**Regular Article**

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**Ultra Cryo-Milling with Liquid Nitrogen and Dry Ice Beads: Characterization of Dry Ice as Milling Beads for Application to Various Drug Compounds**

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Ultra cryo-milling using liquid nitrogen (LN2) and dry ice beads has been proposed as a contamination-free milling technique. The morphological change of dry ice beads was visually monitored in LN2 to clarify their production process and cryo-milling process. We found that dry ice pellets, which are starting material of beads and available on the market, immediately disintegrate in LN2, resulting in the spontaneous production of dry ice beads. In addition, the resultant beads maintain their size and shape even under vigorous agitation in LN2, demonstrating that they could play a role of milling media in the milling process. The driving conditions of this cryogenic milling process including beads size were optimized to enhance the milling efficiency. Dry ice beads provided superior milling efficiency compared to original pellets. The milling efficiency increased as the size of the dry ice beads decreased; furthermore, the larger the amount of beads used, the finer the milled particles. Any crystals of three drug compounds were effectively pulverized to the sub- or single-micron range. Cryo-milling with dry ice beads is valuable on pharmaceutical field because it does not contaminate the product with fractured and/or eroded beads.

**Key words** cryo-milling; nanosuspension; nanoparticle; bead milling; nanomilling; contamination

Dry and wet milling processes are widely applied to drug compounds to develop the pharmaceutical products. Dry milling may cause decomposition and polymorph transition of pharmaceutical compounds due to the thermal energy created by collisions, friction, and grinding between particles and the milling material. And also, achievable smaller limit of milled particle size by common dry milling is considered around 3 µm or more. On the other hands, wet milling could provide particles smaller than 1 µm in diameter, in contrast to dry processes. Wet milling is categorized into two types based on the mechanism: 1) wet media milling and 2) high-pressure homogenization. Wet media milling, also called bead milling, is more popular because no organic solvent is required; suspended material with a high drug concentration could be applied; little excipient is required; and materials in various fields and industries are widely applicable. The particles to be pulverized are placed in a dispersing phase containing small beads and a dispersing agent. The beads are given kinetic energy by the roll, disk or propeller in the equipment rotating at high speed. The particle size is gradually reduced by physical stress such as impacts by collision and friction generated by shear stress. The mechanisms of wet milling are considered to be collisions and grinding between small-size beads or vessel wall as reported in the previous article. The particle size and shape of milled material are dependent on the frequency of mechanical impact on the materials. The repeated collision and grinding with hard beads during running in the process would successfully promote the production of the smaller particles. As a result, wet milling could attain the micronization to submicron size.

Furthermore, decomposition risk, which is a disadvantage of dry milling, may be avoided in wet milling because the temperature of the system unit can be controlled easily and accurately. However, wet milling requires removal of the milling media before the subsequent manufacturing process. In addition, a solidification process is required after milling for solid dosage form.

Many materials have been used as milling media for wet milling. Zirconia (zirconium oxide) is the most popular material for beads because of its high milling power due to its high density and hardness. Zirconia has also high abrasion resistance property, then it is used commercially in jewellery and ornaments as imitation diamond. Submicron-sized drug particles can be successfully manufactured using zirconia beads, but it is well known that zirconia beads are chipped or worn during the milling process. The mixing of fragments generated from broken beads results in contamination of the drug product, leading to safety concerns especially in the pharmaceutical field. To overcome such contamination issue, the novel beads milling technique, commonly known as Nanocrystal technique, has been developed by using highly cross-linked polystyrene resins as bead materials. Several commercial products (Rapamune®, Emend®, Tricor®, Megace ES®, and SILCRYST™) are currently on market in high-value-added pharmaceutical products as Nanocrystal product.

We have previously developed the ultra cryo-milling technique as one of wet milling, in which liquid nitrogen (LN2) was used as a dispersing solvent instead of water. It was reported that the milling efficiency was much higher than jet milling because dispersing medium (LN2) would actively disturb the coaggregation between milled particles. It was also advantageous that the dried products could be directly available due to spontaneous vaporization of LN2. Drying process is not required after the process. Furthermore, our subsequent report showed that the release property of poorly water-soluble model drugs, phenytin, was significantly improved by cryo-
generically co-ground with hydrophilic excipients in LN2. However, the methods described in both reports have used the zirconia beads as a milling media, so the contamination risk could not be avoided. Therefore, we have developed the advanced ultra cryo-milling technique to address the contamination issue, in which milling media were changed from zirconia beads to dry ice beads. The crystals were pulverized by collision with the dry ice beads under cryogenic conditions. Because dry ice and liquid nitrogen spontaneously sublimate and vaporize at ambient conditions, both materials could be easily removed after the milling process, resulting in no residual solvent or bead material in the milled product. Even if beads are broken or eroded during the milling process, there is no concern about contamination. Such cryogenic combination is an ideal contamination-free milling system and the milled material is easily and efficiently recovered because the separation process of beads is not necessary. Sugimoto et al. also reported that milling in liquid nitrogen using dry ice beads provides smaller particles than dry milling using a jet mill, and the obtained products after the solvent removal by the spontaneous vaporization are directly available without a drying process. Thus, this approach therefore encompasses the both advantages of dry and wet milling as mentioned above.

In order to clarify the process and mechanism of the advanced cryogenic milling technique in detail, the morphological changes of dry ice solid were visually observed and quantified in the following two points of view in this study: 1) production process of beads from raw material of cylindrical dry ice pellets in LN2, and 2) milling process under rigorous agitation in LN2. In addition, the optimum operational conditions using dry ice beads (e.g., diameter and quantity of beads, agitation speed, and time) were investigated to enhance milling efficiency. Furthermore, milling experiments using other drug particles than phenytoin were also performed to expand pharmaceutical applications.

The aims of the present study were focusing on the clarification of how dry ice beads are formed from cylindrical pellets and why the materials could be pulverized by light and apparently not hard dry ice beads. The final goal of this research is to develop a contamination-free cryo-milling technique which can produce submicron-sized drug particles and is applicable to the pharmaceutical industry.

**Experimental**

**Chemicals** Phenytoin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fexofenadine hydrochloride and (R)-(−)-ibuprofen were obtained from Osaka Synthetic Chemical Laboratories, Inc. (Osaka, Japan) and BASF Japan, Co., Ltd. (Tokyo, Japan), respectively. 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). Zirconia (ZrO2) beads with a nominal diameter of 0.6mm (YTZ-0.6) were purchased from Nikkato Co., Ltd. (Osaka, Japan). All other chemicals and solvents were analytical reagent grade and distilled-deionized water was used throughout the study.

**Generation of Beads from Dry Ice Pellets** Dry ice pellets placed on a wire mesh were immersed in LN2 without shaking, vibration or agitation, then the mesh was raised after pre-set periods to collect the dry ice disintegrated particles (beads). Photographs of the disintegrated dry ice beads were immediately taken using a CCD camera (Science scope BS-2, Kenis Ltd., Osaka, Japan). Each photo was processed by image analysis as described in “Evaluation of Morphological Changes in Dry Ice Beads during the Preparation Process” to obtain information on the size and shape of the dry ice particles and morphological changes were monitored for up to 120min.

**Evaluation of Morphological Changes in Dry Ice Beads during the Preparation Process** Photographs of the dry ice pellets or particles disintegrated (beads) were taken using the CCD camera under ambient conditions immediately after removing the particles from LN2. The sizes of the dry ice particles were measured as Heywood diameters using image analysis equipment (Luzex-AP, Nireco Corporation, Tokyo, Japan) and the volumetric mean diameters were calculated from the images of more than 500 particles. The volumetric mean diameters of zirconia beads were also determined similarly as a reference and their shape indices were estimated from photo images as described above. Spherical index (ML2/A) was defined according to the following equation;

\[
\text{Spherical index: ML2/A} = \frac{\pi}{4} \times \left( \text{Maximum length} \right)^2 \div \text{area (1)}
\]

where maximum length was the longest distance between arbitrary two points on the peripheral edge of the observed image. Area was occupied area of the projected image. Roughness index (PM2/A) was also defined according to the following equation;

\[
\text{Roughness index: PM2/A} = \frac{1}{4\pi} \times \left( \text{Perimeter} \right)^2 \div \text{area (2)}
\]

where perimeter was the peripheral length of the observed image. Area was same as mentioned above. The spherical index (ML2/A) and roughness index (PM2/A) for the dry ice and zirconia beads were calculated according to Eqs. 1 and 2, respectively. Both indices approach 1.0 as the particle shape becomes a perfect sphere, and the indices increase as the shape departs from a sphere. The images of zirconia beads were also quantified as a reference with uniform size and spherical shape.

**Classification of Dry Ice Beads** Dry ice pellets immersed in LN2 for 1h were recovered to obtain dry ice particles to be used as milling beads and were graded by size using standard sieves (180, 355, 710, and 1700μm mesh openings). Three fractions of beads (small fraction: 180–355μm, medium fraction: 355–710μm, large fraction: 710–1700μm) were used in further studies, especially for evaluating the effect of bead size on milling efficiency. The particle size distributions of the disintegrated dry ice beads and the three sieved fractions were evaluated by image analysis as described in “Evaluation of Morphological Changes in Dry Ice Beads during the Preparation Process.”

**Morphological Evaluation of Dry Ice Beads during Milling Process** Batch-type media milling equipment (RMB-04, Aimex Co., Ltd., Tokyo, Japan) was used to evaluate the morphological change of dry ice beads during the milling process under agitation. An aliquot of the medium fraction of sieved beads (150mL bulk volume) was immersed in LN2 in a vessel (400mL) and agitated using four rotation disks at a speed of 1600rpm for 4h. Dry ice beads were periodically...
removed from the milling vessel and photos of the beads were taken immediately to evaluate the morphological indices by image analysis as described in “Evaluation of Morphological Changes in Dry Ice Beads during the Preparation Process.” No drug was added to allow clear observation of the beads. LN2 was added to the vessel as necessary to compensate for evaporation loss.

**Milling Performance of the Dry Ice Beads** Phenytoin was milled with dry ice beads in LN2 using the same equipment and vessel as described in “Morphological Evaluation of Dry Ice Beads during Milling Process.” Milling conditions were chosen based on the knowledge from our previous reports. Ten grams of drug powder and 150 mL bulk volume of dry ice beads (medium fraction) were placed in the vessel filled with LN2 and agitated at a speed of 100 rpm for 1 min to cool the vessel, beads and powder. Then, a suitable volume of LN2 was poured in to fill the vessel and the agitation speed was increased to 1600 rpm. LN2 was periodically added to maintain the volume of the dispersing liquid throughout the milling process. The agitation was stopped at pre-set time points up to 4 h. The dried drug powder consisting of milled particles was collected by spontaneously evaporating the LN2 and sublimating the dry ice beads at ambient conditions. Cumulative percent in volumetric particle size distribution curve 10, 50, and 90% values and the submicron ratios of milled products were measured at several time-points as described in “Particle Size Distribution of the Milled Particles.” Milling efficiency was compared to that obtained by conventional cryogenic milling with 0.6-mm diameter zirconia beads in liquid nitrogen for 0.25 h, then the slurry of drug particles was separated from the beads by passing through appropriate sieves.

**Optimization of the Milling Conditions** Process parameters such as 1) size and shape of the dry ice particles, 2) quantity of dry ice beads, and 3) agitation speed were varied to optimize the operational conditions for the performance. The other milling conditions were chosen based on the knowledge from our previous articles, which reported the ultra cryo-milling using zirconia beads. Dry ice pellets or beads from the three fractions described in the “Classification of Dry Ice Beads” were used to investigate suitable morphologies of dry ice as a milling medium. Similar size of disintegrated dry ice portion to that of zirconia beads in our previous report were selected to compare between their results. In addition, the bulk volume of the beads was varied from 75 to 300 mL to understand the effect of the quantity of dry ice beads on milling efficiency. Furthermore, the lower and higher disk rotation speed (1600, 2760 rpm) were investigated to optimize the agitation speed. Milling was performed as described in the “Milling Performance of the Dry Ice Beads” unless otherwise stated.

**Physicochemical Properties of Milled Drug Crystals**

**—Scanning Electron Micrographs of the Milled Particles—**

The appearance of the milled particles was observed using a scanning electron microscope (SEM, JSM-6060, JOEL Ltd., Tokyo, Japan) after coating the particles with platinum using a sputter-coater (JFC-1600, JEOL Ltd.).

**—Particle Size Distribution of the Milled Particles—**

The particle size distribution of the milled particles was measured using a laser diffraction scattering instrument (LMS-30, Seishin Enterprise Co., Ltd., Tokyo, Japan) using 0.4 MPa of pressurized air for sample dispersions. The representative particle size, D10, D50, and D90, were defined as the 10, 50, and 90% values in the cumulative volume distribution curve, respectively. In addition, the fraction ratio of particles smaller than 1 µm in diameter was defined as the submicron ratio to assess milling efficiency.

**Application to Other Drug Compounds**

**—Milling of Drug Compounds—**

Fexofenadine hydrochloride and ibuprofen were milled in addition to phenytoin using the same equipment and conditions as described in “Milling Performance of the Dry Ice Beads.” Ten grams of drug powder was milled using dry ice beads (medium fraction, 150 mL of bulk volume) at 1600 rpm for 2 h. The SEMs and particle size distribution of the milled particles were obtained as described in the “Scanning Electron Micrographs of the Milled Particles” and “Particle Size Distribution of the Milled Particles.”

**Results and Discussion**

**Generation of Beads from Dry Ice Pellets**

We previously confirmed that dry ice pellets spontaneously disintegrate into bead-like fine particles immediately after immersion in LN2 without any physical stress. In the present report, the disintegration process of pellets into beads was monitored by taking photographs of the dry ice pellets before and after immersion in LN2 (Fig. 1). The dry ice pellets were disintegrated into small, spherical and uniformed bead-shaped particles within 10 s after immersion in LN2. The size and shape of the beads produced at 10 s seems to be little changed until 60 min of...

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Fig. 1. Changes in the Appearance of Dry Ice Pellets after Immersion in LN2

a) Dry ice pellet before immersion; b) immersion for 1 s; c) immersion for 10 s; d) immersion for 30 s; e) immersion for 1 min; f) immersion for 60 min.
observation. So, the particle size and shape were quantified by image analysis to assess the morphological changes precisely. The representative particle sizes and the shape indices at various time points are plotted in Fig. 2a) and b), respectively. The particle sizes (D10, D50, and D90) drastically decreased during first 10 s and then gradually reached plateau at 60 min (D50 = 769 µm) or 120 min (D50 = 761 µm). The D10 and D90 values of resultant beads at 60/120 min were around 600 and 1000 µm, respectively. In spite of spontaneous collapse of the original dry ice pellets, the size distribution of resultant beads was not wide. This spontaneous phenomenon should motivate us to apply them as milling beads.

The shape indices expressing the degree of sphericity and roughness also rapidly decreased within the first 10 s and then remained steady values for at least 120 min (approx. 1.4 of spherical index and approx. 1.2 of roughness index). Both shape indices approached 1.0, which expresses a perfectly smooth sphere, from the starting point of the pellets. According to the definition of spherical index in this study, the values of equilateral triangle, square, pentagon, and hexagon are calculated at 2.42, 1.57, 1.32, and 1.21, respectively. The shape with approx. 1.4 of spherical index from the visual image was considered to be nearly pentagon. The image analysis data indicated that the cylindrical pellets were transformed to fine polyhedral beads with rugged surface. The spherical and roughness indices of zirconia beads were assigned as 1.06 and 1.08, and not changed, demonstrating the validity of our measurements. The results of present study showed that the dry ice product obtained by the dipping process in LN2 was fine beads with nearly spherical shape and an average diameter of 761 µm, which was close to the 0.6 mmφ spherical zirconia beads. The disintegration of the pellets occurred within first 10 s and the disintegrated particles slowly became smaller without further disintegration.

**Mechanism of Dry Ice Bead Formation** The reason why the cylindrical dry ice pellets were immediately transformed to bead-like round particles in LN2 without physical stress was considered based on the state of carbon dioxide (CO$_2$) under various temperature-pressure conditions. There was a hint to understand the mechanism in its preparation process of dry ice pellets. The dry ice pellets used in this study are manufactured through a compression process. In the first step, purified CO$_2$ gas is converted to the liquid state under pressurized conditions, as shown in the phase diagram (Fig. 3). Once this pressurized liquid CO$_2$ is depressurized to atmospheric pressure, the liquid CO$_2$ is immediately scattered into small droplets. Instantaneous depressurization would suddenly remove the thermal energy from the resultant CO$_2$ droplets and decrease the temperature to the solidification point, resulting in solidification of the CO$_2$ droplets while maintaining their size and shape. Thus, the spherical dry ice particles are generated. In the final step, these particles are compressed and moulded in a die called a pelletizer to form cylindrical pellets. Thus, the dry ice pellets are obtained as the rigid aggregates compressed with the spherical particles. Many cracks on the surface of a pellet indicated by arrows in Fig. 4 are boundaries between individual dry ice particles which would support our suggestion above.
Next, the preparation process of the dry ice beads is considered. The dry ice pellets are placed in LN2, which state is assumed to be at \(-196^\circ C\) and atmospheric pressure, indicated as immersed region in the CO\(_2\) phase diagram (Fig. 3). Phase changes, for example solid/liquid or solid/gas, are not believed to occur in this region. The placement of dry ice pellets into LN2 results in intense boiling of the LN2 because the temperature of dry ice (sublimation point: \(-78^\circ C\)) is much higher than that of LN2 (boiling point: \(-196^\circ C\)). The immersed dry ice pellets are subjected to physical stress caused by convection and fluidity from the generated bubbles of nitrogen gas, boiled LN2. As described above, dry ice pellets have cracks on their surface, which indicates the boundaries between individual dry ice particles. These boundaries may be less rigid than the CO\(_2\) crystal body because they would be contact surface between the crystals. The cracks likely become a trigger to break the pellets transformed to the original primary particles, resulting in spontaneous generation of the uniform-sized spherical beads. The drastic temperature change when dry ice is placed in LN2 (from \(-78^\circ C\) to \(-196^\circ C\)) also might assist breakage. The reduction in volume due to the steep temperature drop may give a strain inside of the pellet. Both extrinsic stress and intrinsic physical strain would induce breakage at the boundaries between primary particles, resulting in the formation of dry ice beads.

**Particle Size Distribution and Fractionation of Dry Ice Beads**

Dry ice beads prepared by the immersion of pellets in LN2 for 1 min appeared suitable as a milling medium under
cryogenic conditions. These beads before grading by sieves were ranged in diameter from 350 to 2000 µm (Fig. 5a). They were sieved to three isolated fractions for the investigation of milling performance as a milling media. The median diameters (D50) of the small, medium, and large fractions were 297, 469, and 1141 µm, respectively (Fig. 5b).

**Morphological Observation of Dry Ice Beads during Milling Process** In the conventional wet milling using zirconia, alumina, and steel beads and so on, milling beads maintain their original size and shape during the milling process. However, it was doubtful whether dry ice beads would have satisfactory toughness, i.e., strength and durability, as milling media, because they seem to be brittle. So, the dry ice beads in the medium fraction (355–710 µm) were examined under agitation in the milling process to assess the strength and durability. Their periodical photographs are exhibited in Fig. 6. The visual observation suggested that the dry ice beads were not broken and fractured during operation and their size/shape were maintained under mechanical agitation in LN2 for up to 4 h. The median and the representative diameters as well as the shape indices of the dry ice beads measured by image analysis were plotted against the milling time (Fig. 7). The particle size of the dry ice beads steeply decreased during the first 30 min, and then decreased more gradually throughout the milling process. The weight ratio of beads fraction ranging in 355–710 µm was gradually reduced and small fraction passing through 355-µm sieve was increased (data not shown). Whereas, the shape indices also slightly decreased and plateaued, which means multi-edged angular beads became sphere but never reached to perfect spherical shape as the values of zirconia beads. These data indicated that the dry ice beads were worn and rounded to approach to spherical shape, resulting in slight size reduction, during the milling process due to collisions between the beads and vessel wall, particularly in the early stage of the milling process.

The uniform size and spherical shape of the beads were subsequently maintained without fracture or aggregation even under vigorous agitation, suggesting that the dry ice beads have adequate strength and durability as a milling medium. In summary, round dry ice beads with an average diameter of around 400 µm were intensively agitated in a vessel filled with LN2 throughout the milling process. In contrast to conventional milling beads such as zirconia, alumina or steel, the dry ice beads gradually became smaller and rounded with time. Bitterlich et al. reported that the milling process is more efficient with spherical beads than with irregular-shaped beads.5) The shape index value getting closer to sphere (σ=1.0) demonstrated that the dry ice beads would have adequate properties as milling media in morphological perspectives. In this study, the drug particles were not added in the milling vessel because the white opaque caused by drug powder disturbed the visual measurement. Even if the drug powder is existed in the system, the morphological change behaviour of beads would not be different from Fig. 7. Although the collision power between dry ice beads would be slightly mitigated by existence of drug particles, the power collided to the disk or vessel wall would be much more intense. In other words, the size reduction of dry ice beads would not be controllable in both cases with and without existing drug powder. Furthermore, the continuous size change of dry ice beads might be applicable to continuous size-reduction operation. In general, the large-sized balls are suitable for rough crushing operation and the small-sized beads are suitable for the fine milling/grinding operation. The dry ice beads with changeable size may have potential to act as crushing ball at early stage and as milling beads at later stage in one pot.

**Milling Performance of Dry Ice Beads** The milling performance of dry ice beads (medium fraction; 355–710 µm) was investigated using phenytoin crystals as the milled material. The sizes of milled particles were plotted against the milling time as shown in Fig. 8. The results obtained using zirconia beads with 0.6 mm in diameter, which are widely used in bead milling, were co-plotted for reference. The results after 0.5 h clearly revealed that the micronization by the dry ice beads was much slower compared to the zirconia beads. It was estimated that 4 to 6 h of dry ice milling would be required to achieve the equivalent reduction in particle size as achieved after 0.25 h of zirconia milling, suggesting that dry ice is an inferior milling material compared to zirconia in LN2.

Mechanism of wet media milling was reported as the collision between the beads and vessel wall12,13) The milling efficiency was mainly dependent on collision energy. Heavy zirconia (density: 6.0 g/cm³) would likely provide a higher collision energy to the subject than light dry ice (density: 1.56 g/cm³). In addition, zirconia beads have more uniform size, smooth surface and rigid body than dry ice beads. Effective milling power would be given by collision between heavier, similar-sized and smooth-surfaced beads. However, SEM photograph of particles after dry ice milling for 6 h (Fig. 8, insert) showed that the phenytoin particles were satisfactorily micronized to submicron particles indicating that dry
Fig. 7. Time Course of the Particle Size and Shape Indices of Dry Ice Beads (Medium Fraction: 355–710 µm) and Zirconia Beads Used as a Reference during the Cryo-Milling Process in LN2
   a) Particle size and residual weight ratio (%) of beads fraction those diameters ranging from 355 to 710 µm; b) shape indices (ML2/A: spherical index, PM2/A: roughness index). Each error bar means standard deviation.

Fig. 8. Time Course of the Particle Size of Phenytoin Cryo-Milled with Dry Ice Beads (Medium Fraction: 355–710 µm) and Zirconia Beads (0.6 mm ø) in LN2
   The inserted photo shows phenytoin particles after 6h milling with dry ice beads. Each error bar means standard deviation.
ice beads have potential as a milling media in LN2. It was assumed that micronization was surely caused by collision between beads in case of dry ice beads, too. When the drug particles would be located between the beads, the impact or shear stress are provided to them, resulting in fracture and grinding of drug particles. Because the beads are vigorously circulating vertically through the disk holes in the vessel of the wet milling equipment used in this study, the collision impact would be main driving force for micronization rather than the shear stress. The impact energy of dry ice beads per one collision was much smaller than that of zirconia beads due to their different properties (density, size uniformity, surface smoothness), thus much frequent collision, that is long milling time, would be required to attain the equivalent size reduction.

**Optimization of Milling Conditions**

Various milling conditions such as bead size, bead quantity, and agitation speed were investigated to optimize the process and enhance milling efficiency.

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**Size and Shape of Dry Ice Beads**

The median size (D50) of phenytoin particles milled for 2 h using the small, medium, and large fractions of dry ice beads were 1.8, 1.9, and 2.1 µm and the submicron ratios were 27, 22, and 19%, respectively (Table 1). Although the three distribution curves were almost overlapped, the smallest phenytoin particles with the highest submicron ratio were attained using the smallest beads (D50: 1.8 µm, submicron ratio: 27%). Several articles have reported that milling with smaller beads provides smaller milled particles in conventional wet milling.\(^{12-14}\) Our findings in the presented report were well consistent with the knowledge in these articles. Takatsuka et al. reported that 0.1 mm of zirconia beads was the optimum to provide the smallest milled particle.\(^{15}\) Both the smaller (0.05 mm) and larger ones (1 mm) did not provide the smallest milled particle. The authors concluded that both collision impact and collision frequency may become the critical factor for the fine milling. Because of the total weight of the beads were unified in this study, small number of 1-mm beads could not give the frequent collision. On the contrary, 0.05-mm beads had less collision impact. As a result, the middle-sized beads (0.1 mm) could provide the finest product. On the other hand, Tanaka reported that milling process was dominated by the collision frequency rather than the collision impact between the media (beads) especially in case of nanomilling.\(^{16}\) He concluded that the representative ball diameter defining the probability is correlated with the size, strength, and the density regardless of grinding mechanisms. Our study results that smaller size of beads provided smaller milled particles were consistent with the part of these reports above. The collision frequency is considered to dominate the milling performance rather than the collision impact. It means that larger number of beads lead to more frequent collision between beads and target material.

We also investigated the effect of bead size distribution on the milled particle size using dry ice pellets. The median size and the submicron ratio of phenytoin milled with dry ice pellets were 4.4 µm and 4%, respectively. The milling efficiency with dry ice pellets was much lower than that from beads with any size. Monitoring of the appearance of dry ice pellets immersed in LN2 showed that the pellets immediately disintegrated and formed beads with wide size distribution (Fig. 1). Whereas, the beads used in this optimization study were further grading by sieving. The size-controlled beads with narrow size distribution had higher milling efficiency than the disintegrated crude beads with broad size distribution. The present results suggest that the collision between two beads with similar size could provide higher collision energy to the drug particle, resulting in higher milling efficiency, compared to the collision between beads with significant size gap. It is concluded that bead size is a primary factor for milling efficiency, and the beads size uniformity would be supplemental factor in the present cryo-milling process.

The balls/beads with uniform size were used in almost all research, and there are few articles investigating the effect of beads size distribution on wet milling performance. If based on the theory of probability, the collision frequency would be higher in broad size distribution rather than uniform size of beads. However, Tanaka introduced the index expressing the wideness/sharpness of beads size distribution, which was defined as the ratio of smallest size against the largest size.\(^{16}\) He researched the wet milling efficiency using beads with 0.01, 0.1, and 0.3 of the distribution index. The article reported that the beads with broad distribution, 0.01 as the index proposed by him, did not provide smaller particles than the beads with narrow distribution, 0.1 and 0.3 as the index. Applying the distribution index to our results, the S, M, L-fractioned dry ice beads have 0.56, 0.53, 0.36 of the index and the crude beads generated from the pellets have 0.16 of the index. Although the crude beads did not have such wider size distribution as reported, our results were well consistent with the Tanaka’s proposal. That is, the fractioned beads (S, M, and L) with narrow size distribution could provide the higher milling efficiency than the crude beads from the pellets with broad distribution.

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**Quantity of Dry Ice Beads**

The milling performance of the dry ice beads was investigated by changing the quantity of medium-fractioned dry ice beads. The initial bulk volume of beads was set to 150 mL.

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**Table 1. Particle Size and Submicron Ratio of Phenytoin Milled with Various Milling Media**

<table>
<thead>
<tr>
<th>Milling media</th>
<th>Particle size (µm)(^{21})</th>
<th>Submicron ratio(^{10})</th>
</tr>
</thead>
<tbody>
<tr>
<td>- (Intact phenytoin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry ice pellets</td>
<td>4.8 (0.8)</td>
<td>10.4 (0.8)</td>
</tr>
<tr>
<td>Dry ice beads (small fraction: 180–355 µm)</td>
<td>1.7 (0.5)</td>
<td>4.4 (0.3)</td>
</tr>
<tr>
<td>Dry ice beads (medium fraction: 355–710 µm)</td>
<td>0.73 (0.0)</td>
<td>1.8 (0.0)</td>
</tr>
<tr>
<td>Dry ice beads (large fraction: 710–1700 µm)</td>
<td>0.82 (0.0)</td>
<td>1.9 (0.1)</td>
</tr>
</tbody>
</table>

\(^{a}\) The particle size indices D10, D50, and D90 were defined as the 10, 50, and 90% values in the cumulative volume distribution curve. \(^{b}\) Each value was presented as average (standard deviation).
according to our prior knowledge acquired using zirconia beads, and the volume was doubled and halved. The particle size evaluation showed that the median size of phenytoin particles milled with 75, 150, and 300 mL of dry ice beads were 3.8, 1.9, and 1.5 µm, respectively, and the submicron ratio increased 7, 22, and 32% in response to beads volume. These results indicated that the larger the loading of beads became, the finer the milled particles obtained. The enhancement of milling efficiency by increasing bead loading has been also reported previously and is likely due to an increase in collision frequency. An increase in bead quantity may lead to reduced recovery of product in the conventional bead milling system due to increased loss by adhesion to the beads. In the current cryo-milling system using dry ice, the adhesion to beads does not lead to manufacturing loss because the dry ice beads were spontaneously sublimated and completely disappeared after LN2 evaporation and not required to be separated from the product. This is an innovative advantage of this advanced cryo-milling technique especially for mass production.

—Agitation Speed—

The effect of agitation speed on the milling efficiency of phenytoin particles was investigated. The two different disk rotation speeds were investigated to optimize the agitation speed. In our previous article for cryo-milling with zirconia beads, it was reported that the more powerful collision between beads generated by higher agitation speed at 1600 rpm resulted in successful pulverization rather than slower agitation at 500 rpm. In order to increase the collision power of lighter dry ice beads, much higher agitation speed (2760 rpm) was additionally investigated together with the standard speed (1600 rpm). The D50 values of phenytoin particles milled at 1600 and 2760 rpm was 1.9 and 2.5 µm, and the submicron ratio was 22 and 14%, respectively. The particle size measurement revealed that increased agitation speed could not promote the milling efficiency. It was observed that some amount of LN2 was spilled out from the vessel at 2760 rpm of agitation speed. The over-speed might bring about turbulent flow in the milling vessel and thus reduced milling efficiency in the current rotating disk equipment. Optimum agitation speed has to be determined in the consideration of material/size of the beads, shape/size of the vessel, and the agitation disk used.

Application to Other Drug Compounds  In addition to phenytoin, other two drug substances with various crystal sizes and shapes were subjected to the present cryogenic milling technique to assess its wide application in pharmaceutical field. As shown in the microscopic photographs (Fig. 9, left), the morphology of intact crystals was significantly different each other; small-sized cubic phenytoin, large-sized cylindrical fexofenadine hydrochloride, and middle-sized rectangular ibuprofen. All crystals were successfully micronized to sub- or single-micron size with dry ice beads under cryogenic condition regardless of their original size and shape (Fig. 9, right). The size distribution curves presented some amount of milled particles being less than 1 µm in diameter (Fig. 10). These results indicated this technique is widely applicable not only for phenytoin but also the other drugs with various sizes and shapes.

The fracture toughness of milled materials has been reported in several articles. Drory et al. introduced Vickers indentation method to estimate the material hardness using single crystal of gallium nitride. Taylor et al. have introduced the nanoindentation method to predict the milling propensity of pharmaceutical materials and suggested fracture toughness as a new index calculated from Young’s modulus, hardness of

Fig. 9. Scanning Electron Micrographs of Intact (before Milling) and Drug Compounds Cryo-Milled in LN2 Using Dry Ice Beads

a1) intact phenytoin; a2) milled phenytoin; b1) intact fexofenadine hydrochloride; b2) milled fexofenadine hydrochloride; c1) intact ibuprofen; c2) milled ibuprofen.
material, and crack lengths. They concluded that the ratio of hardness to fracture toughness had clear discrimination between materials for the breakage propensity. They also introduced the relation between the brittleness index and actual milling result (% size reduction ratio). Masterson and Cao estimated the hardness of various pharmaceutical materials including ibuprofen using Nanoscope atomic force microscopy nanoindentation which allows a high-resolution imaging, composition mapping with spatial resolution in nanometres. The author presented the hardness ranked as: ascorbic acid > sucrose > lactose ≈ ibuprofen from their different extents of indentation size or peak load observed. Ibuprofen was included in our study as one of the target materials because of the well investigated hardness as reported above. Cao et al. also measured the hardness of various materials to investigate the correlation to their compaction profiles. In the article, ibuprofen was introduced as the particle ranked in the class with relatively low hardness. In our study, we tried milling of ibuprofen using dry ice beads in LN2 and confirmed the feasibility to obtain submicron size of ibuprofen particles. However, it was not sufficient to prove that the proposed technique is widely applicable to various materials in only case of ibuprofen.

In the present study, the fine milling was successfully achieved in all case of active pharmaceutical ingredients with diverse sizes and shapes as shown in Fig. 10. In particular, phenytoin and ibuprofen particles were micronized to partially submicron/single-micron size. It was assumed that the milling to the submicron particles was surely achieved under the optimized condition. In case of fexofenadine hydrochloride, the size distribution curve of the milled product was plotted in slightly larger side (right side) and the submicron ratio was lower (7%) compared to ibuprofen and phenytoin, seemed to be insufficient milling result. However, the microscopic observation clearly indicated that the particle sizes of the milled products were not inequivalent among all three drugs (Fig. 9, right). In addition, the minimum size of the milled products, which was expressed by the left end of the distribution curve, was well matched as 0.4 μm among all three drugs (Fig. 10). These results suggest that the milled particles of fexofenadine hydrochloride would tend to be aggregated during dry dispersion in the laser diffractometer. Anyway, the particles of all three drugs were successfully pulverized in the present advanced cryo-milling technique with dry ice beads. In this cryogenic milling, the materials suspended in LN2 were cooled at extremely low temperature and could be fractured like a glass when collided with cryogenic beads because of getting brittle property. Although the mechanical property of the materials should be further investigated under cryogenic condition in LN2, this technique would be advantageous to produce the micronized or nanosized particles of various types of pharmaceutical ingredients.

The crystal form of milled phenytoin under cryogenic condition has already been reported in our previous study. In addition, other two drug crystals were also investigated. X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) profiles indicated that the crystal properties of milled all three drugs were not changed from those of the intact ones (data not shown). The crystal forms of these milled drug substances were assumed as a complete crystal without amorphous fraction. Target materials would not be activated by thermal energy during the milling process due to the extremely cold condition. The presented milling method provides the option to obtain submicron size of milled particle without any change in its crystal form.

Finally, the suitability of dry ice should be considered as a beads material. Volatile dry ice at ambient condition is only applicable in cryogenic condition such as dispersed in LN2. Apparently brittle dry ice is unlikely to be suitable due to insufficient strength. Mohs hardness is one of the indices for assessing the hardness of materials, especially for comparing abrasion strength when two materials are rubbed together. The larger this number is, the higher the tolerability of the material to being scratched and thus the less likely to be eroded. Mohs hardness of stainless-steel and zirconia, two representative milling media, are 7.5 and 8.5, respectively. Whereas, the hardness of dry ice is reported to be 2, similar to that of rock salt or plaster (calcium sulphate). The readers may judge that dry ice having low hardness would be disadvantageous as a milling media. However, the result in this research demonstrated that the dry ice beads have a potential to play a role of the proper milling media under cryogenic condition. Mohs

<table>
<thead>
<tr>
<th>Drug substance</th>
<th>Intact (μm)</th>
<th>Submicron ratio (%)</th>
<th>Milled (μm)</th>
<th>Submicron ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenytoin</td>
<td>9.2 (0.9)</td>
<td>0% (0%)</td>
<td>1.9 (0.1)</td>
<td>22% (2%)</td>
</tr>
<tr>
<td>Fexofenadine hydrochloride</td>
<td>443 (1.5)</td>
<td>0% (0%)</td>
<td>4.2 (1.1)</td>
<td>7% (3%)</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>32 (2.0)</td>
<td>0% (0%)</td>
<td>2.0 (0.2)</td>
<td>18% (6%)</td>
</tr>
</tbody>
</table>

*Each value was presented with average (standard deviation).*

**Fig. 10.** Particle Size Distribution of Intact (before Milling) and Cryo-Milled Drug Compounds in LN2
values shown above suggest that the disk, shaft, and vessel, which are usually made of stainless-steel or zirconia, would be hard to be eroded by collisions with dry ice beads. Therefore, the lower Mohs hardness of dry ice beads may be an advantage of the current cryo-milling system from the perspective of less contamination from the equipment. Thus, milling with dry ice beads in LN2 could pulverize drug substances without crystal form change in the milling process. On another front, dry ice has to be expected no damage of equipment leading to contamination.

**Conclusion**

It was found that the generation of dry ice beads from cylindrical pellets results from spontaneous retransformation in LN2 to their original particle shape prior to being pressed into pellets. This return to the bead form would be attributed to volumetric changes and physical stress inside the pellets. The monitoring of morphological changes showed that dry ice beads have sufficient mechanical strength and durability as a cryogenic medium for milling since the size and shape were maintained throughout the milling process.

All of the investigated drug compounds could be micronized by the advanced cryogenic milling technique with dry ice beads. Zirconia was superior to dry ice as a milling medium that can rapidly produce fine milled particles. Although milling with dry ice requires a longer time to achieve the same level of milling, there would be no contamination of the product due to bead erosion. Our optimization study revealed that uniform-sized beads are preferable for efficient milling, and smaller beads providing smaller milled particles. We have demonstrated the potential application of the proposed technique. Cryogenic milling using dry ice is a valuable milling technique that solves the contamination issue.

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**Conflict of Interest** The authors declare no conflict of interest.

**References**