BPB Reports

Regular Article

Optimization of Milling Parameters for Low Metal Contamination in Bead Milling Technology

Hironori Tanaka,[a,b,*] Yuya Ochii,[a] Yasushi Moroto,[a] Daisuke Hirata,[c] Tetsuharu Ibaraki,[c] and Ken-ichi Ogawara[b]

[a]*Formulation R&D Laboratory, CMC R&D Division, Shionogi & Co., Ltd., 2-1-3, Kuise Terajima, Amagasaki, Hyogo 660-0813, Japan;* [b]*Laboratory of Pharmaceutics, Kobe Pharmaceutical University, 4-19-1 Motoyamakita-machi, Higashinada-ku, Kobe, Hyogo 658-8558, Japan;* [c]*Hiroshima Metal & Machinery Co., Ltd., 1-2-43 Hiroshiratake, Kure, Hiroshima 737-0144, Japan*

Received March 31, 2022; Accepted May 20, 2022

This study aimed to develop a contamination-less bead milling technology using zirconia beads, by optimizing milling parameters. We evaluated the effects of bead milling parameters on milling time and metal contamination; focusing on bead diameter, rotation speed, and bead filling rate as they are the critical parameters determining bead milling efficiency. We studied the milling time required to grind a drug to 0.2 µm size and quantified the extent of metal contamination that arises from the grinding procedure. With optimal bead milling parameters, the minimum concentration of the metal contaminants was 1.27 ± 0.08 µg/mL (zirconium: 0.73 ± 0.09 µg/mL; yttrium: 0.35 ± 0.03 µg/mL; aluminum: 0.19 ± 0.05 µg/mL), when the rotation speed, bead diameter, and bead filling rate were set to 2 m/s, 0.3 mm, and 75% (v/v), respectively. Additionally, under optimized conditions, it was possible to reduce the metal contamination per weight of the drug by increasing the drug concentration to up to 40% (w/w). Under the optimized conditions for drug concentrations of 30–40% (w/w), metal contamination from the grinding process was minimized and reduced to < 10 µg/g, which is comparable to that achieved by the NanoCrystal® technology. These results indicate that contamination-less bead milling using zirconia beads can be achieved when bead milling parameters are optimized.

Key words bead milling, milling parameters, drug nanocrystal, metal contamination, zirconia beads

INTRODUCTION

In recent years, innovative technologies such as high-throughput screening and combinatorial chemistry have facilitated the rapid development of new drug compounds with excellent pharmacological activity. Nevertheless, approximately 40–70% of all new drug candidates have been estimated to have very low water solubility.[1,2] This may cause undesirable effects and eventually lead to poor patient compliance, poor bioavailability, dosing restrictions due to food effects, and the use of harsh excipients or extreme basic or acidic conditions to enhance solubilization.[3] Hence, new technologies to improve water solubility are being actively investigated.[4]

Nano-milling technology is useful for improving the dissolution rates of drugs with low solubility. It increases specific surface area according to the Noyes–Whitney equation,[6] which can improve drug dissolution rates.[5] Thus, formulations that use nano-milling technology to produce medication with nanosized particles have been demonstrated to have improved dissolution and absorption rates after oral administration.[7–9]

Nanosized particles can be prepared by bead milling,[10,11] high-pressure homogenization,[12,13] antisolvent precipitation,[14] or a combination of these methods. As one of the most widely used nano-milling technologies,[15] bead milling has been adopted by many industries both related and unrelated to pharmaceuticals.[16] In pharmaceutical bead milling applications, a grinding medium such as yttria-stabilized zirconia beads, is typically used to grind and disperse drugs. The process exploits the collision energy between beads as they are stirred in the grinding chamber of a mill; its high grinding efficiency, scalability, and reproducibility are widely recognized.[17]

However, metal contamination arising from the grinding process is considered to limit its application in the pharmaceutical manufacturing process.[18,19] For instance, 100–500 µg/g of zirconium contaminant (per weight of drug) was found in a drug that was manufactured using 0.1 mm zirconia beads as the grinding medium.[20] Nonetheless, several pharmaceutical products that rely on bead milling technology for production are still being marketed to consumers.[21–24] The NanoCrystal® technology applied in the manufacture of these products is widely known as a contamination-less bead milling technology; it uses highly cross-linked polystyrene beads as a grinding medium.[17] A previous report from a different research group demonstrated that the amount of insolubles generated during the NanoCrystal® grinding process was < 0.005% (w/w).[5] The patents associated with this technology also suggest it that can maintain heavy metal contamination at levels < 10 µg/g.[25] Therefore, NanoCrystal® technology is currently the bead milling technology of choice in the global pharmaceutical industry. While several other nano-milling technologies that minimize metal contamination have been investigated, a suitable alternative to NanoCrystal® technology has not yet been developed.

*To whom correspondence should be addressed. e-mail: hironori.tanaka@shionogi.co.jp

Milling and bead material parameters have been reported to be closely tied to metal contamination in bead milling. For instance, Inkyo *et al*.[26] have reported that zirconium contamination can be reduced from 1400 to 220 ppm by reducing bead size from 100 to 15 μm. Li *et al*.[27] evaluated the effect of rotation speed on zirconium contamination and demonstrated that contamination can also be reduced by lowering rotation speeds from 14.7 to 11.7 m/s. However, these previous reports only evaluated the effect of milling parameters on metal contamination within a limited range, and the majority of them have focused on evaluating the corresponding effects on the stability of the nanocrystal drug suspensions;[28] either disregarding or paying little attention to the metal contamination aspect. Therefore, in this study, the effects of bead milling parameters on milling time and metal contamination were systematically evaluated, using zirconia beads as a grinding medium. We also evaluated various permutations of the parameter configurations to identify the parameter sets that produce minimal metal contamination.

MATERIALS AND METHODS

Materials Phenytoin (D50 = 8 μm, D90 = 14 μm) was purchased from Shizuoka Caffeine Industries (Shizuoka, Japan), polyvinylpyrrolidone (PVP) K-25, and sodium dodecyl sulfate (SDS) from BASF Japan Ltd. (Tokyo, Japan). Yttria-stabilized zirconia beads with diameters of 0.1, 0.2, 0.3, 0.5, 0.8, and 1.0 mm were purchased from Nikkato Corporation (Osaka, Japan). All other chemicals and solvents were of analytical reagent grade, and purified water was used for solution preparation.

Preparation of Suspensions for Bead Milling A previously optimized formulation for phenytoin containing PVP K-25 and SDS was selected to evaluate the effect of milling parameters on the milling time and metal contamination.[29] Phenytoin at 5–50% (w/w) in purified water was stabilized with 3% (w/w) PVP K-25 and 0.25% (w/w) SDS. The dispersion medium was prepared using a stirrer, at 250 rpm for 20 min (SM-103, AS ONE Corporation, Osaka, Japan), to dissolve PVP K-25 and SDS in purified water. Phenytoin was dispersed in a dispersion medium with the stirrer, at 500 rpm for 30 min, to form a suspension. A total of 500 g of phenytoin suspension was prepared.

Procedure for Bead Milling with Apex-Mill Type-015 Wet milling was performed using an Apex-mill type-015 apparatus (Hiroshima Metal & Machinery Co., Ltd., Hiroshima, Japan). Yttria-stabilized zirconia beads were used as the grinding medium. Bead milling was carried out at a rotation speed of 0.5–12 m/s, bead diameter of 0.1–1.0 mm, and bead filling rate of 25–90% (v/v). The process was performed in the re-circulation mode using a Masterflex 7554-80 (Yamato Scientific Co., Ltd., Tokyo, Japan). Flow rate was set to 10 L/h. During the bead milling process, the suspension in the tank was stirred using a stirrer (SM-103, AS ONE Corporation, Tokyo, Japan) for uniformity. The grinding chamber was connected to an external cooling device RKE7500A-V (ORION machinery Co., Ltd., Nagano, Japan) to dissipate the heat generated during milling. Samples were taken from the outlet of the grinding chamber at pre-determined time intervals, for particle size measurement and the determination of metal contamination.

Determination of Particle Size Distribution in the Nanosized Particle Suspension Particle size distribution was measured by the laser diffraction method (LA-950, HORIBA, Ltd., Kyoto, Japan). Measurements were performed at room temperature using purified water as the diluent. The volumetric median particle size was calculated using a refractive index value of 1.61 for phenytoin and 1.33 for the measurement medium (water).

Determination of Metal Contamination in the Nanosized Particle Suspension The metal contamination in the nanosized particle suspension was determined by inductively coupled plasma-mass spectrometry (ICP-MS, iCAPQ, Thermo Fisher Scientific, MA, Waltham, USA). The samples were placed in a metal-free chamber and an internal standard substance (Co) and an NMP/HCl/HNO_3 mixture (90:5:5) were added. The samples were completely dissolved via ultrasonic irradiation. Elemental analysis was performed with four different calibration solutions and an internal standard. The range of the calibration was 0.5–2.0 μg/mL. In this study, the units of the metal contamination are described as μg/mL per volume of suspension and μg/g per weight of the drug in the suspension.

RESULTS AND DISCUSSION

Effects of Bead Diameter and Rotation Speed on Milling Time Because particle size reduction by bead milling is essentially determined by the collision energy[30,31] and collision frequency of beads,[32] bead milling parameters such as bead diameter, rotation speed, and bead filling rate[28] are critical for optimizing bead milling conditions. Rotation speed and bead diameter have been recognized as two of the major parameters that determine the extent of metal contamination during the grinding process.[33] Considering the influence of these factors on the grinding process, the bead diameter, rotation speed, and bead filling rate, had to be carefully determined because they substantially affect milling time and the extent of metal contamination.

The particle size distribution of drug nanocrystals was suggested to be 0.2–0.6 μm with an average particle size.[34] Therefore, in our study, the target particle size of phenytoin in D50 after milling was < 0.2 μm. Considering D50 and D90 after treatment of wet milling were in the range of 0.1854–0.1998 μm, and 0.2486–0.2732 μm, respectively, the particle size distributions with < 0.2 μm (D50) were expected to be equivalent among used treatments. The milling time required to reach the target particle size was < 120 min, given the practical considerations for wet milling at production scales. The influence of bead diameter on the time (milling) taken to grind phenytoin to 0.2 μm was evaluated for each rotation speed; the results are shown in Fig. 1. At a bead diameter of 0.5 mm, the milling time shortened as the rotation speed increased. The same tendency was observed for bead diameters of 0.2 and 0.3 mm, suggesting that higher rotation speeds reduce the milling time required to obtain the target size of the drug particle. Additionally, when the rotation speed was set to 4–8 m/s, the milling time required shortened as the bead diameter decreased. When the rotation speed was set to 2 m/s, the milling time required shortened as the bead diameter decreased over the range of 0.2–1.0 mm. However, the milling time increased for the 0.1-mm bead diameter, compared to that required for the 0.2-mm diameter. These results indicated that for the various bead diameters tested, smaller bead diameters required shorter milling times, except for the combination of 0.1-mm bead diameter and 2 m/s rotation speed. This last result was attributed to a reduction in per bead collision energy, due to the small-

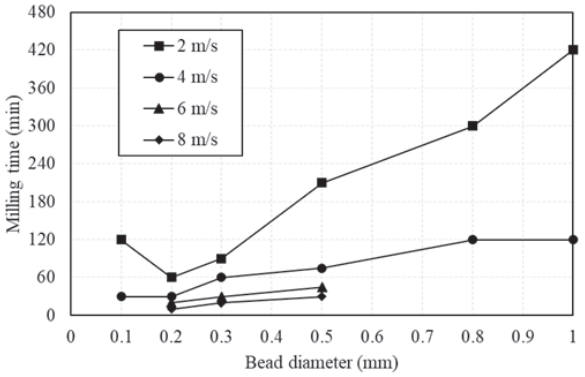

Fig. 1. Effect of Bead Diameter on Milling Time Required for Grinding Drug Particles to 0.2 μm for Each Rotation Speed of the Grinding Process

Drug concentration and bead filling rate were set to 5% (w/w) and 75% (v/v), respectively ($n = 1$).

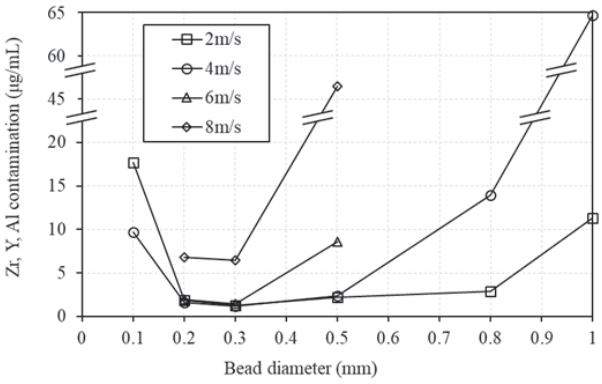

Fig. 2. Effect of Bead Diameter on Metal Contamination for Each Rotation Speed of the Grinding Process

Drug concentration and bead filling rate were set to 5% (w/w) and 75% (v/v), respectively ($n = 1$).

er bead mass and lower rotation speed.

It is worth noting that phenytoin particles could be ground to < 0.2 μm in D50 even at an ultra-low rotation speed of < 4 m/s (Fig. 1). Recently, horizontal bead mills have been commonly used for nano-milling[28] and the typical rotation speed has been reported to be 5–15 m/s.[35] The Apex-mill used in the present study is a vertical mill, which allows the zirconia beads to flow downwards uniformly in the grinding chamber, even at ultra-low rotation speeds. This could be one of the reasons behind the efficient milling of the phenytoin particles observed in this study.

Effects of Bead Diameter and Rotation Speed on Metal Contamination In this study, zirconia-toughened alumina, the material of the grinding chamber, and yttria-stabilized zirconia beads were considered as possible sources of metal contamination. The amounts of zirconium (Zr), yttrium (Y), and aluminum (Al) contaminates were measured after the grinding process, and we evaluated the effects of the bead diameter on the metal contamination (per volume of suspension) at each rotation speed (Fig. 2). When the bead diameter was 0.5 mm and rotation speeds were 2 and 8 m/s, the minimum and maximum metal concentrations of metal contamination were 2.21 and 46.5 μg/mL, respectively. Metal contamination was minimal for all bead diameters, except that of 0.1 mm, with rotation speeds of 2 m/s. This result suggests that lower rotation speeds would ensure lower metal contamination. Across all the rotation speeds tested, the lowest metal contamination concentration was obtained with a 0.3 mm bead diameter. In summary, the combination of 0.3 mm bead diameter and 2 m/s rotation speed produced the lowest metal contamination across all tested configurations.

Theoretically, the number of beads per volume is inversely proportional to the cube of the bead diameter, and the collision energy of the beads is proportional to the cube of the bead diameter. Therefore, the effective number of 0.1-mm beads per volume increased approximately 27 times over that of the 0.3-mm beads. The frequency of collisions between the beads increased accordingly, producing the observed increments in metal contamination. In configurations with bead diameters of 1.0 mm, the collision energy was approximately 37 times higher than that of similar configurations with bead diameters of 0.3 mm. Thus, the metal contamination increased because the zirconia beads experienced higher mechanochemical stresses. Although a suitable mechanism that supports this observation has not been elucidated, the minimum metal contamination with 0.3-mm diameter beads indicates that an optimum balance between the energy and frequency of collisions can be obtained. Particle simulation analyses, such as the discrete element method (DEM),[36,37] are necessary to investigate this, and will be the subject of our further studies.

Effects of Bead Filling Rate on Milling Time and Metal Contamination We evaluated the effects of the bead filling rate on the milling time and metal contamination (per volume of suspension), when the bead diameter and rotation speed were 0.3 mm and 2 m/s, respectively (Fig. 3). When the bead filling rate was set to 25% (v/v), the metal contamination was minimized, with the concentration of metal contamination being 0.66 μg/mL. Conversely, the required milling time was the longest (600 min). However, although the milling time was the shortest (90 min) with a bead filling rate of 90% (v/v), the maximum metal contamination (2.08 μg/mL) was observed under this combination of parameters. A different research group investigating the optimum bead filling rate has also reported that there is a trade-off between metal contamination and milling time.[38]

In this study, we considered a milling time of > 120 min to be impractical. It was < 120 min when the bead filling rate was 75 and 90% (v/v). However, the concentrations of the metal

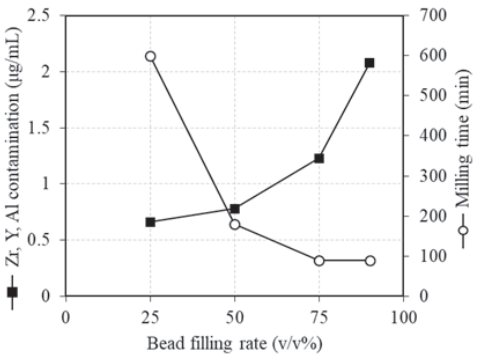

Fig. 3. Effect of Bead Filling Rate on Metal Contamination and Milling Time Required for Grinding to 0.2 μm in the Grinding Process

Drug concentration, rotation speed, and bead diameter were set to 5% (w/w), 2 m/s, and 0.3 mm, respectively ($n = 1$).

Table 1. Effect of Drug Concentration on Production Rate and Metal Contamination During the Grinding Process (n = 1)

Drug concentration [% (w/w)]	Production rate (g/h)	Particle size distribution		Contamination				
		D50 (μm)	D90 (μm)	Zr (μg/mL)	Y (μg/mL)	Al (μg/mL)	Total (μg/mL)	Total (μg/g drug)
5	17	0.1998	0.2732	0.65	0.35	0.24	1.24	24.31
30	100	0.1941	0.2576	0.77	0.32	0.20	1.29	4.02
40	133	0.1907	0.2534	0.84	0.33	0.21	1.38	3.14

contaminants corresponding to these filling rates were 1.24 and 2.08 μg/mL, respectively, with the former being 40.9% lower than the latter. Therefore, the 75% (v/v) bead filling rate was the more optimal configuration for this parameter.

Based on the obtained results shown in Figs. 1, 2 and 3, the following optimized bead milling parameters were determined, for minimal metal contamination: bead diameter, 0.3 mm; rotation speed, 2 m/s; bead filling rate, 75% (v/v)—these optimized parameters were used hereafter. First, the reproducibility of the results for these optimized parameters was evaluated in triplicate, and the results were found to be highly reproducible, with the average concentration of the metal contaminants being 1.27 ± 0.08 μg/mL (Zr: 0.73 ± 0.09 μg/mL, Y: 0.35 ± 0.03 μg/mL, Al: 0.19 ± 0.05 μg/mL). Furthermore, our unpublished data revealed that these optimized bead milling parameters allow the grinding of other poorly water-soluble drugs with different physicochemical properties (itraconazole, fenofibrate, sulfamethoxazole, and mefenamic acid) to < 0.2 μm (manuscript in preparation). The reduced metal contamination results of our optimized zirconia bead milling parameters demonstrate comparable performance to alternative bead milling technologies. For instance, a patent specification for a bead milling technology using polycarbonate beads reported a zirconium contamination concentration of 0.7 μg/mL for materials ground to 225 nm[39]—only about 0.03 μg/mL lower than our average result.

Effects of Drug Concentration on Productivity and Metal Contamination During the Grinding Process The effect of phenytoin concentration on the productivity of the ground phenytoin under the optimized bead milling conditions was evaluated. In this experiment, the milling time was set to 90 min because a drug concentration of < 50% (w/w) did not affect the time required to grind phenytoin to < 0.2 μm (D50). Considering D50 and D90 after treatment of wet milling, the particle size distributions with < 0.2 μm (D50) would be equivalent among drug concentration applied (Table 1). To gauge the effect of the drug concentration on the productivity of the entire manufacturing process, for nanosized particle suspensions, the production rate was defined and calculated as the amount of phenytoin ground to < 0.2 μm per hour (Table 1). When the drug concentration was low, that is 5% (w/w), the production rate was 17 g/h. However, when the drug concentrations were increased to 30 and 40% (w/w), the production rate drastically improved to 100 and 133 g/h, respectively. These production rates were equivalent to those previously reported by another research group, who milled a 20% naproxen suspension at the production scale.[40]

The effect of phenytoin concentration on metal contamination was also evaluated, with the total metal contamination during the grinding process was normalized by the suspension volume and weight of phenytoin; the results obtained are listed in Table 1. When the drug concentration was set to 5% (w/w), the total concentration of the metal contaminants was 1.24 μg/mL, and the metal contamination per weight of phenytoin was calculated as 24.3 μg/g. Increasing the drug concentration to 30 and 40% (w/w) only slightly increased the contamination per suspension volume over the 5% (w/w) treatment. However, the total concentration of the metal contaminants per weight of phenytoin was determined to be 4.02 and 3.14 μg/g. The bead wear was reported to be lower for materials with lower hardness than the grinding medium.[41] The particle hardness of pharmaceutical materials is considered to be approximately 10 times lower than that of zirconia beads.[42,43] Therefore, it has been suggested that metal contamination will not increase even if the concentration of phenytoin increases, and thus, that the metal contamination per weight of drug would be drastically reduced with the suspensions of higher concentrations of drugs. The observed values (4.02 and 3.14 μg/g) were comparable to those observed for NanoCrystal® technology, a world-leading bead milling technology.[25] However, unlike NanoCrystal® technology, for which polymeric contamination level are < 1000 μg/g,[25] there is no risk of non-metallic contamination from the erosion of resin beads in our system.

Conclusion Based on the results obtained in this study, the optimization of bead milling parameters (bead diameter, rotation speed, and bead filling rate) can minimize metal contamination. Our optimized bead milling configuration using zirconia beads minimizes metal contamination and demonstrates performance comparable to the NanoCrystal® technology. Further studies are still required to elucidate the detailed mechanisms driving the influence of milling parameters on bead milling processes. Nevertheless, the results reported herein provide valuable information for the development of a contamination-less bead milling technology with zirconia beads.

Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Loftsson T, Brewster ME. Pharmaceutical applications of cyclodextrins: basic science and product development. *J. Pharm. Pharmacol.*, **62**, 1607–1621 (2010).
2) Di Maio S, Carrier RL. Gastrointestinal contents in fasted state and post-lipid ingestion: *in vivo* measurements and *in vitro* models for studying oral drug delivery. *J. Control. Release*, **151**, 110–122 (2011).
3) Merisko-Liversidge EM, Liversidge GG. Drug nanoparticles: formulating poorly water-soluble compounds. *Toxicol. Pathol.*, **36**, 43–48 (2008).
4) Kaptay G. On the size and shape dependence of the solubility of nanoparticles in solutions. *Int. J. Pharm.*, **430**, 253–257 (2012).
5) Merisko-Liversidge E, Liversidge GG, Cooper ER. Nanosizing: a formulation approach for poorly-water-soluble compounds. *Eur. J. Pharm. Sci.*, **18**, 113–120 (2003).
6) Noyes AA, Whitney WR. The rate of solution of solid substances in their own solutions. *J. Am. Chem. Soc.*, **19**, 930–934 (1897).
7) Komasaka T, Fujimura H, Tagawa T, Sugiyama A, Kitano Y. Practical method for preparing nanosuspension formulations for toxicology studies in the discovery stage: formulation optimization and *in vitro*/in vivo evaluation of nanosized poorly water-soluble compounds. *Chem. Pharm. Bull. (Tokyo)*, **62**, 1073–1082 (2014).
8) Yuminoki K, Tachibana S, Nishimura Y, Mori H, Takatsuka T, Hashimoto N. Scaling up nano-milling of poorly water soluble compounds using a rotation/revolution pulverizer. *Pharmazie*, **71**, 56–64 (2016).
9) Kumar S, Jog R, Shen J, Zolnik B, Sadrieh N, Burgess DJ. Formulation and performance of danazol nano-crystalline suspensions and spray dried powders. *Pharm. Res.*, **32**, 1694–1703 (2015).
10) Yao Q, Tao X, Tian B, Tang Y, Shao Y, Kou L, Gou J, Li X, Yin T, Tang X. Improved oral bioavailability of core-shell structured beads by redispersion of the shell-forming nanoparticles: preparation, characterization and *in vivo* studies. *Colloids Surf. B Biointerfaces*, **113**, 92–100 (2014).
11) Figueroa CE, Bose S. Spray granulation: importance of process parameters on *in vitro* and *in vivo* behavior of dried nanosuspensions. *Eur. J. Pharm. Biopharm.*, **85** (3 Pt B), 1046–1055 (2013).
12) Romanski FS, Jayjock E, Muzzio FJ, *et al*. Important factors in the size reduction of polymer-stabilized drug particle suspensions using high-pressure homogenization. *J. Pharm. Innov.*, **6**, 97–106 (2011).
13) Sun W, Mao S, Shi Y, Li LC, Fang L. Nanonization of itraconazole by high pressure homogenization: stabilizer optimization and effect of particle size on oral absorption. *J. Pharm. Sci.*, **100**, 3365–3373 (2011).
14) Beck C, Dalvi SV, Dave RN. Controlled liquid antisolvent precipitation using a rapid mixing device. *Chem. Eng. Sci.*, **65**, 5669–5675 (2010).
15) Bhakay A, Rahman M, Dave RN, Bilgili E. Bioavailability enhancement of poorly water-soluble drugs via nanocomposites: formulation–processing aspects and challenges. *Pharmaceutics*, **10**, 86 (2018).
16) Weber U. The effect of grinding media performance on milling a water-based color pigment. *Chem. Eng. Technol.*, **33**, 1456–1463 (2010).
17) Merisko-Liversidge E, Liversidge GG. Nanosizing for oral and parenteral drug delivery: a perspective on formulating poorly-water soluble compounds using wet media milling technology. *Adv. Drug Deliv. Rev.*, **63**, 427–440 (2011).
18) Rahman M, Hussain A, Iqbal Z, *et al*. Nanosuspension: a potential nanoformulation for improved delivery of poorly bioavailable drug. *Micro Nanosyst.*, **5**, 273–287 (2013).
19) Patel VR, Agrawal YK. Nanosuspension: an approach to enhance solubility of drugs. *J. Adv. Pharm. Technol. Res.*, **2**, 81–87 (2011).
20) Juhnke M, Märtin D, John E. Generation of wear during the production of drug nanosuspensions by wet media milling. *Eur. J. Pharm. Biopharm.*, **81**, 214–222 (2012).
21) Goel S, Sachdeva M, Agarwal V. Nanosuspension technology: recent patents on drug delivery and their characterizations. *Recent Pat. Drug Deliv. Formul.*, **13**, 91–104 (2019).
22) Van Eerdenbrugh B, Van den Mooter G, Augustijns P. Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. *Int. J. Pharm.*, **364**, 64–75 (2008).
23) Junghanns JU, Müller RH. Nanocrystal technology, drug delivery and clinical applications. *Int. J. Nanomedicine*, **3**, 295–309 (2008).
24) Patel M, Shah A, Patel NM, *et al*. Nano suspension : a novel approach for drug delivery system. *J. Pharm. Sci. Biosci. Res.*, **1**, 1–10 (2011).
25) Czekai DA, Reed RG. inventor; Elan Pharmainternational Ltd, assignee. Apparatus for sanitary wet milling. United States patent US 6,582,285 B2. 2003 Jun 24.
26) Inkyo M, Tahara T, Iwaki T, Iskandar F, Hogan CJ Jr, Okuyama K. Experimental investigation of nanoparticle dispersion by beads milling with centrifugal bead separation. *J. Colloid Interface Sci.*, **304**, 535–540 (2006).
27) Li M, Yaragudi N, Afolabi A, *et al*. Sub-100 nm drug particle suspensions prepared via wet milling with low bead contamination through novel process intensification. *Chem. Eng. Sci.*, **130**, 207–220 (2015).
28) Li M, Azad M, Davé R, Bilgili E. Nanomilling of drugs for bioavailability enhancement: a holistic formulation-process perspective. *Pharmaceutics*, **8**, 17 (2016).
29) Tanaka H, Ochii Y, Moroto Y, Ibaraki T, Ogawara KI. Development of novel bead milling technology with less metal contamination by pH optimization of the suspension medium. *Chem. Pharm. Bull. (Tokyo)*, **69**, 81–85 (2021).
30) Kano J, Miyazaki M, Saito F. Ball mill simulation and powder characteristics of ground talc in various types of mills. *Adv. Powder Technol.*, **11**, 333–342 (2000).
31) Yoden H, Itoh N. Effect of small size beads on dispersion of nanometer-sized silica particle by wet beads mill process. *J. Soc. Powder Technol. Japan*., **41**, 457–464 (2004).
32) Kwade A. Determination of the most important grinding mechanism in stirred media mills by calculating stress intensity and stress number. *Powder Technol.*, **105**, 382–388 (1999).
33) Hennart SLA, Domingues MC, Wildeboer WJ, *et al*. Study of the process of stirred ball milling of poorly water-soluble organic products using factorial design. *Powder Technol.*, **198**, 56–60 (2010).
34) Geetha G, Poojitha U, Arshad K, *et al*. Various techniques for preparation of nanosuspension—a review. *Int. J. Pharma Res. Rev.*, **3**, 30–37 (2014).
35) Kesisoglou F, Panmai S, Wu Y. Nanosizing--oral formulation development and biopharmaceutical evaluation. *Adv. Drug Deliv. Rev.*, **59**, 631–644 (2007).
36) Hisatomi S, Fukui S, Matsuoka M, *et al*. Design for separating performance of grinding media in bead mill by DEM simulation. *J. Soc. Powder Technol. Japan*., **54**, 377–383 (2017).
37) Yamamoto Y, Soda R, Kano J, *et al*. DEM simulation of bead motion during wet bead milling using an enlarged particle model. *Int. J. Miner. Process.*, **114–117**, 93–99 (2012).
38) Peltonen L. Design space and QbD approach for production of drug nanocrystals by wet media milling techniques. *Pharmaceutics*, **10**, 104 (2018).
39) Bruno JA, Doty BD, Gustow E, *et al.* inventor; Nano Systems L.L.C., assignee. Method of grinding pharmaceutical substances. United States patent US 5,518,187. 1996 May 21.
40) Hagedorn M, Liebich L, Bögershausen A, Massing U, Hoffmann S, Mende S, Rischer M. Rapid development of API nano-formulations from screening to production combining dual centrifugation and wet agitator bead milling. *Int. J. Pharm.*, **565**, 187–198 (2019).
41) Kwade A. Prediction of energy-effective grinding conditions. *Miner. Eng.*, **43–44**, 36–43 (2013).
42) Cao X, Morganti M, Hancock BC, Masterson VM. Correlating particle hardness with powder compaction performance. *J. Pharm. Sci.*, **99**, 4307–4316 (2010).
43) Shao L, Jiang D, Gong J. Nanoindentation characterization of the hardness of zirconia dental ceramics. *Adv. Eng. Mater.*, **15**, 704–707 (2013).