The Importance of an Optimum Freezing Rate when Freeze Drying Nanosuspensions

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INTRODUCTION

Freezing is the first step in a freeze drying cycle and involves the transformation of a liquid into a complete solid system. It has been reported previously for freeze drying of nanosuspensions that freezing should be performed as quickly as possible to maintain the particle size distribution. This step might foster the probability of aggregation or even particle fusion of the suspended nanoparticles due to freeze concentration [1]. Therefore, aggregation could be avoided if the freezing rate is much faster than the ideal random movement of the particles, i.e. when the particles have not enough time to aggregate during freezing. However, shelf-freezing in a freeze dryer is typically limited to < 4 °C/min (or less). In this study, freeze-thaw experiments were performed to investigate if fast freezing rates are superior over slow freezing rates in a (typical) freeze dryer. Moreover, the influence of the concentration of the suspended nanoparticles mixed with a cryoprotective excipient during this step has been thoroughly investigated.

MATERIALS & METHODS

Three nanosuspensions stabilized with either Poloxamer 188 (50 mg/mL), Tween 80 (50 mg/mL) or Cremophor EL (30 mg/mL) were prepared by wet bead milling in a high-shear media mill (Netzsch Minimec®) using 0.5 mm yttria-stabilized zirconia beads to achieve stable nanosuspensions (Table 1). Since these surfactants are classified as “non-ionic”, their mechanism of stabilization is considered as “steric” stabilization. A water insoluble API was used since the concentration in all nanosuspensions after milling was kept at 100 mg/mL. Since it was known from recent experiments that freezing of above mentioned API nanosuspensions led to strong particle aggregation, trehalose was added in three varying concentrations (100 mg/mL, 200 mg/mL and 300 mg/mL) to compensate for instability issues [2]. For the freeze-thaw experiments half of all nanosuspensions (now consisting of the cryoprotectant trehalose) were further diluted with water for injection (1:9), i.e. a total of 18 formulations were investigated in the present study (cf. Table 3, 4 and 5 for more details). The resulting formulations were tested in a full factorial design with the two factors “freezing rate” (0.2°C/min and ~4°C/min) and “concentration” of the drug compound (10 mg/mL, 100 mg/mL). The experimental design is illustrated in Figure 1. All freeze-thaw experiments were performed in triplicate on the temperature controlled shelf of a laboratory-scale freeze dryer (FTS Lyostar II, SP Industries). Note that the high freezing rate of 4 °C/min was obtained a “pre-cooled” shelf method, whereby the vessels were placed on a -50°C cooled shelf. The freezing rate was calculated from the product based temperature measurements by 36 gauge thermocouples. The particle size distribution of the nanosuspensions was determined by laser diffraction on a Malvern MasterSizer® after milling and freeze-thawing. The acceptance criteria was the d99-value (< 900 nm).

RESULTS & DISCUSSION

The results obtained indicate that a higher freezing rate was never superior over slow freezing to maintain the original particle size distribution. In the case that slow freezing of a nanosuspension results in a bimodal particle size distribution, about the same distribution is observed with the high ramp rate (Figure 2). In contrast, the degree of dilution of the drug compound in the formulation along with a minimum concentration of trehalose was found to be more critical for stabilization. Here, the diluted nanosuspensions with 10 mg/mL API were not able to resist the freezing stress except for the formulations with Cremophor EL and Tween 80 with a minimum concentration of 20 mg/mL trehalose as a cryoprotectant (Table 2 and 4). In turn, the undiluted nanosuspensions containing 100 mg/mL drug compound were more resistant against freeze concentration. Surprisingly, only Poloxamer 188 failed to preserve the particle size distribution, unless the concentration of trehalose was high enough (i.e. 300 mg/mL) as illustrated in Table 3. Statistical data analysis using MODDE revealed that the two factors “freezing rate” and “concentration” of the drug compound show no significant correlation, i.e. neither a fast nor a slow freezing rate was found critical to preserve the original particle size distribution. Apart from that it was investigated that dilution of the nanosuspension made the suspension more prone to freezing stress. Interestingly, trehalose was not always suitable to preserve the suspended nanoparticles from aggregation, even at high total solid contents of the cryoprotectant. Note that a correlation could be observed between concentration of trehalose and dilution of the formulation: the higher the concentration of trehalose and the lower the dilution of the formulation, the more stable the nanoparticles after freeze-thawing. The type of the steric stabilizer, however, plays a critical role and is found to highly contribute to the resistance of the nanoparticles against freezing stress.

CONCLUSIONS

It was recently reported that a fast freezing rate is beneficial when nanosuspensions are intended for freeze drying. This observation was made using a custom made apparatus for the freezing process, where extreme freezing rates could be obtained [1]. However, this study clearly shows that the freezing step as performed on a more representative temperature controlled shelf does not have significant influence at all on the preservation a the particle size distribution. More attention must be paid to (1) dilution and (2) type of steric stabilizer used to protect the suspended nanoparticles. Further, a sufficient amount of a cryoprotective agent is crucial, unless the steric stabilizer has already cryoprotective properties.

REFERENCES


Table 1: Nanosuspensions after wet bead milling

<table>
<thead>
<tr>
<th>Formulation 1</th>
<th>Formulation 2</th>
<th>Formulation 3</th>
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<tbody>
<tr>
<td>Poloxamer 188</td>
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<td>100 mg/mL</td>
</tr>
<tr>
<td>Tween 80</td>
<td>50 mg/mL</td>
<td></td>
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<tr>
<td>Cremophor EL</td>
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<tr>
<td>d99-value (µm)</td>
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<td>0.73</td>
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</tbody>
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Table 2: Measured d99-values of all formulations before freeze-thawing (144 h).

Table 3: Measured d99-values of all formulations after freeze-thawing experiments.

Table 4: Measured d99-values of all formulations after freeze-thawing experiments.

Figure 1: Experimental design used in this study.

Figure 2: Example of a bimodal particle size distribution before freeze-thawing (red line).