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PERSPECTIVES

Semi-Automated Nanoprecipitation-System—An Option for Operator Independent, Scalable and Size Adjustable Nanoparticle Synthesis

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ABSTRACT The preparation of nano-sized carrier systems increasingly moved into focus of pharmaceutical research and industry in the past decades. Besides the drug load and properties of the selected polymer/lipid, the size of such particles is one of the most important parameters regarding their use as efficient drug delivery systems. However, the preparation of nanoparticles with different sizes in a controlled manner is challenging, especially in terms of reproducibility and scale-up possibility. To overcome these hurdles we developed a system relying on nanoprecipitation, which meets all these requirements of an operator independent, scalable and size-adjustable nanoparticle synthesis—the Semi-Automated Nanoprecipitation-System. This system enables the adaption of the particle size to specific needs based on the process parameters—injection rate, flow rate and polymer concentration—identified within this study. The basic set-up is composed of a syringe pump and a gear pump for a precise control of the flow and injection speed of the system. Furthermore, a home-made tube-straightener guarantees a curvature-free injection point. Thus it could be shown that the production of poly(lactide-*co*-glycolide) nanoparticles from 150 to 600 nm with a narrow size distribution in a controlled semi-automatic manner is possible.

KEY WORDS continuous nanoparticle preparation · nanoprecipitation · PLGA · SAN-System · scale-up

ABBREVIATIONS

DLS	Dynamic light scattering
DMSO	Dimethyl sulfoxide
FR	Flow rate
IP	Injection position
IR	Injection rate
PDI	Polydispersity index
PLGA	Poly(lactide-*co*-glycolide)
PVA	Polyvinyl alcohol
SAN-System	Semi-Automated Nanoprecipitation-System
SEM	Scanning electron microscopy

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INTRODUCTION

Nano drug carrier systems increasingly gain influence in the field of galenic drug formulations. By the use of drug carrier systems in general, problems associated with the free drug or therapeutic ineffectiveness can be solved. Problems that can be addressed, are for example an unfavorable pharmacokinetic, a faster *in vivo* degradation, poor biodistribution or even a lack of selectivity for the target tissue (1). In short, these carrier systems can improve the bioavailability and significantly reduce the toxicity of the free drug (2). Especially great potential promises nanoscale carrier systems, so-called nanocarriers. The term nanoscale defines a size range of approximately 1–100 nm. However, the term nanocarriers describes also systems in the sub-micrometer up to 1000 nm in the context of nanomedicines (3). A major disadvantages of most nanocarriers are the poor scale-up possibility and

bad reproducibility of particle production precluding a commercial or clinical use of nanoparticles (4). Depending on the encapsulated drug, especially colloidal systems based on lipids or polymers are most likely suitable. Consequently, such systems are well studied in recent years (5–7). The enormous potential of nanocarriers is due to their small size and large surface-to-volume ratio enabling a good tissue penetration and a high cellular uptake. Both are important aspects in order to allow a carrier system to effectively deliver a drug to the site of action. For the preparation of such nano-scaled carrier systems the nanoprecipitation method is one of the most prevalent and commonly used methods (5). The nanoprecipitation method, investigated by Fessi in the end of the 1980s (8), is a simple, rapid, inexpensive and due to the low energy input an especially gentle manufacturing process for polymer-based nanoparticles. Specific for the nanoprecipitation method is the precipitation of a dissolved polymer forming particles, after mixing the solvent with a non-solvent for the polymer containing stabilizing agents. The solvent is thereby totally miscible with the non-solvent. The nanoparticles are formed immediately by fast diffusion of the solvent into the non-solvent forming small nano-droplets, which are directly coated by stabilizer (9–11). This method is not limited to a specific polymer but can be transferred to many synthetic or natural polymers which are relevant as drug carrier materials (12). In literature already a multitude of parameters influencing the nanoparticle sizes are extensively discussed. Besides the polymer, solvent and non-solvent, also process parameters, like injection speed of the solvent, FR of the non-solvent and the hydrodynamic forces and their distribution in the non-solvent with respect to IP influence the particle formation. Most of the parameters are considerably depending on the operator and are difficult to control (12). This operator dependency can be avoided using an automated system for nanoparticle preparation which would also allow for scale-up. Therefore, a fluidic nanoprecipitation system was established by Xie and Smith in 2010 (13) without evaluation and identification of production-influencing process parameters. The present work, focus on a broader understanding of the SAN-System and the impact of the process parameters on the formation of a model drug carrier system. Hence the preparation of blank PLGA particles was investigated analyzing size, size distribution, and morphology of the particles. This system can be a viable step on the road to commercialized nanoparticle production for nanomedicines of sufficient pharmaceutical quality.

MATERIALS AND METHOD

Materials

PLGA—Resomer RG 503 H was supplied by Evonik Industries AG (Darmstadt, Germany). DMSO (HPLC grade) and Spectra/Por® 7—dialysis membrane was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). PVA (Mowiol 4–88) was purchased from Kuraray Europe GmbH (Hattersheim, Germany). Water used for all preparations and investigations was Millipore Q-Gard 2 (Merck Millipore, Billercia, United States). All other chemicals used were of analytical grade.

Experimental Setup—SAN-System

The developed SAN-System is composed of several modules (Fig. 1). The relevant parts of the system are the syringe pump (Harvard Apparatus PHD ULTRA, Harvard Apparatus Inc., Holliston, USA), the syringe (Hamilton 1005 TLL, CS-Chromatographie Service GmbH, Langerwehe, Germany), and the gear pump (Ismatec SA MC-Z, IDEX Health & Science GmbH, Wertheim, Germany) with the respective tubing ($Ø_{inner}$=4.8 mm, Norprene Chemical, VWR, Darmstadt, Germany). The syringe pump is controlling the IR of the polymer solvent, by a needle with a straight needle head (Metal (N) Hub Needle 0.72×0.41 ps 3, CS-Chromatographie Service GmbH, Langerwehe, Germany), into the tubing. The gear pump is controlling the FR of the polymer non-solvent within the tubing (0.1 to 100 ml/min). The beginning and end of the tubing's are placed into a beaker with stir bar, for sampling. To avoid bending influenced turbulences (14) at the injection point and to keep the injection point and angle always constant a tube straightener module is used. The needle penetration depth is thereby controlled by a spacer bar (Fig. 1a).

Preparation and Washing of Nanoparticles

Nanoparticles were prepared with by the SAN-System. Different concentrations of PLGA polymer (16.6–100 mg/ml) were dissolved in 3 ml DMSO, drawn up into a 5 ml syringe with a straight needle head and plugged in a syringe pump. Forty milliliters of a 2% PVA solution was used to fill the tubing beaker and gear pump. The resulting nanoparticle solution was purified by dialysis (Molecular weight cut off 15,000 Da) in water (in 2 l for 2 h) to reduce the amount of DMSO for improved lyophilization. Finally, the nanoparticles were purified by centrifugation at 15,000 g for 10 min (Rotina 420 R, Hettich Lab Technology, Tuttlingen, Germany) and washed twice with water to remove excess PVA. After purification Trehalose (w/w) was added as a cryoprotectant for lyophilization. Three independent batches for each formulation were prepared and stored at 6°C after lyophilization.

Characterization of Nanoparticles

The average size (in nm) and size distribution (PDI) of the polymeric nanoparticles were measured using DLS (Zetasizer Nano ZSP, Malvern Instruments, Herrenberg, Germany) at

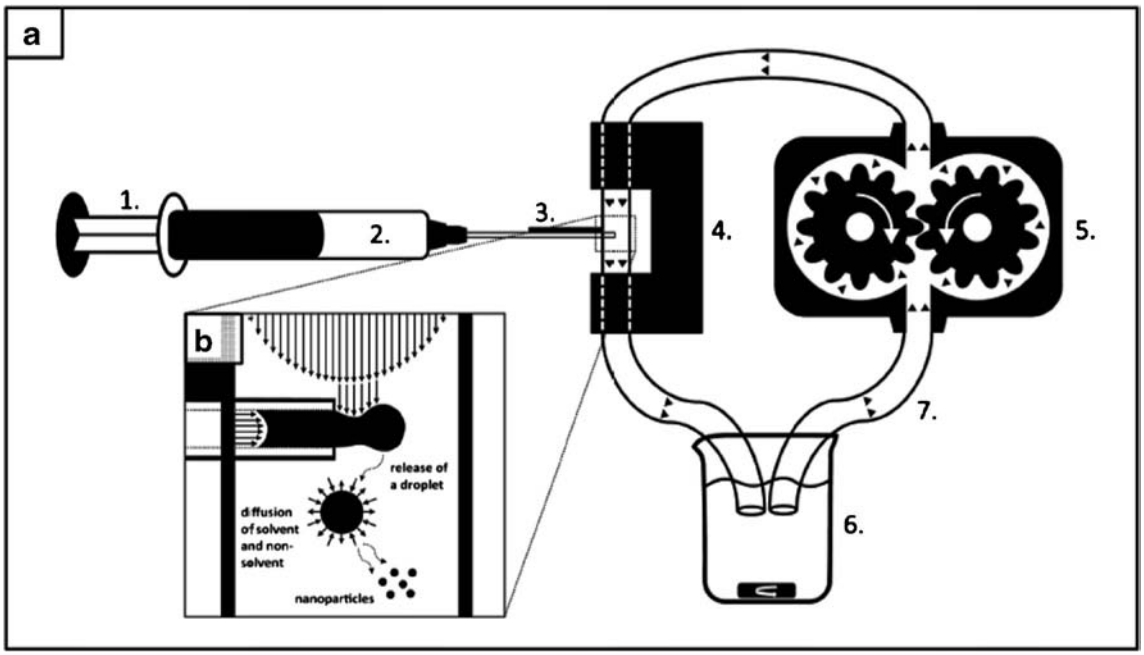

Fig. 1 (**a**) Schematic presentation of the Semi-Automated Nanoprecipitation-System consisting of a syringe pump with a syringe (1), polymer solution to inject (2), a spacer at a needle with a straight head (3), a tube straightener module (4) a gear pump (5), a beaker with stir bar (6), and the tubing (7) (**b**) Schematic presentation of the nanoparticle formation.

25°C. The measurements were performed with aqueous dispersions of nanoparticles (~0.1 mg/ml) before lyophilization. The surface morphology was determined by SEM (Zeiss EVO HD15, Jena, Germany) after sample coating with a 10 nm gold layer (Q150R Rotary-Pumped Sputter Coater, Quorum Technologies, UK) at a focal distance of 10 mm and an acceleration voltage of 5 kV.

RESULTS AND DISCUSSION

The primary objective of our study is the evaluation of the relevant parameters of the SAN-System to control particle size. Several parameters were investigated to show the influence on particle formation. These are the needle position inside of the tube moving the drop formation to different flow conditions of the non-solvent, the IR of the solvent from the syringe, the FR of the non-solvent in the tubes and the polymer concentration. As standard parameters an aqueous 2% PVA solution as non-solvent with a FR of 20 ml/min 50 mg PLGA in 3 ml DMSO as solvent for injection into the non-solvent with an IR of 0.05 ml/min at a fixed needle position in the center of the tubing was used. The size and PDI of the resulting nanoparticles were evaluated as key parameters for comparing the particles supported by scanning electron microscope micrographs.

Based on the laminar flow profile inside the tubes which was estimated by calculating the Reynolds number (< critical Reynolds number of ~2300 for all flow rates investigated (15)) it was assumed that the drop formation and as a consequence particle formation is influenced by the IP. The reason for this assumption is the different flow speed in the center or near the edge of the tube. The head of the needle was placed in the center or near the tube wall at the injection point of the tube or the opposite side of the IP. The penetration depth of the needle was controlled by a spacer and all other parameters, IR, FR and PLGA concentration were kept constant. Smaller and more uniform droplets/particles are estimated for the centered position because of the higher flow speed in the center of the tube. The difference between the centered and the edge position was 1.5 mm. Independently from the needle position no significant smaller particles for the centered position (size 208.1±4.7 nm, PDI 0.09±0.03) compared to the edge positions (size 213.4± 14.7 nm, PDI 0.07±0.03) were found. This is supported by calculations of flow speeds in the center and near the edge of the tubes that show only a difference of 5%. Due to the better handling and no significance of the IP, for all other experiments the center position was used.

To clarify the influence of different IR of the syringe pump, 0.01, 0.05 and 0.25 ml/min were used, with a constant FR of 20 ml/min and PLGA concentration of 50 mg/ml. The needle diameter was also kept constant, because bigger or smaller diameter would only lead to higher or lower speed using the same IR. In detail, the bypassing circulation of non-solvent in the tubes tears off a solvent droplet from the needle exit (Fig. 1b). With higher IR bigger droplets will be formed and as a consequence bigger particles as the FR was kept constant and hence the force to tear off a drop. As expected

an increased size from 145 nm at 0.01 ml/min IR, 154 nm for 0.05 ml/min IR up to 179 nm for 0.25 ml/min IR, with a PDI for all results always below 0.1 was found. Consequently by using different IR the experimental time increases. With the same amount of 50 mg PLGA polymer in 1 ml DMSO, with a decreasing IR from 0.25 to 0.01 ml/min, the experimental time increases from 4 min up to 100 min. As a perspective also a run with an IR of 0.1 ml/min, PLGA concentration of 50 mg/ml and FR of 20 ml/min with an extended time over 8 h was performed. Compared to our short runs, we found no major difference in size (148.1 ± 2.5 nm), by scaling up the amount of polymer and injection time.

For the control of the FR a gear pump was used. The use of a peristaltic pump was excluded due to the non-constant and pulsatile flow which would have complicated or even dismantled the analysis of this parameter. The use of different tube diameters was also not necessary, because thinner or thicker tubes would only result in a changed overall flow speed. Representatively the adjusted flow rates of 1, 4, 8 and 20 ml/min lead to flow speeds of 5.76, 23.06, 46.11 and 115.28 cm/min. The FR used—1, 4, 8 and 20 ml/min for 50 mg/ml PLGA resulted in a steady increase of particles size from 155.6 nm for 1 ml/min up to 228.3 nm for 20 ml/min. This trend is also observed for other PLGA concentrations (Fig. 2). Furthermore it is evident, that with a decrease in FR the particle distribution is increasing. Starting from a PDI with 0.03, which represents a uniform distribution the PDI raises up to 0.15 indicating a more polydisperse size distribution (16). Due to the higher polydispersity of particles prepared with the FR of 1 ml/min this FR was not used for later experiments.

Regarding the influence of the polymer concentration six different PLGA concentrations from 16.6 to 100 mg/ml dissolved in DMSO were tested, while keeping the other parameters constant. The effect of different PLGA concentrations in

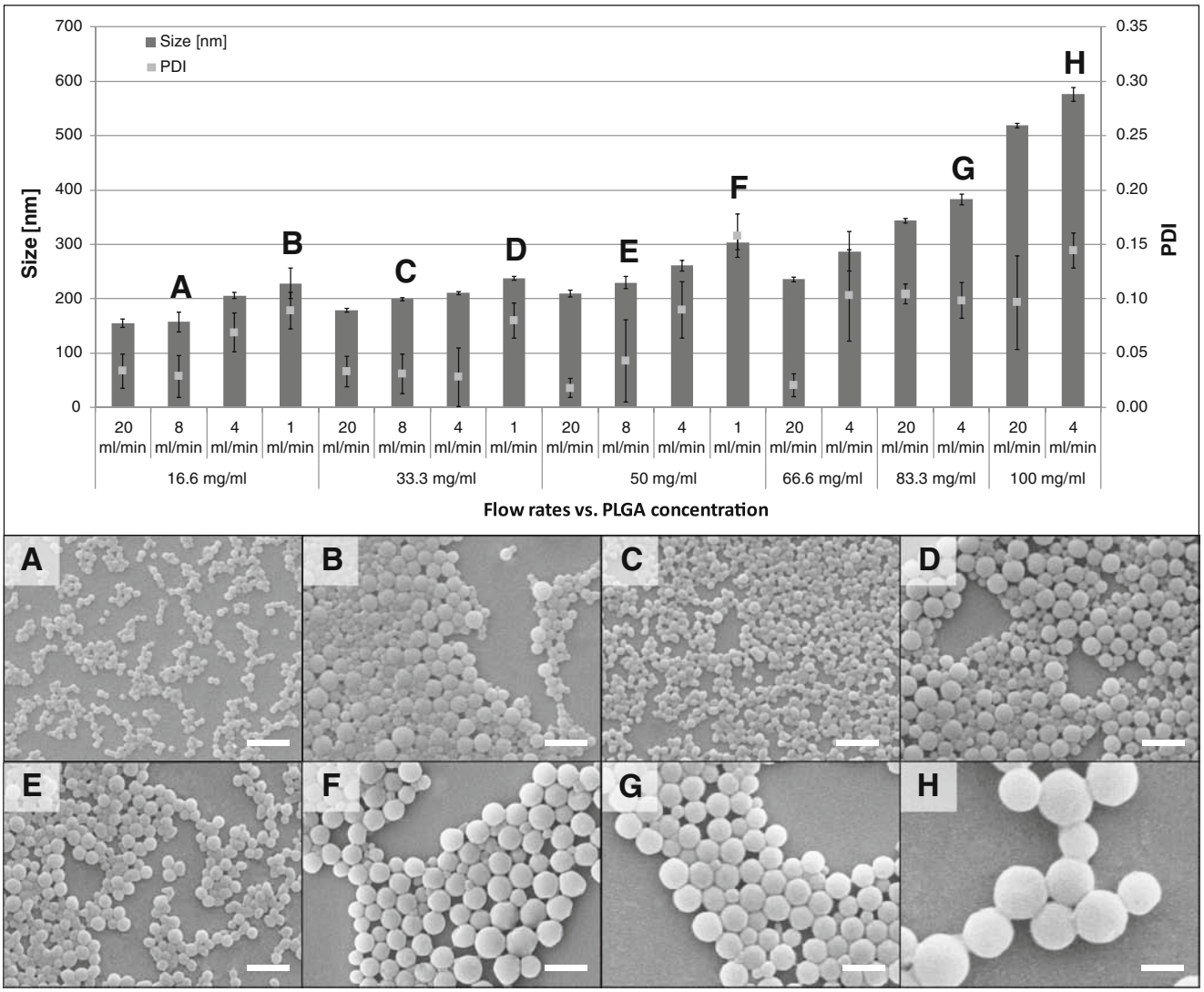

Fig. 2 Bar chart with particle size and PDI as a function of FR and polymer concentration. SEM images (**A**-**H**) to the corresponding DLS measurements. Size of the scale bar = 500 nm.

combination with different FRs for the non-solvent can be seen in Fig. 2. For lower PLGA concentrations 16.6 and 33 mg/ml no change in the size was observed. By increasing the PLGA concentration further from 50 to 100 mg/ml the size of the particles increased up to 580 nm. For higher PLGA concentrations 1 ml/min FR were excluded due to the too high polydispersity. The effect of the concentration on particle size is most likely due to an increase of the viscosity of the solvent, with a higher amount of dissolved polymer. The injected polymer stream from the syringe is still the same, but, with a higher viscosity of the solvent more force is needed to tear off a drop from the stream.

Additionally to DLS the size measurements were evaluated with SEM images (Fig. 2). The particles are spherical and image analyses by Image J revealed 10–20 nm smaller particles. This difference is due to hydrodynamic diameter obtained in DLS, compared to diameters measured under dried conditions with SEM. To summarize, it was possible to generate particles from 150 to 600 nm. The strong influence of the polymer concentration by bench-top (manual) performance of nanoprecipitation was already claimed in literature (17,18).

CONCLUSION

In summary, it can be concluded that the particle size and distribution of the PLGA nanoparticles can be controlled by varying the FR, IR and polymer concentration using the SAN-System in a large range from 150 to 600 nm. The influence can be ranked from IP with no influence under the experimental conditions chosen and to an increasing influence for IR, to FR to polymer concentration. With increasing FR, the resulting particles are smaller. By increasing the IR, the resulting particle size of the prepared PLGA nanoparticles increases (increase of nanoparticles particle size: $0 \sim IP < IR < FR^{-1} < c_{polymer}$). In the present work the polymer concentration was identified as the most relevant process parameter regarding size and size distribution of the particles. The results demonstrate that the developed and validated SAN-System is able to produce particles in a reproducible and controllable manner by the principle of nanoprecipitation without any influence of the operator. Additionally a larger scale-up for the production of nanoparticles with no restrictions is possible, because the quantities of solvent and non-solvent are not limited to a certain extend. Sizes between 100 and 600 nm can be specifically produced for the respective application requirement.

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