Macedonian pharmaceutical bulletin, 69 (Suppl 1) 21 - 22 (2023)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2023.69.03.010
Short communication

Establishment of a continuous inline-monitored nano-production line using the Microfluidizer® technology for the fabrication of safe lipid-based nanoparticles

Christina Glader[1], Ramona Jeitler[1,2], Yan Wang[3], Carolin Tetyczka[1], Manuel Zettl[1], Johannes Khinast[1], Manon Rossano[4], Steve Mesite[5], Matthias Lübbert[6], Eva Roblegg[1,2]

[1]*Research Center Pharmaceutical Engineering GmbH, Inffeldgasse 13, 8010 Graz, Austria*
[2]*University of Graz, Institute of Pharmaceutical Sciences, Pharmaceutical Technology & Biopharmacy, Universitätsplatz 1, 8010 Graz, Austria*
[3]*In-Process LSP, Kloosterstraat 9, 5349 AB Oss, The Netherlands*
[4]*Gattefossé SAS, 36 chemin de Genas, 69800 Saint-Priest, France*
[5]*Micofluidics International Corporation, 90 Glacier Drive, Suite 1000 Westwood, Massachusetts, USA*
[6]*Knauer Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin, Germany*

Introduction

Nano-drug delivery systems (NDDS) are a promising key platform in modern medicine for encapsulating and transporting drugs to the desired site of action. As NDDS are versatile in size, structure and composition, a bandwidth of different manufacturing techniques exists. Currently, industrial production of NDDS is based on time-consuming and error-prone multi-step batch processes that are costly, difficult to scale and hard to control. Beyond this, a lack of process understanding of the individual manufacturing steps and no or insufficient control of the influential process parameters and product quality bear the risk of producing products of poor quality and safety (Operti et al., 2021; Sheybanifard et al., 2022).

To fully exploit nano-systems` potential and to keep pace with advances in medicine, the control on production at an industrial scale needs to be improved. This study focuses on the development and testing of a continuous top-down production line using the scalable microfluidizer® technology. Apart from scalability, different classes of nano-systems (e.g., lipid- and polymer-based) can be produced either with organic solvents or solvent-free and the use of interaction chambers with different geometries further extends the flexibility of this technology. Here we focus on the solvent-free preparation of lipid-based nanoparticles (i.e., solid lipid nanoparticles (SLN), composed of a solid lipid and a stabilizer and nano-structured lipid carriers (NLC), composed of a solid and a liquid lipid plus a stabilizer) using the LM20 Microfluidizer® processor (Microfluidics, Westwood, USA) equipped with precise thermoregulation. Product cooling and particle formation were achieved through linkage with a continuous cooling unit. Since particle size is a critical product attribute, the NanoFlowSizer (InProcess-LSP, Oss, The Netherlands) was installed for in-line monitoring (see Figure 1). For evaluation of the established set-up, SLN and NLC were prepared, considering material properties determined in preliminary Design of Experiments (DoE) studies.

Materials and methods

The critical product-contacting parts of the LM20 Microfluidizer® processor (i.e., the feeder, the Interaction Chamber™, and the conveying/feeding lines) were heated with electrically heated copper plates to achieve microfluidization (MF) temperatures above 50 °C. To avoid temperature fluctuations, the unheated areas of the system were thermally insulated. MF was followed by cooling and solidification of the nano-lipid droplets, which involved pumping the dispersions into the cooling unit via

* eva.roblegg@uni-graz.at

a temperature-controlled high-performance liquid pump (Knauer, Berlin, Germany). The cooling unit comprises a cooling coil with defined dimensions whose cooling capacity is controlled by an external circulating thermostat. In-line monitoring of particle sizes was performed during MF and before and after product cooling via spatially resolved dynamic light scattering (SR-DLS) using the NanoFlowSizer. In addition, the zeta potential was determined on-line by electrophoretic dynamic light scattering using the Litesizer 500 (Anton Paar GmbH, Graz Austria). SLN and NLC were prepared with different solid lipids (i.e., Precirol® ATO 5, Gelucire® 43/01, both from Gattefossé, Saint Priest, France), a liquid lipid (i.e., Labrafac™ lipophile WL 1349, Gattefossé) and a stabilizer (Tween® 80). Material attributes relevant for processability (i.e., thermal behavior, material interactions, miscibility) were determined via Differential Scanning Calorimetry (DSC, 204F1 Phoenix, Netzsch GmbH, Selb, Germany). DoE studies were performed as suggested by the MODDE® software (Satorius AG, Göttingen, Germany) using Central Composite Face-centered (CCF) quadratic experimental design and the multiple linear regression (MLR) projection. For particle preparation, hot mixtures of the molten solid lipid or the molten mixture of solid and liquid lipid (9:1 w:w) were pre-emulsified with the aqueous stabilizer solution (4% w/w) via high-shear mixing (8000 rpm; 30 sec) considering process temperatures of 10 °C above the melting temperature (T_m) of the solid lipid. Resulting pre-emulsions were transferred to the MF set-up. The MF process temperatures were fixed at 10 °C above the T_m of the used solid lipid, whereas the MF pressure and cycle number were chosen as process input parameters and were thus varied (i.e., 500-1500 bar; 2-10 cycles). Moreover, the influence of different cooling rates (i.e., 4-10 °C/min) and the final product temperature (i.e., 4-25 °C) on particle size were investigated and were determined via SR-DLS. In-line acquired results were compared to off-line data measured via dynamic light scattering (DLS; Litesizer 500). Statistical analysis was performed via the MODDE® software, considering values for R^2 (i.e., model fit), Q^2 (i.e., prediction precision), model validity and reproducibility. Moreover, coefficient plots were created to identify most influencing process parameters. Finally, the established strategy for the on-line measurement of the zeta potential was evaluated with regard to robustness and applicability.

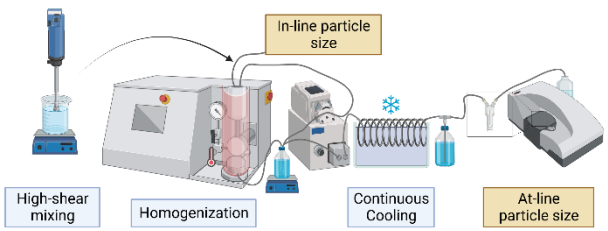

Fig. 1. Current workflow (created with BioRender)

Results and discussion

DSC studies revealed that the required process temperatures were dominated by the T_m of the solid bulk materials. Both the T_m of Precirol® (60.3 ± 0.5 °C) and Gelucire® 43/01 (43.1 ± 0.3 °C) were shifted by less than 2 °C through the addition of the liquid lipid. As a result, the required MF temperatures of 70 °C for Precirol® and 55 °C for Gelucire® 43/01 formulations were chosen. For the analysis of the DoE studies, the obtained models were simplified by excluding insignificant terms. The coefficient plots showed that the particle size was dependent on the matrix composition and MF cycles, whereas the MF pressure had only a minor effect. The cooling strategy (i.e., cooling rate, temperature) of the tested compositions did not show a significant influence on the product particle size. Overall, meaningful results were obtained as the statistical analysis showed R^2 and Q^2 values of more than 0.5 and a difference of less than 0.3. Moreover, via performing center point experiments in triplicate, reproducibility of the models was proven to be high (i.e., >0.9). Optimized process conditions defined from the sweet spot contour computed by MODDE® yielded particles with a size <200 nm, a narrow distribution (i.e., PdI <0.2) and a negative zeta potential (i.e., -25-30 mV). In addition, in-line measured particle sizes were in accordance with off-line data. Finally, the established concept for in-line zeta potential measurements proved to be a valuable tool for in-process product monitoring, as the data obtained in-line and off-line correlated well.

Conclusion

In summary, the newly established continuous nanomanufacturing line successfully produced solvent-free SLN and NLC that were monitored in- and online with respect to size and zeta potential. Accordingly, this approach is promising for the fabrication of safe and high-quality nano-systems for targeted delivery of drugs.

References

Operti M.C., Bernhardt A., Grimm S., Engel A., Figdor C.G., Tagit O., 2021. PLGA-based nanomedicines manufacturing: Technologies overview and challenges in industrial scale-up. International Journal of Pharmaceutics. Vol. 605

Sheybanifard M., Guerzoni L.P.B., Omidinia-Anarkoli A., De Laporte L., Buyel J., Besseling R., et al., 2022. Liposome manufacturing under continuous flow conditions: towards a fully integrated set-up with in-line control of critical quality attributes. Lab Chip. 23(1).