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The importance of solidification stress on the redispersibility of solid nanocrystals loaded with harmine

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ABSTRACT

Due to limited understanding about effect of solidification stress on the redispersibility of drug nanocrystals, the impact of the different type and concentration of stabilizers and cryoprotectants, as well as the solidification temperature on the redispersibility of nanocrystals were systematically investigated. Harmine nanosuspensions were transformed into harmine solid nanocrystals (HAR-SNC) via different stress of solidification process including freezing, lyophilization and spray-drying. The effect of different concentrations of stabilizers and cryoprotectants on redispersibility of HAR-SNC was also investigated, respectively. The results showed that the redispersibility of HAR-SNC at the aggressive freezing temperature stress was better more than those of conservative and moderate stress condition. The HPMC was effective enough to protect HAR-SNC from damage during lyophilization, which could homogeneously be adsorbed into the surface of nanocrystals to prevent the agglomerates. The sucrose and sorbitol achieved excellent performance that protected HAR-SNC from crystal growth during lyophilization. The CMS-Na played an outstanding role in protecting the HAR-SNC from breakage during spray-drying, due to the steric barrier effect of high viscosity polymeric stabilizers. It was concluded that HAR-SNC was subjected to agglomeration or crystal growth during solidification, and the degree of agglomeration or crystal growth varied with the type and the amounts of stabilizers used, as well as stress conditions applied. The polymeric stabilizers were more effective to protect HAR-SNC from the damage during solidification process.

1. Introduction

Nanosuspensions (NS) or nanocrystals suspension (NCS) is colloidal dispersion system with particles size of less than 1 μm, which is generally produced in liquid media and stabilized by surfactants or polymers. Nanosuspensions possess some unique advantages that enhance the solubility and dissolution velocity of poorly soluble drugs due to their small particle size and large surface area (Kocbek et al., 2006). And based on the increased specific surface area of the particles, they can strengthen the adhesion to biological membrane and improve the bioavailability of poorly soluble drug (Muller and Katrin, 1998). And furthermore, NS can also selectively target to special tissue and organ if conducting a particular surface modification (Muller et al., 2011). However, NS are essentially thermodynamically unstable systems. The enormous surface area and the small size of these particles results in high interfacial tension, which in turn results in an increase in the free energy of the system (Rabinow, 2004). Hence, NS would tend to generate flocculation, aggregation or crystal growth to decrease their free energy.

In order to improve the physical stability of liquid NS, it has to be transformed into solid nanocrystals and then processed further into tablets or capsules. Solid nanocrystals(SNC) is composed of drug as well as stabilization agent, and can be easily recovered back to original NCS states instantaneously after rehydration with aqueous media in vitro or gastrointestinal tract (redispersibility), if they did not go through irreversible aggregation during solidification (Yue et al., 2012). Freezing-drying or spray-drying technology can be used to transform liquid NS into solid nanocrystals (Wang et al., 2005; Muller et al., 2006; Lee and Yu, 2006; Kim and Lee, 2010; Yue et al., 2013; Van Eerdenbrugh et al., 2008; Lai et al., 2011; Chaubal and Popescu, 2008; Iskandar et al., 2003). The drying process consists on removing water from NS sample by sublimation and desorption under vacuum, or evaporation under low

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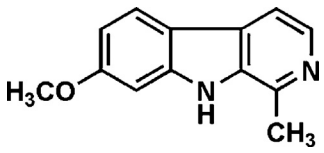

Fig. 1. Chemical structure of harmine.

temperature. Nevertheless, this process generates a series of stresses (due to freezing for lyophilization or heat for spray drying), which could inevitably destabilize nanocrystals and impact on the redispersibility of nanocrystals. For example, if the NS are coated with polymeric surfactants such as poloxamers, drying may lead to crystallization of the polymer, thereby compromising their ability to prevent aggregation. So far, literature about impact of solidification process conditions on redispersibility characteristics of nanocrystals is lacking (Van Eerdenbrugh et al., 2007), and there are no generally accepted views on the formation of hard agglomerates of nanocrystals. In view of these considerations, understanding solidification stress conditions, which have a strong impact on redispersibility of drug nanocrystals, is important.

Furthermore, the cryoprotectant for freeze-drying or dispersants for spray-drying is often added into the NCS prior to solidification, which can be used to protect the NCS from solidification damage. Typical cryoprotectants added prior to freeze-drying are water-soluble materials or sugar alcohols (Kesisoglou et al., 2007). The dispersants were usually polymers such as HPC and HPMC (Kim and Lee, 2010). However, if a cryoprotectant or dispersant is inappropriate for drug nanocrystals, even excessive amounts can not prevent the system from freezing and drying damage. Therefore, the influence of type and concentration of cryoprotectant or dispersants on redispersibility of nanocrystals after solidification is needed to systematically evaluate.

This paper is to provide a case study for elucidate the importance of different solidification temperature strength on the redispersibility of solid nanocrystals. Harmine (HAR) was chosen as the model drug (Fig. 1), a typical compound with poor aqueous solubility, which had been studied for potential of anti-Alzheimer's disease in the past (Sourkes, 1999; Zhao et al., 2013; Zheng et al., 2011, 2009). The main objective was as follows: (1) to prepare harmine nanocrystals suspensions (HAR-NCS) respectively stabilized by a series of stabilizers, such as Tween 80, TPGS, RH40 and polymer stabilizers like HPMC and CMS-Na. And the concentration of each stabilizer employed (relative to the weight of harmine) was 50% (high), 25% (medium) and 10% (low), respectively; (2) to converse HAR-NCS into harmine solid nanocrystals (HAR-SNC) via freezing-drying and spray-drying, respectively. Each method was applied with three temperature strength conditions defined as “conservative”, “moderate” and “aggressive”, respectively; (3) to investigate the effect of different concentrations of cryoprotectants (sucrose, glucose, trehalose, manitol and sorbitol) on protecting HAR-SNC from thermal stress from lyophilization, respectively; (4) to evaluate the characterization of HAR-SNC obtained at predetermined stress condition by means of laser light scattering and scanning electron microscopy, and elucidate the evidences for redispersibility/aggregation of HAR-SNC induced by solidification temperature.

2. Materials and methods

2.1. Chemicals

Harmine (HAR) was purchased from Zelang Co. (Nanjing, China). D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) was purchased from Xi'an Healthful Biotechnology Co., Ltd. (Xi'an, China). Polysorbate 80 (Tween 80) and sodium carboxymethyl starch (CMS-Na) were commercially obtained from Sunhere Pharmaceutical Excipients Co., Ltd. (Anhui, China). Polyoxyethylene hydrogenated castor oil (RH40, Cremophor® RH 40) was kindly donated by BASF (Ludwigshafen, Germany). Hydroxypropylmethylcellulose (HPMC, Methocel E15LV PremiumEP®, Colorcon, Dartford, UK) was commercially obtained.

2.2. Nanosuspensions production

HAR-NCS were prepared by high pressure homogenization technology as follows:

(1) before producing nanosuspensions, suspensions of 0.5 g harmine coarse powder were dispersed into 100 mL water, dependent on different types of stabilizers with different concentration (relative to the drug weight, m/m) like 50% (high), 25% (medium) and 10% (low);
(2) the resultant mixture was disintegrated into coarse suspensions via a high shear homogenizer (FLUKO®FA25, Essen, Germany) at 16,000 rpm for 5 min;
(3) the resultant 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 run as pre-milling step, and then 30 cycles at 1200 bar were applied to obtain the fine nanosuspensions.

2.3. Solidification process of HAR-NCS

2.3.1. Freeze-drying

2.3.1.1. Freezing process. The HAR-NCS stabilized by different polymeric dispersants were frozen at different freezing stress conditions generated from different temperatures. The HAR-NCS (3 mL) in a 10 mL vial were respectively frozen under three conditions: −20 °C for 12 h (“conservative”), −80 °C for 6 h (“moderate”), −196 °C for 2 h (“aggressive”). Then, the system was thawed at room temperature. The average particle sizes were determined. Measurements were made in triplicate for all the measurement runs.

2.3.1.2. Lyophilization process. The HAR-NCS stabilized by different stabilizers were dried by lyophilization. Each HAR-NCS (3 mL) was freeze-dried in a 10 mL vial using freeze dry system (FreezeZone® Stoppering Tray Dryers, LABCONCO Corporation, Kansas, USA). The applied cycle conditions were as follows: freezing was performed at −40 °C for 60 min. The shelf temperature ramp rates from the freezing step into the primary drying step were 1 °C/min for all cycles performed. Three sets of primary drying conditions were employed according to Table 1. The sample temperatures during

Table 1
The applied lyophilization process with different stress conditions.

Conditions	Lyophilization				
	Freezing	Ramp rate	Primary drying	Ramp rate	Secondary drying
“Conservative”	−40 °C for 60 min	1 °C/min	−20 °C for 8 h; −10 °C for 6 h; 0 °C for 5 h	0.05 °C/min	10 °C for 6 h
“Moderate”	−40 °C for 60 min	1 °C/min	−10 °C for 10 h; 0 °C for 8 h	0.2 °C/min	10 °C for 6 h
“Aggressive”	−40 °C for 60 min	1 °C/min	0 °C for 12 h	0.8 °C/min	10 °C for 8 h