplicate measurements and the associated low error demonstrates the high reproducibility of the Nova BT platform. By effectively replicating the PNP results of the original manuscript (Pagels 2018) and combined with our previous results on other lipid and nucleic acid formulations (see other Application Notes), we have demonstrated the Nova BT platform's versatility in handling diverse starting materials to generate nanoparticles with desirable critical quality attributes. We have also expanded on the original Pagels et al. manuscript by testing the effect of TFRs on PNP size and PDI with the Nova BT system, where increasing TFR led to smaller PNPs.

The Nova BT platform represents a significant step forward in the development of PNP formulation for a wide range of applications. The Nova BT platform was shown to be a robust and reliable tool for small-scale screening and optimization of PNP formulations. In addition, our platform offers several advantages over existing manufacturing platforms, including greater control over the manufacturing configuration and process via modular plug-and-play design, and better scalability. Each dual pump module accommodates two syringes with volumes from 1-60 mL, providing a total sample production capacity of <1 mL to over 100 mL. Pump flow rates range from 0.1-100 mL/minute, and reproducibility of the process allows production of over 1 L via multiple runs. The modular platform design also allows for easy implementation of additional pumps and mixers for in-line dilution or nanoparticle modification, or increased throughput. The ability to rapidly and efficiently screen different compositions (lipid, polymer or hybrid), optimize, and scale PNP formulations using our platform will enable researchers to more effectively develop and refine their formulations, potentially leading to more successful clinical outcomes.

Conclusion

In conclusion, polymer nanoparticles have emerged as a promising platform for drug delivery and as imaging agents with potential applications in a wide range of diseases and conditions. Our study demonstrates the effectiveness of our novel Nova BT platform in producing high-quality polymer nanoparticles, with the ability to optimize formulation and process parameters to achieve desired nanoparticle properties. Our platform offers faster and more efficient production, greater control over the manufacturing process, and better scalability than existing platforms. By achieving finer control over particle size, size distribution, and composition, we have paved the way for the design and production of nanomaterials optimized for specific applications, including drug delivery and diagnostics.

Materials and Methods

Materials

Polystyrene (PS, 1.8 kDa) (P4688-S) and Polystyrene-b-poly(ethylene glycol) (PS-b-PEG, 1.6 kDa-b-5 kDa) (P13141-SEO) were purchased from Polymer Source (Quebec, Canada) and used as supplied. Tetrahydrofuran (THF, HPLC grade) was purchased from Fisher Scientific (Pittsburgh, PA). NORM-JECT luer lock solo 2mL syringes were purchased from Grainger (Lake Forest, IL). Note the NORM-JECT syringes are labeled as 2mL but have graduations up to 3 mL. Sterile syringe filters with a 0.22 µm PTFE membrane were purchased from Biomed Scientific (Part No. SFPT-FE025022).

Methods

Nanoparticle Formulation and Manufacturing

Total Mass Concentration and PS % Core Analysis

PS and PS-b-PEG were weighed into glass vials at 320.5 and 320.6 mg, respectively. 4 mL of THF was added to each glass vial using a Hamilton glass syringe. Both solutions were subjected to two rounds of five-minute sonication using a DK Sonic sonicator at 25°C. Some particulate was noticed in each vial after sonication. To remove the particulate, each solution was filtered using a sterile syringe filter with 0.22⌠m PTFE membrane. Final concentration of each polymer solution was 80 mg/mL. Five conditions were examined with samples prepared in triplicate using the polymer stocks as described in Table 1.

Table 1. Polymer+THF Stock solution preparation calculations.

Case	PS Stock Volume (80 mg/mL)	PS-b-PEG Stock Volume (80 mg/mL)	THF, µL (neat)	Sample Stock THF Volume
5 mg/mL, 50% Core	125 uL	125 uL	3750 uL	4000 uL
20 mg/mL, 25% Core	250 uL	750 uL	3000 uL	4000 uL
20 mg/mL, 50% Core	500 uL	500 uL	3000 uL	4000 uL
20 mg/mL, 75% Core	750 uL	250 uL	3000 uL	4000 uL
80 mg/mL, 50% Core	2000 uL	2000 uL	0	4000 uL
Total Stock	320mg PS + 4mL THF = 4mL 80mg/mL PS Stock (Weighed 320.5mg)	320mg PS-b-PEG + 4mL THF = 4mL 80mg/mL PS-b-PEG stock (Weighed 320.6mg)		

A Nova Benchtop Impinged Jet Mixer (Nova BT IJM, Helix Biotech, Knoxville, TN) was used to manufacture the polymer nanoparticles using a Size 1 mixer. Solvent compatible lines, fittings, and mixing chamber (PEEK) were used in the Nova BT IJM system. The system was thoroughly cleaned using deionized H_2O in the upper syringe and THF in the lower syringe. For all samples, 1 mL of deionized H_2O was loaded into the upper syringe and 1mL sample in THF was loaded into the lower syringe. The instrument was initially primed with a 100 L injection. The manufacturing settings were as follows: start waste was 650 L, sample collection was 1 mL, end waste was 150 L, flow rate ratio (FRR) was 1:1, and total flow rate (TFR) was 18 mL/min. Each sample was collected into a 15 mL conical tube with 4 mL of deionized H_2O preloaded, giving a final 1:9 ratio of THF:H_2O. Note that after sample collection, two phases were noticed in the sample. To alleviate this, the sample was gently inverted a few times to mix. Each sample was run in triplicate. Cleaning, as described previously, was performed between each composition for all samples. Note that for the 5 mg/mL 50% Core

Methods, Continued

Total Flow Rate Analysis

PS and PS-b-PEG were weighed into glass vials at 302 mg and 288.2 mg, respectively. 3.78 mL (PS) and 3.6 mL (PS-b-PEG) of THF was added to the appropriate glass vials using a Hamilton glass syringe. Both solutions were subjected to two rounds of five-minute sonication using a DK Sonic sonicator at 25°C. Some particulate was noticed in each vial after sonication. To remove the particulate, each solution was filtered using a sterile syringe filter with 0.22 µm PTFE membrane. Final concentration of each polymer solution was 80 mg/mL. One condition was examined at varying TFR (1, 3, 5, 10, 15, and 18 mL/min) with samples prepared in triplicate using the polymer stocks as described in Table 1.

Table 2. Polymer+THF Stock solution preparation calculations.

Case	PS Stock Volume (80 mg/mL)	PS-b-PEG Stock Volume (80 mg/mL)	THF, µL (neat)	Sample Stock THF Volume
20 mg/mL, 50% Core	500 µL	500 µL	3000 µL	4000 µL

Again, a Nova BT IJM was used to manufacture the polymer nanoparticles via a Size 1 mixer. The cleaning and preparation steps are as previously described above. The manufacturing settings were as follows: start waste was 650µL, sample collection was 1 mL, end waste was 150 µL, flow rate ratio (FRR) was 1:1, and total flow rate (TFR) was varied from 1,3,5,10,15,18 mL/min. Each sample was collected into a 15 mL conical tube with 4 mL of deionized H_2O preloaded, giving a final 1:9 ratio of THF:H_2O. Note that after sample collection, two phases were noticed in the sample. To alleviate this, the sample was gentle inverted a few times to mix. Each sample was run in triplicate. Samples were run in series with new loaded syringes added after each run. Start 650 uL waste is used for cleaning between runs.

Nova BT IJM Formulation Parameters	
Polymer Type	PS, PS-b-PEG
Start Waste	650 mL
Sample Collection Volume	1 mL
End Waste	150 mL
Flow Rate Ratio (FRR)	1:1
Total Flow Rate (TFR)	1, 3, 5, 10, 15, 18 mL/min

Table 3. Formulation Parameters

Dynamic Light Scattering

The hydrodynamic diameter of each sample was measured using an Anton Paar LiteSizer 500 dynamic light scattering instrument (DLS). 10 µL of each sample was diluted into 1 mL of deionized H_2O and mixed directly in a plastic cuvette. At this 100x dilution, all sampled were clear in the DLS cuvette. Measurement parameters were as follows: 25°C, 173° detection angle, and Normal Mode analysis with intensity average peak 1 diameters reported. The particle size in nm and polydispersity index (PDI) were reported. DLS measurements were performed immediately after manufacturing and post three days to access particle stability. Samples were stored in a 4°C fridge during the stability period. DLS was also attempted in backscattering mode, however, the measurement failed due to low scattering intensity.