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ARTICLE

Dispersion-Based Methods for the Engineering and Manufacture of Polymeric Nanoparticles for Drug Delivery Applications

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ABSTRACT

Polymeric nanoparticle is a general platform for drug delivery that has been receiving extensive attention in both academia and industry. With the ability to load drugs at high encapsulation efficiency, to release drugs at a sustained and controllable manner, and to be functionalized with targeting agents for cell-specific drug delivery, polymeric nanoparticle-based drug formulations show great potential for improving disease therapeutics. One great advantage of polymeric nanoparticles is that many of the methods used for their fabrication are simple and safe. However, in order to bring promising polymeric nanoparticle platforms from the lab to the clinic, these fabrication technologies need to be scaled up several orders of magnitude in terms of production yield. This review details the most popular methods for synthesizing polymeric nanoparticles in recent literature. Current lab-scale techniques are examined before going in to an evaluation of the promising technologies for large, clinical-scale nanoparticle production. This review article concludes with an outlook on the future of polymeric nanoparticle fabrication as it relates to clinical translation.

KEYWORDS: Polymeric Nanoparticle, Nanoprecipitation, Emulsion, Microfluidic Device, Self-Assembly, Drug Delivery.

1. INTRODUCTION

The onset of the nanotechnology revolution in recent decades has allowed for a myriad of novel drug delivery platforms that are ushering in a new generation of therapeutic treatments for diseases such as cancer.[1,2] With a vast array of nanoparticle-based drug delivery products on the market, in clinical trials, and in pre-clinical tests, the prospect of being able to significantly improve upon the efficacy of traditional free drug formulations is becoming an ever-increasing reality.[3–7] As our understanding of the biology of human diseases improves, it is important to be able to intelligently engineer solutions that tailor to this new knowledge. One of the great appeals of nanoparticles, which generally refer to particles in the 1–100 nm size range, is that they provide inherent advantages that are not manifested in larger-sized platforms. These include the ability to passively target tumor vasculature due to the enhanced permeability and retention (EPR) effect[8] and an increased surface area for functionalization with active targeting ligands.[8–10] Nanoparticles can also efficiently incorporate hydrophobic compounds, which allows for the delivery of many promising drugs that would otherwise be rendered useless by their physicochemical properties.[8] Additionally, multi-model nanoparticle systems have the potential to increase treatment efficacy by simultaneously delivering multiple active agents at once.[11–13] This can allow for novel applications such as simultaneous drug delivery and imaging[14] as well as the co-delivery of multiple drugs using the same particle.[15–20]

Of particular interest in this review is a class of nanoparticles known as polymeric nanoparticles. These nanoparticles are generally composed of a biodegradable polymeric core, which can encapsulate active agents, and a stabilizing outer layer for solubilization in aqueous solution. Of the different polymeric nanoparticle systems, two of the most popular in recent literature have been block copolymer nanoparticles[21] and hybrid nanoparticles.[22] Block copolymers generally consist of a hydrophobic polymer conjugated to a hydrophilic polymer. Under the correct conditions, these copolymers self-assemble into micelle structures that can serve as drug delivery vehicles. Copolymer systems have been shown to efficiently incorporate drugs and imaging agents into their solid core.[21,23] Additionally, *in vivo* studies have proven that functionalizing the outer stabilizing layer with active targeting ligands can improve localization in tumor vasculature.[24,25] Hybrid

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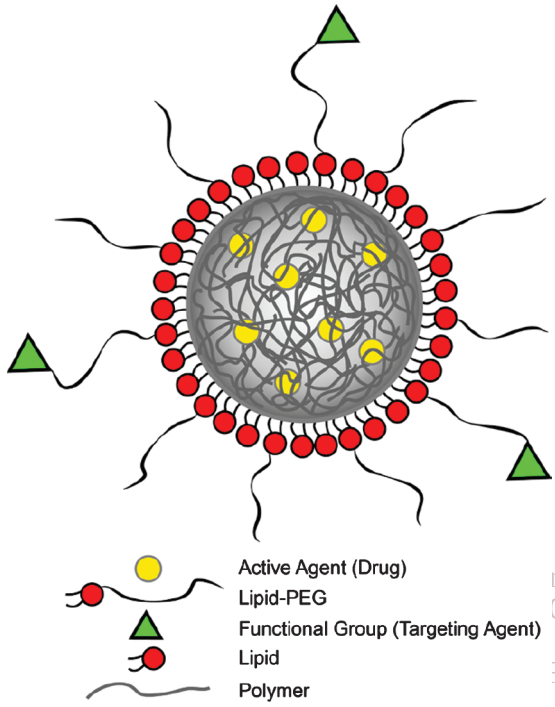

Fig. 1. Hybrid nanoparticle. Illustration of a functionalized and drug-loaded lipid-polymer hybrid nanoparticle with a core-shell structure. The polymeric core, with drug loaded into it, is surrounded by a lipid/lipid-polyethylene glycol (lipid-PEG) monolayer. The nanoparticle is functionalized by conjugating ligands onto the PEG.

nanoparticles (Fig. 1) typically consist of a solid polymeric core on the inside and a lipid shell on the outside.[26] These particles are generally synthesized using a modified, single-step nanoprecipitation process that results in particles with good drug delivery characteristics and tunable physical properties.[22, 27, 28] Hybrid nanoparticles, like copolymer nanoparticles, have also shown the ability to incorporate drugs at high efficiency[26] as well as the ability to be functionalized with active agents for targeted drug delivery.[29–31]

This review details dispersion-based methods for the synthesis of polymeric nanoparticles from pre-synthesized polymers. First, we will explore current lab-scale techniques for nanoparticle fabrication. The following section then details the path to scaling up nanoparticle production and current technologies that have the potential for manufacturing the gram-sized quantities required for eventual clinical use. Finally, we will present our vision on the hurdles that need to be overcome to make polymeric nanoparticles ubiquitous in medical technology.

2. LAB-SCALE SYNTHESIS

Various dispersion-based methods have been employed to synthesize polymeric nanoparticles in the laboratory (Fig. 2). Some of the most popular techniques include the emulsification-diffusion, salting-out, and nanoprecipitation methods.[32, 33] While all technically different, these techniques share the same basic principle, which is to evenly precipitate and stabilize the polymers in an anti-solvent. Galindo-Rodriguez et al. performed an in depth study on the effects of the physicochemical properties of the aqueous and organic phases on nanoparticle formation.[34] Their results showed that a wide array of factors, including solvent, polymer, and salt concentration could be systematically varied to fine-tune the final nanoparticle characteristics. In this section, each of these three techniques will be examined.

2.1. Emulsification-Diffusion

Emulsion-based processes are a powerful tool employed in many forms of nanoparticle synthesis.[35–37] In terms of polymeric nanoparticles, the emulsification-diffusion process, first reported by Leroux et al., was an improvement over the original emulsification-evaporation method in that it did not require the use of toxic, water-immiscible solvents.[38] The method involves water as the anti-solvent and a partially water-soluble solvent phase. Oil-in-water emulsions are created by over-saturating the water phase with the solubilized polymer solution and using high-speed homogenization in the presence of an emulsifier/stabilizing agent. The polymer, which preferentially resides in the oil phase, is contained within the resulting emulsions. In order to form the polymeric nanoparticles, water is added such that the solvent concentration is decreased below saturation. This allows the solvent to diffuse out of the emulsions, resulting in precipitated nanoparticles. It is believed that this diffusion of solvent out of the emulsions creates small, localized regions of polymer super-saturation that promote particle formation.[39]

Quintanar-Guerrero et al. studied the effect of different formulation and synthesis parameters on the size and distribution of poly(D,L-lactic acid) (PLA) nanoparticles produced by the emulsification-diffusion method.[40] They found that PLA polymer concentration, stirring rate, and stabilizer concentration all had an influence on the final nanoparticle size. For example, by varying the stabilizer concentration from 1 to 15% (w/v), they were able to decrease the mean particle size from 450 nm to almost 100 nm. Delmas et al. recently investigated the preparation of nano-scale emulsions through the use of sonication.[41] Their findings indicate that emulsion size can be significantly reduced by introducing energy into the system via sonication.

One of the problems with o/w emulsion systems is that it is difficult to incorporate hydrophilic active agents into the core of the nanoparticles in an efficient manner. In order to solve this problem, water-in-oil-in-water (w/o/w) double emulsion systems have been employed.[42, 43] This method differs from the original

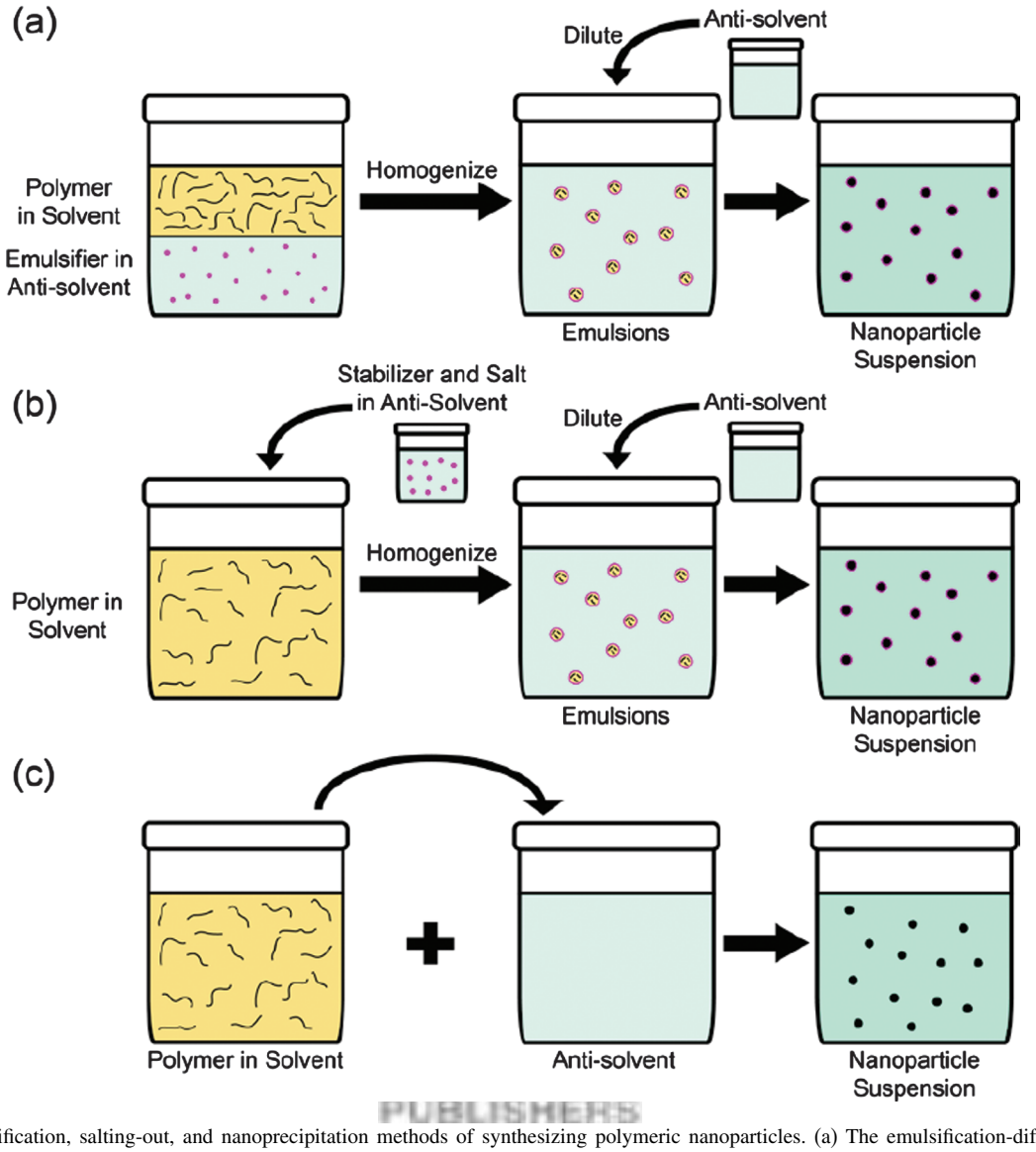

Fig. 2. Emulsification, salting-out, and nanoprecipitation methods of synthesizing polymeric nanoparticles. (a) The emulsification-diffusion method involves creating oil-in-water emulsions. The polymer is located in the oil (solvent) phase and the emulsions are stabilized by emulsifiers. Upon diluting the emulsions with water, the solvent diffuses out of the emulsions and nanoparticles form. (b) The salting-out method is a modified emulsification technique in which emulsions are formed as a result of the decreased miscibility of the solvent and anti-solvent at high salt concentrations. The resulting emulsions are then diluted with anti-solvent such that the two phases are once again fully-miscible, causing the solvent to diffuse out of the emulsions and nanoparticles to form. (c) The nanoprecipitation method involves dissolving polymer into a solvent and precipitating that solution into an anti-solvent, causing the spontaneous formation of nanoparticles.

method in that a water-in-oil (w/o) emulsion is formed first with the hydrophilic payloads present in the water phase and the polymer in the oil phase. The w/o emulsion is then added into water with an emulsifier to form a w/o/w emulsion, which eventually leads to the final nanoparticle formation. Zambaux et al. investigated the loading of protein C, a plasma inhibitor, into PLA nanoparticles formed by the double emulsion method.[42] In their experiments, they were able to successfully confirm the loading of protein C by running activity assays. They also found that a decrease in PLA molecular weight led to an increase in the release of protein C over time.

2.2. Salting-Out

The salting-out method to prepare polymeric nanoparticles is a unique variation of the emulsification process that was first detailed by Ibrahim et al. in their exploration of cellulose acetate phthalate nanodispersions.[44] The major difference when comparing to traditional emulsification methods is that it is possible to use non-emulsifier stabilizing agents such as poly(vinyl alcohol) (PVA). In short, the method involves the formation of oil-in-water (o/w) emulsions through the salting-out of a water-miscible solvent under vigorous stirring. The resulting emulsions are

then diluted with water to reverse the salting-out process, allowing the solvent to diffuse into the aqueous phase and nanoparticles to form.

Allémann et al. studied the effect of various process parameters on the final size of particles produced by the salting-out method.[45] They found that increasing the stirring rate during the emulsification step from 500 to 1400 rpm decreased the final size of the particles from 450 to 250 nm. Likewise, increasing the amount of PVA in the external phase from 5 to 12% (w/v) also decreased particle sizes significantly. The same group also evaluated the drug loading and drug release of savoxepine-encapsulated PLA nanoparticles in a later study.[46] They were able to achieve up to 16.7% drug loading by weight and an entrapment efficiency of 94.6%. The nanoparticles also exhibited sustained drug release over long periods of time, with an initial burst during the first few days followed by gradual release over a period of up to 30 days. More recently, Mccarron et al. corroborated these results by examining celecoxib-loaded poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles prepared using a modified salting-out procedure.[47] They were able to produce 200 nm particles at an entrapment efficiency of more than 97%. In the same study, the authors also determined that employing sonication in place of homogenization produced nanoparticles much more efficiently.

2.3. Nanoprecipitation

Perhaps the simplest and one of the most widely used modes for polymeric nanoparticle synthesis in recent literature is the nanoprecipitation method. By dissolving a polymer in a solvent, usually organic, and transferring the solution to an anti-solvent, usually aqueous, nanoparticles are formed as the polymer solution diffuses into the anti-solvent. Bilati et al. reported on a variety of factors that affect the size and distribution of nanoparticles formed by nanoprecipitation.[48] Relevant factors included choice of solvent and anti-solvent, solvent to anti-solvent ratio, polymer choice, and polymer concentration. In one experiment, by varying the types of solvent and anti-solvent chosen for nanoprecipitation, they were able to produce particles ranging in size from 84 to 525 nm. Additionally, Legrand et al. have more recently shown that the interaction between solvent and polymer is also an important determinant of nanoparticle quality.[49]

Although nanoprecipitation is a relatively simple process, there have been intensive studies on ways to improve final particle characteristics. While no stabilization agents or emulsifiers are required to make bare nanoparticle dispersions,[48] the use of block copolymers[50] or the addition of lipid to the aqueous phase before nanoprecipitation[26] has shown to result in particles with excellent stability in solution. Thevenot et al. have also demonstrated that it is possible to stabilize nanoprecipitated particles by adsorbing premade liposomes onto the surface of bare polymeric nanoparticle cores.[51]

A challenge of using nanoprecipitation is that the polymers used are generally hydrophobic, leading to low encapsulation efficiencies when trying to load hydrophilic drugs.[52] To address this, Govender et al. have demonstrated that by varying the pH of the aqueous phase from 5.8 to 9.3, it was possible to increase drug entrapment from 11.0 to 58.2% for procaine hydrochloride, a water soluble drug.[53] Finally, it is interesting to note that, while most nanoprecipitation platforms have focused on the polymers such as PLA and PLGA, Hornig et al. have proven that it is possible to make poly(styrene) (PS), poly(methyl methacrylate) (PMMA), and poly(ε-caprolactone) (PCL) nanoparticles through the nanoprecipitation method as well.[54]

3. THE ROAD TO LARGE-SCALE SYNTHESIS

In order to bring the promise of polymeric nanoparticles to fruition, it is highly needed to manufacture them in large, gram-scale quantities. One of the most attractive features of the dispersion-based synthesis methods described in the previous section is that they have great potential to be scaled up due to their simplicity and reproducibility.[55] Galindo-Rodriguez et al. performed initial studies at 20 times lab-scale production for the emulsion, salting-out, and nanoprecipitation methods, demonstrating that high quality nanoparticles could be made in each case.[56] It is important to note, however, that for the emulsification-diffusion and the salting-out techniques, they discovered that nanoparticle sizes were not constant between the large pilot-scale and the lab-scale methods at the same stirring rate. The nanoprecipitation technique proved to be the most promising for large-scale synthesis as it required the least amount of time and steps for synthesis and could be scaled up in a reproducible way by adjusting the smallest number of parameters compared with the other methods. It is perhaps for this reason that many of the recent studies with large-scale potential have been based off of nanoprecipitation techniques, which will be the focus of this section.

3.1. Optimization of Parameters

In order to more effectively design systems for large-scale synthesis, it is important to understand the different parameters that can be altered to optimize nanoparticle fabrication. Fang et al. have recently reported that the use of sonication in conjunction with a modified nanoprecipitation technique could result in a 20-fold reduction in the time required for synthesis of lipid-polymer hybrid nanoparticles.[57] By creating a cocktail of the

required materials and sonicating for 5 min, they were able to produce sub-100 nm nanoparticles with very low polydispersity values. In terms of the mechanics of particle formation, Chen et al. have shown through Brownian dynamics simulations that super-saturation is a major driver in the formation of nanoparticles via their novel Flash Nanoprecipitation (FNP) method.[58] In a study done by the same group, Johnson et al. have demonstrated that mixing time is a major determinant of particle size down to a certain point.[59] As the characteristic mixing time of the FNP reactor decreased below the characteristic aggregation time of the polymer in anti-solvent, they found that the final particles were the same size. For characteristic mixing times above the characteristic aggregation time, final particle sizes became larger. These studies showed that particle formation in an FNP system is a kinetically driven, diffusion-limited process. In terms of maintaining particle stability, Liu et al. have shown that having a high ratio of solvent to anti-solvent in the product solution is an important factor for the prevention of particle growth via Ostwald ripening.[60]

3.2. Fluidic Devices

Stainmesse et al. performed mechanistic studies on nanoparticle formation via nanoprecipitation and concluded that only a very limited region on a ternary diagram of solvent, anti-solvent, and polymer composition could result in the formation of a nanoparticle suspension.[61] The region of interest corresponded to low polymer concentrations, which presents a problem for scaling up due to the large volumes of solvent and anti-solvent required for nanoparticle synthesis. The use of continuous synthesis methods such as those based on fluidic devices.[59, 62–64] can potentially circumvent this problem. It has been shown that, by shooting the polymer solution and anti-solvent rapidly into a mini/micro-reaction chamber, fluidic devices can aid in the synthesis of nanoparticles via nanoprecipitation with final sizes that are independent of initial concentration. As previously mentioned, this is done by optimizing reaction parameters such that the mixing rate is faster than the characteristic aggregation time of the particles.[59] Additionally, because these fluidic systems perform the mixing in a very small chamber, there is no need for reactors that are the same size as the final volume of the product.

The ability of fluidic devices to manufacture nanoparticles has been confirmed for both block copolymer nanoparticles.[62, 63] and lipid-polymer hybrid nanoparticles.[64] Valencia et al. have shown that sub-100 nm hybrid nanoparticles can be made with a three-inlet microfluidic system.[64] By fine-tuning parameters such as polymer concentration, flow rates, and polymer/stabilizer ratios, they were able to control the size and surface zeta potential of the final nanoparticles. Likewise, Karnik et al. and Johnson et al. have shown that block copolymer nanoparticles can be made using a three-inlet microfluidic system.[63] and a cross-impinging jet (CIJ) system,[62] respectively. The use of rapid mixing allowed for the synthesis of homogeneous populations of sub-100 nm nanoparticles in both cases.

It has also been demonstrated that it is possible to functionalize the nanoparticles synthesized using these fluidic devices and encapsulate active agents into them. Karnik et al. explored the loading and release of docetaxel for PLGA-polyethylene glycol (PLGA-PEG) block copolymer nanoparticles.[63] Compared to traditional synthesis techniques, which resulted in an average particle size of almost 200 nm, the use of microfluidics resulted in nanoparticles with sizes around 100 nm for the same initial drug input. Additionally, the drug loading yield was slightly increased and the rate of drug release over time was marginally prolonged for those nanoparticles synthesized using microfluidics. In a separate study, Kolishetti et al. demonstrated the ability to differentially deliver drug-loaded nanoparticles using the A10 aptamer, which binds preferentially to a specific antigen on the membrane of prostate cancer cells.[16] Using a post-synthesis step, the A10 aptamers were conjugated via 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) chemistry to the carboxyl terminal group of the PEG end of the block copolymer.

3.3. Multi-Inlet Vortex Reactor

The multi-inlet vortex reactor (MIVR) (Fig. 3), first reported by Liu et al., is a novel type of device that has been proven both experimentally and in simulation to provide very efficient micro-mixing for nanoprecipitation-based particle synthesis.[65] The device can be fabricated with either two or four circularly symmetric inlets; the outlet is located at the center of the device. The arrangement of the inlets allows each input stream to contribute independently to the mixing performance of the MIVR. This flexibility in relative input rates allows for the fine-tuning of solvent to anti-solvent ratios, which play an important role in determining final particle characteristics.

In a separate study by Liu et al., the MIVR was used to successfully fabricate block copolymer nanoparticles loaded with the pesticide bifenthrin, demonstrating that it is possible to load organic actives into the cores of nanoparticles synthesized using this approach.[66] For 120 nm nanoparticles, they were able to achieve a drug loading of up to 90%. Gindy et al. demonstrated a post-synthesis process for conjugating ligands to the surface of MIVR-prepared nanoparticles.[67] In this study, bovine serum albumin (BSA) acted as the model ligand and was attached to the surface of PCL-PEG block copolymer nanoparticles via a maleimide-thiol reaction. By varying the amount of maleimide-terminated PEG versus the

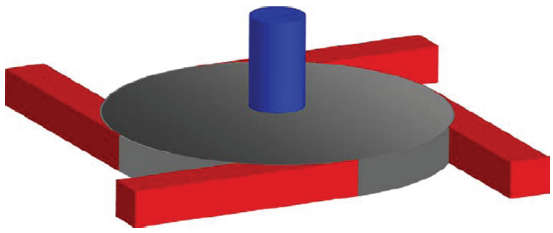

Fig. 3. Multi-inlet vortex reactor (MIVR). The red channels indicate the four inlets and the blue channel indicates the outlet of the device. The gray disk indicates the reaction chamber.

amount of methoxy-terminated PEG in the formulation, it was shown that the ligand surface coverage density could be fine-tuned. Akbulut et al. leveraged the findings of the previous two studies to produce multifunctional block copolymer nanoparticles with fluorescent agents and/or drugs in the core and targeting agents on the surface, proving that all the modifications that are performed on traditionally synthesized nanoparticles can be applied to those synthesized using an MIVR system.[68]

4. CONCLUSIONS AND OUTLOOK

Polymeric nanoparticles are a promising group of drug delivery vehicles not only because of their functionality, but also for their potential scalability. The most common modes of polymeric nanoparticle synthesis have proven to be very simple to carry out and give reproducible results from batch to batch. Using the methods previously described, polymeric nanoparticles can have tunable size, surface zeta potential, drug loading yield and drug release kinetics among many other characteristics. In terms of large-scale synthesis, the most promising methods currently involve the use of fluidic devices such as the multi-inlet vortex reactor. Using these systems, it has been proven that nanoparticles can be made that have characteristics at least on par with those made using traditional lab-scale techniques.

Despite the great promise of fluidic devices, current studies have been more proof-of-concept in terms of large-scale manufacturing. Microfluidic devices are promising in that they can continuously synthesize nanoparticles, but the flow rates are so low[63, 64] that hundreds of devices would need to be run in parallel to achieve the productivity that is desired. Larger systems like the MIVR have flow rates on the correct order, but employ the use of syringe pumps,[65] which have limited volume per injection. In order to convincingly prove the scalability of these systems, these limitations need to be addressed. Possible solutions could involve the fabrication of microfluidic chips with hundreds of parallel reaction chambers or to adapt the MIVR for pumps that can draw from continuously replenishable reservoirs.

Furthermore, although the ability to functionalize nanoparticles made using fluidic systems has been proven,[16, 67, 68] the current methods employ the use of a post-precipitation step after the nanoparticles have already formed. One important consideration when scaling a formulation up is the number of synthesis steps. The beauty of nanoprecipitation is that it requires only a single step; adding another step could easily double the complexity of the synthesis. One way to address this is to adapt the MIVR system for making hybrid nanoparticles. In hybrid nanoparticle synthesis, the final particle can be functionalized by adding functional building blocks to the aqueous phase before nanoprecipitation. In doing so, the complete, functionalized particles should be able to be synthesized in a single-step, thereby preserving the simplicity of the original method.

Finally, in order to make nanoparticles ready for clinical use, the issues of sterilization and purification must be considered. As lab-scale synthesis shifts towards large-scale manufacturing, more questions will arise on how to obtain pure and sterile nanoparticle suspensions. To address this, collaboration between academia and industry is highly encouraged, as the biotech and pharmaceutical industries have much more experience in addressing these types of issues. Seeing as how polymeric nanoparticle platforms have great potential for commercialization, it makes sense to have more industry involvement in the development of this technology as it matures.

The challenges noted above are important items that need to be addressed before polymeric nanoparticles can reach ubiquity in disease therapeutics, but none of them are issues that cannot be overcome with further investigation. The polymeric nanoparticle remains a platform that shows tremendous potential for use in drug delivery applications and could very well one day reside at the forefront of medical technology.

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