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Study on formability of solid nanosuspensions during solidification: II novel roles of freezing stress and cryoprotectant property

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ABSTRACT

The freezing stress and cryoprotectants were known to be the crucial factors for solidification formability of nanosuspensions during freeze-drying. However, there has been controversy as to whether an aggressive or conservative freezing stress (freezing temperature or freezing rate) prevents from irreversible aggregation of nanosuspensions. And the screening of cryoprotectants for solidification formability of nanosuspensions has largely relied on empirical approaches. A systematic investigation was presented herein regarding the effect of both the freezing stress and property of cryoprotectants on solidification formability of drug nanosuspensions during freeze-drying. It was found that at different freezing stresses (−20 °C, −80 °C, and −196 °C), the redispersibility of BCN, NGN, RCN, and RVL nanosuspensions stabilized, respectively, by seven stabilizers, was $RDI_{-20\,°C} > RDI_{-80\,°C} > RDI_{-196\,°C}$. But the redispersibility of UDCA and OCA nanosuspensions stabilized, respectively, by seven stabilizers, was $RDI_{-20\,°C} < RDI_{-80\,°C} < RDI_{-196\,°C}$. These phenomena could be explained by proposed novel crystal-stabilizer separation hypothesis in nano-concentrated phase during freezing, based on freezing rate, the diffusion characteristics of the drug crystals and stabilizer molecules. The two key properties of cryoprotectant that played an important role on the formability of nanosuspensions during freeze-drying were glass transition temperature and osmotic pressure. The cryoprotectant candidates with moderate π and T_g values, sucrose achieved the excellent solidification formability of drug nanosuspensions. And the firstly proposed classify guideline based on roles of freezing stress and cryoprotectant property could give a reference for process screening of solid nanosuspensions. This study will facilitate an in-depth understanding of formability of solid nanosuspensions during freeze-drying.

1. Introduction

Nanosuspensions are colloidal dispersions of nanosized drug particles (drug nanocrystals) stabilized by surfactants. They can also be defined as a biphasic system consisting of pure drug particles dispersed in an aqueous media (Kesisoglou et al., 2007). A significant drawback of liquid nanosuspensions (LNS) is its limited long-term stability, which is often found in the range of several months (Abdelwahed et al., 2006a,b; Chacon et al., 1999). There is ,however, a general preference for solid oral dosage forms, from a marketing (patient convenience) and physical stability perspective (Müller et al., 2006). Therefore, solidification of nanosuspensions (solid nanocrystals, SNS) should be considered as an almost essential step in the production of a final nanosuspension dosage form intended for oral delivery. Solidification transformation methods for nanocrystals include freeze-drying, spray-drying, vacuum drying, pelletization or granulation (Wang et al., 2005; Müller et al., 2006; Lee and Yu, 2006; Kim and Lee, 2010; van Eerdenbrugh et al., 2008a,b; Lai et al., 2011). A commonly used drying technology applied to nanosuspensions is freeze-drying (Abdelwahed et al., 2006a,b). During solidification of nanosuspensions, it was found that the surface hydrophobicity and cohesive energy of drug were responsible for the formability of the solid nanosuspensions during solidification, which was concluded to have a direct correlation on the feasibility of formation of stable solid nanosuspensions (Yue et al., 2013).

During freeze-drying of nanosuspensions, agglomeration is an inevitable phenomenon that has been reported to be able to

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profoundly impact the properties of products intended for a diversity of applications (Wang et al., 2005). Solidification process consists on removing water from nanosuspensions sample by sublimation and desorption under vacuum, or evaporation under low temperature. Nevertheless, this process generates various stresses, especially from freezing steps, which could influence on the formability of solid nanocrystals. These freezing stresses included increase of nanosuspensions concentration that enhances the interaction between them leading to their aggregation or fusion (Abdelwahed et al., 2006a,b). Extensive research has been conducted on the relationship between processing variables and the redispersibility of final solid nanocrystals (Ho and Lee, 2011; Kesisoglou et al., 2007; Vergote et al., 2001a,b; Kim and Lee, 2010). However, controversy also exists regarding the freezing stress, a low freezing temperature may allow for better redispersibility, but in other cases, a high freezing temperature yields better results. The importance of the freezing stress in the freezing process, and the mechanism behind the phenomenon was not yet well-understood.

Furthermore, prior to freeze-drying, the cryoprotectant is often added into the nanosuspensions, which can be used to protect the nanosuspensions from solidification damage. Typical cryoprotectants added prior to freeze-drying are water-soluble materials such as sugars (e.g., sucrose, saccharose, and lactose), sugar alcohols (e.g., mannitol, sorbitol) and water-soluble polymers (e.g., PVP, polyvinylalcohol, long chained PEG) (Kesisoglou et al., 2007). In current literature, there is only a limited number of reports dealing with cryoprotectants study of solid nanocrystals (Lee, 2003; Konan et al., 2002; Moschwitzer and Muller, 2006; Vergote et al., 2001a, b). For example, in the case of freeze-drying of a loviride nanocrystal formulation, it was found that sucrose was proved to be successful in the conversion of a semisolid product with poor dissolution into a fast-dissolving solid product (van Eerdenbrugh et al., 2007). However, freeze-drying of an itraconazole–sucrose system, lead to the opposite effect. Although sucrose acted as cryoprotectant, increasing amounts in the formulation resulted in more pronounced agglomeration during the last phase of the drying process. Therefore, if the nature of a cryoprotectant is inappropriate for drying of drug nanocrystals, even excessive amounts of cryoprotectants are unable to prevent the system from freezing and drying stresses. Therefore, whatever method used for the production of drug nanocrystals, a systematic evaluation of type and concentration of cryoprotectant used is key factor to the successful production of nanocrystals.

As so far, the selection of related freeze-drying process conditions and cryoprotectants has largely relied on empirical approaches. No attempt has ever been made to understand the feasibility of nanocrystals production in terms of freeze-drying stress conditions and characterizations of cryoprotectants. As a continuation of our previous work (Yue et al., 2013), this manuscript would fill this gap and present an approach for rational design of stable nanocrystals during solidification of freeze-drying. So the objectives of this study were: (1) to evaluate the effect of freeze stress conditions on the formability of drug nanocrystals during freeze-drying, and elucidate the importance of freezing stress on the redispersibility of drug nanocrystals and (2) to investigate the influence of seven different classes of cryoprotectants on formability of drug nanocrystals during freeze-drying.

2. Materials and methods

2.1. Chemicals

Baicalin (BCN), ursodeoxycholic acid (UDCA), rubescensin (RCN), oleanolic acid (OCA), rutacarpine (RPN), resveratrol (RVL), and naringenin (NGN) were purchased from Zelang Co. (Nanjing, China). D-a-tocopherol polyethylene glycol 1000 succinate (TPGS) was purchased from Xi'an Healthful Biotechnology Co., Ltd. (Xi'an, China). Poloxamer 188 (P188, Lutrol® F 68), poloxamer 407 (P407, Lutrol® F 127) and polyoxyethylene hydrogenated castor oil (RH40, Cremophor® RH 40) were kindly donated by BASF (Ludwigshafen, Germany). Povidone 30 (PK30, Plasdone® K-29/32) were kindly donated by JSP (New Jersey, USA). Hydroxypropylmethylcellulose (HPMC, Methocel E15LV Premium EP®, Colorcon, Dartford, UK), sodium dodecyl sulfate (SDS, SHANHE, China), Tween 80 (TW80, SHANHE, China), polyethylene glycol 4000 (PEG4000, SHANHE, China) trehalose (Asahi KASEI, Japan), sodium carboxymethyl starch (CMS-NA, SHANHE, China) and microcrystalline cellulose and carboxymethyl cellulose sodium (MCC, Ceolus™ RC-A591NF, Asahi KASEI, Japan) were commercially obtained. Glucose, sucrose, mannitol, sorbitol, and lactose were obtained from DAMAO Chemical Co., Ltd. (Tianjin, China).

2.2. Nanosuspensions production

2.2.1. Production of nanosuspensions for freezing study

Drug nanosuspensions for seven nature compounds were prepared by high pressure homogenization, respectively. Firstly, coarse drug powder 1% (w/v) was dispersed in the solution with different types of stabilizer (0.1%, w/v), respectively (Table 1). The obtained mixture was disintegrated into coarse suspensions by a high shear homogenizer (FLUKO® FA25, Essen, Germany) at 16,000 rpm for 5 min. Secondly, then obtained coarse suspensions were homogenized at high pressure using a piston-gap high pressure homogenizer (AH- 1000D, ATS Engineering Inc., Seeker, Canada). 5 cycles at 500 bar were employed as pre-milling step, and then 20 cycles at 1000–1500 bar were applied to obtain the fine nanosuspensions. The experiment design for 70 formulations was illustrated in Table 1.

2.2.2. Production of nanosuspensions for cryoprotectants study

Drug nanosuspensions for seven nature compounds were prepared by high pressure homogenization, respectively. Before producing nanosuspensions, coarse drug powder 1% (w/v) was dispersed in 0.1% (w/v) concentration of representative stabilizer solution, respectively. Firstly, the obtained mixture was disintegrated into coarse suspensions by a high shear homogenizer (FLUKO® FA25, Essen, Germany) at 16,000 rpm for 5 min. Secondly, then obtained coarse suspensions were homogenized at high pressure using a piston-gap high pressure homogenizer (AH-1000D, ATS Engineering Inc., Seeker, Canada). 5 cycles at 500 bar were conducted as pre-milling step, and then 20 cycles at 1000–1500 bar were applied to obtain the fine nanosuspensions. The experiment design for 147 formulations was settled in Table 2.

2.3. Solidification of drug nanosuspensions

2.3.1. Freezing process study

LNS prepared according to Section 2.2.1 were frozen at different freezing stress conditions generated from different temperatures. LNS in a 10 mL vial were frozen according to the process temperature conditions in Table 3. Then, the system was thawed at room temperature. The average particle sizes were determined. The redispersibility index was calculated in order to evaluate the redispersibility of SNS after freeze–thawing. Measurements were made in triplicate for all the measurement runs.

2.3.2. Lyophilization process study

As illustrated in Table 2, the different amount of cryoprotectants was added into LNS prepared according to Section 2.2.2, respectively. LNS were freeze-dried at −40 °C using freeze dry system