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Development of a novel ultra cryo-milling technique for a poorly water-soluble drug using dry ice beads and liquid nitrogen

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ABSTRACT

A novel ultra cryo-milling micronization technique has been established using dry ice beads and liquid nitrogen (LN2). Drug particles were co-suspended with dry ice beads in LN2 and ground by stirring. Dry ice beads were prepared by storing dry ice pellets in LN2. A poorly water-soluble drug, phenytoin, was micronized more efficiently using either dry ice beads or zirconia beads compared to jet milling. Dry ice beads retained their granular shape without pulverizing and sublimating in LN2 as the milling operation progressed. Longer milling times produced smaller-sized phenytoin particles. The agitation speed for milling was optimized. Analysis of the glass transition temperature revealed that phenytoin particles co-ground with polyvinylpyrrolidone (PVP) by dry ice milling were crystalline, whereas a planetary ball-milled mixtures process with zirconia beads contained the amorphous form. The dissolution rate of phenytoin milled with PVP using dry ice beads or zirconia beads was significantly improved compared to jet-milled phenytoin or the physical mixture. Dry ice beads together with LN2 were spontaneously sublimated at ambient condition after milling. Thus, the yield was significantly improved by dry ice beads compared to zirconia beads since the loss arisen from adhering to the surface of dry ice beads could be completely avoided, resulting in about 85–90% of recovery. In addition, compounds milled using dry ice beads are free from abraded contaminating material originating from the beads and internal vessel wall.

1. Introduction

Classification of active pharmaceutical ingredients (API) according to the Biopharmaceutics Classification System (Amidon et al., 1995) places more than 35% of commonly prescribed drugs into the poorly water-soluble category (Wu and Benet, 2005). Lipinski et al. (2001) pointed out that lead compounds obtained through high-throughput screening (HTS) tend to have higher molecular weights and greater lipophilicity than those from the pre-HTS era. To address this, many technologies for aiding the solubilization of poorly water-soluble APIs have been developed, such as salt formation (Agharkar et al., 1976), pro-drugs, API particle size reduction (Junghanns and Muller, 2008; Merisko-Liversidge et al., 2003; Van Eerdenbrugh et al., 2008a,b; Wu et al., 2004), semi-solids (Cole et al., 2008), solid dispersion of the amorphous form (Curatolo et al., 2009), lipid-based formulation, and complexation with cyclodextrin (Rajewski and Stella, 1996). Of these technologies, milling to reduce the size of API particles is conventional, is utilized for a broad range of APIs, and is the approach tried first by pharmaceutical companies.

We previously reported a novel micronization technique for pharmaceutical powders using liquid nitrogen (ultra cryo-milling) (Niwa et al., 2010). Unlike conventional dry milling where the milling pot is cooled by liquid nitrogen, in our ultra-cryo milling technique the materials are suspended directly in liquid nitrogen together with hard small spherical balls (e.g., zirconia beads), and are broken down by intensive agitation. The original phenytoin crystals were effectively broken down into submicron particles that were much finer and more uniform in size and shape than conventional jet-milled particles. The spontaneous vaporization of liquid nitrogen at ambient temperature and pressure is very convenient because dry powders of the API are obtained after the milling process. Moreover, compared to jet milling, the dissolution rate of phenytoin was dramatically improved by co-grinding with pharmaceutical excipients using ultra-cryo milling (Sugimoto et al., in press). The submicron % of the particle size of phenytoin and excipient mixtures correlated well with the initial dissolution rate.

However, milling with beads leads to two concerns for pharmaceutical applications: contamination of the API by the material used for the beads and mixing vessel, and insufficient recovery of the ground materials. These beads are driven by agitation disk with the high-velocity revolution, given the corresponding momentum, and moves in the suspension at the corresponding speed. The beads collide with the rotation axis, disk, the inner wall of the vessel, and

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the other beads. As a result, the ground material can become contaminated with bead or vessel material. Contamination during the homogenization of food materials due to the abrasion of grinding tools has been reported (Cubadda et al., 2001). Although zirconia is chemically stable and hard, some abrasion cannot be avoided. Strict quality controls for pharmaceutical drug products are required for pharmaceutical companies. A second area requiring improvement is the recovery of the ground material. Since the ground materials are suspended together with the beads after milling, separation of the beads from the suspension is necessary. The ground materials adhere strongly to the beads as a result of the high-velocity collisions. The beads are rubbed each other well on a sieve while being flushed with liquid nitrogen in order to retrieve the adhered ground powders to the surface of the beads. However, 20–50% could not be retrieved.

We report herein an improvement to our novel cryo-milling technology which addresses these concerns. We have replaced the zirconia beads with beads of dry ice (solid carbon dioxide). At atmospheric pressure and ambient temperature, solid carbon dioxide sublimates directly to vapor (sublimation temperature: −79 °C; (Bailey, 1949). Dry ice is used for cooling foods, industrial cleaning, blasting, and for stripping paints, oils and biofilms (Silverman, 2006). Instead of using hard abrasive media such as sand to grind a surface, dry ice blasting uses soft dry ice accelerated to supersonic speeds to create mini-explosions on the target surface and lift the contaminant from the underlying substrate (Imura and Anezawa, 2006). The low temperature of liquid nitrogen (boiling point: −196 °C) (Brovik, 1960; Vesserman, 1966) allows dry ice to remain in the solid state, allowing the fracturing of materials under super cold conditions. Furthermore, following milling, there is no need to separate the beads from the ground material since the dry ice beads sublimate at ambient temperature and pressure. Thus, the ground material adhered to the beads is retrieved. To our knowledge, there have been no previous reports of the use of dry ice beads for milling.

In this study, dry ice beads were prepared, their utility for producing finer milled particles was evaluated, and the dissolution of the ground drug was determined and compared to particles ground by zirconia bead milling.

2. Materials and methods

2.1. Chemicals and materials

Phenytoin was purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Polyvinylpyrrolidone K-30 (PVP) was provided by BASF JAPAN, Co., Ltd. (Tokyo, Japan). Liquid nitrogen (LN2) was purchased from Iwatani Industrial Gases Co., Ltd. (Osaka, Japan). Dry ice pellets (shot dry®) were purchased from Iwatani Carbonix Co., Ltd. (Osaka, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized-distilled water was used throughout the study. Zirconia hard small balls (0.6 mm diameter; YTZ-0.6) were purchased from Nikkato Co., Ltd. (Osaka, Japan).

2.2. Manufacturing equipment

A commercially available batch-type wet milling machine (RMB-04, Aimex Co., Ltd., Tokyo, Japan) generally used for wet-milling with beads was used for ultra cryo-milling in LN2, as illustrated in our previous paper (Niwa et al., 2010). LN2 was refilled during milling operation to compensate volatilized LN2: The weight of total milling machine was monitored on a scale and LN2 was added by every 50 g reduction to keep the constant volume of LN2 in the vessel. About one litter of LN2 was needed for processing for 15 min. A four hundred-milliliter-capacity vessel, rotation shaft and disks made of zirconia (zirconium oxide) were used for zirconia bead milling, while all equipment for dry ice bead milling was made of stainless steel.

2.3. Ultra cryo-milling using zirconia beads (zirconia milling)

Ultra cryo-milling was carried out by colliding and grinding the suspended materials with zirconia beads, as reported previously (Niwa et al., 2010). In brief, liquid nitrogen was used as the dispersing medium. Around 15 g of phenytoin or phenytoin premixed with pharmaceutical excipient was suspended in LN2. The rotation disks were spun at 1700 rpm. At appropriate intervals, LN2 was added to the vessel to compensate for evaporation. After 15 min of agitation, the drug slurry was separated from the beads by passing through a 212-μm sieve. The dried milled particles were collected after spontaneous evaporation of the LN2 under ambient conditions. In order to avoid adsorption of moisture from the air, the whole operation was performed under a flow of nitrogen gas.

2.4. Ultra cryo-milling using dry ice beads (dry ice milling)

Ultra cryo-milling with dry ice beads was conducted in the same manner as with zirconia beads, except for the following changes. The rotation disks were spun at 1660 rpm (tip speeds; 4.69 m/s), 2230 rpm (tip speeds; 6.41 m/s), and 2880 rpm (tip speeds; 8.28 m/s), and for longer time (e.g., 30, 60, 120, and 360 min. The resultant slurry of the drug was not sieved; rather, the dried milled particles were collected after spontaneous evaporation of the LN2 and dry ice beads under ambient conditions. The vessel was made of stainless steel.

2.5. Jet milling and planetary ball milling

Jet milling and planetary ball milling were also conducted to provide reference milled material. Fifteen grams of phenytoin was size-reduced using a jet mill machine (A-O, Seishin Enterprise Co., Ltd., Tokyo, Japan) operated at 0.7 MPa air pressure and a feed rate of 0.4 – 0.8 g/min. Five grams of phenytoin was milled with a planetary ball mill (PM100, Retsch, Germany) operated at 400 rpm with 10 numbers of 10 mm diameter stainless steel balls for 120 min.

2.6. Zirconia assay of zirconia-milled phenytoin

The zirconia in ground phenytoin generated by zirconia milling was quantified by high-resolution inductively coupled plasma mass spectrometry. Ground phenytoin mixed with sulfuric acid and nitric acid was heated at 80 °C and degraded until the phenytoin dissolved completely. The residual zirconia (ZrO_2) was calculated as the zirconium (Zr) content in the milled particles.

2.7. Morphology and particle size distribution (PSD)

The morphology and size of the milled particles were compared to the original bulk particles using a scanning electron microscope (SEM, JSM-6060, JEOL Ltd., Tokyo, Japan). The particles were coated by platinum sputtering (JFC-1600, JEOL Ltd.). The particle size distribution of the original and milled phenytoin dispersed in dry air, 0.4 MPa pressure, was measured by laser diffraction scattering using a diffractometer with a dry dispersing unit (LMS-30, Seishin Enterprise Co., Ltd., Tokyo, Japan). The diameters of the particles at 10%, 50%, and 90% of the cumulative volume distribution ($D_{10\%}$, $D_{50\%}$, $D_{90\%}$, respectively) were represented as the size distribution. In addition, the cumulative weight percentage of particles less than 1 μm in diameter was defined as "submicron %" in order to assess the milling efficiency.

2.8. Microscopic observation and particle size of dry ice beads

The dry ice beads were agitated in LN2 without adding the phenytoin particles, called pseud-milling, to clearly observe them, avoiding the opaque by drug powder. The morphology and size of the dry ice beads were observed under a digital microscope (VHX-500, Keyence, Osaka, Japan). The dry ice beads were placed on the microscope stage and photographed as quickly as possible after removal from the milling unit. The particle size of the dry ice beads was measured using the image analyzer equipped the digital microscope. The data from more than 200 particles were collected and averaged.

2.9. Modulated DSC

Thermal analysis was performed using a modulated DSC instrument (Q2000, TA Instrument Inc., New Castle, DE, USA). Around 5 mg of each test sample was weighed and hermetically sealed in aluminum pans. The samples were heated by modulating the temperature at 0.5 °C/min in 60-s cycles while increasing the average temperature at a rate of 3 °C/min from −10 °C to 350 °C. The glass transition temperature (T_g) was identified as the midpoint between the shifted phases in the thermogram. The intrinsic T_g of phenytoin and of the physical mixture with PVP was measured after melting at 290 °C and quenching in liquid nitrogen.

2.10. Dissolution testing

Dissolution studies of phenytoin and mixtures with pharmaceutical excipients were performed using a USP type XXIV II dissolution apparatus (Evolution 6100, Distek Inc., USA) for 60 min. The dissolution medium was 900 mL of USP pH 6.8 phosphate buffer maintained at 37 °C. The paddle speed was set to 75 rpm. Around 34 mg of phenytoin or an equivalent amount of the mixtures were tested, and represent an excess over the thermodynamic solubility of phenytoin in the medium. At specified times, 10-mL samples were collected through a 0.2-μm filter and an equal volume of dissolution medium was added to the reaction mixture. The samples were analyzed by HPLC (LC-2010, Shimadzu Co., Kyoto, Japan) at 258 nm. The dissolved % was evaluated as the dissolved percentage vs. the thermodynamic solubility.

3. Results and discussion

3.1. Preparation of dry ice beads

The dry ice beads were prepared by storing dry ice pellets in liquid nitrogen, as shown in Fig. 1. The purchased dry ice pellets were made by compressing carbon dioxide at 206.8 bar using a Dry Ice Pelletizer (HP-1000, TOMCO2 Equipment Company, Loganville, GA). Large compressed rod-type dry ice particles (diameter of ca 3 mm and length of between 5 and 30 mm) transformed to small grainy particles following storage in liquid nitrogen for over 12 h. There are highly and slightly compressed regions in the dry ice. The crack would be formed in the less compressed regions. Dry ice loses plasticity in liquid nitrogen (below −196 °C) and this promotes the crack formation. Liquid nitrogen evaporates gradually, resulting in slight flow of the liquid during storage. The breaking and cracking of dry ice occurs continuously, resulting in the dry ice forming small clusters. Storage time and the amount (bulk) of the dry ice affect the particle size of the resulting dry ice beads. This process is currently under investigation in our laboratory. In this study, dry ice beads were prepared using purchased bulk compressed dry ice stored in liquid nitrogen for 12 h in order to obtain the consistency results.

3.2. Morphology and particle size distribution of jet-milled, zirconia-milled, and dry ice-milled phenytoin

The morphological appearance of the original particles, jet-milled particles, zirconia-milled particles, and dry ice-milled particles were observed by SEM, as shown in Fig. 2. The original phenytoin was composed of angular particles around 1–10 μm in size (A). Jet milling reduced the particle size of phenytoin (B), but zirconia milling produced much finer particles that were more uniform in size and shape (C). Dry ice milling for 30 min (D) produced a mixture of smaller particles mixed with unmilled particles and it was similar to jet milling. Phenytoin milled with dry ice beads for 120 min (E) and 360 min (F) became increasingly smaller. Zirconia milling for 15 min (C) and dry ice milling for 360 min (F) produced small particles of equivalent size. The dry ice milling required a longer time to produce particles equivalent in size to that obtained by zirconia milling, but dry ice beads did eventually break phenytoin particles into submicron particles. The milling effect of beads milling arises from the frequent collisions between beads and ground material, and the intensity of collision of ground material (Kwade et al., 1996). Those conditions are different from zirconia milling and dry ice milling in this study, so the more investigation was required to explain the milling effect between them.

Particle size measurements by laser diffraction also showed that dry ice milling breaks phenytoin particles. The *D*50% of original phenytoin was 8.9 μm (Table 1), with a particle size distribution between 5 and 20 μm (Fig. 3). The dry ice-milled particles became smaller as milling time increased (Fig. 3 and *D*50%, 4.0 μm after 30 min milling and 1.7 μm after 360 min milling, Table 1). The particle size distributions were slightly broadened compared to the SEM results, presumably due to agglomeration of the particles, since some of the milled particles might not be fully dispersed in air.

Fig. 4 shows the submicron % value (an indication of the extent of nano-milling) of the original bulk, jet-milled, zirconia-milled, and dry ice-milled phenytoin powders. Despite incomplete dispersion

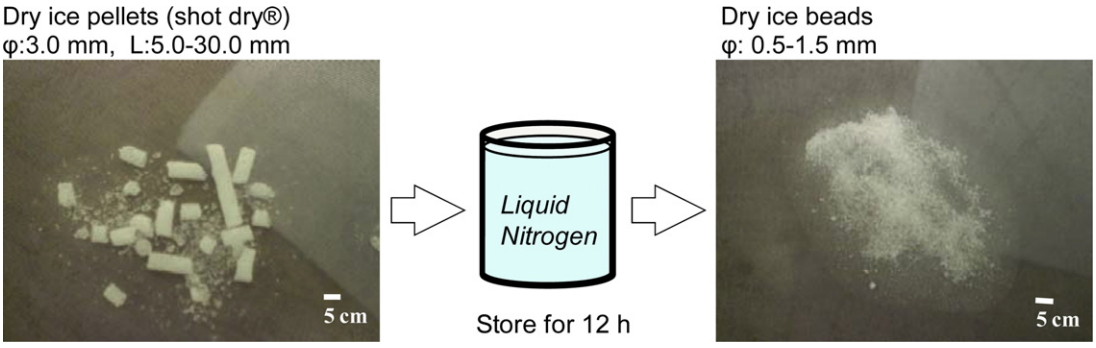

Fig. 1. Preparation of dry ice beads. Dry ice rods (shot dry®) were stored in liquid nitrogen for 12 h, resulting in the production of dry ice beads.

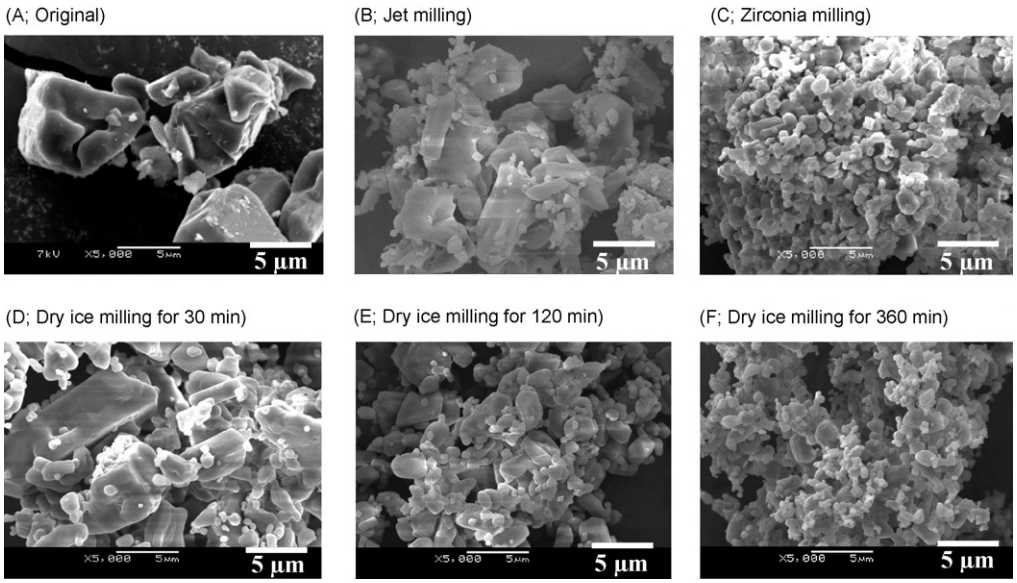

Fig. 2. Scanning electron microphotographs of phenytoin particles. Key: (A) original phenytoin, (B) jet-milled phenytoin, (C) phenytoin ground with zirconia beads, (D) phenytoin ground with dry ice beads for 30 min, (E) phenytoin ground with dry ice beads for 120 min, (F) phenytoin ground with dry ice beads for 360 min. Dry ice milling was conducted at an agitation speed of 1660 rpm.

Table 1
Representative particle sizes of the original and milled particles of phenytoin measured by laser diffraction using a dry dispersion unit.

Samples	$D_{10\%}$ (μm)[a]	$D_{50\%}$ (μm)[a]	$D_{90\%}$ (μm)[a]
Original particles	4.4	8.9	15.6
Jet-milled particles	1.3	3.9	8.2
Zirconia bead-milled particles	0.7	2.0	5.3
Dry ice bead-milled particles (30 min)[b]	1.5	4.0	8.7
Dry ice bead-milled particles (60 min)[b]	1.1	2.7	5.8
Dry ice bead-milled particles (120 min)[b]	1.0	2.7	9.5
Dry ice bead-milled particles (360 min)[b]	0.8	1.7	3.5

[a] $D_{10\%}$, $D_{50\%}$, $D_{90\%}$ are the diameters at 10%, 50% and 90% of the population distribution.
[b] Dry ice milling was conducted at an agitation speed of 1630 rpm.

of the milled particles, the submicron % of dry ice-milled particles significantly increased to 24.2% compared to the original (0%) and jet-milled particles (6.9%). As demonstrated by the SEM photographs, dry ice milling for 30 min was equivalent to jet milling, and dry ice milling for 360 min was equivalent to zirconia milling for 15 min. In summary, it was demonstrated that dry ice milling is a powerful technique for making submicron-sized particles.

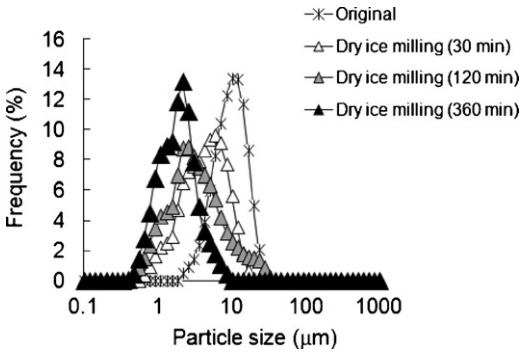

Fig. 3. Particle size distribution of phenytoin ground by LN2 milling with dry ice beads. Key: (cross) original powder, (white triangle) powder ground for 30 min, (gray triangle) powder ground for 120 min, (black triangle) powder ground for 360 min. The size measurements were performed by dispersing the powder in dry air. Dry ice milling was conducted at an agitation speed of 1660 rpm.

3.3. Microscopic observation and particle sizes of dry ice beads

The morphology and size of dry ice beads were observed under a digital microscope (Fig. 5). The dry ice beads after milling for 120 min (C) showed the white phenytoin powder was opaque and did not allow visualization of the dry ice beads clearly. So, the pseudo-milling without adding drug powder was conducted.

The dry ice was observed before and after pseudo-milling (A and B respectively). The photograph (A) indicates that the dry ice was present mainly as particles, or "beads". The shape was polyhedral and angular particles. As described in Section 3.1, these dry ice beads were formed by multiple fragmentations of large masses of dry ice in liquid nitrogen, producing multi-edged particles. Table 2 shows the particle sizes of the dry ice beads, evaluated by image analysis software. The mean particle size of dry ice particles before milling was 375.4 μm, and the maximum diameter was 648.9 μm. The photos were taken after pseudo-milling in the absence of phenytoin (B). The dry ice beads were smaller than before milling, and more spherical particles. But the particle size seemed to be bigger than in the photograph (B). It was conjecturing that the dry ice beads became smaller by collision and abrasion mainly with phenytoin. The mean particle size of dry ice particles after pseudo-milling was 143.9 μm, smaller than the particles before milling by ca 200 μm.

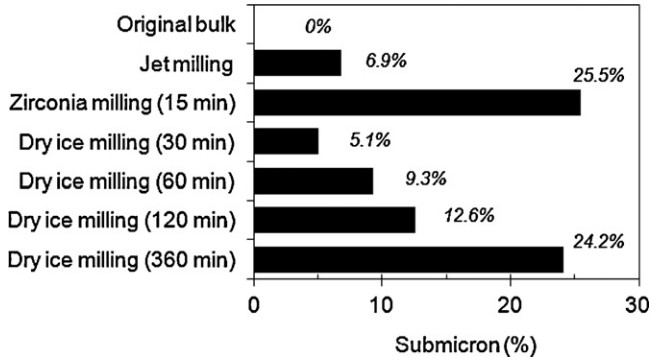

Fig. 4. Percentage of submicron-sized particles of the original and milled phenytoin. Dry ice milling was conducted at an agitation speed of 1660 rpm.

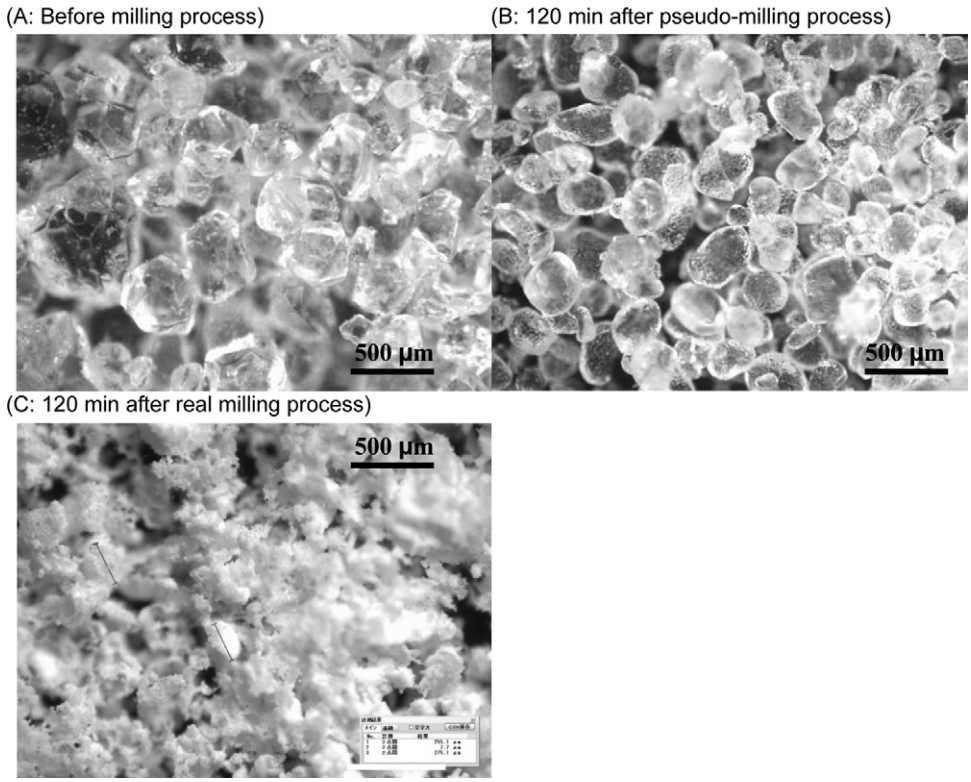

Fig. 5. Digital microscopic photographs of dry ice beads before and after the milling process. (A) Before the milling process, (B) after 120 min pseudo-milling process (the milling operation was conducted without phenytoin in order to allow clear observation of the dry ice beads) and (C) After 120 min real milling process.

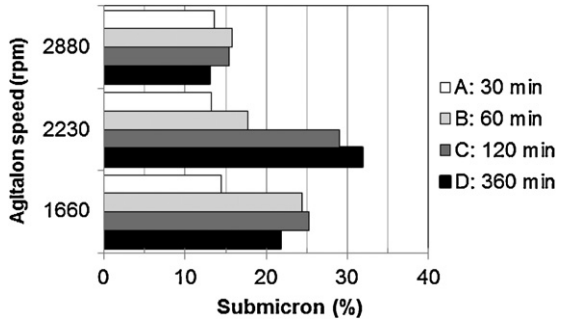

Fig. 6. Percentage of submicron-sized particles of ground phenytoin by dry ice beads milling at agitation speeds of 1660, 2230 and 2880 rpm. Key: (white) milling for 30 min, (light gray) milling for 60 min, (dark gray) milling for 120 min, (black) milling for 360 min.

3.4. Optimization of agitation speed

Longer milling time produced smaller phenytoin particles, as described in Section 3.2. Agitation speed is another milling condition which presumably affects ground particle size. Fig. 6 shows the effect of the agitation speed of dry ice milling on the submicron % of ground phenytoin. In our previous study, an agitation speed of about 1600 rpm provided ground phenytoin particles with a submicron ratio of about 24% (Sugimoto et al., in press). Fig. 6 shows that 2230 rpm generated the largest submicron % ratio. Generally speaking, higher agitation speed would make the beads and ground particles move faster and collide more frequently. However, the overall submicron % value decreased when the particles were agitated at 2880 rpm. In order to investigate this further, a clear vessel made of acrylic polymer was prepared to allow observation inside the vessel during the milling process. It was observed that most of the dry ice beads floated when the agitation speed was 2880 rpm, due to the high speed to the weight of dry ice beads. These floating beads had fewer collisions with the phenytoin, resulting in decreased milling at 2880 rpm. In the further co-grinding study, the mixture was co-ground at 2230 rpm.

3.5. Particle size distribution of co-ground mixtures

Phenytoin was co-ground with PVP by jet milling, zirconia milling, and dry ice milling at a weight ratio of 50:50. Our previous report showed that the grinding of phenytoin alone by zirconia milling did not improve its dissolution rate due to strong agglomeration of the submicron particles in the dissolution medium. Co-grinding with pharmaceutical excipients such as PVP, Eudragit L100, hypromellose, hypromellose acetate-succinate, microcrystalline cellulose, hydroxypropylcellulose and carboxymethyl cellulose effectively avoid the agglomeration of submicron-sized phenytoin by improving wettability and dispersity in the medium, and a larger effective surface area (Sugimoto et al., in press). In this study, phenytoin was ground with a

Table 2
Particle sizes of the dry ice beads measured by visual observation.

Material	Samples	Minimum (μm)[a]	Average (μm)[a]	Maximum (μm)[a]
Dry ice beads	Before milling process	169.6	375.4	648.9
	After pseudo-milling for 120 min	114.0	143.9	452.1

[a] The sizes were determined using the Feret diameter.

Table 3
Representative particle sizes of a physical mixture and co-ground particles of phenytoin and polyvinylpyrrolidone (1:1, weight ratio) by jet milling, zirconia milling, and dry ice milling measured by laser diffraction using a dry dispersion unit.

Material	Samples	$D_{10\%}$ (μm)[a]	$D_{50\%}$ (μm)[a]	$D_{90\%}$ (μm)[a]	Submicron (%)[b]
Mixture of Phenytoin and PVP	Physical mixture	4.9	14.4	79.2	0.0
	Jet-milled mixture	2.4	6.6	18.9	0.0
	Zirconia-milled mixture	0.9	3.6	14.7	14.4
	Dry ice-milled mixture (30 min)[c]	1.7	7.4	70.7	6.0
	Dry ice-milled mixture (60 min)[c]	1.2	6.5	62.6	8.1
	Dry ice-milled mixture (120 min)[c]	1.1	5.2	75.7	9.4
	Dry ice-milled mixture (360 min)[c]	1.4	16.7	67.0	6.9

[a] $D_{10\%}$, $D_{50\%}$, $D_{90\%}$ are the diameters at 10%, 50% and 90% of the population distribution.
[b] Submicron (%) is the weight ratio of particles smaller than 1 μm in size in the cumulative distribution curve.
[c] Dry ice milling was conducted at an agitation speed of 2230 rpm.

representative excipient, PVP, by dry ice milling. Table 3 showed the resulting representative particle size distribution and submicron % level. In the co-ground mixture, the particle sizes became larger due to the presence of PVP. However, zirconia milling produced submicron-sized particles at a 14.4% ratio, compared to 0% of the physical mixture and mixture co-ground by jet milling. Dry ice milling also produced submicron-sized particles. However, the submicron % and the particle size, $D_{10\%}$, $D_{50\%}$, $D_{90\%}$ were inconsistent with the results of phenytoin single milling, which showed that particle size decreased as the milling time increased (Section 3.2). The *D*50% value of the 360 min dry ice-milled mixture was comparatively large, perhaps due to moisture. We noticed the presence of frost on the upper portion of the shaft when longer milling operations were conducted. In the ultra cryo conditions, the surrounding air was cooled and became saturated with humidity. Even if the milling operation was conducted in the dry air, the frost formation was inevitable since 0% of relative humidity was not achieved practically. Condensed water dropped into the liquid nitrogen, sometimes contaminating the sample. The negative impact on particle size reduction of phenytoin by water ice during milling might be none as long as a excess amount of water ice do not contaminate. The risk of water contamination increases as the milling operation lengthens. However, it is convinced that the influence of moisture on quality could be fully controlled by improved design of milling equipment in the future.

3.6. Crystalline state of phenytoin in co-ground mixtures

The crystalline state of phenytoin was investigated by modulated DSC. X-ray powder diffraction (XRPD) is a representative method for evaluation of the crystalline state. However, a partial phase transition to the amorphous state in the co-ground mixtures would likely not be detected by XRPD, due to its low resolution. Furthermore, conventional DSC showed that the endothermic peak derived from the melting of phenytoin is shifted due to co-melting of the co-existing PVP. Therefore, glass transition temperature (T_g) analysis by modulated DSC was conducted to investigate the presence of amorphic material (Fig. 7). To identify the T_g of phenytoin and PVP, each material was first evaluated individually. Phenytoin melted at 290 °C in the DSC; it was immediately quenched in liquid nitrogen to obtain the amorphous state of phenytoin. The T_g of amorphous phenytoin was observed at around 68 °C (B) and that of PVP was observed at around 164 °C (C). Similarly, a quenched physical mixture of phenytoin and PVP was prepared and evaluated. The mixture of amorphous phenytoin and PVP showed a T_g of 127 °C. Next, phenytoin ground by dry ice milling was evaluated (A). No transition peak was observed, showing that the milled phenytoin was totally crystalline. Furthermore, the T_gs of co-ground mixtures generated by planetary ball milling (E), jet milling (F), zirconia milling (G), and dry ice milling (H) were evaluated. The T_g of ball

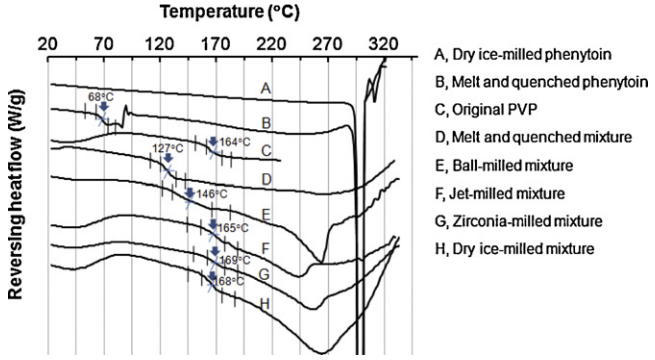

Fig. 7. Reverse heat flow of phenytoin, PVP and the mixture, evaluated by modulated DSC. Arrows show the glass transition temperature (T_g), taken as the midpoint of the transition. Key and T_g: (A) Phenytoin ground by dry ice beads milling, not detected (B) Melted and quenched phenytoin, 68 °C (C) Original PVP, 164 °C (D) Melted and quenched mixture of phenytoin and PVP, 127 °C (E) Milled mixture ground by ball milling, 146 °C, (F) Mixture ground by jet milling, 165 °C, (G) Mixture ground by zirconia milling, 169 °C (H) Mixture ground by zirconia milling, 168 °C, Dry ice milling was conducted at an agitation speed of 2230 rpm for 360 min.

milled mixtures was 146 °C, which is lower than PVP, indicating that some amorphous phenytoin was present in the mixture. On the other hand, the T_g of jet-milled, zirconia-milled, and dry ice milled mixtures were 165 °C, 169 °C and 168 °C, respectively, the almost same as PVP alone, indicating that phenytoin in these mixtures remained completely crystalline. Thus, jet milling, zirconia milling and dry ice milling do not alter the crystallinity of phenytoin.

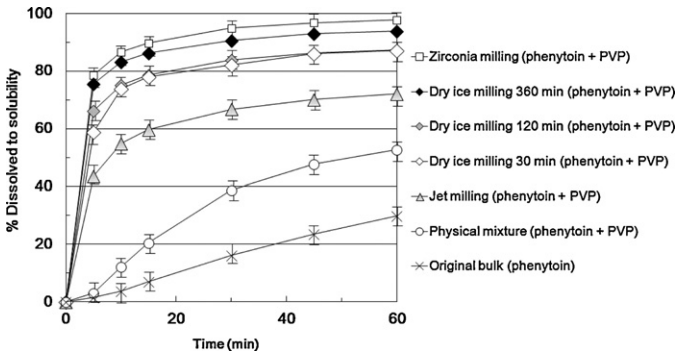

Fig. 8. Dissolution profiles of original and milled phenytoin. Key: (cross) Original phenytoin, (circle) Physical mixture of phenytoin and PVP, (triangle) Mixture co-ground by jet milling, (white triangle) Mixture co-ground by dry ice bead milling for 30 min, (gray triangle) Mixture co-ground by dry ice bead milling for 120 min, (black triangle) Mixture co-ground by dry ice milling for 360 min, (square) Mixture co-ground by zirconia bead milling for 15 min. Dry ice milling was conducted at an agitation speed of 2230 rpm for 360 min.

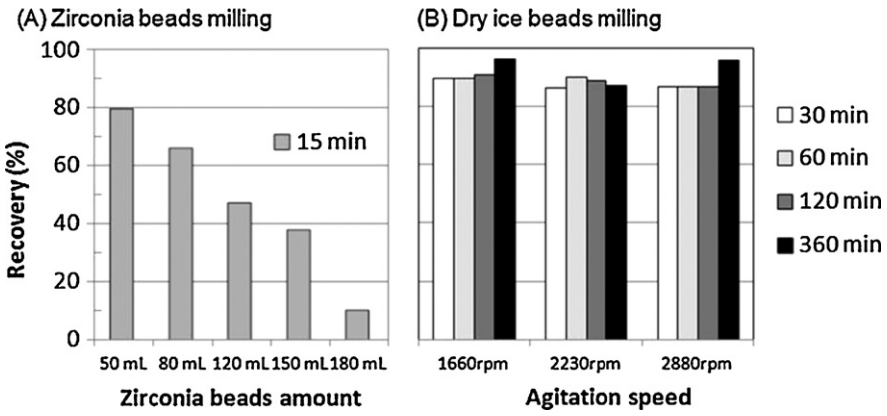

Fig. 9. Comparison of recovery percentage of dry phenytoin powder after milling. (A) Zirconia bead milling, (B) dry ice bead milling; (A) Recovery % of phenytoin ground using different amounts of zirconia beads following milling for 15 min, (B) Recovery % of phenytoin ground at several agitation speeds using dry ice bead milling for 30, 60, 120 and 360 min.

3.7. Dissolution of co-ground mixtures

The dissolution profiles of mixtures co-ground by dry ice milling were compared to original phenytoin and mixtures co-ground by jet milling and zirconia milling, as shown in Fig. 8. The dissolution rate of phenytoin was slightly improved by physical PVP mixing, attributed to the wettability of PVP. Co-grounding of the mixture by jet milling improved the dissolution profile further: more than 40% of the phenytoin dissolved in 5 min, whereas less than 5% of the physical mixture dissolved after 5 min. In addition to the wettability provided by PVP, the larger surface area of phenytoin (due to reduction of the particle size by jet milling) would contribute to the rapid dissolution. Moreover, the mixture co-ground by zirconia milling exhibited the most dramatic improvement of all the samples tested: 78% of the phenytoin was dissolved after 5 min, and more than 90% was dissolved after 60 min. This is attributable to the efficiency of milling, resulting in more submicron-sized phenytoin particles than produced by jet milling. Furthermore, co-grinding the phenytoin mixture by dry ice milling was more rapid than jet milling, and the mixture co-ground for 360 min dissolved almost as readily as the mixture co-ground by zirconia milling. The particle size of the mixture co-ground by dry ice milling was larger, due to contamination with water, but the milling nonetheless produced the desired large surface area. Formation of amorphous phenytoin did not play a role in this dissolution, as described in Section 3.6. The improvement of dissolution by co-grinding by dry milling has been reported frequently, but generally involves the formation of amorphous material (Bahl et al., 2008; Chono et al., 2008; Crowley and Zografi, 2002; Jagadish et al., 2010; Suzuki et al., 2001; Vadher et al., 2009; Vogt et al., 2008; Watanabe et al., 2003; Wongmekiat et al., 2002). This is undesirable because the quality of the powder can easily change during storage. We have demonstrated that dry ice milling is a powerful tool for improving dissolution, and is as effective as zirconia milling if the milling is performed for a long enough time.

3.8. Recovery of phenytoin ground by zirconia milling and dry ice milling

This study examined the utility of dry ice bead milling, and compared the technique to jet milling, and in particular to zirconia milling. Although our results show that zirconia bead milling in liquid nitrogen is a powerful approach for improving dissolution, the yield of the ground material requires improvement. Fig. 9(A) shows the poor recovery of phenytoin after zirconia bead milling. The recovery tended to decrease as the amount of zirconia beads increased. Phenytoin adheres to the surface of the zirconia beads, leading to difficult separation from the beads and low recovery. On the other hand, more than 80% of the ground phenytoin was consistently recovered when dry ice bead milling was used. Fig. 9(B) shows examples of the recovery after dry ice milling at 3 agitation speeds for 30–360 min. Recovery could not be compared between dry ice milling and zirconia milling because different amounts of dry ice beads were used but Fig. 9(B) shows that recovery from dry ice milling is obviously good. Separation of the product from the dry ice beads is not necessary since dry ice sublimates after the liquid nitrogen is evaporated, which is an important advantage when used on an industrial scale.

3.9. Assay of zirconia in ground mixtures following zirconia milling

The ziroconia in phenytoin ground by zirconia milling was quantified using high-resolution inductively coupled plasma mass spectrometry. The residual zirconia concentration was 0.32 ppm. To our knowledge, zirconia is not classified as a heavy metal. However, we have demonstrated that ultra milling with dry ice beads completely avoids contamination by zirconia. Reduction of the risk of contamination is very important for pharmaceutical development.

4. Conclusions

A novel ultra cryo-milling micronization technique for pharmaceutical powders has been established using dry ice beads and liquid nitrogen. Dry ice beads 400 μm in diameter were prepared by storing compressed dry ice pellets in liquid nitrogen. Poorly water-soluble phenytoin could be micronized effectively by both dry ice beads and zirconia beads, and resulted in more submicron-sized particles compared to jet-milled phenytoin. Phenytoin co-ground with polyvinylpyrrolidone (PVP) by dry ice bead and zirconia bead milling was crystalline. The dissolution rate of phenytoin co-milled with PVP using dry ice beads or zirconia beads was significantly improved compared to jet-milled powder and the physical mixture. However, the yield ratio was significantly improved by dry ice bead milling compared to zirconia bead milling since the ground powder adhering to the dry ice beads could be completely retrieved, and there is no contamination by material abraded from the beads.

In summary, ultra cryo-milling using dry ice beads and liquid nitrogen has the following advantages: (1) the particles produced are submicron-sized, (2) the dissolution rate is enhanced, (3) the dry powder is obtained in one step, (4) the crystal form is unaltered, (5) there is no risk of contamination by the bead material, and (6) the ground material is obtained in high yield.

This technique has potential for industrial applications, and further development is ongoing. The authors plan to perform scale-up studies. The results of these studies will be reported in the future.

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