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Large-Scale Synthesis of Lipid–Polymer Hybrid Nanoparticles Using a Multi-Inlet Vortex Reactor

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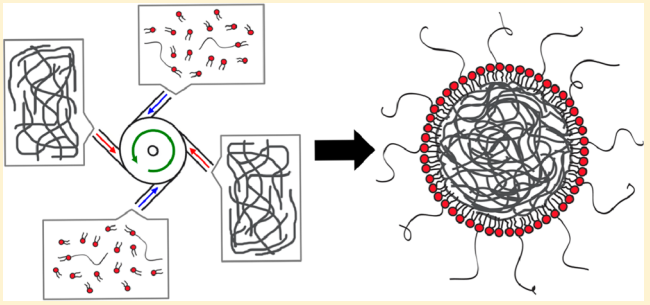
ABSTRACT: Lipid–polymer hybrid nanoparticles combine the advantages of both polymeric and liposomal drug carriers and have shown great promise as a controlled drug delivery platform. Herein, we demonstrate that it is possible to adapt a multi-inlet vortex reactor (MIVR) for use in the large-scale synthesis of these hybrid nanoparticles. Several parameters, including formulation, polymer concentration, and flow rate, are systematically varied, and the effects of each on nanoparticle properties are studied. Particles fabricated from this process display characteristics that are on par with those made on the lab-scale such as small size, low polydispersity, and excellent stability in both PBS and serum. Using this approach, production rates of greater than 10 g/h can readily be achieved, demonstrating that use of the MIVR is a viable method of producing hybrid nanoparticles in clinically relevant quantities.



INTRODUCTION

Nanoparticle drug delivery has shown the potential to enable more effective treatment and management of diseases such as cancer.[1−6] To this end, lipid–polymer hybrid nanoparticles[7−9] represent a distinct class of drug nanocarriers that combine the advantages of both polymeric[10−12] and liposomal[13,14] delivery platforms. The hybrid nanoparticles consist of three distinct components: a biodegradable polymeric core for the efficient loading of poorly water-soluble drugs; a lipid monolayer surrounding the core that enhances the particle's stability in salt solutions and helps to reduce outward diffusion of drug; and a lipid-PEG outer corona that protects the particle from the immune system in vivo. These hybrid nanoparticles display many characteristics of a reliable drug carrier, including excellent stability,[15] the ability to load multiple drugs,[16] and the ability to be functionalized with targeting ligands.[17] One aspect that still needs further exploration is the scaling up of the production of these particles to clinically relevant quantities.[18]

On the lab-scale, the hybrid nanoparticles are typically made using a modified nanoprecipitation method.[7] This involves addition of an organic phase containing dissolved polymer into a heated aqueous phase containing lipid and lipid-PEG. This is then followed by mechanical vortexing to complete self-assembly and evaporation to remove excess organic solvent. A sonication-based protocol has also been reported that streamlines the lab-scale synthesis process, allowing for a 20-fold reduction in time required for nanoparticle fabrication.[15] Most recently, large-scale synthesis has been accomplished through the use of a microfluidic device that generates controlled microvortices.[19] In contrast to previous microfluidic devices that rely on diffusive mixing,[20] the approach reported in this study is able to facilitate the production of hybrid nanoparticles with desirable characteristics for drug delivery (small size and narrow distribution) through efficient mixing at a high Reynolds number.

Multi-inlet vortex reactors (MIVRs) are macro-scale devices that have been extensively studied for the large-scale fabrication of polymeric nanoparticles.[21−29] The device can be made with either two or four radially symmetric inlets that lead into a circular reaction chamber. By inputting an organic phase containing dissolved polymer and an aqueous phase acting as the antisolvent into separate inlets, rapid nanoprecipitation to form polymeric particles can be achieved. With this design, each input stream can contribute independently to the mixing parameters of the device, allowing for fine-tuning of organic and aqueous phase flow rates. Additionally, the device allows for highly efficient mixing with Reynolds numbers reaching into the thousands depending on the flow rate that is used. Because it has also been shown that, beyond a certain threshold, an increase in Reynolds number no longer results in a reduction of particle size,[24,26] this macro-scale mixing device should enable

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sufficient mixing for the production of high-quality polymeric nanoparticles at a large throughput.

In this study, we adapted an MIVR device for use in the production of lipid−polymer hybrid nanoparticles. Because of both the structural and the synthetic similarities between polymeric nanoparticles and hybrid nanoparticles made via nanoprecipitation, we hypothesized that it should be possible to fabricate hybrid nanoparticles on a large scale using this macroscale mixing device while still maintaining the desired particle characteristics for drug delivery applications. Several variables, including lipid-to-polymer ratio, polymer concentration, and flow rate, are systematically manipulated to maximize output while at the same time maintaining particle size and distribution. Ultimately, the physicochemical properties and stability characteristics of these particles fabricated using an MIVR are compared to those made using a lab-scale sonication technique to assess the viability of such an approach for high rate production of lipid−polymer hybrid nanoparticles.

EXPERIMENTAL SECTION

Materials. Ester-terminated poly(DL-lactic-*co*-glycolic acid) (PLGA) (inherent viscosity = 0.82 dL/g) was obtained from LACTEL Absorbable Polymers (Pelham, AL). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxy(poly(ethylene glycol)) 2000 (DSPE-PEG) and L-α-phosphatidylcholine (Egg PC) were obtained from Avanti Polar Lipids (Alabaster, AL). Fetal bovine serum, PBS buffer, acetonitrile, and all other solvents were purchased from Sigma-Aldrich (St. Louis, MO).

Design and Manufacture of Multi-Inlet Vortex Reactor (MIVR). A four-inlet MIVR was fabricated according to previous specifications[24] using two blocks of stainless steel. The top block contained all of the reactor geometry along with the outlet port, while the bottom block contained the four inlet ports. The inlet channels were 1.1 mm in width. The reaction chamber was 6 mm in diameter and 1.5 mm in height. The outlet was 1.3 mm in diameter. A piece of rubber with holes corresponding to the four inlets was sandwiched between the two blocks to prevent leakage. Input streams were manipulated using syringe pumps (NE-300) from New Era Pump Systems equipped with 50−60 cc syringes from EXELint. Because only two phases were used in the experiments, a T-shaped connector was used to split the input streams, and each of the resulting streams was fed into an inlet opposite the other.

Large-Scale Synthesis of Hybrid Nanoparticles. To determine the optimal parameters for large-scale synthesis using the MIVR device, the shell material (lipid and lipid-PEG) to core material (PLGA) mass ratio, the PLGA concentration in acetonitrile, and the total flow rate through the device was systematically varied. For all experiments, an equal flow rate of the organic and aqueous phases was used. At the beginning of each run, 5 mL of leading flow-through was allowed to pass out of the device before collecting the sample. The samples were then allowed sufficient time for the organic solvent to evaporate off before adjusting to 1X PBS with a 20× stock and using dynamic light scattering (DLS) to measure the size and polydispersity index (PDI). Herein, PDI is a measure of particle size distribution with a value ranging from 0 (monodisperse) to 1 (highly polydisperse).

To optimize the outer shell content, the Egg PC to DSPE-PEG ratio was fixed at 1:2.5 molar ratio. A stock solution of PLGA in acetonitrile at 1 mg/mL was used for all samples. A stock solution of 0.25 mg/mL Egg PC with DSPE-PEG was prepared at the 1:2.5 molar ratio and then serially diluted 2× five times, resulting in a total of six different formulations. The total flow rate used was 2 mL/min.

To determine the effect of the PLGA concentration in the organic phase on particle characteristics, solutions were prepared at 1, 2.5, 5, and 10 mg/mL. Solutions of Egg PC with DSPE-PEG were also prepared individually such that the final shell/core mass ratio would be 0.075 for each sample. The total flow rate used was 2 mL/min.

To determine the effect of the total flow rate through the device on particle synthesis, the PLGA concentration was set at a constant 2.5 mg/mL, and the aqueous phase was prepared such that the final shell/core mass ratio would be 0.075. The total flow rate was set at 2, 10, 20, and 50 mL/min to produce each sample.

Lab-Scale Synthesis of Hybrid Nanoparticles. Lipid−polymer hybrid nanoparticles were made on the lab-scale using an adapted version of a previously published sonication-based protocol.[15] Briefly, stock solutions of PLGA in acetonitrile at 2.5 mg/mL, Egg PC in water at 1 mg/mL, and DSPE-PEG in water at 1 mg/mL were prepared. 250 μL of DSPE-PEG solution and 12.5 μL of Egg PC solution were added into a 7 mL glass scintillation vial, and the volume was brought up to 4 mL with water. 400 μL of the PLGA solution was then pipetted into the aqueous solution. The resultant mixture was sonicated for 5 min in a bath sonicator (FS30D) from Fisher Scientific. After sonication, the samples were purified by washing three times using 10 kDa MWCO Amicon centrifuge filters from Millipore and then resuspended in water.

Nanoparticle Characterization. To determine nanoparticle size, PDI, and surface zeta potential, samples were suspended at 1 mg/mL in water and measured by DLS using a Zetasizer (ZEN3600) from Malvern. Clear disposable capillary cells (DTS1061) from Malvern were used for all samples. All measurements were conducted at a backscattering angle of 173° at room temperature. The average of three subruns was used for each measurement. To visualize nanoparticle morphology, scanning electron microscopy (SEM) was conducted on an XL30 ESEM from Phillips at a beam intensity of 10 kV. Samples were prepared by diluting 1 mg/mL nanoparticle solutions 1000 times and then air drying 1 μL of the resulting dilution on silicon wafers. Each sample was coated with iridium at 85 mA for 7 s on a K575X Sputter Coater from Emitech and then imaged by SEM.

Nanoparticle Stability Studies. For PBS stability studies, the hybrid nanoparticles were suspended at 1 mg/mL in water and then adjusted to 1X PBS using a 20× concentrated PBS stock. One milliliter of each sample was stored in a capped low volume sizing cuvette (ZEN0040) from Malvern. Over a period of 2 weeks, the size and PDI of the samples were recorded every 2 days.

For serum stability studies, fetal bovine serum was first concentrated to double the concentration. 100 μL of both the concentrated serum and the nanoparticle samples at 1 mg/mL was added into a clear 96-well plate, and aggregation was assayed by measuring the absorbance at 560 nm every 1 min for a period of 3 h following a published protocol.[15,30]

RESULTS AND DISCUSSION

A four-inlet MIVR was machined from stainless steel, and the final assembled device along with all of the relevant instrumentation and tubing are depicted in Figure 1A. Two inlets were dedicated to each of the two phases required for nanoparticle precipitation to occur. The organic phase consisted of PLGA dissolved in acetonitrile, while the aqueous phase consisted of the lipid (Egg PC) and lipid-PEG (DSPE-PEG) dissolved in water. Syringe pumps were used to accurately control the flow rate of each phase into the reaction chamber in the configuration depicted in Figure 1B. Upon mixing within the reaction chamber, the different components from the two phases self-assemble into lipid-polymer hybrid nanoparticles with a core−shell structure. The PLGA forms the core, while the lipid and lipid-PEG form the shell by orienting their hydrophobic tails toward the hydrophobic polymer.

To optimize the large-scale production of the hybrid nanoparticles using this MIVR setup, several variables were explored. First, to optimize the formulation, the mass ratio of the shell material (lipid and lipid-PEG) to the core material (PLGA) was varied (Figure 2). Using a constant molar ratio of lipid to lipid-PEG and setting all other parameters constant, including PLGA concentration and flow rate, it was shown that there was little change in the particles' size and size distribution beyond a certain shell/core mass ratio. At low ratios, there was

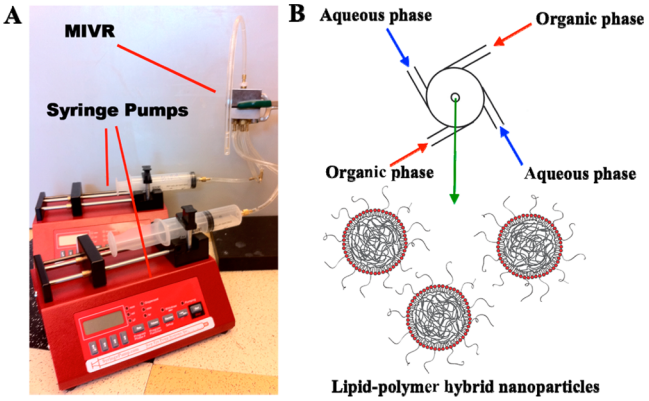

Figure 1. (A) Experimental setup of the multi-inlet vortex reactor (MIVR) device. Two syringe pumps are each split to feed four separate streams into the device. The produced nanoparticles are pushed out of the device through outlet tubing. (B) Schematic working mechanism of the MIVR device. The organic phase contains the dissolved polymer, while the aqueous phase contains the lipid and lipid–PEG mixture. The streams are fed into a circular reaction chamber that facilitates nanoparticle self-assembly, and the product is collected from the outlet.

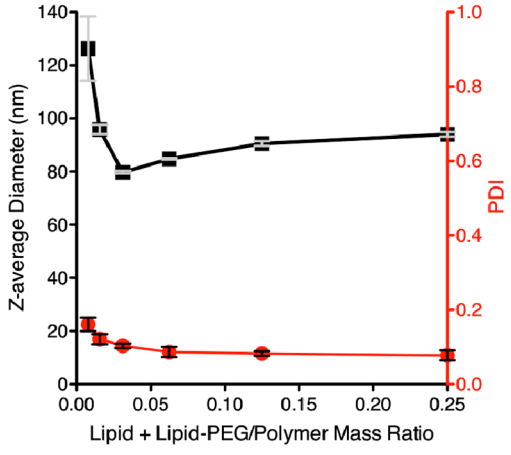

Figure 2. Effect of the shell material (lipid + lipid-PEG) to core material (PLGA polymer) mass ratio on the production scalability of the hybrid nanoparticles. Lipid/lipid-PEG mass ratio, PLGA concentration, and flow rate were kept constant, while the mass ratio of the shell material to the core material was varied. All samples were adjusted to 1X PBS. Nanoparticle size (diameter, nm, ■) and polydispersity index (PDI, red circle) were determined by DLS.

not enough lipid and lipid-PEG to cover all of the PLGA cores, and the resulting particles were unstable in PBS. This led to an increase in both the average size of the particles as well as the particle polydispersity due to aggregation. When the shell/core mass ratio was greater than 0.05, there was little change in particle size and PDI, signifying that at that point there was enough lipid and lipid-PEG to coat all of the PLGA cores.

After the formulation parameters of the hybrid nanoparticles were optimized, the PLGA polymer concentration in organic phase was varied. This parameter was an important determinant of final particle size in the synthesis of hybrid nanoparticles on the lab-scale,[8] and thus it was believed that manipulating the polymer concentration in an MIVR system could allow for the same fine-tuning of particle properties. Fixing the shell/core mass ratio at 0.075, the concentration of PLGA in the organic phase was varied from 1 to 10 mg/mL (Figure 3). As observed

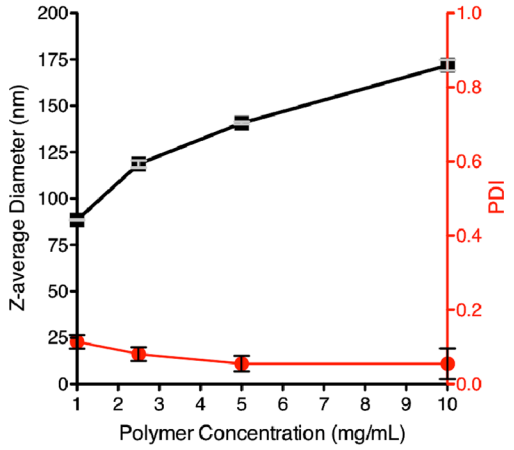

Figure 3. Effect of the polymer (PLGA) concentration on the production scalability of the hybrid nanoparticles. The shell/core mass ratio and flow rate were kept constant, while the concentration of PLGA in the organic phase was varied. All samples were adjusted to 1X PBS. Nanoparticle size (diameter, nm, ■) and polydispersity index (PDI, red circle) were determined by DLS.

in the case of lab-scale synthesis, the higher was the polymer concentration, the larger was the resulting particle size. The particle size varied from about 100 to 200 nm as the concentration of PLGA in acetonitrile was increased across the specified range. Additionally, the PDI dropped marginally from 0.10 to 0.05 across the same range. These results demonstrated it is indeed possible to manipulate particle size by varying the concentration of polymer. Furthermore, polymer concentration is an important parameter in large-scale synthesis because it scales directly with production yield; choosing the highest possible polymer concentration for synthesis while still maintaining acceptable particle parameters is a significant consideration.

The last synthesis parameter that was explored was the effect of the total flow rate through the MIVR system on the production scale-up of the hybrid nanoparticles. Increasing the flow rate alters the mixing efficiency of the system while concurrently boosting the production yield. Using a shell/core mass ratio of 0.075 and a PLGA concentration of 2.5 mg/mL, the total flow rate was varied from 2 mL/min to as high as 50 mL/min. As shown in Figure 4, it was observed that the particle size decreased from about 130 to 80 nm as the flow rate was increased within this range. The decrease in size can be attributed to the increase in Reynolds number, and the corresponding increase in mixing efficiency that occurs as flow rate is increased through the device. It should be noted that there was also a slight increase in PDI as the flow rate increased, which could possibly be explained by the increase in turbulence within the mixing chamber at higher Reynolds numbers. Overall, there seems to be a trade-off between a decrease in size and an increase in PDI as production is scaled up via an increase in flow rate.

By manipulating the concentration of PLGA in the organic phase and the flow rate through the MIVR, it is possible to maximize the production rate while still obtaining particles with desirable characteristics for drug delivery. As shown in Table 1, the maximum output that can be achieved using the ranges studied above for these two parameters is 15 g/h. This represents an over 10 000-fold increase in production rate as compared to the original nanoprecipitation-based lab-scale

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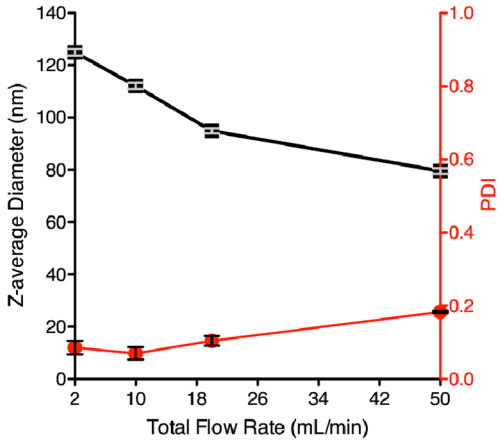

Figure 4. Effect of the total flow rate on the production scalability of the hybrid nanoparticles. The concentration of PLGA in the organic phase as well as the overall formulation were kept constant, while the total flow rate (organic/aqueous = 1:1) through the MIVR was varied. All samples were adjusted to 1X PBS. Nanoparticle size (diameter, nm, ■) and polydispersity index (PDI, red circle) were determined by DLS.

Table 1. Nanoparticle Production Yield (g/h) as a Function of Polymer Concentration (i.e., PLGA) and Total Flow Rate (Organic/Aqueous = 1:1)

	total flow rate (mL/min)			
PLGA concentration (mg/mL)	2	10	20	50
1	0.06	0.3	0.6	1.5
2.5	0.15	0.75	1.5	3.75
5	0.3	1.5	3	7.5
10	0.6	3	6	15

method, which only allows for yields on the order of 1 mg/h. Note that there appears to be a balance between production yield, particle size, and polydispersity that needs to be managed when scaling up the production of hybrid nanoparticles using an MIVR device. Increasing the polymer concentration within the organic phase increases production yield, but at the same time there is also an increase in the average particle size. Increasing the flow rate through the device increases production yield and decreases particle size, but it at the same time also produces a corresponding increase in the polydispersity of the resulting particle population. Thus, the maximum production rate depends on the size and size distribution requirements that are dictated by eventual downstream applications.

Several steps were taken to confirm that the particles synthesized using the MIVR were on par with those fabricated via lab-scale methods. To carry out this comparison, hybrid nanoparticles were made using a sonication process that was previously reported to produce high-quality particles in a single-step process.[15] In this process, the organic and aqueous phases are mixed together in a cocktail fashion, and the resulting mixture is bath sonicated for 5 min to promote self-assembly. These particles were compared to those made using the MIVR with the following parameters: 0.075 shell/core mass ratio, 2.5 mg/mL PLGA concentration in acetonitrile, and 50 mL/min total flow rate. As shown in Figure 5, the physicochemical characteristics of large-scale particles were very similar to those prepared on the lab-scale. Both were well under 100 nm in size with zeta potentials of around −40 mV. Visualization by scanning electron microscopy (SEM) revealed that both particle populations were fairly monodisperse and that the particles were both spherical in shape.

Particle stability is an important factor to consider both for long-term storage and to predict longevity in physiological conditions. To test the shelf life of hybrid nanoparticles made on the large-scale, the particles were fabricated and adjusted to 1X PBS. The size and PDI were then measured every 2 days for a period of 2 weeks (Figure 6A). There was no perceptible change in both the average size and the PDI throughout the duration of the study. This matched what was observed for the particles' lab-scale counterparts, which have been shown to be stable in PBS previously. Additionally, the stability of the particles was assayed in 100% fetal bovine serum. Particles were synthesized and added to an equal volume of 2X serum. The absorbance of the particles was then measured at 560 nm every minute for 3 h to detect for any aggregation that was occurring within the solution (Figure 6B). Again, there was no perceptible change for the large-scale sample, which corresponded well to what was observed for the lab-scale sample. On the other hand, bare PLGA particles with no surface coating of lipids aggregated quickly, which was detected as a quick rise in the absorbance of the sample over the first hour of the study. Taken together, these studies demonstrate that hybrid nanoparticles fabricated on a large-scale using the MIVR not only have physicochemical characteristics on par with those made on the lab-scale, but also are just as stable.

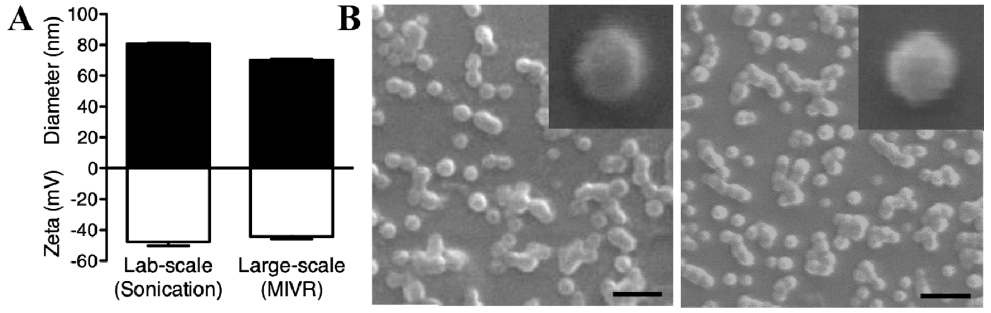

Figure 5. Comparative physicochemical characterization of hybrid nanoparticles synthesized on lab-scale (using sonication) and large-scale (using MIVR). (A) Nanoparticle size (diameter, nm) and surface zeta potential (mV) were measured by DLS. (B) Scanning electron micrographs of hybrid nanoparticles synthesized using lab-scale (left) and large-scale (right) methods. Insets show a single nanoparticle ~80 nm in size. Scale bar = 200 nm.

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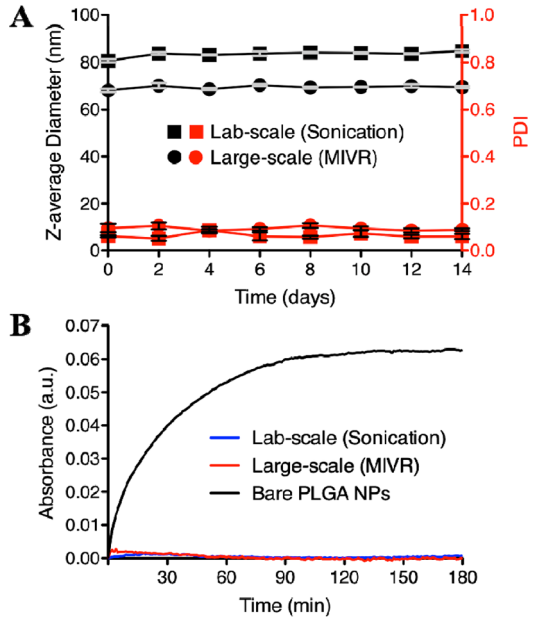

Figure 6. Comparative stability of hybrid nanoparticles synthesized on lab-scale (using sonication) and large-scale (using MIVR). (A) Stability of the two samples in PBS. Samples were adjusted to 1X PBS, and their size (diameter, nm) and PDI were measured every 2 days over a period of 14 days using DLS. (B) Stability of the two samples in 100% serum. Each sample was mixed with an equal volume of 2X fetal bovine serum, and the degree of aggregation was determined by the absorbance reading of the mixtures at 560 nm over a period of 3 h.

CONCLUSIONS

We have adapted a multi-inlet vortex reactor (MIVR) for use in the large-scale synthesis of lipid−polymer hybrid nanoparticles. Using this device, it was demonstrated that several different variables, including formulation, PLGA concentration, and flow rate through the device, had marked effects on the final particle characteristics. Hybrid nanoparticles synthesized using the MIVR were comparable to lab-scale particles in both their physicochemical properties and their stability in PBS and serum. When scaling up the formulation, there is a trade-off between production yield and the desired particle characteristics. This needs to be considered when tailoring the large-scale production to meet the requirements of specific applications. There remains work to be done to show that both nanoparticle functionalization and drug loading can be incorporated into the large-scale synthesis process. Nonetheless, use of the MIVR appears to be a viable strategy for producing hybrid nanoparticles in clinically significant quantities.

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Notes

The authors declare no competing financial interest.

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