Effect of temperature, vacuum, and process duration on the freeze-dry of PEGylated Solid Lipid Nanoparticles

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Introduction

In the last three decades, the development of several nanostructures with the potential to provide controlled drug release and targeted delivery of active agents has gained great interest. Thus, the use of nanoparticles (NPs) has become a promising tool to establish new therapeutic routes for clinical use, such as gene and RNA therapies.

One of the types of nanoparticles that has become more important in recent years are Solid Lipid Nanoparticles (SLN), thus being one of the most promising mechanisms for gene therapy. Cationic solid-lipid nanoparticles (cSLNs) are biodegradable and biocompatible non-viral lipid-based nanoparticles with a positive surface charge, capable of forming SLN complexes with DNA/RNA.

However, complex, and difficult-to-scale manufacturing processes, high cost, and low transfection efficiency, compared to viral vectors, continue to hamper the widespread use of nanotechnology for clinical purposes in humans, and greater efforts are needed to improve these issues.

In this sense, one of the great challenges in the field is to achieve adequate colloidal stability of nanoparticles (NPs) over time. To achieve this goal, the freeze-drying process is the main technique used, since NP-based formulations are currently available in liquid suspensions and require very cold temperatures to prevent particle aggregation and/or fusion (COVID-19 vaccines), which limits its transportation and storage [1].

Nonetheless, both processes, the complexity of the lyophilization and the development of NPs, are highly challenging, due to the lack of universal rules and the low colloidal stability that NPs have. In addition, the lyophilization cycle (freezing, primary drying, and secondary drying) generates stressful conditions for NPs, especially the freezing step, which drastically affects their physicochemical properties, such as particle size and polydispersity index.

It is essential to establish the correct parameters, such as temperature, vacuum, and process duration to achieve good lyophilization results to avoid nanoparticle aggregation [2]. Therefore, this study seeks to evaluate the effect of the parameters mentioned above on two different PEGylated cSLNs based on cholesteryl-oleate matrix core, which have demonstrated their efficacy and safety *in vitro* [3] and thus develop an optimal, reproducible, and successful freeze-drying process.

In this poster, the impact of the lyophilization process on the physicochemical characteristics of two different formulations incorporating different types of PEG excipients will be presented and compared.

Materials and methods

PEG-cSLNs production
The following materials were used to synthesize the nanoparticles: poloxamer 188, octadecylamine, stearic acid, cholesteryl oleate, ultrapure water, and two different PEGylated excipients (Myrj 52 and Myrj Tefose 1500). PEG-SLNs were prepared using an oil-in-water emulsion technique based on the hot microemulsification method.

Freeze-drying of PEG-cSLNs
The glass transition temperature (Tg) was determined by Differential Scanning Calorimetry (DSC) before lyophilizing the PEG-cSLNs to establish the appropriate parameters for the process. The SLNs were freeze-dried using a trehalose solution (5%, w/v) as a cryoprotectant, and were performed in a pilot LyoLab C85 20 (Coolvacuum, Barcelona, SPAIN) freeze-drying system.

We performed two main experiments involving temperature (ºC), vacuum (mbar), and process duration (hours). The first corresponds to three different assays modifying temperature and duration in the primary drying (ramps) with a previous fast and constant freezing step. In the second, we tested different vacuum values also in the primary drying with the best conditions previously obtained, thus the correct parameters for a quality freeze-drying process can be established.

Physicochemical characterization of PEG-cSLNs
The physicochemical properties of both suspended nanoparticles in aqueous medium and freeze-dried SLNs reconstituted with 4 ml of MiliQ Water were

analyzed. Particle size (PSD) and polydispersity index (PdI) were determined by dynamic light scattering on a Zetasizer Nano ZS90 (Malvern Instruments, UK). The surface charge (zeta-potential) of all formulations was measured by laser Doppler microelectrophoresis using a Zetasizer Nano-Z (Malvern Instruments, UK).

Table 1. Polydispersity index (PdI), particle size (Size), zeta potential (ZP), and size difference (Diff. S) between the suspended (S) SLNs vs. unfiltered (UF) and filtered (F) SLNs of PEG Tefose 1500.

PEG	Vacuum (mbar)	SLNs	PdI	Size (nm)	ZP (mV)	Diff. S vs. UF/F
Tefose 1500	0,3	S	0,184	196,4	34	
		UF	0,321	203,3	NA	6,9
		F	0,217	196,4	44,8	0

Results and Discussion

The results showed that the optimization and standardization of the freeze-drying process are crucial to avoid irreversible SLNs aggregation, due to the unavoidable stress produced in the freeze-drying cycle. The adjustment of the several parameters that the process has, is very important to protect the nanoparticles from both the low temperatures and the extreme vacuum that is required for the lyophilization to take place (Table 1).

It is well known that the most aggressive step in the process is freezing due to the formation of ice crystals that exert mechanical stress on the molecules. To avoid crystal formation, we performed a quick freezing in the three temperature ramps carried out with the two PEG-cSLNs.

Thus, the implementation of temperature ramps in the primary drying, which is the most transcendental step in the freeze-drying process, contributes to avoid the nanoparticle agglomeration and therefore the increase in particle size (Figure 1). It is important to know the glass transition temperature (Tg) of the samples since it is recommended that the temperature established in the primary drying be below Tg.

The parameters (temperature and process duration) of the temperature ramp # 3 resulted in being the most suitable procedure to lyophilize the SLNs, indicating a minimum increase in particle size, which means that the agglomeration of nanoparticles was low. This ramp with a vacuum of 0,3 mbar proved to be the best freeze-drying cycle to maintain the physicochemical properties of the SLNs similar to those in suspension (Figure 2).

However, in all assays, the reconstituted freeze-dried SLNs were filtered through 43–48 µm filter papers to remove all agglomerates found in the resuspension medium. This implies that the lyophilization process is not yet fully standardized, so other changes to the procedure will be tested to achieve optimal freeze-drying.

Conclusions

It was not possible to correctly resuspend the lyophilized nanoparticles, and both PEG formulations had to be filtered to remove any agglomerates that might exist. Nevertheless, a freeze-drying cycle was established that turned out to be effective in obtaining resuspended SLNs with acceptable physicochemical characteristics. This indicates that the optimization of the lyophilization process is not yet fully standardized, so it is necessary to carry out tests involving a reduction in freezing time, to test different types of cryoprotectants and/or lyoprotectants, as well as different concentrations, and if necessary, carry out tests to evaluate different concentrations of the surfactant used in the formulations.

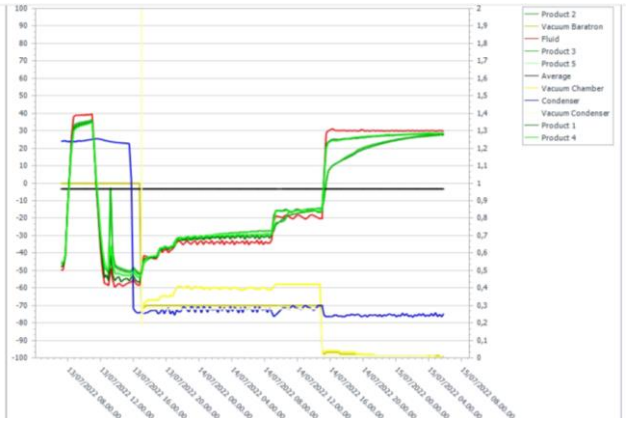

Figure 1. Freeze-drying graph of ramp # 3. Product 3 and Product 2 correspond to both PEGs: Myrj 52 and PEG Tefose 1500 respectively. Freezing (-55 ºC, 2 h), Primary drying (-45 ºC, 2 h; -40 ºC, 2 h; -35 ºC, 2 h; -30 ºC, 12 h; -15 ºC, 8 h), vacuum (0,3 mbar), Secondary drying (25 ºC, 16 h).

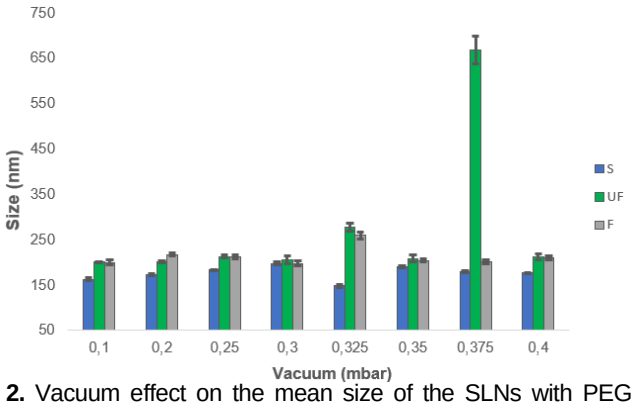

Figure 2. Vacuum effect on the mean size of the SLNs with PEG Tefose 1500 at different pressures. The blue bars indicate SLNs suspended in water; the green bars indicate SLNs unfiltered; the grey bars indicate SLNs filtered.

References

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