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Mechanism of freeze-drying drug nanosuspensions

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ABSTRACT

Drug nanoparticles prepared in a liquid medium are commonly freeze-dried for the preparation of an oral dosage in solid dosage form. The freezing rate is known to be a critical parameter for redispersible nanoformulations. However, there has been controversy as to whether a fast or slow freezing rate prevents irreversible aggregation. A systematic investigation is presented herein regarding the effect of both the molecular weight of the cryoprotectant and the freezing rate in order to elucidate the mechanism underlying irreversible aggregation. It was found that irreversible aggregation occurred during drying rather than freezing, although a proper freezing rate is critical. A more homogeneous distribution of the cryoprotectant and drug nanoparticles led to more redispersible powders. Thus, keeping the local concentration distribution of the nanoparticles and cryoprotectant fixed during the freezing step plays a critical role in how the freezing rate affects the redispersibility. The kinetic approach of excluding the tendency of ice crystal growth permitted an explanation of the controversial results. This study will facilitate an in-depth understanding of the aggregation process of nanoparticles or proteins during freeze-drying.

1. Introduction

Freeze-drying, or lyophilization, is known to be the most effective unit operation to preserve perishable materials. The process was developed to preserve serum during WWII. Protein drugs are popular materials that require freeze-drying, and recently the extensive use of drug nanoparticles has increased the importance of drying technologies (Tang and Pikal, 2004; Haugh et al., 2010; O'Brien et al., 2004; Abdelwahed et al., 2006). The intrinsic energy penalty associated with nanosized dimensions requires special nanotechnologies for external energy and stabilization, which mostly produce liquid state products, i.e., nanodispersions or nanoemulsions (Abdelwahed et al., 2006; Jacobsand and Muller, 2002; Hill, 2001; Lee, 2004; Ploehn and Russel, 1990; Philip et al., 2003; Berglund et al., 2003a,b; Zhang et al., 2011; Wang et al., 2009; Zhou et al., 2010; Vyas et al., 2008). Therefore, a drying step is commonly needed to convert the liquid products into solid dosage forms. Unfortunately, phase and composition changes that occur in the conversion process often nullify the effects of external energy and stabilization (Jacobsand and Muller, 2002; Hill, 2001; Lee, 2004; Ploehn and Russel, 1990; Philip et al., 2003; Berglund et al., 2003a,b) in the preparation steps, resulting in the aggregation of nanoparticles. Irreversible aggregates of drug nanoparticles are unable to redisperse into nanoparticles upon dissolution and thus, the advantages of nanoformulation can be lost. Indeed, the development of suitable pharmaceutical unit operations for nanoparticles, such as drying, granulation, and compaction, is still a challenging task.

The successful preparation of *redispersible* nanopowders that readily revert back to nanosuspensions upon reconstitution in water requires delicate process control and certain processing windows. Extensive research has been conducted on the relationship between processing variables and the redispersibility of final solid powders (Ho and Lee, 2011; Kesisoglou et al., 2007; Vergote et al., 2001; Kim and Lee, 2010). However, similar to protein drug lyophilization, the formulation of a detailed mechanism and an in-depth understanding of this relationship has yet to be achieved. Most research has focused on the choice of cryoprotectant, temperature profile, concentration and vacuum pressure.

In the freeze-drying process, a wide variety of cryoprotectants, such as glycerol, quaternary amines, carbohydrates, and synthetic polymers are used to protect nanoparticles from stresses and subsequent aggregation. It is generally known that a higher concentration of cryoprotectant and faster freezing result in better nanoparticles redispersibility. However, it has been reported that the use of a common cryoprotectant destabilized nanoparticles in some cases. Kamiya et al. investigated the effect of interactions between lipid nanoparticles and saccharides on freeze-drying (Kamiya et al., 2010) and found that the crystallization of a saccharide excluded drug-lipid nanoparticles, resulting in irreversible

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aggregation. With an increase in the cryoprotectant concentration, the irreversible aggregation of nanoparticles may either increase or decrease depending on the system of nanoparticles and the freeze-drying conditions. Controversy also exists regarding the freezing rate; a fast freezing rate (low freezing temperature) may allow for better redispersibility, but in other cases, a slow freezing rate yields better results. Thus far, the selection of cryoprotectants and related processing conditions has largely relied on empirical approaches.

Previous reports focused on the freezing rate, which had mostly been neglected in earlier research. To facilitate an in-depth understanding, a control method was developed for the freezing rate by adjusting the temperature gradient. The freezing rate is closely related to the temperature profile of the actual industrial unit operation. By systematically controlling the freezing rate for nanocrystalline systems of water-insoluble drugs, it was found that, without any other changes, only variations in the freezing rate could result in significantly different redispersibilities. We defined a critical freezing rate above which nanoparticles can retain their redispersibility (Lee and Cheng, 2006). However, depending on the cryoprotectant and processing conditions, the successful processing of redispersible nanopowders requires faster or sometimes slower freezing rates (Lee et al., 2009). The choice of cryoprotectant is also critical. In the case of carrageenan, only 0.5 wt% could successfully protect naproxen nanocrystals (Kim and Lee, 2010).

Although we revealed the importance of the freezing rate in the freeze-drying process, the mechanism behind the phenomenon is not yet well-understood. During freezing, ice crystals nucleate and grow while excluding most of the solutes into a cryoconcentrate phase. In the cryoconcentrates, further phase separation or crystallization of a component can occur. Therefore, by focusing on the parameters related to the phase separation and crystallization phenomena, a more in-depth mechanism may be elucidated. First of all, kinetic phenomena are most likely dependent on the diffusion characteristics of the nanoparticles and cryoprotectant. Therefore, in addition to the control of the freezing rate, the molecular weight of the cryoprotectant was systematically varied in this study to investigate its effect on diffusivity. The diffusivity issue was highlighted from the perspective of frozen structure development (internal spatial distribution) in order to reveal the mechanism of irreversible aggregation among nanoparticles. In particular, we hypothesized that morphological characteristics developed during freezing influence subsequent irreversible aggregation.

2. Materials and methods

2.1. Materials

Naproxen, a relatively insoluble crystalline drug compound (API, M_w = 452.8 g/mol, purity >95%, Tokyo Chemical Industry, Japan) was used as a model drug, while hydroxypropyl cellulose (HPC, M_w = 60 kg/mol, surface energy = 45 mN/m, FMC, Philadelphia, PA, USA) was employed as a steric stabilizer for wet comminution. Ramda-carrageenan (non-gelling at 1% in a 0.2 M KCl solution), sucrose (purity >98%), and polyethylene glycol (PEG, Mn = 200–35,000 g/mol) obtained from Aldrich (St. Louis, MO, USA) were used as cryoprotectants. HPLC-grade water from Aldrich and yttria-stabilized zirconia beads (1 mm diameter, Performance Ceramics, OH, USA) were employed without further purification.

2.2. Preparation of nanocrystal dispersions

Low energy comminution was used to produce nanocrystal dispersions with 1 mm zirconia beads (50 mL) as the grinding media. API was mixed with HPC previously dissolved in water (100 mL), and the beads and additional water were put into a glass bottle.

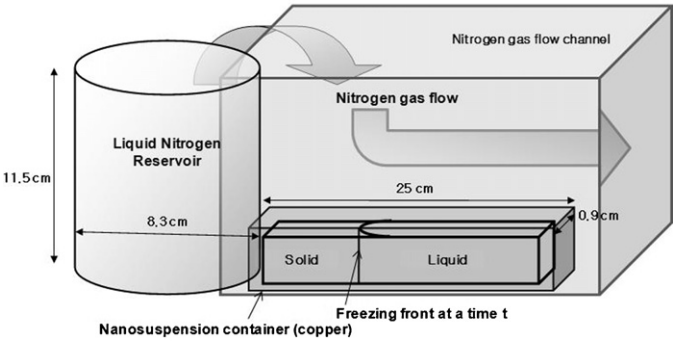

Fig. 1. Schematic illustration of a controlled freezing device with a temperature gradient, which decreases as a function of distance from the liquid nitrogen reservoir. Freezing starts at the region close to the reservoir, and the freezing rate decreases as the freezing front moves away from the reservoir.

The total weight of the slurry (API + stabilizer + water) was 8.4 g, and the concentration of API in water was 15 wt%. The weight ratio of HPC:drug was kept constant at 1:6. The comminution speed and time were 125 rpm and five days, respectively. After comminution at room temperature, the media beads were filtered out and the suspension was stored at 5 °C. The cryoprotectants were then introduced into the nanocrystal suspension 24 h before the subsequent experiment. The solution concentration of cryoprotectant was varied from 5 to 7.5 wt%.

2.3. Controlled freeze-drying

Nano-suspensions were frozen using a custom-made apparatus (Fig. 1) following a previously outlined procedure (Lee and Cheng, 2006; Lee et al., 2009). The freezing rate was varied as a function of the distance from the liquid nitrogen reservoir in the sample, thus reflecting changes in the temperature gradient, which was repeatedly checked as outlined in earlier studies (Lee and Cheng, 2006; Lee et al., 2009). As shown in Fig. 1, nano-suspensions were first put into a long tube, and the tube was subsequently placed in contact with a liquid nitrogen reservoir. After contact, freezing began from the region near the liquid nitrogen reservoir. Both the container and the reservoir were made of copper to enable rapid heat transfer.

In addition to heat transfer through the copper, cold nitrogen gas flow aided in the propagation of the freezing front. Because the frozen portions of the suspension had different turbidities when compared to the liquid portions, the freezing interface between the frozen and liquid portions was distinct (Lee et al., 2009). Freezing rates could be calculated by monitoring the change in position of the freezing front with respect to time. To obtain reproducible results, all other experimental factors, such as the amount of liquid nitrogen, were kept constant. The frozen suspension was vacuum-dried using the FD-1000 bench top freeze-dryer (EYELA, Tokyo, Japan, trap chilling temperature of −55 °C, 6.1 Pa) for 3–24 h (unless otherwise indicated).

2.4. Characterization

The particle size was measured with a Horiba LA 910 laser light scattering analyzer (632.8 nm He-Ne laser) after dispersing the particles in 150 mL of water and sonicating the mixture for 1 min. A relative refractive index of $1.2 \pm 0.00i$ and a 1 min sonication of 40 W and 39 kHz were employed. After freeze-drying, disk-shaped powder samples (5.1 mm diameter) taken at different locations along the long tube were used for characterization (Fig. 1). The volume-averaged particle sizes of the powders were used in this study.