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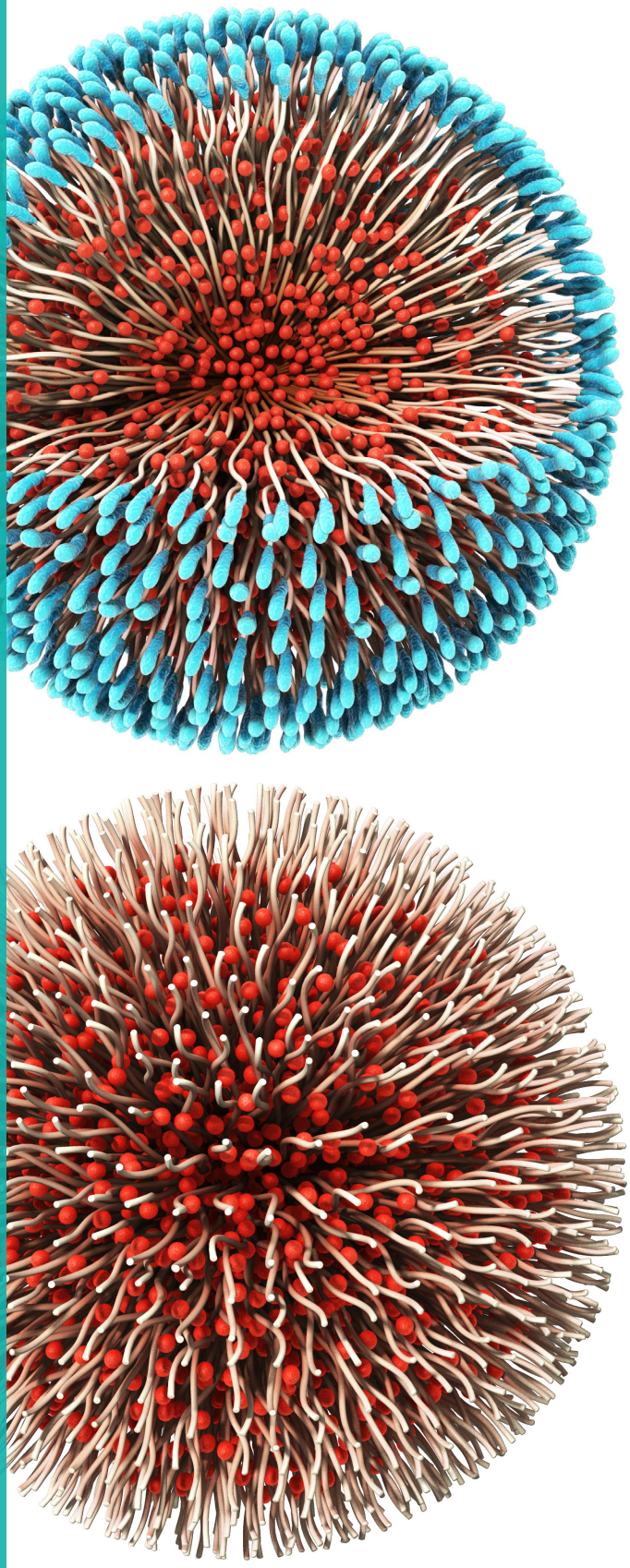
PLGA Nano-particles

Production and *In Situ* Drug Loading Using the NanoAssemblr® Benchtop Instrument and the Impact of Solvent Removal Methods



S.M. Garg, A. Thomas, G. Heuck, A. Armstead, J. Singh, T.J. Leaver, A.W. Wild, S. Ip, R.J. Taylor, E.C. Ramsay

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Precision NanoSystems Inc., Vancouver, BC, Canada

Abstract

There is extensive interest in using polymer-based nanoparticles as drug delivery agents due to the range of suitable payloads, extended release characteristics, and high biocompatibility of these polymeric systems. One of the challenges associated with nano-based drug carriers is ensuring that the nanoparticles (NPs) are of a desired size (at or below 100nm) and have high drug encapsulation efficiency, to ensure that the nanoparticle delivers sufficient amounts of drug to its intended target to achieve the desired therapeutic effect. Here, we present a novel approach for manufacturing poly(lactide-co-glycolide) or PLGA nanoparticles encapsulating coumarin-6 as a model hydrophobic drug using the NanoAssemblr® Benchtop instrument. Two methods of removing the solvent, namely dialysis and centrifugal filtration, were compared to determine the effects on particle size, uniformity (polydispersity index, PDI), and drug encapsulation efficiency. An encapsulation efficiency of 75% was achieved, which is higher than reported in literature using traditional production methods. Additionally, the choice of solvent removal method was found to influence PDI and encapsulation efficiency.

Introduction

Polymeric nanoparticles (NPs) are gaining major interest in the field of nanomedicine, because polymeric carriers are suitable for delivering a range of payloads (i.e. small molecules, nucleic acids, peptides, etc.) and offer sustained release properties, high biocompatibility and low toxicity.[1-3] In particular, poly(lactide-co-glycolide) or PLGA is a top candidate for NP-based drug delivery applications and has already been approved by the US Food and Drug Administration (FDA) for a number of therapeutic applications.[2] While a number of methods are currently used to manufacture PLGA NPs for drug delivery, these NPs are large (> 100 nm), and one of the challenges is to manufacture drug-loaded NPs smaller than 100 nm[4] as these have more desirable biodistribution profiles.[5] Microfluidic methods for NP manufacture have recently gained momentum as a suitable manufacturing method for producing sub-100 nm NPs.[6]

Encapsulation of drug molecules with a polymeric carrier can change the pharmacokinetic profile of the drug, enabling it to reach its target site. For these drug delivery applications, it is critical that the NPs are loaded with sufficient amounts of the payload (e.g. hydrophobic small molecules) so that the intended dose is delivered and the therapeutic action is attained.[7] However, high drug encapsulation efficiencies may be difficult to achieve with current techniques, depending on the limitations of the production method as well as the formulation of the NP itself and how the components interact with the intended payload.

Herein, we describe the production of sub-100 nm PLGA NP encapsulating Coumarin-6 (C6) as a model hydrophobic drug molecule using the NanoAssemblr Benchtop microfluidic instrument. C6 is a low molecular weight (MW: 350.43 g/mol) fluorescent probe that, like many drug molecules, is insoluble in water but soluble in water-miscible organic solvents such as ethanol, methanol, N,N-dimethylformamide, and acetonitrile. The effects of NanoAssemblr system parameters, formulation parameters, drug loading and choice of solvent removal methods are explored for this model PLGA-C6 NP system.

Result

PLGA NPs were formulated on the NanoAssemblr benchtop using the microfluidic mixing approach, illustrated in ***Figure 1.*** This process promotes rapid and even mixing of the aqueous and solvent phases, driving controlled precipitation of the PLGA NP at the desired size.

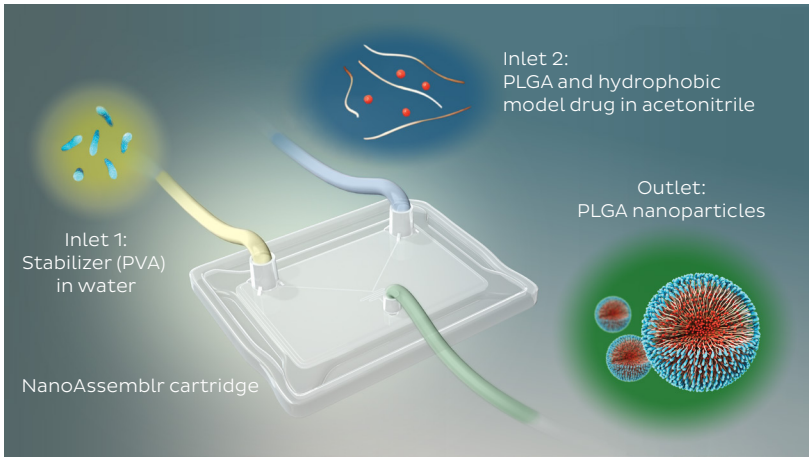
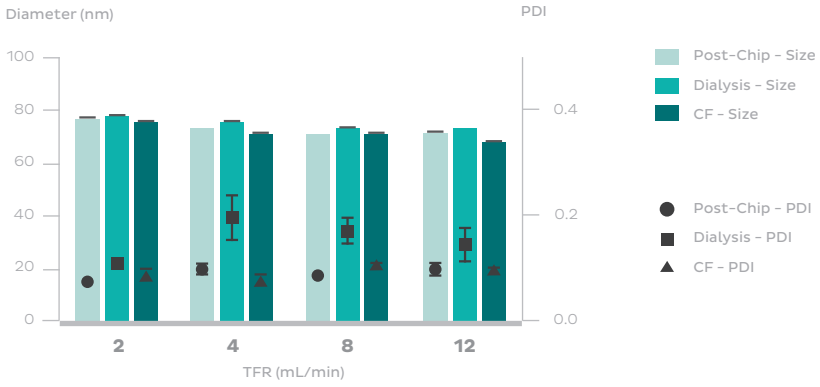


Figure 1. *Microfluidic mixing process employed by the NanoAssemblr Benchtop Instrument provides rapid, homogeneous mixing and controls the nanoprecipitation of PLGA NPs with in situ drug loading.*

EFFECT OF SOLVENT REMOVAL METHODS ON THE SIZE AND PDI OF EMPTY PLGA NP

After formulation of PLGA NPs on the NanoAssemblr Benchtop, the solvent (acetonitrile) was removed using either dialysis or centrifugal filtration. As shown in ***Figure 2***, NP that underwent dialysis had a small increase in the PDI as compared to the NPs before dialysis (Student's t-test, $P < 0.05$). For NPs that underwent centrifugal filtration, no change in the PDI after solvent exchange can be seen. In the case of nanoparticle size, both dialysis and centrifugal filtration caused no change in the size of the particles.



Polymer (PLGA) concentration	5 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 solvent removal method on the size and PDI of PLGA nanoparticles at polymer concentrations of 5 mg/mL (CF, centrifugal filtration). Each bar/plot represents the mean ± SEM for 3 independent size/PDI measurements on three independent samples (n = 3).*

IN SITU ENCAPSULATION OF THE MODEL DRUG COUMARIN-6 WITHIN PLGA NANOPARTICLES

Encapsulation of C6 within PLGA NPs was achieved by dissolving the C6 in the solvent phase along with the PLGA polymer and formulating the NPs with poly(vinyl alcohol) (PVA) as a stabilizer in the aqueous phase. The effect of total flow rate (TFR) on the encapsulation efficiency of C6 in PLGA NPs is shown in ***Figure 3.*** C6 was successfully encapsulated at high efficiencies (> 50%) at varying total flow rate (TFR). The maximum encapsulation was 75% w/w when formulated at a TFR of 12 mL/min.

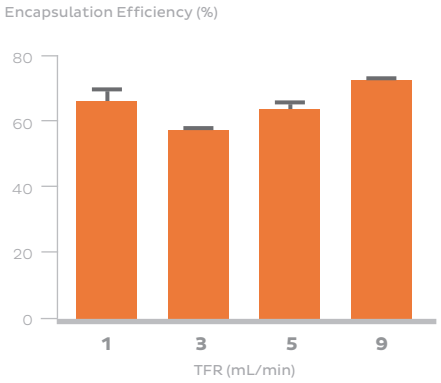
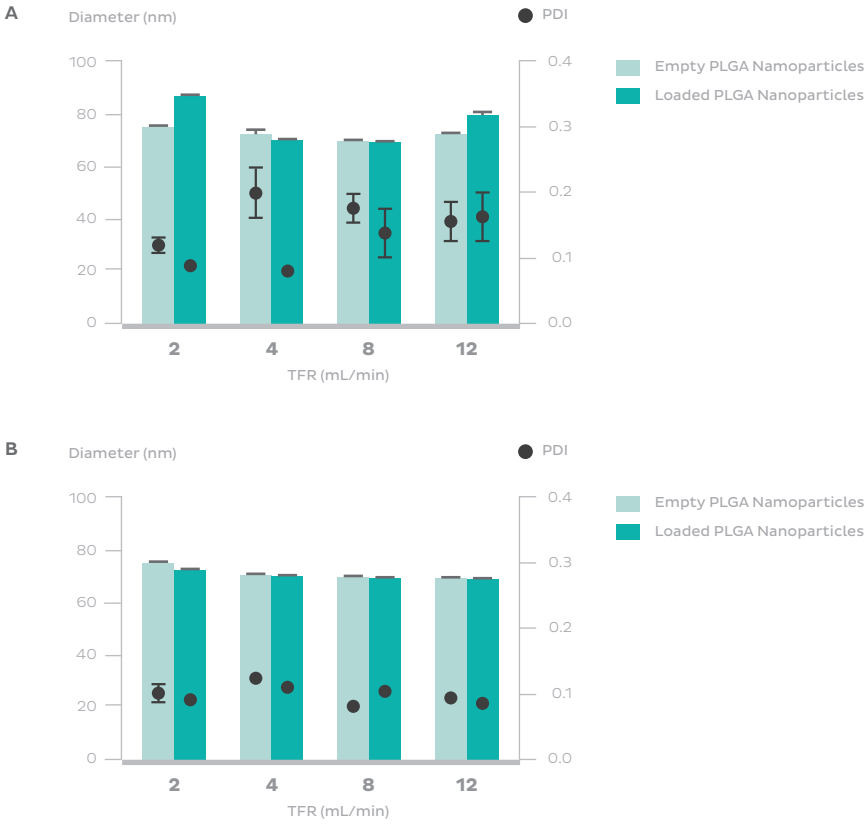


Figure 3. *Encapsulation of coumarin-6 in PLGA NPs as a function of total flow rate (TFR). Each bar represents the mean ± SEM for 3 independent measurements on three independent samples (n = 3).*

EFFECT OF COUMARIN-6 ENCAPSULATION AND SOLVENT REMOVAL METHODS ON THE SIZE AND PDI OF C6-LOADED PLGA NP

The effects of drug encapsulation and downstream processing on the size and PDI of the PLGA NP is shown in ***Figure 4.*** The encapsulation of C6 into PLGA NPs did not significantly increase the size when compared to empty PLGA (polymer only) NPs formulated under identical conditions. At the same time, the buffer exchange method did not significantly change the size of the loaded nanoparticles (Student's t-test, $P > 0.05$), but significantly reduced the PDI in case of nanoparticles processed using CF for most flow rates (Student's t-test, $P < 0.05$).

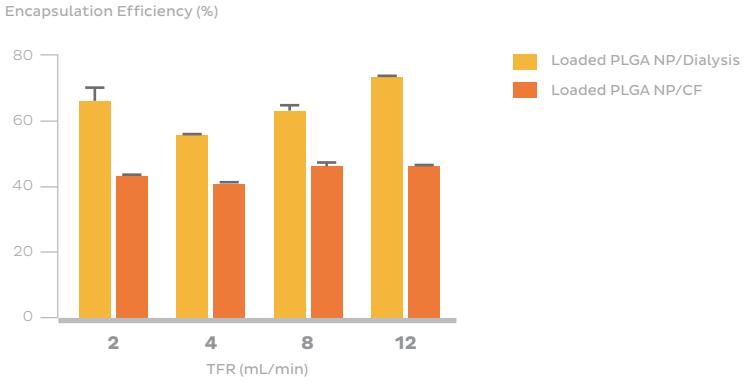


Polymer (PLGA) concentration	5 mg/mL
Stabilizer (PVA) concentration	2.0 % w/v
Total flow rate (TFR)	2 - 12 mL/min
Aqueous: solvent flow rate ratio (FRR)	1:1

Figure 4. *The effect of coumarin-6 encapsulation on the size and PDI of PLGA NP at polymer concentrations of 5 mg/mL buffer-exchanged by either A) dialysis or B) centrifugal filtration. Each bar/plot represents the mean ± SEM for three independent size/PDI measurements on three independent samples (n = 3).*

EFFECT OF THE SOLVENT REMOVAL METHOD ON THE ENCAPSULATION OF COUMARIN-6

The effect of the solvent removal method on the final encapsulation of C6 is shown in ***Figure 5.*** NPs processed using centrifugal filtration showed lower encapsulation efficiencies as compared to the NPs processed using dialysis. Maximum C6 encapsulation of 75% was achieved at a TFR of 12 mL/min using dialysis for solvent removal.



Polymer (PLGA) concentration	5 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 5. *Encapsulation of C6 (0.25 % w/w of the polymer) in PLGA nanoparticles at polymer concentrations of 5 mg/mL either dialyzed or buffer exchanged by centrifugal filtration (CF). Each bar represents the mean ± SEM for 3 independent measurements on three independent samples (n = 3).*

Discussion

In addition to size, particle stability is also an important consideration when developing NP based drugs for the clinic. During the manufacturing process, certain solvents are required in order to successfully formulate the NP (i.e. acetonitrile, ethanol, methanol, etc.), but these solvents must be removed for downstream in vitro and in vivo testing.[8] This process not only increases the long-term stability of the NP, but is also required to minimize toxicity due to residual solvent content in the final NP product. For the PLGA NPs formulated as described here, we employed two different solvent removal methods: dialysis and centrifugal filtration (CF).

The solvent removal method employed can influence the size, PDI, and the total drug encapsulation of the final formulation. As shown in ***Figures 2***, dialysis led to a slight increase in the PDI of the PLGA NPs whereas centrifugal filtration showed no such change in the PDI. This could be due to differences in the speed at which the solvent removal takes place during centrifugal filtration and during dialysis. It is possible that the slow removal of solvent during dialysis facilitates an uneven amount of particle growth and increase in PDI during the acetonitrile removal process.

Coumarin-6, selected as a model hydrophobic small molecule, was successfully encapsulated within PLGA NPs at high efficiencies, shown in ***Figure 3.*** The maximum C6 encapsulation obtained under these conditions on the NanoAssemblr Benchtop was 75% w/w, with an initial drug loading of 0.25% w/w of the PLGA polymer. This value was considerably higher than that reported in literature (60% w/w C6 encapsulation) for a similar PLGA polymer and stabilizer system, using a similar theoretical drug loading and a single-emulsion method to formulate the NPs.[9] In addition to achieving a higher C6 encapsulation, the size of the PLGA NP produced on the NanoAssemblr were much smaller than those reported in the literature, 80 nm vs. 177 nm, respectively.[9] This indicates the ability of the NanoAssemblr microfluidic mixing platform[6] to produce smaller NP with higher encapsulation efficiencies, a promising find for the field of nanomedicine. [4, 6]

However, we found significant differences in the final C6 NP encapsulation between the two methods employed for removal of acetonitrile, shown in ***Figure 5.*** C6 NPs that were processed using CF had a lower final encapsulation of C6 compared to NPs processed using dialysis. This could be due to the increased stress and force that the PLGA NPs undergo during the CF process itself, leading to lower encapsulations when compared to NP processed via dialysis. Alternatively, solvent exchange by dialysis is a slower process than CF, allowing solvent exchange to occur under equilibrium conditions where structural relaxation of the PLGA core can occur, trapping more drug molecules. These findings highlight the importance of selecting a suitable solvent removal method for a given NP application, depending on the final goals for the NP drug product.

Conclusion

These data indicate that the microfluidic approach utilized by the NanoAssemblr Benchtop instrument successfully generates PLGA NPs less than 100 nm in size, with higher encapsulation efficiencies of Coumarin-6. The encapsulation of Coumarin-6 did not change the NP size, however, the solvent removal method used was found to influence the PDI and the encapsulation efficiency of drugs in PLGA NPs. In conclusion, the NanoAssemblr platform offers an attractive solution for manufacturing drug-loaded nanoparticles with small size and high encapsulation efficiencies.

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 concentration of 5 mg/mL, whereas, PVA was dissolved in deionized water at a concentration of 2% 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 aqueous:solvent FRR of 1:1 and various TFR from 2-12 mL/min. 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 further processed using one of two methods to remove the organic solvent. For dialysis, samples were 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. For solvent exchange using centrifugal filtration, samples were diluted 3 times followed by centrifugation at 1500 - 1600× g for 30 min using Amicon® Ultrafiltration tubes (MWCO – 10,000). The samples were washed 3 times during this process until the original formulation volume was reached.

For encapsulation of Coumarin-6, the drug was dissolved in a stock solution of PLGA in acetonitrile such that the concentration of PLGA is 5 mg/mL and the drug/polymer w/w ratio is 1/400. The Coumarin-6 - PLGA solution in acetonitrile was then injected through one inlet of the microfluidic mixer whereas, the 2% w/v PVA solution was injected through the other inlet of the microfluidic mixer to form nanoparticles using the same parameters as listed above at different flow rates from 2 - 12 mL/min. The samples were dialyzed as mentioned above, followed by centrifugation at 8000 × g for 5 minutes to remove and/or precipitate the free drug.

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.

Encapsulation of C6 was measured in PLGA nanoparticles by first adding ethanol to solubilize the drug followed by precipitation of the solution at 15000 × g for 10 minutes. The solution was then measured against a standard curve of C6 in ethanol using fluorescence spectroscopy at an excitation and emission wavelength of 450 nm and 505 nm, respectively.

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Precision NanoSystems Inc.
50 - 655 West Kent Ave. N.,
Vancouver, BC, V6P 6T7
Canada

Precision NanoSystems Inc.
395 Oyster Point Boulevard, Suite 145
South San Francisco, CA, 94080
USA

phone: 1-888-618-0031
info@precision-nano.com

precisionnanosystems.com