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Review

Overview of milling techniques for improving the solubility of poorly water-soluble drugs



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ABSTRACT

Milling involves the application of mechanical energy to physically break down coarse particles to finer ones and is regarded as a "top-down" approach in the production of fine particles. Fine drug particulates are especially desired in formulations designed for parenteral, respiratory and transdermal use. Most drugs after crystallization may have to be comminuted and this physical transformation is required to various extents, often to enhance processability or solubility especially for drugs with limited aqueous solubility. The mechanisms by which milling enhances drug dissolution and solubility include alterations in the size, specific surface area and shape of the drug particles as well as millinginduced amorphization and/or structural disordering of the drug crystal (mechanochemical activation). Technology advancements in milling now enable the production of drug micro- and nano-particles on a commercial scale with relative ease. This review will provide a background on milling followed by the introduction of common milling techniques employed for the micronization and nanonization of drugs. Salient information contained in the cited examples are further extracted and summarized for ease of reference by researchers keen on employing these techniques for drug solubility and bioavailability enhancement.

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1. Introduction

The process of drug dissolution is critical to the therapeutic efficacy of a medicinal product regardless of its route of administration. Dissolution involves the transfer of a solid drug into solution in the surrounding physiological fluid. The rate of dissolution of a drug is affected by factors embodied in the Noyes—Whitney equation [1]. The extent to which drug dissolution proceeds under prevailing physiological conditions is governed by its aqueous solubility. Drug solubility is defined as the amount of drug that passes into solution when an equilibrium is established between the drug solute in solution and any excess, un-dissolved drug to produce a

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saturated solution at a specified temperature [2]. The solubility and dissolution rate of a drug are often positively correlated. The bioavailability of a drug is defined as the rate and extent to which a dissolved drug is absorbed and becomes available at its target site of action [3]. The bioavailability of a drug is thus dependent not just on its dissolution and solubility characteristics, but also on its membrane permeability and associated absorption-related degradation.

Currently, the pharmaceutical industry faces considerable challenges associated with the increasing number of poorly water-soluble drugs coming through the drug discovery pipeline [4,5]. Despite promising pharmacological activity, many of these drug candidates fall under class II of the Biopharmaceutics Classification System (BCS), characterized by high membrane permeability but low aqueous solubility [6]. These drugs exhibit erratic or incomplete absorption often leading to unsatisfactory drug exposure in vivo and poor bioavailability. Biopharmaceuticals and biotechnologyderived therapeutic agents face similar challenges [7]. For BCS class II drugs, the dissolution step is the rate-determining factor in drug absorption. Pharmaceutical scientists are constantly seeking new approaches to facilitate and enhance the solubility and thus dissolution rate of BCS class II drugs. Current strategies employed to improve the apparent solubility of a drug include the use of: (i) co-solvents (e.g. low molecular weight polyethylene glycols and propylene glycol) in combination with water to dissolve the drug; (ii) complexing agents (e.g. cyclodextrins and its derivatives) to form watersoluble inclusion complexes of the drug [8] or (iii) hydrophilic excipients (e.g. polyvinylpyrrolidones and high molecular weight polyethylene glycols) as drug carriers for the preparation of solid dispersions in which the drug is dispersed

molecularly or as ultrafine crystals [9]. Alternatively, the drug molecule may be modified chemically by the syntheses of suitable pro-drugs [10] or salt forms of the drug that often exhibit greater aqueous solubility than the parent molecule. However, drug precipitation is a common threat faced by some of these formulations [11]. Precipitation may arise from excess drug coming out of solution when a previously supersaturated drug solution is diluted upon administration. For oral formulations, drug precipitation maybe triggered by the changing pH environment of the gastro-intestinal tract. To ensure that a drug stays in solution up till the point of absorption, lipids and oils have been employed as drug carriers. Lipid formulations of drugs basically comprise drugs dispersed, but more often, dissolved, in lipids or oils. These formulations improve drug bioavailability by exploiting the innate lipid digestion and absorption mechanisms in the body. Depending on the chemical nature of the lipid, the formulation may also selfemulsify in the gastro-intestinal tract to facilitate lipid digestion and maximize drug absorption. The advantage of lipid formulations is that the drug is maintained in a solubilized state prior to absorption. A comprehensive reference on oral lipid-based formulations can be found in the book edited by David J. Hauss [12]. The key advantages and disadvantages of the different strategies employed to improved drug dissolution and bioavailability are highlighted in Table 1.

Milling 2.

Apart from the techniques aforementioned, another strategy employed to improve solubility and ultimately, bioavailability of poorly water-soluble drugs is milling. The terms milling,

Table 1 — Key advan bioavailability.	tages and disadvantages of common strategies em	ployed to improve drug dissolution and
Technique	Advantages	Disadvantages
Use of co-solvents	Simple techniqueLower costs involvedApplicable for a wide range of drugs	 Toxicity of solvents Risk of drug precipitation in-vivo Limited to liquid formulations
Complexation using cyclodextrins	 Improves the chemical stability of the drug May potentially enhance drug absorption by modification of lipid barrier 	 Successful complexation depends on both chemical and geometrical properties of drug molecule Large amounts of cyclodextrins may be required due to low complexation efficiencies Higher costs involved
Solid dispersions	 Creates fine drug particles without excessive application of energy Fine particles are readily wetted with minimal risk of agglomeration Wide range of hydrophilic polymers are available as drug carriers 	 Preparation method is difficult to scale up Amorphous drug forms created are physically unstable and may convert to crystalline forms during storage, accelerated by moisture absorption by the hydrophilic carrier
Chemical modification (e.g. prodrugs)	 Prodrugs may enable drug targeting and improve drug stability 	 Toxicity potential of prodrugs Fate of prodrugs is difficult to predict <i>in-vivo</i> due to biological variations in the way they are handled in the body
Lipid formulations	 Exploits the innate lipid digestion mechanisms of the body to enhance drug bioavailability Emulsifiable lipid formulations further enhance lipid digestion and drug bioavailability Diversity of lipid excipients allow formulation flexibilities Lower risks of drug precipitation <i>in-vivo</i> 	 Amount of lipids typically present in the formulation may be insufficient to trigger an appropriate physio- logical response to enhance drug bioavailability Quality control of lipid-based formulations is challenging due to the complex and diverse physicochemical properties of lipids and the lack of standardized testing methods

Table 1 – Key advantages and disadvantages of common strategies employed to improve drug dissolution and
bioavailability.

size reduction, comminution, grinding and pulverization are often used interchangeably. Milling is a unit operation where mechanical energy is applied to physically break down coarse particles to finer ones and hence, is regarded as a "top–down" approach in the production of fine particles [13]. As virtually every drug can be comminuted to fine particles regardless of its solubility in aqueous or non-aqueous solvents, the "top– down" approach has wider commercial and industrial applications than the "bottom-up" approach (e.g. precipitation) where fine particles are constructed from their dissolved molecular state and suitable solvents/anti-solvents of the drug need to be selected.

Traditionally, milling is carried out to facilitate the extraction of crude drugs or to improve their bulk processing properties. Cutter mills, roller mills, pestle and mortars and runner mills may be employed for this purpose. In these milling operations, the dried crude drug may be cut by sharp blades (cutter mill), impacted by hammers or crushed/compressed by the application of pressure (roller mill, pestle and mortar). As a limited amount of energy is imparted, the milled particles remain relatively coarse. Technological advancements in milling equipment now enable the production of ultrafine drug particles down to the micron or even sub-micron dimensions. Griseofulvin, an anti-fungal drug, represents one of the pioneering examples of drugs where solubility and absorption were enhanced by milling. Milling of carbamazepine was found to be more effective in enhancing drug dissolution than formulating the drug as a solid dispersion due to polymorphic transformation of the drug (from the β to α form) in the solid dispersion system [14]. Other early examples of drugs where milling has resulted in enhanced dissolution include nitrofurantoin, nifedipine, ibuprofen and spironolactone [3].

Milled drug particles are rarely used as it is and are intermediates in the production of pharmaceutical dosage forms. Oftentimes, they are cohesive and exhibit poor flow properties, largely due to their higher surface energies compared to their coarser counterparts. To alleviate this problem, inert pharmaceutical excipients or fillers, e.g. calcium phosphate, lactose, mannitol and other sugars etc., are often added and mixed with the milled drug particles to improve powder flow. Alternatively, the drug particles may be granulated with these fillers to form granules which typically exhibit improved flow properties and content uniformities than the corresponding physical mixtures. Apart from improving flow during manufacturing, these fillers may also serve other functions e.g. modifying drug release, enhancing drug stability and dissolution as well as taste-masking.

3. Mechanisms by which milling improves drug dissolution and solubility

Milled products possess specific physical attributes that contribute to improved drug dissolution and solubility. Milling reduces the size and alters the size distribution of the drug particles. These properties may be measured by light scattering techniques such as photon correlation spectroscopy (5 μ m down to 0.001 μ m) and laser diffraction (0.05 μ m–2000 μ m), respectively [15]. By virtue of their smaller size, milled particles possess larger specific surface area compared to their unmilled

counterparts. Based on the Noyes—Whitney equation, this is likely to increase the dissolution rate of the milled drug particles if the particles can also be adequately wetted. Milled particles possess higher surface free energies and this, coupled with their thinner diffusion boundary layers [16] further enhance the dissolution rate of the milled drug substance.

Apart from size, milling also alters the surface roughness and shape of particles. This has been demonstrated in many studies on the development of inhalable dry powder formulations. It has been shown that the surface properties of milled particles can affect wetting [17] and dissolution behavior [18]. Furthermore, milled particles are rarely isometric or spherical in shape. Compared to particle size, considerably lesser attention has been devoted to the impact of particle shape [19-23] on drug dissolution, solubility and bioavailability although early studies have demonstrated that when particles are platelet-like or possess needle shapes, the shape factors of particles are closely related to their dissolution rates and profiles [24–28]. Particle shape may be determined by image analysis techniques, laser diffraction [29], scanning electron microscopy, transmission electron microscopy and atomic force microscopy.

In a milling operation, particle size reduction ceases at a practical limit [30] beyond which the material becomes progressively difficult to comminute even when milling time is prolonged. When particle size reduction has reached a critical threshold, the continued transfer of mechanical energy from the mill to the drug substance leads to the accumulation of defects on the drug crystal and disordering of the crystal structure, eventually bringing about the disappearance of the order in the positions of atoms or molecules in the crystal [31]. These defects may manifest throughout the entire crystal resulting in complete amorphization of the drug or be restricted to the crystal surfaces in which case a thin, amorphous (disordered) layer may be formed around a crystalline (ordered) core [32,33]. Under these circumstances, the drug is said to be "mechanochemically-transformed" or "activated" by the milling process. Drug amorphization as a result of milling improves the aqueous solubility and dissolution characteristics of the drug. It may also confer additional benefits such as improved compressibility [34]. Milling-induced amorphization of drug substances have been reported for piroxicam [35], budesonide [36], naproxen [37] and indomethacin [38] amongst many others. However, the disadvantage of these solid state transformations is that amorphous regions or crystal defects created may be thermodynamically unstable, leading to amorphous-crystalline inter-conversions of the drug during storage, alteration of particle size distribution, specific surface area, chemical and physical reactivity, dissolution or overall performance of the drug product [39].

An approximate measure of the extent of milling-induced drug activation may be obtained experimentally by determining the amorphous content or residual crystallinity before and after milling the drug substance using standard solidstate characterization tools such as x-ray powder diffraction (XRPD), Raman spectroscopy or differential scanning calorimetry. As the information obtained from these different techniques complement each other, a combination of techniques is often desired to fully elucidate the solid-state condition of the milled drug substance. The solubility of many drugs e.g. griseofulvin [40], chloramphenicol palmitate, indomethacin and phenylbutazone have been enhanced by mechanochemical activation. Comprehensive information on the mechanochemical activation of drugs can be obtained in the review by Boldyrev [31].

4. Milling adjuvants

The micro or nanoparticles produced from milling possess a large surface/interfacial area, increased free energy and decreased thermodynamic stability. These factors promote particle agglomeration. Mechanochemically-activated particle surfaces and amorphous regions generated during milling also increase the surface free energy of the particles, favoring agglomeration. In practice, it has been suggested that particle agglomeration arising from van der Waals' and other forces (e.g. electrostatic forces) become significant at particles sizes of about 30 µm and below [41]. Fine, hydrophobic drug particles less than 5 μ m in size are known to be exceptionally prone to agglomeration and this is attributed to the inter-particulate cohesive forces between them. Hence, when milling is prolonged, particle agglomeration may supersede particle fracture and this severely reduces the efficiency of the mill over time. Agglomeration occurring during or after milling reduces the effective surface area of the drug particles, with their resultant dissolution rate and bioavailability, being comparable or even less than their untreated counterparts. It was observed that continued milling of ketoconazole in a cryogenic impact mill led to apparent particle size growth by fine particle mechanofusion [42]. Aspirin, phenacetin and phenobarbital are known to be prone to the effects of aggregation during particle size reduction.

In most cases, drugs are co-milled together with certain adjuvants to minimize the conditions promoting agglomeration. These adjuvants are inert, non-toxic pharmaceutical excipients that function as a carrier and/or stabilizer of the drug in the milled product. There is considerable variation in the amount of excipient employed, with drug to excipient ratios ranging from 1:3 to 50:1 w/w being reported in the literature [43]. Typically, the excipient employed is hydrophilic in nature and notable examples are hydrophilic polymers such as polyvinylpyrrolidone, cellulose ethers, polyethylene glycol, polyvinyl alcohol or poloxamers; surfactants, ionic or non-ionic; inorganic materials like magnesium aluminometasilicate [44] and cyclodextrins. By conferring hydrophilicity to the hydrophobic drug particle surfaces, the added excipient also enhances the wettability, solubility and bioavailability of the poorly water-soluble drug.

The efficiency of a particular stabilizer depends on its potential for interaction with the drug compound. Generally, milling may be conducted with the drug in its dry state (dry milling) or suspended in a liquid medium (wet milling). In dry milling, the mechanical energy imparted fosters drug-excipient interactions via van der Waals forces or hydrogen bonding. The resultant drug-excipient composite particles are often stable, exhibit low tendencies to agglomerate and retain the activated status of the drug [31,34]. In wet milling, the addition of surfactants (e.g. sodium lauryl sulfate and polysorbate 80) and polymers (e.g. hydroxypropylmethyl cellulose, hydroxypropyl cellulose, polyvinylpyrrolidone and poloxamer), singly or in combination, helps to minimize the agglomeration of suspended particles via electrostatic and steric mechanisms. Steric stabilization is achieved when long chain polymers are adsorbed onto the surfaces of the drug particles, forming a physical barrier that prevents the close approach of the particles. The chain length, molecular weight [45], hydrophobicity [46,47], concentration, shape and surface energy [48] of the polymer will influence the efficiency of adsorption. The method of adding the polymer (periodic additions or addition at the start of milling process) also affected its stabilizing properties [49]. Electrostatic stabilization is achieved when charged polymers or ionic surfactants become Adsorbed on the surfaces of the drug particles and lower their apparent charge. Apart from preventing agglomeration, these stabilizing molecules may also aid in preventing crystal growth (Ostwald ripening) that could adversely alter the dissolution and bioavailability of the drug suspension after storage. Hydroxypropylmethyl cellulose (3 cps) was found to stabilize and minimize crystal growth in a nanosuspension of an unidentified drug compound NSV-102 (Novartis Pharma) produced by media milling, an example of wet milling. This was attributed to improved surface coverage owing to its stronger interaction with the drug in comparison with other stabilizers, pluronic F-68, pluronic F-127, sodium lauryl sulfate and polyvinylpyrrolidone K-30, investigated [50]. A screening study of polymers, copolymers, and surfactants has revealed that the stabilizing performance of surfactants to be the best followed by linear synthetic polymers and semisynthetic polymers for the 9 drug compounds (cinnarizine, griseofulvin, indomethacin, mebendazole, naproxen, phenylbutazone, phenytoin, itraconazole and loviride) investigated [51]. Common adjuvants employed in the dry and wet milling of drugs are summarized in Tables 2-4.

Achieving the desired size, shape and activation of drug particles in a milling process often requires extensive optimization of a multitude of process and material-related variables. In terms of processing, a prudent selection of the type of milling equipment is required, followed by the adjustment of the conditions of milling such as the duration of milling, material feed rate and other operational or equipment parameters. Occasionally, a combination of milling techniques may be necessary to achieve the desired outcomes. When such combination techniques are used, numerous processrelated variables need to be adjusted and fine-tuned as this allows the unique advantages of each milling technique to be synergistically combined for the desired outcome. Suitable and compatible adjuvants have to be selected to minimize agglomeration, improve wetting, stability and resultant solubility of the milled drug particles. The following sections will provide the background from literature on the common milling techniques employed for size reduction.

5. Milling techniques for the production of microparticles

5.1. Fluid energy milling

Fluid energy milling, sometimes referred to as air jet milling, effectively reduces the size of drug particles from the range of

Table 2 – Summary of examples obtained from scientific literature on the use of media milling for the production of drug nanoparticles. Drug Adjuvants Variables Mean particle size or particle size range attained Equipment Authors

			range attained		
Naproxen	Vit E TPGS, pluronic F127, SLS, DOSS, HPMC 3 cps	Different stabilizers	<500 nm	Planetary mill (Retsch PM 400 MA, Retsch, Haan, Germany)	[122]
Griseofulvin	HPC, SLS	Concentration of HPC	50-250 nm	Wet-stirred media mill (Microcer, Netzsch Fine Particle Technology, PA, US)	[123]
Unidentified compound NVS-102	Vit E TPGS	Concentration of Vitamin E TPGS	<300 nm	Planetary mill (PM400, Retsch, Newtown, PA, US)	[124]
Fenofibrate	HPMC, DOSS	Milling speed	<700 nm	Agitator ball mill (Dyno Mill Research Lab, WAB, Muttenz, Switzerland)	[125]
Ibuprofen (transdermal)	Pluronic F127, Vit E TPGS, PEG, PVP	Different stabilizers	123 ± 4 nm	Planetary mill (PM400, Retsch, Haan, Germany)	[83]
Paclitaxel (intraperitoneal)	Pluronic F68 and F127	Different stabilizers and stabilizer ratios	307 ± 12 nm	Roller-mill (Peira, Beerse, Belgium)	[126]
Compound A (oral)	Vit E TPGS, HPMC 3cps	Stabilizer level and drug loading	138.6 nm	Media mill (Netzsch Labstar Mill, Exton, PA, USA) with a zeta agitator in the recirculation mode	[127]
Griseofulvin, naproxen, fenofibrate (oral)	HPMC (E15LV), SLS	Effect of different drugs on film properties	163 nm (griseofulvin); 201 nm (fenofibrate); 144 nm (naproxen)	Wet-stirred media mill (Microcer, Netzsch Fine Particle Technology, LLC, PA, US)	[73]
Indomethacin (inhalation)	Poloxamer 188	-	485 nm	Planetary ball mill (Pulverisette 7 Premium, Fritsch, Germany)	[86]
Phenytoin (oral)	PVP, SLS	-	~300 nm	Oscillating beads-milling apparatus (Multi-Beads Shocker, Yasui Kikai, Osaka, Japan)	[84]
Itraconazole, fenofibrate, griseofulvin, ibuprofen, azodicarbonamide, sulfamethoxazole	HPMC (4000 –5600 cp), Tw80, SLS, sodium alginate	Different drugs and stabilizers	8.72 \pm 5.66 μ m (itraconazole); 3.37 \pm 2.48 μ m (fenofibrate); 2.65 \pm 1.12 μ m (griseofulvin) 3.30 \pm 2.18 μ m (ibuprofen) 0.67 \pm 0.52 μ m (azodicarbonamide) 0.63 \pm 0.56 μ m (sulfamethoxazole)	Wet milling machine (Micros- 0 Ring Mill, Nara Machinery, Tokyo, Japan)	[49]
Unidentified compound NVS 102 (oral)	Vit E TPGS, pluronic F68 and F127, HPMC 3cps, SLS, PVP K-30	Effect of different stabilizing agents on crystal growth	230.2 ± 0.257 nm	Media mill (Netzsch Labstar Mill, Exton, PA, US) with a zeta agitator in the recirculation mode	[50]
Phenytoin, nifedipine, pranlukast (oral)	PVP, HPC, HPMC, PVA, SLS, Tw80	Different scales of preparation	292 nm (phenytoin) 353 nm (nifedipine) 334 nm (pranlukast)	Oscillating beads-milling apparatus (Multi-Beads Shocker, Yasui Kikai, Osaka, Japan)	[128]
Miconazole (ophthalmic)	SLS, sodium docusate, benzalkonium chloride, HPC-LF, HPC-EF, PVP, poloxamer, HPMC	Formulation variables	140 ± 1 nm	Media mill (Netzsch Labstar Mill Selb, Germany)	[129]
Candesartan cilexetil (oral)	SLS, poloxamer 188, PVP K-30, HPMC 6 cps	Drug and stabilizer concentrations	127 nm	Heidolph mixer (RZR2051Control, Rose Scientific, Alberta, Canada)	[85]
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Table 2 – (continued)						
Drug	Adjuvants	Variables	Mean particle size or particle size range attained	Equipment	Authors	
Cilostazol (oral)	HPC, DOSS	Different milling processes	260 nm	Agitator ball mill (Dyno-Mill KDL Type-A, Willy A. Bachofen AG Maschinenfabrik, Basel, Switzerland)	[130]	
Zn-insulin (subcutaneous and intraduodenal)	Sodium deoxycholate, pluronic F68	Different stabilizers	<150 nm	Low energy wet media mill (Stoneware, East Palestine, OH, US)	[131]	
Ethyl diatrizoate (radio- contrast medium)	Poloxamine 908	Different formulation variables	230 ± 10 nm	Roller mill (Stoneware, East Palestine, OH, US)	[132]	
Abbreviations: DOSS, dioctyl sulfosuccinate sodium; HPC, hydroxypropylcellulose; HPMC, hydroxypropylmethyl cellulose; PEG, polyethylene						

Abbreviations: DOSS, dioctyl sulfosuccinate sodium; HPG, hydroxypropylcellulose; HPMC, hydroxypropylmethyl cellulose; PEG, polyethylene glycol; PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; SLS, sodium lauryl sulfate; TPGS, d-alpha tocopheryl polyethylene glycol 1000 succinate; Tw80, Tween 80 (polysorbate 80); Vit, vitamin.

20–100 μ m to less than 10 μ m. In this micronization method, high velocity compressed air streams are injected into a chamber where the starting raw materials are fed by a ratecontrolled feeder (Fig. 1). As the particles enter the air stream, they are accelerated and caused to collide with each other and the wall of the milling chamber with high velocities. Particle size reduction is brought about by a combination of impact and attrition. Impacts arise from collisions between the rapidly moving particles and particles onto the wall of the milling chamber. Attrition occurs at surfaces of particles as they move rapidly against each other, resulting in shear forces that may break them up. A classifier may be integrated into the milling system such that only particles that are sufficiently fine or have acquired dimensions below the predefined cut-off size are entrained in the exhausting air stream and removed from the milling chamber. Classification may be effected by a spinning wheel classifier where the centrifugal force generated by the high spinning speed of the fluted wheel limits the cut-off size of particles that can accompany the exhausting air through the wheel to the air exhaust outlet. Alternatively, a large tubular shaped milling chamber, circular or oblong, may be used, with colliding air jets at the periphery and the exhaust air leaving centrally, causing a centrifugal classification system for the milled particles. Longer durations of milling are required when finer particles are desired. This process is suitable for meltable materials [52] and drugs that are heat-sensitive. It is also capable of manufacturing large quantities of powder continuously.

Fluid energy milling has been successfully employed for the micronization of many drugs for the purpose of improving their dissolution and solubility characteristics. Some examples include ibuprofen [53], salbutamol sulfate [54] and fenoterol hydrobromide [55]. Drugs are commonly milled on their own although occasionally, co-milling with suitable excipients is carried out. It was reported that fluid energy milling of a blend of fenofibrate, a poorly water-soluble drug, together with a mixture of hydrophilic excipients resulted in faster drug dissolution rates from a rapidly disintegrating dosage form compared to a powder formulation of identical composition prepared by mixing pre-milled fenofibrate with the excipient mixture [56]. This was attributed to the persistence of aggregates of pure, jet-milled fenofibrate which retarded drug dissolution. In an attempt to improve the bioavailability of EMD 57033, a poorly water-soluble calcium sensitizing agent, Vogt et al. [57] found that co-grinding a mixture of EMD 57033 with lactose and hydroxypropylmethyl cellulose using a fluid energy mill was more effective than micronizing the drug alone or spray drying a nanosuspension of the drug. Fluid energy milling of ibuprofen together with nanosilica was carried out by Han and co-workers [58]. It was found that fluid energy milling not only decreased the size of drug particles from 102 to <10 μ m but also facilitated the coating of nanosilica on the surfaces of the milled drug particles. This surface modification brought about by milling reduced particle agglomeration and improved powder flow.

However, the popularity of fluid energy milling has somewhat dipped following the development of other milling techniques capable of effecting greater extents of size reduction, enabling the production of sub-micron or nanoparticles at commercial scale. The lowest mean particle diameters achievable by fluid energy milling is 3-5 µm with size distribution ranging from a few hundred nanometers to about 25 µm and a very low fractional content of nanoparticles [59]. Nonetheless, this milling technique remains as a benchmark for the evaluation and development of new milling methods and strategies. In the context of drug solubility enhancement, fluid energy milling may be employed in combination with other particle design techniques (e.g. "bottom-up" approaches such as precipitation, crystallization) to produce drug microparticles with desirable morphological characteristics. Fluid energy milling of ibuprofen was investigated and found generally hard to mill in its dry state due to its ductility and low melting point [53]. In the study, ibuprofen crystals of different sizes (<40 µm or 50-250 µm) and morphologies (plate-like and needle-like crystals) were first produced by controlled crystallization. It was reported that the drug could be milled down to less than 5 μ m, which is below the reported particle size for brittle-ductile transition of the drug. Furthermore, it was observed that the size and morphology of the starting drug crystals influenced the milling outcome. Compared to plate-like crystals, needle-like crystals were more susceptible to micronization. Milled

Table 3 – Summary of examples obtained from scientific literature on the use of high pressure homogenization for the production of drug nanoparticles.

Drug	Adjuvants	Operating parameters	Minimum/avg particle size	Equipment	Ref
Telmisartan (oral)	β-cyclodextrin, meglumine	15 cycles at 1200 bar	698 ± 23 nm	High pressure homogenizer (Niro Soavi, Italy)	[133]
Nitrendipine (oral)	PVA	20 cycles at 1000 bar	175 nm	High pressure homogenizer (AH100D, ATS Engineering, Shanghai, China)	[99]
Ketoprofen	PLGA, CO_2 , PVA, ethyl acetate	Pressure drop of 900 bar & up to 150 passes	1.34 μm	High pressure piston pump (Haskel, Burbank, CA, US)	[97]
Quercetin (topical)	Tristearin, hydrogenated phosphatidylcholine	1 or 2 cycles at 1000 bar	486 nm	Hot and cold high pressure homogenizer (Panda 2K GEA; Niro Soavi, Parma, Italy)	[134]
Unidentified compound PIK75 (intravenous)	Pluronic F68, lipoid S75	15 cycles at 7250 psi, 5 cycles at 15 000 psi and 15 cycles at 18,000 psi at $2-4$ °C.	187.6 ± 19.11 nm	High pressure homogenizer (Emulsiflex-C3, Avestin, Canada)	[135]
Curcumin	PVA, PVP, Vit E TPGS, SLS, Na CMC	2 cycles each at 300, 500 & 1000 bar followed by 20 cycles at 1500 bar	500-700 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna, Germany)	[136]
Fish oil (intravenous)	Lecithin and glycerol	5 cycles at 500 –1500 bar	150 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna. Germany)	[137]
Tanshinone IIA (intravenous)	Soybean lecithin, poloxamer 188, glycerol, soybean oil, medium-chain triglyceride	3 cycles at 100 MPa	251.7 ± 16.6 nm	High pressure homogenizer (Avestin, Ottawa, Canada)	[138]
Itraconazole (oral)	Chitosan, N-trimethyl chitosan, polyethyleneimine	2 cycles each at 150, 500, 1000 bar followed by 15 cycles, 1350 bar	267.6 ± 15.8 nm	High pressure homogenizer (AH100D, ATS Engineering, Shanghai, China)	[139]
Emodin (oral)	Poloxamer 188, Tw80, compritol 888 ATO, GMS, stearic acid, lauric acid	500, 700, 900 bar with 3, 4 and 5 cycles	28.6 ± 3.1 nm	High pressure homogenizer (NS1001L, Niro Soavi, Italy)	[140]
Beclomethasone dipropionate (inhalation)	-	2 cycles each at 150 & 300 bar followed by 500 bar for different homogenization cycles	247 nm	High pressure homogenizer (AH110D, ATS Engineering, Italy)	[141]
Baicalein (oral and parenteral)	Tw80, SLS, poloxamer 188	5 cycles at 200 bar followed by 5–35 cycles at 1000 bar	287 ± 5 nm	High pressure homogenizer (NS 1001L, Niro Soavi, Italy)	[142]
Recombinant human epidermal growth factor (topical)	Hydrogenated phospholipid, triglycerides, diethylamine cetylphosphate, butylated hydroxyl toluene in ethanol, Na ascorbyl phosphate, EDTA	2 cycles at 1000 bar	155.57 ± 2.59 nm	High pressure homogenizer (Emulsiflex-C3, Avestin, Canada)	[143]
Atorvastatin (oral)	Chitosan	1–3 cycles at 20,000 –40.000 psi	214.8 ± 15.8 nm	High pressure homogenizer (Nano DeBEE, BEE International, MA, US)	[144]
Pranlukast (oral)	Poloxamer 407, PEG 200	680 bar – 15 circles, 1048 bar – next 9 circles & 1500 bar – last 9 circles	315.4 ± 4.2 nm	High speed homogenizer (Ultra- Turrax T-18 Basic, IKA-Werk, Staufen, Germany)	[145]
Quercetin	Tw80	2 cycles each at 300 & 500 bar, 1 cycle at 1000 bar followed by 20 cycles at 1500 bar	338.3 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna, Germany)	[146]

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Table 3 – (continued)					
Drug	Adjuvants	Operating parameters	Minimum/avg particle size	Equipment	Ref
Fenofibrate (oral)	Soybean protein isolate, whey protein isolate, egg phosphatidylcholine, β-lactoglobulin, cremophor EL and RH 40, poloxamer 188, solutol HS15. Tw80	1 to 10 cycles at100 to 800 bar	200 to 250 nm	High pressure homogenizer (ATS Engineering, Ontario, Canada)	[147]
Astaxanthin (topical)	Tween 60, glyceryl citrate/lactate/ linoleate/oleate, liquid paraffin, ceramide, co- anti oxidants, cholesterol	1000 bar	160–190 nm	High pressure homogenizer (Panda, Niro Soavi, Italy)	[148]
Flurbiprofen (ocular)	Glycerol behenate, saturated fatty acid of C18, Tw80	3 homogenization cycles at 600 & 60 bar in first & second stage, respectively, at 85 °C	<199 nm	High pressure homogenizer (APV- 2000, Invensys, Albertslund, Denmark)	[149]
Puerarin	Lecithin and HPMC	6 cycles at 800 bar followed by 15 cycles at 1500 bar	448.9 ± 15.1 nm	High pressure homogenizer (Emulsiflex-C3 Avestin Inc., Ottawa, Canada)	[150]
Camptothecin	-	2 cycles each at 200 & 400 bar followed by 20 cycles at 1300 bar	125.1 ± 8.6 nm	High pressure homogenizer (NS1001L-Panda 2K, Niro Soavi, Italy)	[151]
Co-enzyme Q10 (oral)	Sucrose monolaurate, mixture of mono, di & triglycerides, medium- chain triglycerides	20 homogenization cycles at 800 bar	282 ± 15 nm (nanocrystals); 143 ± 10 nm (lipid nanoparticles)	Melt high pressure homogenizer (ATS Engineer, China)	[152]
Quercetin	Tw80	2 cycles each at 300 & 500 bar, 1 cycle at 1000 bar & finally, 20 cycles at 1500 bar	338 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna, Germany)	[153]
Lutein (oral and dermal)	Decyl glycoside	2 cycles each at 200, 500 & 1000 bar followed by 25 cycles at 1500 bar	429 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna, Germany)	[154]
Alkylpolyglycoside C8- 10 (dermal)	Neutral oil, glycerol distearate,	3 cycles at 750 bar	150.40 ± 3.79 nm	High pressure homogenizer (EmulsiFlex-C3, Avestin, Germany)	[155]
Piroxicam (oral)	Poloxamer 188	3 cycles at 500 bar and 30 cycles at 1500 bar	414.3 ± 21.1 nm	High pressure homogenizer (EmulsiFlex-C5 Avestin, Ottawa, Canada)	[156]
Glibenclamide (oral)	_	2 cycles at 500 bar followed by 20 cycles at 1500 bar	335 nm	High pressure homogenizer (Micron LAB 40, APV Deutschland, Unna, Germany)	[101]
Itraconazole (inhalation)	Sodium taurocholate, polyethylene 1000 succinate, poloxamer 407, Vit E	300 cycles at 20 000 psi	221 ± 10 nm	High pressure homogenizer (Emulsiflex-C5 Avestin, Ottawa, Canada)	[157]

Abbreviations: CMC, carboxymethyl cellumose; EDTA, ethylenediamine tetraacetic acid; GMS, glycerol monostearate; HPMC, hydroxypropylmethyl cellulose; PEG, polyethylene glycol; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; PVP, polyvinylpyrrolidone; Vit, vitamin; SLS, sodium lauryl sulfate; Tw80, Tween 80 (polysorbate 80).

particles produced from smaller-sized starting materials also exhibited smaller volume mean diameters compared to their coarser counterparts for both morphologies of ibuprofen particles. The authors explained that with proper control of the crystal attributes of the starting material, fluid energy milling might be equally, if not more effective than wet milling in reducing the particle size of drugs. Despite it being a micronization technique, fluid energy milling also plays a significant role in the development of nanoparticulate drug delivery systems. Drug microparticles produced from fluid-energy milling may be subjected to a subsequent milling process to attain particles in nanoscale dimensions. In a study, horseradish peroxidase enzyme as a model drug was loaded in a suitable polymer matrix and pre-micronized using a fluid energy mill [60]. The protein-polymer microparticles were then subjected to a nanonization process (high pressure homogenization) to produce stable, protein-loaded nanoparticles with controlled drug release properties.

nanoparticles.					
Drug	Adjuvants	Variables	Observations	Equipment	Ref.
Phenytoin	PVP	_	Novel ultra cryomilling micronization technique using dry ice beads and liquid nitrogen	Wet milling machine (RMB- 04, Aimex, Tokyo, Japan)	[102]
Phenytoin	PVP, Eudragit L100, HPMC, HPMC acetate-succinate, MCC, HPC, CMC	-	Enhancement of dissolution rate with phenytoin nanoparticles	Wet milling machine (RMB- 04, Aimex, Tokyo, Japan)	[105]
Indomethacin	-	Milling time	Dissolution rate depended on the milling time	Oscillatory ball mill (Mixer Mill MM301, Retsch, Haan, Germany)	[38]
Griseofulvin	_	Milling time	Continued mode of attrition with milling time	Cryogenic impact mill (Spex CertiPrep 6750 Metuchen,	[42]
Ketoconazole			Continued milling caused apparent particle growth	NJ, US)	
Furosemide	PVP, inulin	Milling time	Solid state amorphization and chemical decomposition	Cryogenic impact mill (Spex CertiPrep 6750 Metuchen, NJ, US)	[113]
γ-Indomethacin	_	-	Atomic pair-wise distribution functions was better than x-ray diffractograms in assessing the degree of disorder	Oscillatory ball mill (Mixer Mill MM301, Retsch, Haan, Germany)	[158]
Indomethacin	-	-	Correlation between physical stability and relaxation time was established for the same amorphous systems prepared by different methods	Oscillatory ball mill (Mixer Mill MM301, Retsch, Haan, Germany)	[110]
Phenytoin, ibuprofen, salbutamol sulfate	-	_	No change in crystal form and amorphization after milling	Wet milling machine (RMB- 04, Aimex, Tokyo, Japan)	[114]
Glibenclamide	-	_	Transformation from crystalline to amorphous state without chemical decomposition	Cryogenic freezer/mill (Spex SamplePrep 6770, Metuchen, NJ, US)	[111]
Griseofulvin	-	Milling time	Reduction of crystallinity due to crystal defects rather than amorphization of materials	Cryogenic impact mill (Spex CertiPrep 6750 Metuchen, NJ, US)	[115]
Ranitidine hydrochloride	-	Milling time	Ranitidine hydrochloride polymorphs form 1 and 2 could be fully converted to the amorphous form	Oscillatory ball mill (Mixer Mill MM301, Retsch, Haan, Germany)	[112]
Carbamazepine	Saccharin	-	Higher amorphization with cryogenic cogrinding than room temperature cogrinding	Cryogenic impact mill (Spex CertiPrep 6750 Metuchen, NJ, US)	[106]
Whole inactivated influenza virus	Chitosan, lactose, trehalose	_	Dry powder influenza vaccine successfully formulated	Micro-ball mill (SPEX CertiPrep 3117, Metuchen, NJ, US)	[159]
Indomethacin polymorphs	-	-	Amorphous materials obtained	Cryogenic impact mill (Spex	[109]

Table 4 – Summary of examples obtained from scientific literature on the use of cryomilling for the production of drug nanoparticles.

Abbreviations: CMC, carboxymethyl cellulose; HPC, hydroxypropyl cellulose; HPMC, hydroxypropylmethyl cellulose; MCC, microcrystalline cellulose; PVP, polyvinylpyrrolidone.

physical stability

after milling possessed similar T_g

but significant differences in their

5.2. Ball milling

and solvates

Ball milling is another popular size reduction technique used for the production of microparticles, especially in research laboratories. Fundamentally, a ball mill comprises a vessel or vial filled with balls, or rods, constructed from a variety of materials such as ceramic, agate, silicon nitride, sintered corundum, zirconia, chrome steel, Cr–Ni steel, tungsten carbide or plastic polyamide (Fig. 2). The material to be milled is placed inside the vessel, which is made to rotate or vibrate at a particular speed or frequency. The movement of the vessel causes the balls to cascade or move in a particular pattern, colliding with each other and with the opposing inner wall of the vessel. Size reduction of the drug particles is effected from the impact they receive from the balls as well as attritive forces arising from the movement of the balls relative to each other [61,62].

NJ, US)

CertiPrep 6750 Metuchen,

The quantities of the balls and starting material determine the extent of fill of the vessel and the intensity of the milling process. Typically, the vessel is filled by the balls and starting material to 50% and 25% of the total volume of the vessel,



Fig. 1 – Schematic diagram of a fluid energy mill. Air is introduced through specially-designed fluid inlets creating sonic or supersonic air streams. Raw materials are introduced into the violent and turbulent air stream. High velocity collisions between the raw particles lead to effective pulverization of the feed into smaller particles.

respectively, although variations exist in the literature. In the case of a rotating vessel, rotation is usually carried out at 50–85 % of the critical speed, defined as the speed at which the balls cease to cascade owing to the centrifugal force imparted by the rotating vessel. The critical speed may be estimated based on the following relationship [63]:

Critical speed (rpm) = $54/\sqrt{R_{ft}}$

where $R_{\rm ft}$ is the diameter of the vessel measured in feet. As the rotational speed of the vessel decreases, attrition plays a

more dominant role in particle size reduction relative to impaction and compression, yielding finer particles at the expense of longer processing times. Apart from the speed of rotation or vibration, the size, density and hardness of the balls affect the rate and extent of particle size reduction. Decreasing the size and/or increasing both the density and hardness of the balls often increase the rate and extent of particle size reduction.

The use of ball milling as a micronization technique for enhancing drug solubility is well supported by literature dating as far back as the 1970s. Apart from its comminution function, ball milling also serves as an intensive mixing technique capable of producing co-ground drug-excipient mixtures comprising amorphous drug forms intimately mixed with suitable hydrophilic excipients at the molecular level. This interaction between the drug and hydrophilic excipient enhances the wetting and dissolution of the drug. Studies on the vibrational ball milling of griseofulvin and phenytoin have demonstrated that milling these drugs in combination with microcrystalline cellulose resulted in amorphization of the drug, enhancing its dissolution and bioavailability [64,65]. Ball milling of ibuprofen and aluminum hydroxide was carried out to facilitate complex formation between ibuprofen and aluminum hydroxide as well as drug amorphization which enhanced the dissolution of ibuprofen [66]. The incorporation of excipients may, in some cases, mitigate milling-induced drug amorphization. It was demonstrated that the co-milling of salbutamol sulfate with crystalline excipients (a-lactose monohydrate, adipic acid, magnesium stearate) in a ball mill was effective in reducing milling-induced amorphization or structural disorder of salbutamol sulfate [67]. Ball milling of pure drug mixtures have also been investigated. More recently, ball milling of a combination of two BCS Class II drugs, simvastatin and glipizide, resulted in the formation of stable co-amorphous mixtures [68].

Despite the efficiency of ball milling for size reduction or amorphization, it is less amendable to scale up. By a flowthrough method, with vibrating balls or discs in the milling chamber, milling efficiency can be improved. External jacketing for heat removal allows the mill to be used continuously and will limit the rise in temperature within the milling chamber.



Fig. 2 – Schematic diagram of a ball mill. The balls make up the grinding media and drive rollers help to rotate the milling chamber.

6. Milling techniques for the production of nanoparticles

6.1. Wet milling

Both fluid energy and ball milling techniques involve size reduction of drug particles in their dry state. The extent of size reduction achievable in these dry milling techniques is limited to a few micrometers [69]. It was reported that when the solubility of a drug is very low, down-sizing it to the micrometer range is insufficient to increase its dissolution rate and gastrointestinal absorption [70]. In the last decade, significant advancements and evolutions in milling processes have enabled the production of submicron-sized (<1 µm) or nanoparticles [43]. This process may be termed as nanonization. Nanoparticles, sometimes termed as nanocrystals, are typically 200-500 nm in size [71], and are particularly suited for the formulation of parenteral preparations. Nanoparticles possess significant advantages over microparticles in enhancing drug solubility. Most notably, the process of nanonization increases not only the surface area and dissolution rate of the drug particles, but also the saturation solubility of the drug. Ordinarily, the saturation solubility of a drug is dependent on the temperature and solvent used for dissolution. When the size of drug particles falls below 1 μ m, dissolution pressure increases due to the strong curvature of the particle surface. This leads to an increase in saturation solubility in accordance to the Ostwald-Freundlich and Kelvin equations. Hence, at the nanoparticulate level, the saturation solubility of a drug becomes a function of particle size. The increase in saturation solubility of the drug will increase the concentration gradient for drug diffusion and promote drug absorption. Additionally, it was reported that orally-delivered nanoparticles displayed strong adhesive properties to the mucosal surfaces of the gastro-intestinal tract, arising from the increased van der Waals forces of attraction between the nanoparticles and the gut wall. This would further contribute to the increased absorption and bioavailability of drugs administered as nanoparticles.

Drug nanoparticles are most commonly produced by wet milling. As the name suggests, wet milling involves size reduction of drug particles suspended in a liquid medium that may be aqueous or non-aqueous in nature. Wet milling is particularly suited for potent drugs and drugs which possess high residual moisture contents (>50% moisture) because dry milling may be problematic for drugs of this nature. In wet milling, a drug nanosuspension is produced as the end product although for improved product stability (minimization of Ostwald ripening and possible hydrolytic degradation of drug), patient convenience and the drive towards green or sustainable manufacturing processes, the nanosuspension may subsequently be transformed into a solid dosage form (e.g. tablets and capsules) by granulation, freeze drying and spray drying [43] using suitable excipients or "matrix formers" like mannitol or lactose. The dissolution rate of ezetimibe, a lipid-lowering compound, was improved even after nanocrystals of the drug were tableted without the inclusion of any solubilizing agents like sodium lauryl sulfate [72]. Drug nanoparticles may also be incorporated into polymer films

[73] or layered on sugar beads [74]. It has been demonstrated in many studies that these "solidification" processes retain the mechanically-activated status and other desirable physical attributes of the original, milled drug particles.

Two common "top-down" approaches for the production of drug nanoparticles are media milling and high pressure (piston-gap) homogenization. These two milling techniques have been the focal point of research in the past decade due to their ease of scale-up, robust processing, economic advantages and acceptance by regulatory authorities. Since 2000, the US Food and Drug Administration has approved numerous products e.g. Rapamune[®] (sirolimus, Wyeth), TriCor[®] (fenofibrate, Abbott) and Megace[®] ES (megestrol acetate, Par Pharmaceuticals) that have been produced by these techniques [75]. Tables 1 and 2 summarize the current research on the application of media milling and high pressure homogenization in particle size reduction and solubility enhancement of specific drugs.

6.2. Media milling

Media milling can be considered a modernized version of the ball mill (Fig. 3). This technology, first developed by Liversidge and co-workers [76,77], is a classical wet milling technique wherein a sufficiently concentrated dispersion of drug particles in an aqueous or non-aqueous liquid medium is subjected to a traditional ball milling operation [77,78]. The liquid medium prevents adhesion and subsequent compaction of the milled drug particles on the wall of the vessel and/or the surfaces of the milling balls, which is a common occurrence when the drug is milled in its dry state. This improves the yield of nanoparticles. The liquid may also serve additional purposes such as lubrication and coating of the newly-formed particle surfaces through various physicochemical interactions like electrostatic and hydrophobic interactions [79,80].

In media milling, mechanical attrition and impaction of the suspended drug particles are brought about by grinding balls, often termed as the milling media, constructed out of a variety of material such as glass (yttrium-stabilized), zirconium oxide, ceramics or highly cross-linked polystyrene resins [78]. Pearl balls and beads are commonly used as well in which case the techniques are termed pearl and bead milling, respectively. Unlike ball milling where the whole vessel rotates or oscillate/vibrates whilst in operation, the vessel remains stationary in media milling. Movement of the balls is initiated by a stirring or agitating device, often represented by several discs mounted on a central shaft rotating at high velocities, 20 000 rpm and above, within the vessel. For this reason, media milling is sometimes known as "stirred-ball milling". Media milling is a continuous process wherein the drug suspension is pumped through the milling chamber to effect size reduction of the suspended material. Prior to their exit from the milling chamber, the milled particles pass through a screen that serves to separate the suspended, milled particles from the milling media. Media milling has been employed for particle size reduction of loviride [81], ezetimibe [72], alpha-lipoic acid [82], ibuprofen [83], cinnarizine, naproxen [74], ketoconazole, phenytoin [84] and candesartan cilexetil [85]. The majority of these cited studies involve the



Fig. 3 – Schematic representation of the media milling process. The milling chamber is charged with milling media. A crude slurry consisting of drug, water and stabilizer is fed into the milling chamber and processed into a nanocrystalline dispersion.

conversion of the resultant drug nanosuspension into a suitable solid dosage form like dry powders and tablets. Less conventionally, stable nanoparticles of naproxen, fenofibrate and griseofulvin produced from wet-stirred media milling have also been incorporated into hydroxypropylmethyl cellulose polymer films [73]. The dissolution rates of the drugs were improved by nanonization and based on x-ray diffraction and Raman spectroscopic analysis, the process of film formation did not affect drug crystallinity. Another interesting example is the incorporation of crystalline nanoparticles of indomethacin, prepared by media milling, into coated mannitol microparticles using an aerosol flow reactor method. The nanostructured microparticles produced exhibited rapid dissolution properties [86].

In recent years, there is a trend towards the use of milling media of much smaller dimensions (<100 μ m) to bring about the nanonization of drugs. As it is difficult to separate milling media of this size from the milled products using the conventional screen separator without choking or plugging the screen, centrifugal technology has been employed to effect such separations. The Ultra Apex Mill (Kotobuki Industries) is an example where centrifugal technology has been integrated into the design of the mill to effectively separate the milling media, which can range from 15 to 100 μ m, from the milled product. This mill has been successfully employed for the production of nanoparticles of albendazole, danazol and omeprazole as well as probucol [87] for the enhancement of their dissolution and absorption properties.

A major drawback of media milling is the erosion of the balls arising from the intensive mixing forces in the vessel. Residues of the milling media produced from erosion may result in product contamination [71,88,89], leading to chemical destabilization of the newly-formed particle surfaces and possibly affecting critical product attributes such as particle size and size distribution. Monitoring impurity levels in the final milled product is thus warranted. Contamination problems may be mitigated by a prudent choice of the materials such as erosion-resistant polymers and ceramics, used in the construct of the milling media as well as other key equipment components (e.g. inner walls of milling chamber and stirring device) in contact with the milled product. Optimization of key process parameters like stirring speed and in particular, milling time also contributes to reducing the likelihood of erosion. This is because milling durations of up to several days are not uncommon in media milling [77] and such long milling durations are likely to promote erosion of the milling media. Commercially, media milling is exemplified by the Nano-Crystal[®] technology from Elan Pharmaceutical Technologies. To date, NanoCrystal[®]-based products have been approved by worldwide regulatory agencies including the US, Canada, EU and Japan.

6.3. High pressure homogenization

The production of nanosuspensions using high pressure or piston-gap homogenization was first developed by Muller et al. in 1994 [90]. It is a high energy process in which size reduction of drug particles is achieved by repeatedly cycling, to 200 plus cycles, with the aid of a piston, a drug suspension through a very thin gap at high velocity, around 500 m/s, and pressure, 1000–1500 bars (Fig. 4). The width of the gap, which generally falls within the range of 5–20 μ m, may be adjusted according to the viscosity of the suspension and the applied pressure. Pre-micronization of the starting materials using a process like fluid-energy milling may be necessary prior to homogenization. This is to minimize clogging of the homogenization gap and to reduce milling time. When the suspension is forced through the gap at a high flow rate, the static pressure exerted on the liquid falls below the vapor pressure of the liquid at the prevailing temperature (Bernoulli's equation). As a result, the liquid boils and gas bubbles are formed which collapse when the liquid exits from the gap and normal pressure is resumed. The powerful cavitation forces arising from the formation and collapse of the gas bubbles, coupled



Fig. 4 – Schematic diagram of the high pressure homogenization process. The microparticles are forced through a minute gap in the micronizing zone. This creates conditions of high turbulence and shear, combined with compression, acceleration, pressure drop and impact, leading to the formation of a nanosuspension.

with a shearing effect, bring about nanonization of the drug microparticles [91-93]. The extent of subdivision of the nanoparticles depends on the pressure applied as well as the number of passes or homogenization cycles the drug suspension is subjected to during the process. The mechanical properties (hardness) of the drug particles also affect the milling outcome, with mechanically softer drug particles achieving a lower (200-300 nm) nanometer size range eventually [7]. High pressure homogenization is a high energy process. In the course of milling, the drug particles are exposed to a power density of up to 1013 W/m³ which is comparable to power densities observed in nuclear power stations. The high energy imparted mechanically activates the drug particles potentially leading to partial or complete amorphization of the drug [94]. High pressure homogenizers are available in different capacities, ranging from few tens of milliliters for laboratory use to a few thousand liters for large scale production. The process is robust and may easily be adapted for aseptic production of parenteral drug nanosuspensions. High pressure homogenization is employed for the production of parenteral nutrition emulsions (e.g. Lipofundin[®] and Intralipid[®]). High pressure homogenization also enables continuous processing and unlike media milling, contamination problem from milling equipment is much less. In the last decade or so, extensive research has been carried out on the application of high pressure homogenization for particle size reduction and solubility enhancement of drugs. Table 2 summarizes the research reports in the area over the recent years. Commercially, high pressure homogenization is exemplified by the Dissocubes™ technology which has been owned by SkyePharma PLC since 1999. Nanosuspensions prepared using Dissocubes[™] are water-based and are characterized by well-defined particle sizes and narrow particle size distributions, with few particles exceeding 5 µm in size [95]. Many different drugs have been processed by the

 $Dissocubes^{TM}$ technology resulting in bioavailability enhancement.

In 1999, a variant of the high pressure homogenization process, known as the Nanopure[®] (pure nanocrystals) technology, was introduced. This technology is owned and developed by Pharmasol GmbH, Berlin. Homogenization in this case is conducted in a non-aqueous dispersion medium, polyethylene glycols, propylene glycol, water-alcoholic mixtures or suitable oils [96], rendering the process suitable for comminuting drugs susceptible to hydrolysis. This technique is also suitable for drugs that are thermolabile. This stems from the option of conducting homogenization under milder conditions such as reduced temperature [97] and in the event where the nanosuspension has to be converted into a solid form, less heat will be required for solvent removal (compared to homogenization using water) thereby preserving drug stability. Supercritical CO₂ has also been employed as a dispersion medium. In two recent studies, high pressure homogenization of phenytoin [98] and ketoprofen [97] dispersed in supercritical CO₂ was carried out. The use of supercritical CO₂ enhanced the efficiency of homogenization as the liquid medium could be easily evaporated under ambient conditions, allowing the milled product to be readily recovered in the form of a dry powder.

High pressure homogenization has also been combined with precipitation and this is marketed as the Nanoedge™ technology. In this technique, the drug is first dissolved in a water-miscible alcoholic solvent (e.g. methanol, ethanol or isopropanol), then added to water to cause it to precipitate. The precipitated particles are subsequently homogenized. The homogenization step is purported to reduce the size and size distribution of the precipitated particles, thereby minimizing the likelihood of crystal growth and improving the stability of the nanosuspension during storage. Precipitation, in tandem with high pressure homogenization, has been successfully applied for the preparation of nitrendipine nanocrystals to improve drug bioavailability. Chitosan, which was used subsequently to modify the surfaces of the nanocrystals, further enhanced drug bioavailability without bringing about any measurable increase in the size of the original nanocrystals [99].

6.4. Cryogenic milling

In principle, size reduction begins when the externallyapplied stress induces sufficient strain within the particles and causes the formation of cracks. The cracks are then propagated through lines of weaknesses in the material, with new cracks being initiated and perpetuated along the way at other discontinuities. A cascade effect occurs and material fracture results. The mechanical property such as hardness or elasticity of a drug is likely to affect the ease of crack initiation and propagation during milling. Logically, harder materials require greater energy input to effect particle size reduction as they do not yield to the externally applied stress as readily as softer materials. However, the brittleness or plasticity of the material also plays a critical role in rendering the ease of particle size reduction. It is easier to comminute hard and brittle materials, such as chalk, than soft and viscoelastic materials, such as rubber, waxes or natural gums. Instead of brittle fracture, more plastic materials are capable of absorbing large amounts of energy through viscoelastic deformation without crack initiation or propagation. At ambient conditions, these materials resist fracture or may even melt (for waxy materials) when exposed to the heat generated during milling. Drug substances that exhibit such characteristics are not likely to be amenable to the conventional dry or wet milling methods aforementioned. However, there had been few studies on the milling of soft and highly plastic pharmaceutical materials [52].

Cryogenic milling or cryomilling in short, is a size reduction method specially catered to soft, elastic/plastic, nonbrittle and thermolabile materials. Cryomilling may be carried out either by first freezing the materials in liquid nitrogen (-100 to -150 °C) prior to milling or by milling the materials under cryogenic conditions, i.e., in the presence of liquid nitrogen (Fig. 5). Exposure to liquid nitrogen results in the "embrittlement" of the material which facilitates crack propagation, reducing the specific energy required for milling [100] and potentially shortening the milling duration. Although freeze drying performs a similar function and renders a material brittle and porous, by virtue of the fact that it is a drying process implies that it is more suitable for liquids or solid materials containing higher residual moisture contents. Salazar et al. [101] studied media milling and high pressure homogenization of glibenclamide that was first pre-treated by freeze-drying. Freeze-drying rendered the drug brittle and porous, facilitating the subsequent milling process. This combination approach reduced milling time and improved milling efficiency.

Cryomilling enables the production of both micron and nano-sized particles but it has not been widely adopted in the pharmaceutical industry for the milling of drugs. Sugimoto et al. [102] studied the cryogenic co-milling of phenytoin and polyvinylpyrrolidone as a means to improve the dissolution rate of the drug. Phenytoin was dispersed in liquid nitrogen and subjected to media milling using beads constructed out of zirconium or dry ice. Spontaneous sublimation of the dry ice beads together with liquid nitrogen at ambient condition enabled the recovery of a high yield (85–90 %) of dry phenytoin nanoparticles, since material loss arising from adhesion to the surfaces of the milling media was of no consequence. In another study, pluronic F-68, a soft and meltable material that may be used both as an excipient and active ingredient in inhalable dry powder formulations [103], was subjected to a micro-ball milling technique using stainless steel ball bearings as the grinding agent. Ball milling was carried out in the presence of liquid nitrogen vapor. Apart from chilling the chamber, the liquid nitrogen also prevented plastic deformation of the material and improved the particle fracture process [52].

Cryomilling is advantageous in that it minimizes the degradation of thermolabile drug substances and loss of volatile drug compounds. It also reduces the risk of explosion, oxidation of formulation constituents and particle aggregation during the milling [104]. The latter stems from the low surface tension and viscosity of liquid nitrogen which allows it to penetrate into inter and intra-particulate void spaces and micropores of the particles, forming a physical barrier which prevents particle agglomeration [105]. Cryomilling decreases the effect of temperature-induced changes during milling. Jayasankar et al [106], in a study on co-crystal formation between carbamazepine and saccharin, reported that cogrinding the drug and excipient under cryogenic conditions was necessary to prevent the reaction from proceeding through the melt phase which commonly occurs when cogrinding is carried out at ambient conditions. Cryogenic cogrinding also led to higher levels of amorphization than cogrinding at room temperature. These results are echoed in another study involving the ball milling of 3 different crystalline forms of piroxicam [107]. Differing extents of drug amorphization was achieved by ball milling the drug under different temperature conditions (ambient and cryogenic conditions), with cryogenic ball milling being more effective in inducing drug amorphization. This is because milling at cryogenic temperatures effectively 'traps' the milled material in its amorphous state by removing the thermal energy required for re-crystallization to occur [108]. In this regard, cryogenic milling is advantageous as it enables the production of amorphous material without the deleterious effects of solvents or heating [61]. Crowley and Zografi [109] studied the cryogenic grinding of 5 crystal forms of indomethacin and found that amorphization occurred for one of the solvates (indomethacin methanolate) and all the three polymorphs (γ , α , and δ) studied. Recently, γ -indomethacin was subjected to cryomilling and it was reported that the amorphous



Fig. 5 - A) Cryogenic mill, B) Schematic diagram showing a cross-section of the cryogenic milling chamber. Cryogenic atmosphere is supplied in the chamber.

indomethacin produced exhibited enhanced dissolution rates which was positively related to the duration of cryomilling [38]. The same group of researchers had also evaluated the physical stability of the cryomilled amorphous indomethacin samples by measuring the time required for the drug to recrystallize on storage [110]. Cryogenic grinding was successfully used to convert crystalline glibenclamide to its amorphous form, averting possible chemical degradation during the process. The crystalline-amorphous conversion was shown to be connected with the amide-imidic acid tautomerism of glibenclamide [111]. Chieng and co-workers [112] investigated the effect of cryomilling on 2 polymorphic forms of ranitidine hydrochloride and evaluated the physical stability of the milled amorphous drug under different storage conditions.

High impact cryomilling thus enables the production of completely amorphous drugs that may otherwise be difficult to obtain by milling at room temperature. However, drug amorphization may not always be advantageous from a stability point of view. In a recent study on the cryomilling of furosemide, it was reported that the duration of cryomilling and resultant drug amorphization were factors responsible for the chemical decomposition of the drug [113]. Drug amorphization may not occur in all cases. In a study by Niwa et al. [114], the nanocrystals of phenytoin, ibuprofen and salbutamol sulfate produced from an optimized cryomilling process retained their crystalline character. This was explained by the mild processing conditions that prevailed during cryomilling. Feng et al. [115] reported that cryogenic milling of griseofulvin led to a reduction in drug crystallinity due to the increase of crystal defects, rather than the formation of amorphous drug. A body of research on the use of cryomilling to process molecular materials, including model pharmaceutical compounds, has been carried out by Willart and Descamps [108]. Table 3 summarizes the latest research findings on the use of cryomilling for particle size reduction and solubility enhancement of drugs.

7. Process analytical technology (PAT) in milling

In 2004, the Food and Drug Administration (FDA) initiated the quality by design (QbD) concept, in which process analytics are embedded to monitor, in real time, critical process and product attributes rapidly and non-destructively. The aim of the PAT initiative is to diminish the reliance on end product testing to ascertain product quality, improve process understanding as well as develop intelligent sensing and responsive manufacturing processes. It represents the agency's move to a science-based approach to pharmaceutical manufacturing. In line with this initiative, there is a need to implement process analytical tools to achieve better understanding of the particle phenomena during milling. As aforementioned, the size and shape of milled particles can affect the dissolution properties, resultant bioavailability and storage stability of the formulated product. Hence, it is critical to monitor and track the evolution of particle size and shape during milling. Laser diffraction has become the method of choice for particle size measurement during milling due to its versatility, rapid and reproducible particle sizing [116]. It uses light-scattering measurements to calculate the volume-based particle size distribution. In the last decade, alternative techniques to characterize particle size distribution based on focused beam reflectance measurement (FBRM), ultrasonic attenuation spectroscopy (UAS), phase Doppler method (PDA), spatial filtering technique (SFT) and shadow Doppler velocimetry (SDV) have surfaced. FBRM uses a focused beam of laser light that scans across a particle passing in front of the probe window to measure a chord length distribution. UAS measures the volume-based particle size distribution from extinction spectra using the fundamental equations of mass, momentum and energy balance describing the interaction between an ultrasonic wave and suspended particle. The PDA is based upon the principles of light scattering interferometry and measurements are made using the same optical probe as for laser Doppler velocimetry (LDV). SFT uses the measuring principles of the fiber optical spatial filtering velocimetry (SFV) and the fiber optical spot scanning (FSS) in order to determine simultaneously the size and the velocity of particles. SFV is a method of the velocity determination of an object by observing the object through a spatial filter in front of a receiver. FSS is an addition to the SFV to observe the shadow image of a moving particle through a single optical fiber with a small diameter. SDV is based on the imaging of a conventional LDV probe volume onto a linear photodiode array.

Particle size measurements can be made in-line or on-line. In in-line particle sizing, a probe is directly inserted into the process stream for measurements. However, the main process stream often operates at high particle flow rates. It is therefore a common method to measure in a side-stream that can be isolated from the main process flow. Another option is the application of a dilution step between the particle stream and the measuring device. In on-line particle sizing, a sample is diverted from the manufacturing process stream for measurement, to circumvent the high particle flow rate in the main stream.

As particle shape along with size are directly related to the product quality and performance, the use of a single parameter to describe the physical dimension of particles is often insufficient, especially for non-spherical (rod or plate-like) particles. Therefore, in-line or on-line particle sizing together with shape measurement can play a crucial role in controlling milling processes. However, little attention has been given to real-time particle shape measurement due to the lack of available technologies. Laser Doppler methods have been employed to analyze particle shape, but only with limited success [29,117,118]. Dynamic image analysis (DIA) is still the most commonly used technique for particle shape characterization. However, manual operation was required for sample preparation, measurement and data analysis due to low level of automation. Recent advancements in technologies of high speed cameras and computers have increased the level of automation which enabled the measurement of two dimensional images of dynamic particles [119]. DIA was specifically suitable in sizing non-spherical particles [119,120]. However, the use of the DIA systems have been mostly limited to wet analysis, where particles are suspended in a liquid medium to allow easy control of particle flow rate and thus reduction of motion blur during image acquisition. Recently, Sympatec Inc. (Clausthal-Zellerfeld, Germany)

Table 5 – Summary of different PAT approaches in milling.					
Technique	Mechanism	Measurement parameters			
Particle size analysis					
Laser diffraction	Light scattering	Volume-based particle size distribution			
FBRM (focused beam reflectance measurement)	Focused beam laser light scan across a particle	Particle chord length distribution			
UAS (ultrasonic attenuation spectroscopy)	Interaction between ultrasonic wave and suspended particle	Volume-based particle size distribution			
PDA (phase Doppler method)	Light scattering interferometry	Size, velocity and concentration of spherical particles			
SFT (spatial filtering technique)	Combination of spatial filtering velocimetry (SFV) and fiber optical spot scanning (FSS)	Particle chord length and velocity			
SDV (shadow Doppler velocimetry) Particle size and shape analysis	Doppler shift in a laser beam	Particle size and velocity			
DIA (dynamic image analysis)	Image analysis	Particle size and shape analysis in wet environment			
DIA (QICPIC)	Image analysis	Particle size and shape analysis in dry environment			
DIA (Eyecon TM)	Image analysis using illumination technique	3D particle characterization			

commercialized a DIA system (QICPIC) that is capable of capturing images of dry powder particles in a rapidly-moving air stream. Another introduction of DIA system is the noninvasive, real-time 3D particle characterizer capable of giving live particle size and shape information for coarser particles is the Eyecon[™] (Innopharma Labs, Dublin, Ireland) which uses a unique illumination technique to assist in detection of particle boundaries. The different PAT approaches in milling are summarized in Table 5.

8. Conclusion

A feature of advancement in the pharmaceutical industry is the increased use of materials, particularly drugs, in their finest state of subdivision to enhance dissolution, solubility and bioavailability. A myriad of milling techniques and equipment are now available for particle size reduction of drugs, with many capable of scaling up and adaptable to consistent and continuous manufacturing. The application of PAT tools in milling also improves process understanding as well as facilitates the monitoring and control of the particle size reduction process. This enables the production of fine particulates with predictable and controllable physicochemical characteristics. There are other aspects of milling that are not within the scope of this review but nonetheless warrant considerable attention. These include the possible cellular toxicities of drug nanoparticles as well as the increased risks of chemical degradation, polymorphism and loss of the activities of drugs as a result of them being milled to a finelydivided state. Despite recent progress and innovations in the art of milling, much effort is still needed to improve the energy efficiencies of milling operations particularly those designed for ultrafine grinding. Even in the most efficient mills, as little as 2% of the total energy consumption may be channeled to effect particle size reduction, with the remainder being lost via elastic/plastic deformation of particles, inter-particulate and particle-machine friction, heat, sound and vibration [121]. The use of milling as a means to engineer and produce fine, surface-modified particles also represents an exciting area of research wherein the function of milling extends beyond size reduction and offers a more holistic and integrated approach to particle design.

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