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Research Article

The Roles of Vitrification of Stabilizers/Matrix Formers for the Redispersibility of Drug Nanocrystals After Solidification: a Case Study

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Abstract. To elucidate the roles of vitrification of stabilizers/matrix formers for the redispersibility of drug nanocrystal powder after solidification at storage stress, the influence of different drying methods and storage stresses on stability of drug nanocrystals was systemically investigated. A poorly soluble drug, baicalin, used as model drug was converted into baicalin nanocrystals (BCN-NC). The residual moisture contents of BCN-NC were applied at two different stress conditions defined as "conservative" (<1%) and "aggressive" (>1%), respectively. The influence of different stabilizers, matrix formers, and storage stresses on the redispersibility of BCN-NC powder was systemically investigated, respectively. The results showed that storage stresses had significantly influence the redispersibility of BCN-NC. Aggressive storage temperature and residual moisture could be unfavorable factors for stability of drug nanocrystals, due to the exacerbation of aggregation of BCN-NC induced by vitrification. It was demonstrated that vitrification of spray-dried BCN-NC was dependent on temperature and time. The polymeric stabilizers hydroxypropylmethylcellulose (HPMC) and sodium carboxymethyl starch (CMS-Na) with high glass transition temperature (T_g) played more important role in protecting the BCN-NC from breakage during storage, compared to the surfactants Tween 80, D-α-tocopherol acid polyethylene glycol 1000 succinate (TPGS), or RH 40. Besides, the polyvinylpyrrolidone K30 (PVP K30) and lactose with high T_g were effective matrix formers for preserving the redispersibility of BCN-NC. It was concluded that the vitrification transition of stabilizers/matrix formers could be responsible for aggregation of drug nanocrystals during storage, which was a time-dependent process. The suitable residual moisture contents (RMC) and T_g were very important for preserving the stability of drug nanocrystals during storage.

KEY WORDS: baicalin; nanocrystals; redispersibility; storage stress; vitrification.

INTRODUCTION

Nanosuspensions (NS) are generally produced in liquid media in which drug particles size is less than 1 μm and stabilized by surfactants or polymers. NS has some unique advantages that enhance the solubility and dissolution velocity of poorly soluble drugs due to their small particle size and high surface area (1,2). But liquid NS has a significant drawback of poor stability, which is often found in the range of several months (3,4). Nanocrystals (NC) are conversed from NS by means of freeze-drying and spray-drying (5–7), are composed of drug as well as stabilizers, and can be easily reconstituted into original NS states spontaneously followed by rehydration *in vitro* or in the gastrointestinal tract (redispersibility), if they were not subjected to irreversible aggregation during solidification (8).

Redispersibility stability is an important requirement in development of drug nanocrystals, which can provide evidence on how the quality of drug nanocrystals varies with time under the influence of a variety of storage conditions, such as temperature, humidity, and light (9). The drug nanocrystals are usually regarded as having excellent stability if their redispersibility does not change significantly during storage. However, the storage process generates a series of stresses from internal (due to stabilizers or residual moisture) and external factors (due to heat) at various storage conditions, which could inevitably destabilize nanocrystals and impact on the redispersibility of nanocrystals (Fig. 1). Previous research in this field has shown that the stabilizer played an important role in stabilizing drug nanosuspensions (10,11). The key question on what roles a stabilizer must exhibit to destabilize nanocrystals during storage still needs to be systemically investigated.

It was well known that after converting nanosuspensions into nanocrystals, all stabilizers/matrix formers should be theoretically converted into the solid or glassy state where the drug nanocrystals were immobilized and "mobility" might be

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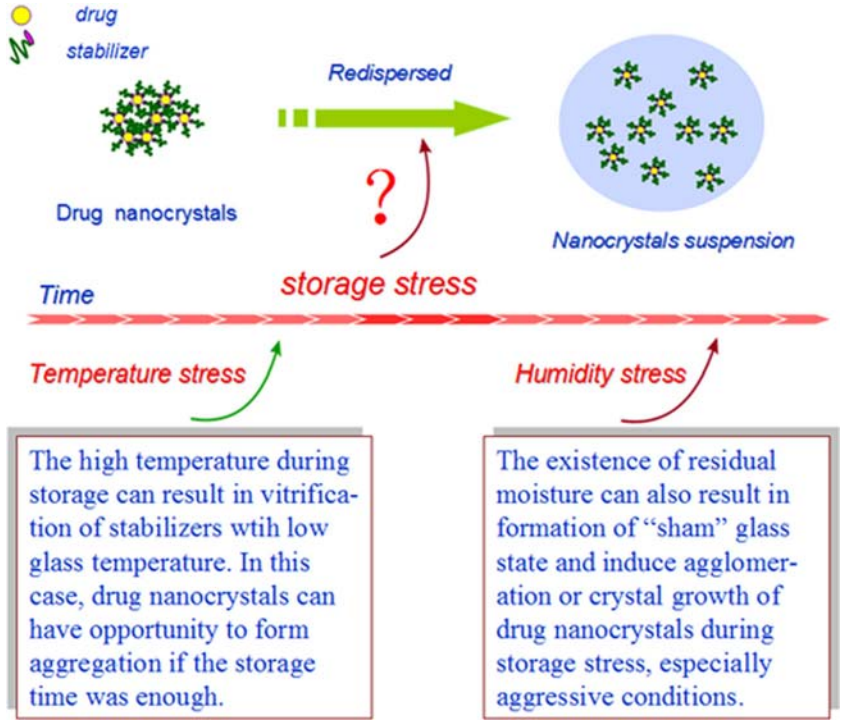

Fig. 1. Schematic illustrations of storage stress involved in storage of drug nanocrystals

reduced to a minimum (12). That is said that the stabilizers/matrix formers should remain as a solid or glassy state, which might be a prerequisite for the stability of drug nanocrystals.

The glass transition temperature (T_g) is defined as the temperature at which an amorphous system changes from the glassy to the rubbery state (13,14). Theoretically, in the glassy state, the high viscosity of the matrix does not allow the aggregation of nanocrystals. However, the stabilizers/dispersants with low T_g could be subjected to various stresses (due to heat or humidity) and convert into the rubbery state during storage, the nanocrystal particles would have opportunity to form some aggregates again. For example, D-α-tocopherol acid polyethylene glycol 1000 succinate (TPGS) and Tween 80 are naturally viscous liquids and have low glass temperature values. If such materials presented in drug nanocrystals can be converted into the glassy state after solidification, but subsequently changed from the glassy to the rubbery state when storage temperature is higher than T_g, then drug nanocrystal particles could not be completely "imprisoned" and again form aggregations. From that point of view, one may expect that even at ambient conditions, individual nanocrystals is mobile, if ambient temperature is more than its glass temperature. The vitrification property of stabilizers/dispersants seems to be responsible for the redispersibility of drug nanocrystals (15,16). But previous study demonstrated that fresh drug nanocrystals stabilized by RH 40 or Tween 80 after spray-drying can completely redisperse into original nanosuspensions, where they did not comprise to high dry temperature (17). A hypothesis is drawn that influences of vitrification of stabilizers/matrix formers on the stability of drug nanocrystals may be dependent on a series of storage stresses, such as time and temperature.

Furthermore, nanocrystals with T_g higher than their storage temperature can be viewed as staying in a stable state. However, a case may be often neglected that drug nanocrystals also may be freely mobile and not completely immobilized, if the drug nanocrystals powder contains relative high residual moisture. The existence of residual moisture can easily result in "vitrification" of nanocrystals system at storage stress, which might induce agglomeration of nanocrystal particles. But the literature about the effect of residual moisture on the redispersibility of drug nanocrystals is lacking. In view of these considerations, understanding storage stress conditions, which have a strong impact on stability of nanocrystals, is important.

As so far, no attempt has ever been made to understand the influence of storage stress on the redispersibility of drug nanocrystals. As a continuation of our previous work (17), this manuscript aimed to fill this gap and to present an approach for rational design of stable NC during storage. Baicalin (7-D-glucuronic acid-5,6-dihydroxy-flavone) was used as a case study, a typical compound with poor aqueous solubility, which had already been used in research in the past (18–21). Baicalin nanocrystal suspensions (BCN-NS) were prepared by high-pressure homogenization. Cremophor EL (RH 40), Tween 80, TPGS, hydroxypropylmethylcellulose (HPMC), and sodium carboxymethyl starch (CMS-Na) were used as steric stabilizers and used either alone or in combination with the matrix formers sucrose, trehalose, lactose, sorbitol, and polyvinylpyrrolidone K30 (PVP K30). The objectives of this manuscript are (1) BCN-NS was converted into baicalin nanocrystals (BCN-NC) *via* freeze-drying (conservative drying method) and spray-drying (aggressive drying method), respectively. The influence of different drying methods on the stability of BCN-NC was investigated. (2) The influence of the residual moisture contents on the stability of BCN-NC was evaluated to elucidate its importance on the redispersibility of BCN-NC during storage. The residual moisture contents

(RMC) of BCN-NC was applied at two different stresses defined as "conservative" condition ($0 < RMC < 1\%$) and "aggressive" condition ($1\% < RMC < 2\%$), respectively. (3) Short-term (6 months) stability testing was performed to investigate the influence of stabilizers' property and storage stress on the redispersibility of BCN-NC. Storage temperature was applied at three different stress conditions defined as conservative (4°C), "moderate" (25°C), and aggressive (40°C), respectively. (4) The influence of matrix formers on the redispersibility of drug nanocrystals at different storage stresses was investigated.

MATERIALS AND METHODS

Materials

Baicalin was purchased from Zelang Co. (Nanjing, China). TPGS was purchased from Xi'an Healthful Biotechnology Co., Ltd. (Xi'an, China). HPMC (Methocel E15LV Premium EP®, Colorcon, Dartford, UK) was commercially obtained. Polyoxyethylene hydrogenated castor oil (RH 40, Cremophor® RH 40) was kindly donated by BASF (Ludwigshafen, Germany). PVP K30 (Plasdone® K-29/32) was kindly donated by JSP (New Jersey, USA). Tween 80 (SHANHE, China), trehalose (Asahi KASEI, Japan), and CMS-Na (SHANHE, China) were commercially obtained. Sucrose and lactose were obtained from DAMAO Chemical Co., Ltd. (Tianjin, China).

Production of BCN-NS I

BCN-NS was prepared by high-pressure homogenization. Before producing BCN-NS, baicalin coarse powder 1% (*w/v*) was dispersed in the solution with 0.5% (*w/v*) of stabilizer (TPGS, RH 40, Tween 80, HPMC, and CMS-Na), 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 using a piston-gap high-pressure homogenizer (AH-1000D, ATS Engineering Inc., Seeker, Canada). Five cycles at 500 bar were conducted as a pre-milling step, and then 20 cycles at 1000 bar were applied to obtain the fine BCN-NS I.

Production of BCN-NS II

For comparison purpose, baicalin coarse powder 1% (*w/v*) was dispersed in the solution with 0.1% (*w/v*) of stabilizer TPGS or HPMC, respectively. BCN-NS II was prepared according to the procedure as mentioned above. The resulting stock nanosuspensions were mixed with 4% (*w/v*) of sucrose, trehalose, lactose and PVP K30, respectively.

Production of Baicalin Nanocrystals

Aggressive Condition via Spray-Drying

The BCN-NC powders were obtained by spraying BCN-NS through the nozzle of a BUCHI mini spray dryer (model B290, Buchi Laboratoriums-Technik AG, Flawil, Switzerland). The process parameters were set to allow a modulation in water content in the samples as follows: inlet temperature at 110°C–150°C, feed flow rate at 1–5 ml/min, aspiration rate at 55%, and atomizing air flow at 50 mmHg. This process allowed a modulation in residual moisture in the spray-dried samples. The dried BCN-NC powders were separated from the drying air in the cyclone (57°C–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.

Conservative Condition via Freeze-Drying

The BCN-NS stabilized by different polymeric dispersants were dried by lyophilization. Each BCN-NS (3 ml) was freeze-dried at −40°C in a 10-ml vial using a freeze dry system (FreezeZone® Stoppering Tray Dryers, LABCONCO Corporation, Kansas, USA) with 50 mbar vacuum for 24 h. The applied cycle conditions were as follows: freezing was performed at −40°C for 60 min. The shelf temperature ramp rate from the freezing step to the primary drying step was 1°C/min for all cycles performed. The primary drying conditions were employed at −20°C for 6 h. The shelf heating rate from the primary drying shelf set point to the secondary drying set point at 20°C was 0.15°C/min. Secondary drying for these cycles was performed over 2–8 h. This process allowed a modulation in residual moisture in the freeze-dried samples. The chamber pressure during primary and secondary drying was controlled at 75 mTorr throughout all experiments.

Stability Evaluation

Immediately after drying, the products ($n = 3$) were sealed and stored at 4°C, 25°C, and 40°C, respectively. After 1, 3, and 6 months, samples were analyzed in terms of the redispersibility index (RDI).

Particle Size Evaluation of BCN-NC

The particle size and distribution of BCN-NC obtained during solidification was evaluated, using a Mastersizer Micro Plus (Malvern Instruments Limited, Worcestershire, UK). Analysis of the diffraction patterns was performed using the Mie model, with a dispersant refractive index of 1.33.

The average 50% and 90% volume percentile D_{50} and D_{90} were determined, as well as the average span of the distribution

$$\text{Span} = {}^{(D_{90}-D_{10})}\Big/_{D_{50}}$$

Determined of Residual Moisture Contents of BCN-NC

The RMC was determined by a thermogravimetric analyzer (TGA, Q5000IR, TA Instruments, New Castle, USA). The sample weight loss was monitored as the furnace temperature increased from room temperature (23°C) to 250°C at a program heating rate of 5°C/min. The weight of the evolved moisture was taken as the difference between the initial sample weight and the sample weight (at approximately 100°C) at the horizontal region of the thermogram indicative of the attainment of constant weight. The ratio of the weight of the

lost residual moisture to the initial sample weight multiplied by one 100 yielded the percentage RMC of the sample.

Redispersibility Index

$$\mathrm{RDI} = \frac{D}{D_0} \times 100\%$$

Where D_0 is the redispersed particle size (D_{50}) of freeze-dried/spray-dried BCN-NC and D is the reconstituted corresponding D_{50} value of BCN-NC post-storage. A RDI of near 100% would generally mean that nanocrystal powder can be completely reconstituted into the original BCN-NS after rehydration.

Scanning Electron Microscopy

The morphological evaluation of representative samples of BCN-NC was performed and compared against each other under a scanning electron microscope (SEM) (Nova Nano SEM45, FEI, USA). All samples were evaluated on a brass stub using carbon double-sided tape. The samples were gold coated (thickness ≈ 15–20 nm) with a sputter coater (Fison Instruments, UK) using an electrical potential of 2.0 kV at 25 mA for 10 min.

Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g) of the samples were performed using a differential scanning calorimeter (DSC) (Diamond DSC, Perkin-Elmer, USA) equipped with an intercooler. Calibration for temperature and heat of fusion was carried out with indium and tin as reference materials. The samples were analyzed in open aluminum pans and scanned under a nitrogen purge with a heating rate of 10°C/min from 10 to 90°C above the expected melting point.

RESULTS

Inherent Damage of Freeze/Spray-Dry Process on the Redispersibility of Drug Nanocrystals During Storage

The particle size of redispersed BCN-NC after drying is listed in Fig. 2. The mean particle size of prepared BCN-NS was in the range of 500~700 nm. The morphology of the freeze-dried/spray-dried BCN-NC stabilized by TPGS/Tween 80 is showed in Fig. 3, respectively. The results showed the morphology of the freeze-dried BCN-NC was different with that of the spray-dried BCN-NC. The freeze-dried BCN-NC appeared to be cotton-shaped, but the spray-dried BCN-NC seemed to form some aggregations. But the particle size of spray-dried BCN-NC did not significantly increase, compared with that of freeze-drying.

The comparison of the redispersibility index of BCN-NC prepared by freeze-drying or spray-drying after 6 months of storage at three stresses conditions of 4°C, 25°C, and 40°C stabilized by TPGS or Tween 80 is showed in Fig. 4. The results showed that the redispersibility index of freeze-dried BCN-NC stabilized by TPGS or Tween 80 was not significantly different compared with that of spray-dried BCN-NC at equivalent storage conditions. It can be observed that the redispersibility of BCN-NC was $\mathrm{RDI}_{40^\circ\mathrm{C}} > \mathrm{RDI}_{25^\circ\mathrm{C}} > \mathrm{RDI}_{4^\circ\mathrm{C}}$ at three stress conditions, which was respectively stabilized by 50% TPGS and Tween 80. But the redispersibility of BCN-NC stabilized by TPGS was better than that of by Tween 80 during storage conditions, which indicated that the stability of BCN-NC during storage was related with property of stabilizers.

The Roles of Different Stabilizers on the Redispersibility of Drug Nanocrystals with Different RMC at Different Storage Conditions

The RDI of BCN-NC stabilized by different stabilizers (Tween 80, TPGS, HPMC, and CMS-Na) at different storage stress conditions (4°C, 25°C, 40°C) is showed in Fig. 5. A series of BCN-NC with lower than 1% RMC was successfully prepared. The results showed that the RDI of BCN-NC respectively stabilized by HPMC and CMS-NA at different storage stress conditions was less than 1.05, which indicated that the redispersibility of BCN-NC respectively stabilized by HPMC and CMS-NA was acceptable. But the RDI of BCN-NC respectively stabilized by TPGS and Tween 80 at different storage stress conditions was significantly increased (1.7–5.7), with higher storage temperature, especially at 40°C, and the RDI of BCN-NC was significantly related with the storage time.

The RDI of BCN-NC stabilized by different stabilizers (Tween 80, TPGS, HPMC, and CMS-Na) at different storage stress conditions (4°C, 25°C, 40°C) is showed in Fig. 6. It was observed that the RMC of BCN-NC stabilized by different stabilizers (Tween 80, TPGS, HPMC, and CMS-Na) was between 1% and 2%. But the RDI of BCN-NC respectively stabilized by TPGS and Tween 80 at different storage stress conditions was significantly increased, respectively, compared with that of BCN-NC ($0 < \mathrm{RMC} < 1\%$) at equivalent storage temperature, especially at 25°C (moderate stress). The DSC curves of BCN-NC stabilized by different stabilizers (TPGS/Tween 80/RH 40/HPMC/CMS-Na) with different stresses of RMC are showed in Fig. 7.

The Roles of Matrix Formers on the Redispersibility of Drug Nanocrystals at Different Storage Conditions

The RDI of BCN-NC/TPGS with different matrix formers (sucrose, lactose, trehalose, and PVP K30) at different storage stress conditions (4°C, 25°C, 40°C) is showed in Figs. 8 and 9. Figure 10 shows the DSC results of BCN-NC/TPGS with different matrix formers, which indicated that BCN-NC/TPGS/lactose and BCN-NC/TPGS/PVP K30 with high RMC had vitrification transformation at 38°C–40°C and the T_g of BCN-NC/TPGS/sucrose and BCN-NC/TPGS/trehalose with higher RMC seemed to be more lower (Fig. 10a), compared with those of low RMC (Fig. 10b). These results illustrated that BCN-NC/TPGS/PVP K30 with relative high RMC had some significant aggregation of crystals, compared with those of low RMC.

DISCUSSIONS

Inherent Damage of Freeze/Spray-Dry Process on the Redispersibility of Drug Nanocrystals During Storage

Compared with freeze-drying, spray-drying was a rapid process for generating nanocrystal powder in which a feed solution containing the drug nanosuspensions is atomized into