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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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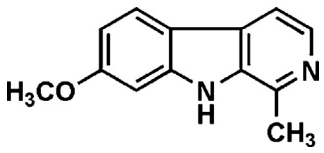


**Fig. 1.** Chemical structure of harmine.

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 |

lyophilization were measured using calibrated thermocouples. Every thermocouple was introduced through a stopper and positioned bottom center of the vial to obtain a representative temperature.

*2.3.1.3. Lyophilization process with cryoprotectant.* The different amount (100%, 200% and 400%, relative to the weight of harmine) of cryoprotectants (sucrose, glucose, trehalose, manitol and sorbitol) was respectively added into HAR-NCS stabilized by 10% concentration (relative to the weight of harmine) of Tween80 or HPMC prepared according to Section 2.2, respectively. HAR-NCS were 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. Primary drying was performed at −20 °C for 8 h; −10 °C for 6 h; and 0 °C for 5 h. The shelf temperature ramp rates from the freezing step into the primary drying step were 1 °C/min. The secondary drying was performed at 10 °Cfor 6 h. The shelf temperature ramp rates from the freezing step into the primary drying step were 0.5 °C/min.

*2.3.2. Spray-drying process*

The HAR-SNC powders were obtained by spraying the HAR-NCS through the nozzle of a Buchi mini spray dryer (model B290, Buchi Laboratoriums-Technik AG, Flawil, Switzerland). The process parameters were set as follows: inlet temperature and feed flow rate according to Table 2, aspiration rate at 60%; and atomizing air flow at 50 mmHg. The dried HAR-SNC powders were separated from the drying air in the cyclone (57–83 °C outlet temperature) and deposited at the bottom of the collector. They were collected and kept at room temperature for future testing and evaluation.

*2.4. Laser diffractometry (LD)*

Laser diffractometry was performed on a Mastersizer Micro Plus (Malvern Instruments Limited, Worcestershire, UK), which has a working range of 0.050–550 μm. Analysis of the diffraction patterns was done using the Mie model. From the resulting volume distributions, the median was calculated (=50% volume percentile, $D_{50}$). All measurements were performed in triplicate.

*2.5. Redispersibility index (RDI)*

$$\mathrm{RDI} = \frac{D_0}{D}$$

where $D_0$ represents the volume-weighed mean particle size of the freshly prepared HAR-NCS directly prior to solidification (freezing, lyophilization, and spray-drying) and $D$ represents the particle size of redispersed HAR-NCS after solidification. An RDI of near 1 would therefore mean that HAR-SNC powder can be completely redispersed back to the original particle size after rehydration.

*2.6. Scanning electron microscopy (SEM)*

Morphological evaluation of representative samples of HAR-SNC powder subjected to different solidification stress conditions was performed and compared against each other under scanning electron microscope (SEM) (Hitachi X650, Tokyo, Japan).

**Table 2**
The applied spray-drying process with different temperature stress conditions.

| | Conditions | | |
|---|---|---|---|
| | "Conservative" | "Moderate" | "Aggressive" |
| Inlet temperature | 110 °C | 125 °C | 140 °C |
| Feed flow rate | 6 ml/min | 6 ml/min | 6 ml/min |

## 3. Results and discussion

*3.1. Preparation of HAR-NCS*

HAR-NCS were prepared by high pressure homogenization. Mean particle size ($D_{50}$) and average span values for all the stabilizers with respectively different concentrations (relative to the drug weight, m/m) were listed in Fig. 2. The particle size of HAR-NCS was in range of 500–700 nm. These results demonstrated that the coarse harmine were completely disintegrated to nano-sized particles by means of high pressure homogenization technology, and successfully formed the different HAR-SNC in terms of different stabilizers.

*3.2. Freeze-drying study of HAR-NCS*

*3.2.1. Effect of freezing process on redispersibility of HAR-SNC*

During freezing of liquid NS, absence of myriad of stabilizers can induce formation of non-frozen NS phase resulting in phase separation into ice and cryo-concentrated solution. This might cause concentration of NS in the small non-frozen phase increasing the probability of particulate aggregation. Once frozen, polymers and nanoparticles hardly have enough mobility for entanglement or crystal fusion (Lee, 2003). Hence, before sublimation step, different freezing processes were employed to investigate the influence of different freezing stress on the redispersibility of HAR-SNC. The freezing conditions and the RDI of HAR-SNC stabilized by low, medium or high concentration of stabilizers respectively after freeze–thawing were gathered in Fig. 3.

As shown in Fig. 3, the redispersibility of frozen HAR-SNC at three freezing conditions was significantly different, which indicated that the different freezing stress would induce various damages to the protection effect of stabilizers and even make them inactive, and then promote the irreversible aggregation of HAR-SNC during freezing step. But the employed types and amounts of stabilizers possessed different effects to resist the damage during freezing under the same condition. It can be observed that, the rank order on redispersibility of frozen HAR-SNC were $\mathrm{RDI}_{-20\,^\circ\mathrm{C}} > \mathrm{RDI}_{-80\,^\circ\mathrm{C}} > \mathrm{RDI}_{-196\,^\circ\mathrm{C}}$ at three stress conditions, which was respectively stabilized by 10% TPGS, RH40, Tween80, HPMC and CMS-Na. And when the application amounts of stabilizers were up to 25% and 50%, there were almost identical trends with the 10% item. The results meant that the redispersibility of HAR-SNC at the aggressive temperature (the highest freezing rate) was better than those at the conservative and moderate conditions. The reason might be that HAR-NCS had sufficient time to allow water molecules to exclude the foreign particles and lead them approach each other and aggregate eventually at low freezing rate (conservative and moderate conditions), and simultaneously the activity of stabilizers was no longer effective because of the phase separation (Lee, 2003). The highest freezing rate could provide favorable condition to prevent aggregation of NS or even fusion of NS occurred during freezing process (Lee and Yu, 2006; Searles et al., 2001).

Besides, it could also be observed that the type and amounts of stabilizers under an equivalent freezing process have different effect on redispersibility of HAR-SNC. Fig. 3 shows the surfactants (TPGS, RH40 and Tween 80) were more effective compared with polymeric stabilizers (HPMC, CMS-Na). And the higher was the concentration of surfactants stabilizers, the more near to 100% was RDI of frozen HAR-SNC at three stress conditions. It meant that redispersibility of HAR-SNC with high (50%) concentration of surfactants or polymers stabilizers became much better than those

with medium and low concentration. Therefore, it was concluded that the redispersibility of frozen HAR-SNC depended on not only the freezing conditions but also the type of stabilizers and their amounts. The redispersibility of HAR-SNC during freezing was most likely dependent on freezing stress (freezing rate), the diffusion characteristics of the drug crystals and stabilizer molecule (Deville et al., 2007).

*3.2.2. Effect of lyophilization process on redispersibility of HAR-SNC*

Lyophilization process after freezing can influence on the redispersibility of HAR-SNC, due to heat from lyophilization). Fig. 4 demonstrates that RDI of lyophilized HAR-SNC respectively stabilized by different concentration of stabilizers at three stress conditions was significant different. It showed that the types and amounts of stabilizers played an important role on the redispersibility of lyophilized HAR-SNC. It was also observed that there was distinct difference in influence of three stress conditions on the redispersibility of lyophilized HAR-SNC. These were consistent with the morphology of HAR-SNC after lyophilization shown in Fig. 5. It can be seen that lyophilized HAR-SNC stabilized by HPMC did not form some aggregation or crystals growth at predetermined conditions (Fig. 5 M–R), but HAR-SNC stabilized by TPGS or Tween80 had some aggregation or crystals growth at three conditions (Fig. 5 A–L). The polymeric stabilizer HPMC possessed better performance on RDI of HAR-SNC than the other stabilizers, besides, the higher the concentration of stabilizers used was, the better protection effect of stabilizers displayed under the three stress conditions (Fig. 5 A–R). It also meant that when the application amounts for all the stabilizers were up to 50% (relate to the weight of drug), the RDI of HAR-SNC respectively stabilized by TPGS, RH40, Tween 80, HPMC and CMS-Na was more nearer to 1, compared to the one of 25% and 10%.

The results demonstrated that compared to the thermal stress generated from lyophilization, the employed amount and type of stabilizers more dramatically affected the redispersibility of HAR-SNC during lyophilization. It could be the reason that HAR-SNC could approach each other and from crystal bridges during drying, and then these crystal bridges combined and large agglomerates could be formed (Wang et al., 2005). However, the aggregation tendency of nanosuspensions can be counterbalanced by the protection effects of stabilizer, such as HPMC (Choi et al., 2005; Ploehn and Russel, 1990). The polymer stabilizers were effective enough to protect the HAR-SNC from damage generated from the various stresses during drying, which could homogeneously absorb into the surface of HAR-SNC and form steric barrier layer to prevent from agglomerates during lyophilization.

*3.2.3. Effect of cryoprotectants on redispersibility of HAR-SNC*

The cryoprotectants is necessary to maintain a good redispersibility during lyophilization, such as the polysaccharides that had been frequently used to prevent the irreversible aggregation of nanosuspensions (Schwarz and Mehnert, 1997; Saez et al., 2000). Therefore, the protection effects provided by cryoprotectants and along with their different concentration during solidification were scientifically investigated.

The RDI of freeze-dried HAR-SNC is shown in Fig. 6. It was showed that the types and concentrations of cryoprotectants played an important role in maintaining the redispersibility features of HAR-SNC. It can be seen that cryoprotectant sorbitol had a better performance on RDI of HAR-SNC than the other cryoprotectants, and the higher the concentration of cryoprotectants used was, the better protection effect of cryoprotectants was under the three concentration conditions. However, the cryoprotectant manitol had a worst performance for HAR-SNC, among all the five cryoprotectants. It also meant that respectively used 10% concentration of TPGS, Tween 80 and HPMC as stabilizer, RDI of HAR-SNC respectively protected by 400% (relate to the weight of drug) concentration of sucrose, trehalose and sorbitol was more

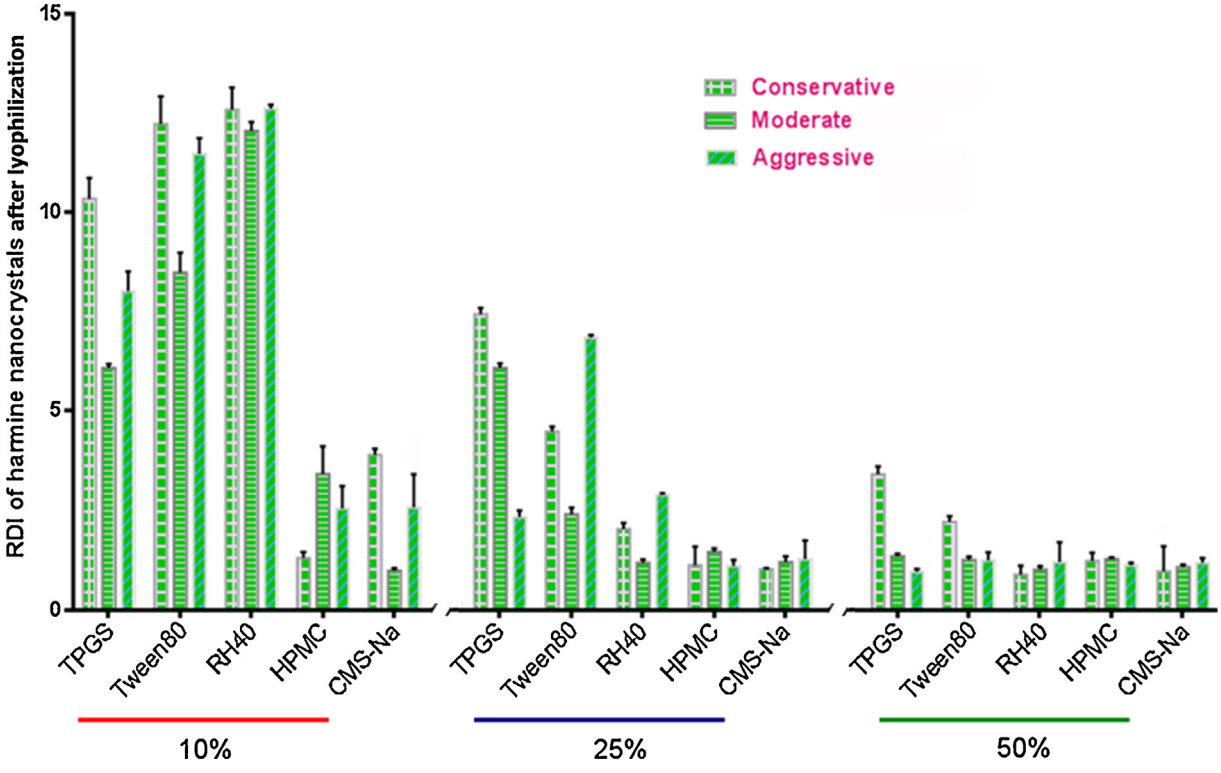


**Fig. 4.** The redispersibility index (RDI) of lyophilized HAR-SNC stabilized by different concentration (relative to the drug weight, m/m) of stabilizers at different lyophilization stress of "conservative", "moderate" and "aggressive", respectively.

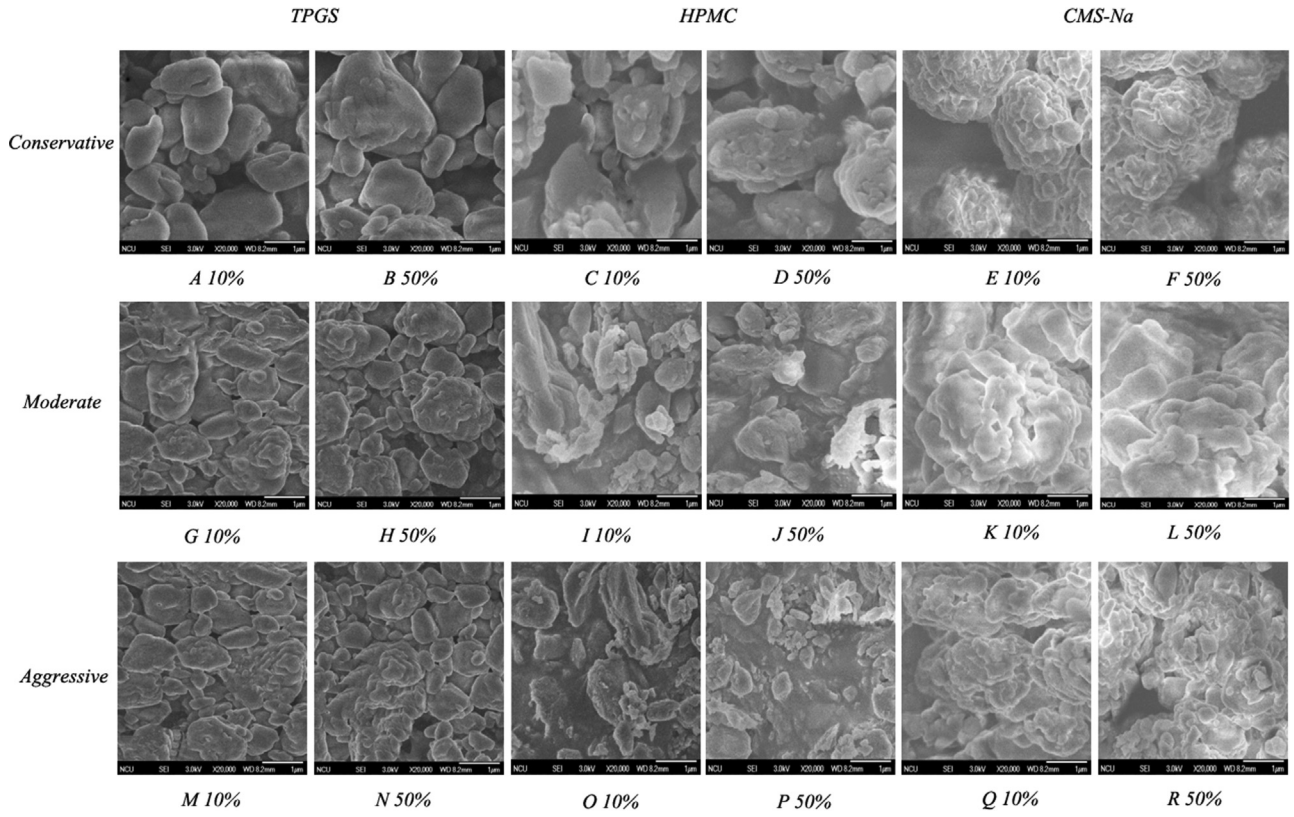


**Fig. 9.** TEM morphology of spray-dried HAR-SNC stabilized by different concentration (relative to the drug weight, m/m) of stabilizers at different spray-drying condition of "conservative", "moderate" and "aggressive", respectively.

However, the temperature strength used in spray-drying is usually more aggressive compared with one in freeze-drying, and the lack of information regarding the effect of different spray-drying temperature strength on the redispersibility of drug nanocrystals hinders further application of this technique. Hence, there is an urgent need for filling this knowledge gap so as to predict their effect.

It was observed that influence of different concentration of surfactants on the redispersibilty of spray-dried HAR-SNC was significantly different compared with those of polymeric stabilizers, as shown in Fig. 8. The redispersibilty of spray-dried HAR-SNC stabilized by polymeric stabilizers was much smaller than the one of HAR-SNC stabilized by surfactants when the concentration was low (10%) and medium (25%) at the three different spray-drying stress conditions. And of all the stabilizers, CMS-Na had a best performance for the redispersibility of HAR-SNC, even if only used at low concentration condition (10%). However, when the amount of stabilizers was up to 50%, the redispersibilty of spray-dried HAR-SNC stabilized by all stabilizers was nearer to 1. These results were consistent with the morphology of spray-dried HAR-SNC shown in Fig. 9, which demonstrated that the protection effect of stabilizer for HAR-SNC during spray-drying could be significantly related with amounts of stabilizer. It can be seen that spray-dried HAR-SNC stabilized by CMS-Na did not form some aggregation or crystals growth at predetermined conditions (Fig. 9 E F, K L, Q R), but HAR-SNC stabilized by TPGS had some aggregation or crystals growth at three stress (Fig. 9 A B, G H, M N). The polymeric stabilizer CMS-Na possessed better performance on RDI of spray-dried HAR-SNC than the other stabilizers, besides, the higher the concentration of stabilizers used was, the better the protection effect of stabilizers was under the three stress conditions (Fig. 9 A–R).

Furthermore, it was also obviously observed that at different stress conditions, the redispersibility of spray-dried HAR-SNC respectively stabilized by TPGS, RH40, Tween 80, HPMC and CMS-Na was $RDI_{110\,^{\circ}C} < RDI_{125\,^{\circ}C} < RDI_{140\,^{\circ}C}$. These results showed that the aggressive spray-drying condition (high spray-drying temperature) could impair the protection effect of stabilizer for HAR-SNC, but there was no obvious difference on the RDI of spray-dried HAR-SNC when the stabilizer concentration was 50%. Therefore, these demonstrated that the stabilizers types and the amounts for HAR-SNC appeared to have the crucial roles on the redispersibility of HAR-SNC during spray-drying.

To sum up, the polymeric stabilizers played more important role in protecting the HAR-SNC from breakage during the spray-drying process, compared to the surfactants. Besides, the high concentration of stabilizers was suitable for maintaining the redispersibility of HAR-SNC, although the medium concentration for the polymeric stabilizers had been enough to protect HAR-SNC from aggregation during spray-drying.

## 4. Conclusions

The aggregation of drug nanocrystals was inevitable due to a series of stresses yielded from freeze-drying/spray-drying. The HAR as a model case was investigated for influence of different solidification stress on the redispersibility of HAR-SNC. The freezing temperature was a crucial role for redispersibility of frozen HAR-SNC, and the redispersibility of frozen HAR-SNC at the aggressive temperature was better more than those of

conservative and moderate conditions. The polymer stabilizers were effective enough to protect HAR-SNC from damage generated from the various stress during lyophilization, which could homogeneously absorb into the surface of nanocrystals to prevent from agglomerates during lyophilization. The types and concentrations of cryoprotectants played an important role in maintaining the redispersibility of lyophilized HAR-SNC. The sucrose and sorbitol achieved excellent performance that protected HAR-SNC from crystal growth during lyophilization, which might be related with its high osmotic pressure. The aggressive spray-drying condition (high spray-drying temperature) could impair the protection effect of stabilizer for HAR-SNC, and stabilizers types and the amounts for HAR-SNC appeared to have the most significant impact on the redispersibility of HAR-SNC compared to the impact occurred from the spray-drying temperature stress. During spray-drying process, the polymeric stabilizers played outstanding role in protecting spray-dried HAR-SNC from breakage, due to the steric barrier effect of polymeric stabilizers. However, the in-depth mechanism behind the phenomenon is not yet well-understood in this study. This further systematically elucidates protection mechanism for drug nanocrystals during solidification.

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## References

Chaubal, M.V., Popescu, C., 2008. Conversion of nanosuspensions into dry powders by spray drying: a case study. Pharm. Res. 25, 2302–2308.
Choi, J.Y., Yoo, J.Y., Kwak, H.S., Nam, B.U., Lee, J., 2005. Role of polymeric stabilizers for drug nanocrystal dispersions. Curr. Appl. Phys. 5, 472–474.
Deville, S., Saiz, E., Tomsia, A.P., 2007. Ice-templated porous alumina structures. Acta Mater. 55, 1965–1974.
Iskandar, F., Gradon, L., Okuyama, K., 2003. Control of morphology of nanostructured particles prepared by the spray dying of a nanoparticle sol. J. Colloid Interface Sci. 265, 296–303.
Kim, S., Lee, J., 2010. Effective polymeric dispersants for vacuum: convection and freeze drying of drug nanosuspensions. Int. J. Pharm. 397, 218–224.
Kocbek, P., Baumgartner, P., Kristl, J., 2006. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int. J. Pharm. 312, 179–186.
Kesisoglou, F., Panmai, S., Wu, Y., 2007. Nanosizing-oral formulation development and biopharmaceutical evaluation. Adv. Drug Deliv. Rev. 59, 631–644.
Lai, F., Pini, E., Angioni, G., Manca, M.L., Perricci, J., Sinico, C., Fadda, A.M., 2011. Nanocrystals as tool to improve piroxicam dissolution rate in novel orally disintegrating tablets. Eur. J. Pharm. Biopharm. 9, 552–558.
Lee, J., 2003. Drug nano-and microparticles processed into solid dosage forms: physical properties. J. Pharm. Sci. 92, 2057–2068.
Lee, J., Yu, C., 2006. Critical freezing rate in freeze drying nanocrystal dispersions. J. Control. Release 111, 185–192.
Muller, R.H., Gohla, S., Keck, C.M., 2011. State of the art of nanocrystals-special features, production, nanotoxicology aspects and intracellular delivery. Eur. J. Pharm. Biopharm. 78, 1–9.
Muller, R.H., Katrin, P., 1998. Nanosuspensions for the formulation of poorly soluble drugs I. Preparation by a size reduction technique. Int. J. Pharm. 160, 229–237.
Muller, R.H., Möschwitzer, J., Bushrab, F.N., 2006. Manufacturing of nanoparti-cles by milling and homogenization techniques. In: Gupta, R.B., Kompella, U.B. (Eds.), Nanoparticle Technology for Drug Delivery, Drugs and the Pharmaceutical Sciences, 159. Taylor & Francis Group, LLC, NewYork, USA, pp. 21–51.
Ploehn, H.J., Russel, W.B., 1990. Interactions between colloidal particles and soluble polymers. Adv. Chem. Eng. 15, 137–228.
Rabinow, B.E., 2004. Nanosuspensions in drug delivery. Nat. Rev. Drug Discov. 3, 785–796.
Saez, A., Guzman, M., Molpeceres, J., Aberturas, M.R., 2000. Freeze drying of polycaprolactone and poly(D,L-lactic-glycolic) nanoparticles induce minor particles size changes affecting the oral pharmacokinetics of loaded drugs. Eur. J. Pharm. Biopharm. 50, 379–387.
Schwarz, C., Mehnert, W., 1997. Freeze-drying of drug-free and drug loaded solid lipid nanoparticles (SLN). Int. J. Pharm. 157, 171–179.
Searles, J.A., Carpenter, J.F., Randolph, T.W., 2001. The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. J. Pharm. Sci. 90, 860–871.
Sourkes, T.L., 1999. Rational hope in the early treatment of Parkinson's disease. Can. J. Physiol. Pharmaeol. 77, 375–382.
Van Eerdenbrugh, B., Froyen, L., Martens, J.A., Blaton, N., Augustijns, P., Brewster, M., Van den Mooter, G., 2007. Characterization of physicochemical properties and pharmaceutical performance of sucrose co-freeze-dried solid nanoparticulate powders of the anti-HIV agent loviride prepared by media milling. Int. J. Pharm. 338, 198–206.
Van Eerdenbrugh, B., Vercruysse, S., Martens Johan, A., Vermant, J., Froyen, L., Van Humbeeck, J., Van den Mooter, G., Augustijns, P., 2008. Microcrystalline cellulose: a useful alternative for sucrose as a matrix former during freeze-drying of drug nanosuspensions – a case study with itraconazole. Eur. J. Pharm. Biopharm. 70, 590–596.
Wang, B.H., Zhang, W.B., Zhang, W., 2005. Progress in drying technology for nanomaterials. Drying Technol. 23, 7–32.
Wolfe, J., Bryant, G., 1999. Freezing, drying and/or vitrification of membrane-solute-water systems. Cryobiology 39, 103–129.
Yue, P.F., Wang, Y., Wan, J., Wu, Z.F., Hu, P.Y., Zheng, Q., Yuan, H.L., Yang, M., 2012. The research progress of preparation methods of solid nanocrystal delivery system. Acta Pharm. Sin. 47, 1120–1127.
Yue, P.F., Li, Y., Wan, J., Yang, M., Zhu, W.F., Wang, C.H., 2013. Study on formability of solid nanosuspensions during nanodispersion and solidification: I. Novel role of stabilizer/drug property. Int. J. Pharm. 454, 269–277.
Zhao, T., Ding, K.M., Zhang, L., Cheng, X.M., Wang, C.H., Wang, Z.T., 2013. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of β-Carboline and quinoline alkaloids derivatives from the plants of Genus *Peganum*. J. Chem., http://dx.doi.org/10.1155/2013/717232
Zheng, X.Y., Zhang, Z.J., Chou, G.X., Wu, T., Cheng, X.M., Wang, C.H., Wang, Z.T., 2009. Acetylcholinesterase inhibitive activity-guided isolation of two new alkaloids from seeds of *Peganum* Nigellastrum Bunge by an in vitro TLC- bioautographic assay. Arch. Pharm. Res. 32, 1245–1251.
Zheng, X.Y., Zhang, L., Cheng, X.M., Zhang, Z.J., Wang, C.H., Wang, Z.T., 2011. Identification of acetylcholinesterase inhibitors from seeds of plants of genus *Peganum* by thin-layer chromatography–bioautography. J. Planar Chromatogr. 24, 470–474.