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RESEARCH ARTICLE

Novel aspects of wet milling for the production of microsuspensions and nanosuspensions of poorly water-soluble drugs

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Abstract

Micronization and nanoparticle production of poorly water-soluble drugs was investigated using single wet milling equipment operating in the attritor and stirred media modes. The drug particles in the median size range of 0.2–2 μm were prepared by changing the milling mode and operating conditions of a Micros mill with a purpose of elucidating the dynamics of the wet milling process. It was determined that particle breakage due to mechanical stresses and aggregation due to insufficient stabilization are two competing mechanisms which together control the wet milling dynamics of the poorly water-soluble drugs. The study in the attritor mode using four different classes of stabilizers with six drugs indicated that steric stabilization worked better than electrostatic stabilization for the drugs studied. In addition, the existence of different minimum polymer concentrations for the stabilization of microsuspensions and nanosuspensions was indicated. The major role of a non-ionic polymer during the production of fine particles is its stabilization action through steric effects, and no experimental evidence was found to support the so-called Rehbinder effect. Periodic addition of the polymer as opposed to the addition of the polymer at the start of milling process was introduced as a novel processing method. This novel method of polymer addition provided effective stabilization and breakage of drug particles leading to a narrower and finer particle size distribution. Alternatively, it may allow shorter processing time and lower overall power consumption of the milling process for a desired particle size.

Keywords: Bioavailability enhancement, nanoparticles, wet stirred media mill, breakage dynamics, Rehbinder effect

Introduction

An increasing number of newly developed molecules exhibit poor water solubility and hence poor bioavailability[1]. One of the approaches to increase the bioavailability of these drugs, in particular the BCS (Biopharmaceutical Classification System) Class II drugs, is by particle size reduction that leads to an increase in the surface area of the particles. A modified version of Noyes–Whitney equation[2]

$$\frac{dm}{dt} = k_0 A(C_S - C)$$

can be used to describe the dissolution rate. Here, k_0 is the overall solute transfer coefficient defined by $1/k_0 = 1/k_i + 1/k_c$, where k_i and k_c are the interface rearrangement constant and the external mass transfer coefficient, respectively, C_S is the saturation solubility, C is the concentration in the bulk solution, A is the total surface area, and m is the amount dissolved at time t.

The increase in dissolution rate of micronized particles, also known as microparticles (particles typically in the size range 1–10 μm) and nanoparticles (typically colloidal particles smaller than 1 μm, in line with the prevalent pharmaceutical terminology) can be explained by three effects. The major contribution to the enhanced dissolution rate of the drug is through the specific surface area and the total area A of the particles, both of which increase due to size reduction. The increase in k_0 leading to increased dissolution rate is explained by a decrease in

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the thickness of the diffusion layer surrounding the particles and associated increase in the external mass transfer coefficient k_c. The saturation solubility C_S increases with decreasing particle size according to the Ostwald–Freundlich equation and results in an increased dissolution rate in the nanoparticle size domain[3]. The increase in saturation solubility and surface area are especially important for dissolution of the nanoparticles, whereas the increase in surface area and decrease in the thickness of diffusion layer around the microparticles[4] contribute to the higher dissolution rates of the microparticles.

Microparticles and nanoparticles can be produced either by size reduction of larger particles, the so-called top-down approach[5,6] or by building up particles via precipitation of dissolved molecules, the so-called bottom-up approach. Top-down approaches include jet milling, rotor-stator milling, high pressure homogenization, stirred media milling, and ball milling. Among the top-down approaches, wet stirred media milling has been one of the most commonly used techniques for preparing microsuspensions (suspensions with particles having a mean or median size in the range 1–10 μm) and nanosuspensions (suspensions with particles having a mean or median size <1 μm)[7–13]. Currently, products such as Rapamune® (Wyeth), Emend® (Merck), Tricor® (Abbott), and Megace® (Par Pharmaceutical) are already on the market manufactured by Elan's Nanocrystal Technology®[14] using media milling. Albeit being very effective at lab-scale, high pressure homogenization[15–17] has not been widely used in commercial production of nanosuspensions.

The production of fine particles also needs an understanding of stabilization since the aggregation of very fine particles is a major issue[5,18,19]. A large number of polymers and surfactants are available for stabilization of drug particles. A few research groups have studied the effects of hydrophobicity of copolymers[20,21], polymer molecular weight[22], and the surface energy of polymers[23] on drug stabilization. A screening study[24] of polymers, copolymers, and surfactants has revealed the stabilizing performance of surfactants to be the best followed by linear synthetic polymers and semisynthetic polymers for the specific drug compounds studied.

Although there are ample experimental data on pharmaceutical nanosuspension production and their stabilization via wet milling, little to no attention has been paid to the dynamics of the wet milling process and the complex interplay between particle breakage (mechanical aspects) and suspension stabilization. Some of these aspects were studied for non-pharmaceutical materials like minerals and pigments (see refs. 5,19). In most cases, no data for the pharmaceutical wet milling dynamics was presented (as in refs. 7,11,13,23–26). In other cases[10,20–22,27], temporal evolution of the median or mean size was presented. However, these studies did not elucidate the aggregation and complex interplay between breakage and aggregation. Hence, an objective of this study is to shed some light on the breakage and aggregation dynamics during the wet nano-milling of drug compounds.

In all wet nano-milling studies that exist in the pharmaceutical literature, the stabilizer(s) are added at the beginning of wet milling and then the milling process is carried out. The stabilizer addition method includes adding the stabilizer from the start of milling, at the end of milling, and periodic addition (step-wise) in multiple steps during milling. A major novelty of this article is the investigation of the effects of the stabilizer addition method and potential decrease in the strength of solid bodies such as particles upon adsorption of surface-active agents, which is commonly known as the Rehbinder effect. Rehbinder effect is described as the facilitation of crystal cleavage and decrease in hardness of crystals caused by surface-active agents, and can be explained by the weakening of bonds in the crystal surface layer due to adsorption and lowering of the specific surface free energy[28–32].

This article is divided into two parts. The first part deals with the study and assessment of the stabilization effectiveness of various stabilizers and extent of breakage of the poorly water-soluble drugs, which were milled in the attritor mode. Based on this study, a model stabilizer-drug system was selected to be considered in the second part that focuses on various processing aspects such as the effects of milling mode, polymer concentration, and method of polymer addition on the particle size distribution (PSD). The concentration of stabilizer required for 7-day stability of the drug microsuspensions and nanosuspensions was also investigated. By switching from attritor milling mode to stirred media milling mode, it was determined that the breakage rate dramatically increased, which allowed us to produce both microsuspensions and nanosuspensions of drugs in a single mill within 75 min. Experimental data suggest no evidence for the Rehbinder effect, and the major role of a non-ionic polymer during the production of fine particles is its dispersion and stabilization action through steric effects. It was also shown that a process change in the method of polymer addition can produce narrower and finer PSDs in the stirred media mill. The competition between breakage and aggregation was elucidated through the dynamic analysis of the data. Furthermore, the influence of milling mode, polymer concentration, and method of polymer addition on breakage and aggregation dynamics was demonstrated.

Materials and methods

Materials

The physicochemical properties of the drugs are provided in Table 1[33]. Six poorly water-soluble drugs with a wide range of water solubility were used in the wet milling study. All drugs studied here belong to BCS Class II, except Azodicarbonamide, for which no BCS class information has been reported in the open literature. Griseofulvin was donated by Johnson and Johnson

Co. Ltd., (New Brunswick, NJ, USA); Fenofibrate was purchased from Sigma Aldrich (St. Louis, MO, USA); Ibuprofen (110 US grade) from Alfa Chem, (Kings Point, NY, USA); Azodicarbonamide from Pfaltz and Bauer Inc., (Waterbury, CT, USA); Sulfamethoxazole from MP Biomedicals, LLC (Solon, OH, USA) and Itraconazole from Hawkins Pharm Group, (Minneapolis, MN, USA) were purchased. The stabilizers hydroxypropylmethyl cellulose (HPMC; high molecular weight [HMW, was purchased from Sigma Aldrich (St. Louis, MO, USA)] 4000–5600 cp), Tween 80 (T80), sodium dodecyl sulfate (SDS) (99% ACS reagent), and sodium alginate (SA) (food grade) were purchased from Sigma Aldrich (Milwaukee, WI, USA). The stabilizer HPMC (Methocel E15LV, low molecular weight) was purchased from Dow (Midland, Michigan, USA). Crosslinked polystyrene beads (Norstone Inc., Bridgeport, PA, USA, 200–350 μm) were used for stirred media milling.

Methods

Equipment

The mill used in this study was a Micros-0 Ring Mill manufactured by NARA Machinery Corp. of Japan (Tokyo, Japan). The mill design was modified to run it in either of the two modes: attritor mill mode (Figure 1A) and stirred media mill mode (Figure 1B). The mill consists of a main shaft rotating in the vessel attached to six equidistant sub-shafts. The vessel wall is lined with zirconia. The vessel has an effective grinding volume of 350 ml and is surrounded by a cooling jacket through which cooling water circulates to maintain a product temperature of 23 ± 3°C. In the attritor mode, zirconia rings were mounted on the sub-collars that were attached loosely around the sub-shafts, which resulted in the formation of four grinding zones: between the rings, between the rings and the vessel wall, between the sub-collars and the rings, and between the sub-shafts and the sub-collars. Particle breakage occurred due to shear (mainly) and compression forces acting on drug particles in these grinding zones[34].

The zirconia rings were replaced with crosslinked polystyrene beads for media milling. The mill was operated as a vertical batch stirred media mill where the main shaft and sub-shafts played the role of agitating the milling media (beads). Particle breakage in this mode occurred due to the bead–bead collisions and bead–wall collisions, which is shortly referred to as the impaction mechanism[5,6]. Due to the turbulent flow induced by high speed rotation of the shaft in the milling chamber, beads have a high average oscillating speed on the order of 0.1–1 m/s and oscillation frequency on the order of 1–10 kHz[35], which can lead to frequent high intensity compressions of drug particles captured between the beads.

Table 1. Physicochemical properties of the drug compounds used.

API	Solubility (mg/L)	Molecular weight	Melting point (°C)	Log P
Itraconazole	4.72×10^{-4}	705.6	166.2	6.5
Fenofibrate	0.50	360.8	80.5	5.3
Griseofulvin	8.99	352.8	220.0	2.2
Ibuprofen	21.0	206.3	76.0	3.6
Azodicarbonamide	35.0	116.1	225	5.8
Sulfamethoxazole	610.0	253.1	167.0	0.7

Preparation of microsuspensions and nanosuspensions

In all experiments, the drug loading was 3.5 g drug/175 g deionized water (2% w/w). In runs that used crosslinked polystyrene beads (stirred media mode), the beads volume fraction in the suspension was 0.5, which was calculated from the ratio of the true volume of the beads (175 ml) to the effective grinding volume (350 ml). No beads were used in the attritor mode. In the first part of this study, the stabilization effectiveness of four different polymers/surfactants and extent of breakage of six poorly water-soluble drugs were investigated using the attritor mode of the mill (see Runs 1–5 in Table 2). Run 1 was a base experiment that was conducted by preparing the aqueous suspension of each drug in the absence of any stabilizer. For Runs 2–5, aqueous solutions of the stabilizers at a concentration of 0.1% w/w were prepared using a magnetic stirrer, Fisher Scientific (Pittsburgh, PA, USA). The drug particles were dispersed in the prepared

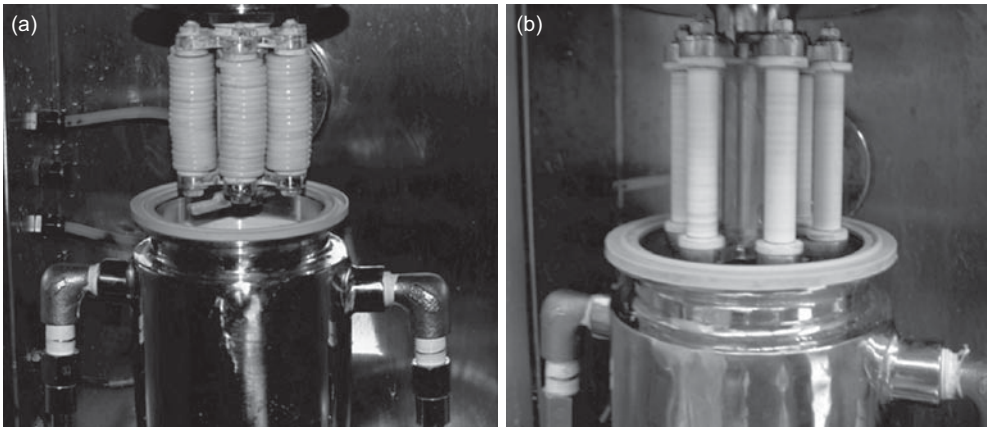

Figure 1. Images of Micros Mill set up for (A) attritor mode of milling and (B) stirred media mode of milling (with rings removed and crosslinked polystyrene beads added).

Table 2. Operating conditions and formulations of the wet milling experiments.

Run No.	Drug(s) milled	Stabilizer	Drug loading (% w/w)	Stabilizer concentration (% w/w)	Method of stabilizer addition	Milling time (min)	Shaft speed (rpm, m/s)	Milling mode
1	All[a]	—	2	—	—	75	1500, 4.97	Attritor
2	All[a]	SA[b]	2	0.1	Initial	75	1500, 4.97	Attritor
3	All[a]	SDS[b]	2	0.1	Initial	75	1500, 4.97	Attritor
4	All[a]	HPMC HMW[b]	2	0.1	Initial	75	1500, 4.97	Attritor
5	All[a]	T80[b]	2	0.1	Initial	75	1500, 4.97	Attritor
6	Griseofulvin	HPMC E15LV[b]	2	1	Initial	75	800, 2.65	Attritor
7	Griseofulvin	HPMC E15LV[b]	2	1	Initial	75	800, 2.65	Media
8	Griseofulvin	HPMC E15LV[b]	2	2	Initial	75	800, 2.65	Attritor
9	Griseofulvin	HPMC E15LV[b]	2	2	Initial	75	800, 2.65	Media
10	Griseofulvin	HPMC E15LV[b]	2	2	After 75 min	75 + 2	800, 2.65	Media
11	Griseofulvin	HPMC E15LV[b]	2	2	After 30 min, periodic[c]	30 + 45	800, 2.65	Media
12	Griseofulvin	HPMC E15LV[b]	2	2	After 30 min, periodic[d]	30	800, 2.65	Media

[a]Each drug from the six poorly soluble drugs was milled in a separate experiment.
[b]SA, sodium alginate; SDS, sodium dodecyl sulfate; HPMC, hydroxypropylmethyl cellulose; HMW, high Molecular Weight; E15LV, low viscosity grade; T80, Tween 80.
[c]Performed in the stirred media mill (SMM) within 45 min.
[d]Performed in the low shear mixer (LSM) within 45 min.

stabilizer solution and stirred for 5 min. The drug suspensions were then poured into the mill vessel and milled under the same milling conditions for 75 min at 1500 rpm shaft speed, which corresponds to a tip speed of 4.97 m/s. Particle size was measured immediately after milling and the samples were stored for 24 h to monitor their physical stability. Runs 1–5 were repeated for each of the six drugs.

In the second part of this study (Runs 6–12 in Table 2), the processing aspects of the wet milling were investigated with Griseofulvin as a model drug and HPMC chosen as the stabilizer based on the findings from the first part of this study. The operating parameters of the mill such as the shaft speed of 800 rpm, which corresponds to a tip speed of 2.65 m/s, and the drug loading of 2% w/w, were kept the same for Runs 6–12. The shaft speed was decreased from 1500 rpm in Runs 1–5 to 800 rpm in Runs 6–12 to prevent any potential damage to the polystyrene beads, vessel wall lining, or the sub-shafts at high speeds in the stirred media milling mode because the Micros mill was not optimally designed for stirred media milling. Runs 6–9 were performed with a milling time of 75 min to study the effect of milling mode and HPMC E15LV concentration (1% and 2% w/w) on the stability and breakage dynamics. The effect of polymer addition method was explored in the stirred media mode (Runs 10–12). Suspensions were prepared by suspending the drug in water without HPMC under magnetic stirring for 5 min which were then milled for 75 min in Run 10 and 30 min in Runs 11 and 12. In Run 10, the mill was stopped after 75 min, HPMC E15LV was added to the milled suspension at 2% w/w concentration and then the suspension with HPMC was milled for 2 more minutes. In Run 11, instead of adding HPMC E15LV all at once, it was added periodically at 30, 40, 50, and 60 min in the mill. In Run 12, the suspension was removed from the mill after 30 min of milling and HPMC E15LV was periodically added to the drug suspension (without beads) in a low shear mixer at the same time intervals as used in Run 11. The low shear mixer used in Run 12 was a laboratory stirrer from Fisher Scientific (Pittsburgh, PA, USA) (Catalog no. 14–503).

Particle size analysis

Particle size analysis of the suspensions was performed by laser diffraction using a Beckmann Coulter (Miami, FL, USA) LS230. The polarization intensity differential scattering was maintained at 45% for the particle size measurement of all samples. A sample of <1 ml was removed from the center of the mill, at the end of milling for Runs 1–5 and at various time points as indicated in the figures for Runs 6–12. Following the sample extraction, samples were diluted with the respective stabilizer solutions and particle size was measured immediately. The samples extracted from the mill were filtered through 35 μm sieve to separate the polystyrene beads from the slurry when stirred media mode was used. The mean particle size and the standard deviation of the PSD were reported for Runs 1–5, whereas the median particle size and the cumulative PSDs were reported for Runs 6–12.

Scanning electron microscopy (SEM)

The morphological evaluation of the drug particles before milling and after milling was conducted by SEM imaging. The suspensions were mounted on a silicon chip (Ted Pella, Inc., Redding, CA, USA), placed on top of carbon specimen holders, and dried in a desiccator. The samples were then sputter coated with carbon and observed under a scanning electron microscope LEO 1530 SVMP (Carl Zeiss, Inc., Peabody, MA, USA).

Results and discussion

Effectiveness of stabilizers and breakage of drug particles

The performance of the polymers and surfactants was evaluated in terms of the drug particle size obtained by wet milling using four different stabilizers and six different poorly water-soluble drugs (refer to Table 3). Long-term stability of the suspensions and optimization of the stabilizers were not considered here because the drug suspensions are intended to be dried shortly following milling for potential solid dosage form preparation. The drug suspensions were milled in the absence of stabilizers as a base experiment in Run 1. In this run, the high surface energy of milled particles led to particle aggregation, which explains the general trend with larger mean particle size in the absence of stabilizers (Table 3). The stabilizers adsorb on the surfaces of the drug particles and provide an ionic or steric barrier[36,37]. SDS, an anionic surfactant, and SA, an ionic polymer, can provide electrostatic stabilization, whereas Hydroxypropylmethyl cellulose (HPMC HMW), a non-ionic polymer, and T80, a non-ionic surfactant, provide steric stabilization.

HPMC is a semisynthetic (polysaccharide based) polymer, has a hydrophobic alkyl chain as well as hydrophilic hydroxyl groups in its side chain, as shown in Figure 2, which can form hydrogen bonds with the drug particles. T80, a non-ionic surfactant, has a long hydrophobic tail containing an unsaturated bond, which gets adsorbed on the drug surface, and has a bulky head group producing steric energy barriers to aggregation. The steric force is caused by the long-tail interference of adsorbed HPMC or T80 molecules, resulting in repulsive forces when sufficiently short distances between particles are reached as shown in Figure 3. SDS, being an anionic surfactant, and SA, being an ionic polymer, may render the particles negatively charged after adsorption on the drug surface (see Figure 3). This may increase the energy barrier by increasing electrostatic repulsion among the particles thereby preventing particle aggregation[38].

It should be noted that different drugs had different extent of breakage depending on their mechanical strength characteristics, their interaction with the stabilizers, and operating conditions in the mill. For a given drug and set of operating conditions in the mill, the stabilization effectiveness of different stabilizers is an important factor[21]. For the same wet milling operating conditions and stabilizer loading, the ratio of volume-mean size of the unmilled particles to that of milled particles, also known as the size reduction ratio, can be taken as a comparative measure of the extent of breakage and can be used to rank-order the stabilizing potential of the polymers and surfactants used. For each drug, four stabilizers were arranged in the decreasing level of associated size reduction ratio (stabilization effectiveness) using the data in Table 3 and assigned points from 4 to 1, respectively. Then, an overall ranking was performed considering all drugs and adding the individual points for each stabilizer. As expected, the overall ranking of four stabilizers does not necessarily follow the same pattern for each specific drug due to specific drug–stabilizer interaction. The overall stabilization effectiveness of the stabilizers was found to be HPMC > T80 > SDS > SA. Thus, steric stabilization was inferred to be more effective than electrostatic stabilization for the drugs studied. HPMC had the highest overall ranking and it appears to be the most versatile stabilizer followed by T80. The use of HPMC resulted in the smallest volume-mean particle size and the highest size reduction ratio for wet milling of five out of six drugs.

It can be seen from Table 3 that Griseofulvin mean particle size was in the range of 2–3 μm irrespective of the stabilizer used. The stabilization for Fenofibrate was not very effective in the presence of SA, SDS, and T80. Ibuprofen milled in the presence of SA was not stabilized as the particle size with SA was greater than that of the milled Ibuprofen in the absence of SA. Nanosuspensions of only two of six poorly water-soluble drugs, i.e. Azodicarbonamide and Sulfamethoxazole, were obtained in the attritor mode within 75 min when HPMC and SA were used. If each drug was considered individually with respect to the effectiveness of the stabilizers, the stabilizers do not exactly follow the same order of overall stabilization effectiveness as mentioned above since this depends on the specific drug–polymer interactions. Overall, HPMC appears to be the most effective stabilizer that can stabilize almost all poorly water-soluble drugs studied here. The use of polymer along with a surfactant can have synergistic stabilizing action and serve as a more effective stabilizing system, but this possibility will be investigated in a future study.

The ratio of volume-mean size of the unmilled particles to that of milled particles was again taken as a

Table 3. Effect of stabilizer type on the particle size of the drugs.

API	Unmilled (μm)	Milled without stabilizer (μm)	Milled with SA (μm)	Milled with SDS (μm)	Milled with HPMC HMW (μm)	Milled with T80 (μm)
Itraconazole	18.03 ± 13.53	15.86 ± 8.24	14.82 ± 9.95	13.45 ± 11.42	8.72 ± 5.66	9.14 ± 6.64
Fenofibrate	35.90 ± 20.94	28.56 ± 15.34	18.32 ± 11.1	11.84 ± 6.45	3.37 ± 2.48	11.15 ± 6.84
Griseofulvin	6.55 ± 5.35	3.02 ± 1.86	3.01 ± 2.13	2.67 ± 1.41	2.72 ± 2.00	2.65 ± 1.12
Ibuprofen	91.31 ± 50.83	35.63 ± 20.84	52.55 ± 23.47	5.70 ± 4.32	3.30 ± 2.18	3.87 ± 2.72
Azodicarbonamide	8.36 ± 5.02	1.85 ± 0.81	0.93 ± 0.76	1.07 ± 0.82	0.67 ± 0.52	2.41 ± 1.04
Sulfamethaxazole	40.55 ± 18.61	12.37 ± 5.78	0.74 ± 0.71	6.25 ± 2.55	0.63 ± 0.56	1.16 ± 0.67

Data shown are the volume-mean particle size and standard deviation of the respective particle size distribution (PSD) obtained from samples that were produced after 75 min of milling in the attritor mill mode.

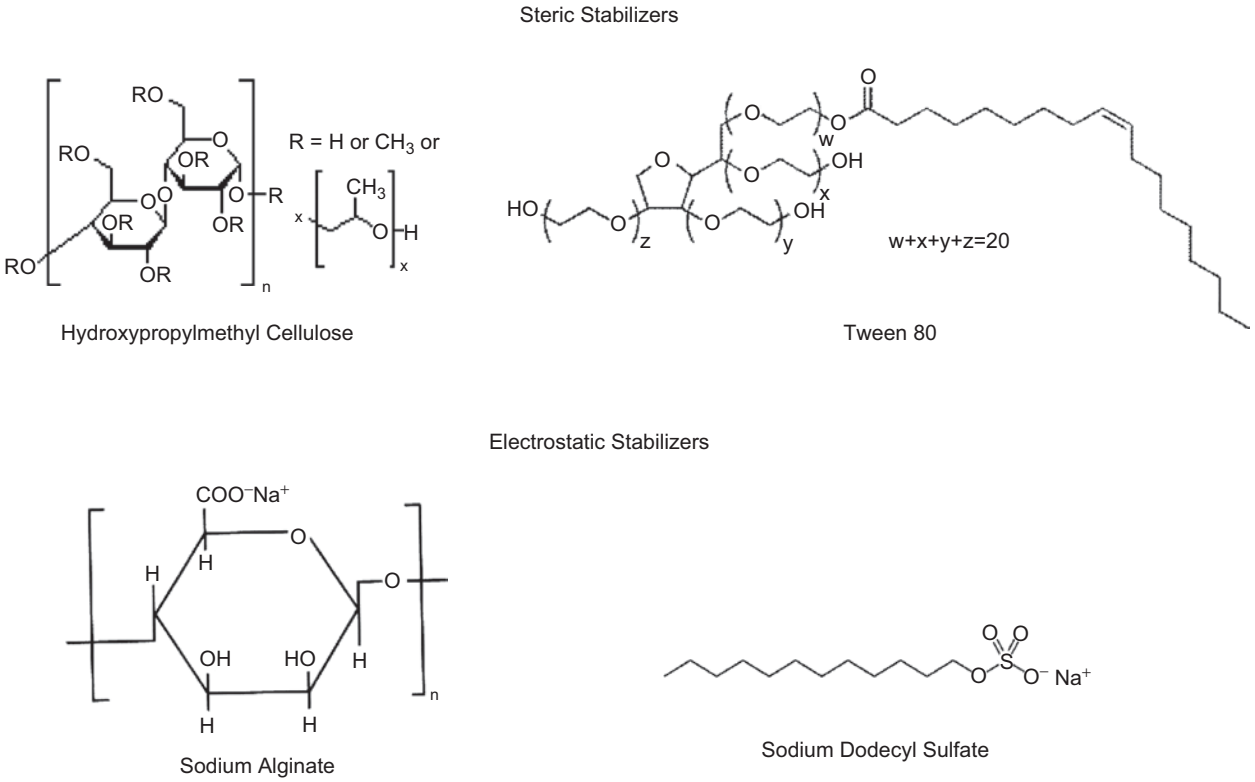

Figure 2. Chemical structures of the stabilizers.

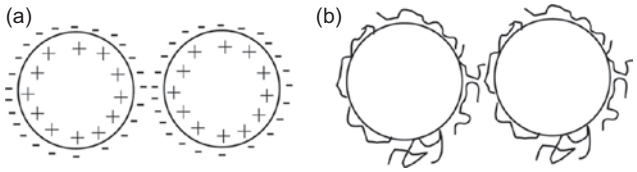

Figure 3. Schematic representing particle interactions after adsorption of stabilizers on the drug surface: (A) electrostatic stabilization and (B) steric stabilization.

comparative measure of the extent of breakage, which is inversely related to particle strength, when HPMC was used as the stabilizer under the same milling conditions. A ratio >10 may be considered to indicate severe particle breakage or high extent of breakage[39]. The data in Table 3 suggest that particles of Azodicarbonamide, Fenofibrate, Ibuprofen, and Sulfamethoxazole were broken to a much greater extent than those of Itraconazole and Griseofulvin. Although no theoretical foundation or strong correlation exists for a particle strength–solubility relation, we note that the former group of drugs, except Fenofibrate, has higher water solubility than the latter group.

Particle growth in suspensions

Particles smaller than a few microns exhibit Brownian motion during storage, which leads to particle–particle collisions and possibly to aggregation if the repulsive forces do not overcome the attractive interparticle forces. In addition, for particles smaller than ~1 μm, significant growth can occur due to Ostwald ripening, which takes place because of the higher solubility of smaller particles (Kelvin effect). The difference between bulk solubility and solubility for particles in a few microns range may be negligibly small, but the difference can be big for nanoparticles. Nanoparticles thermodynamically favor reduction of surface energy either via aggregation or via dissolution and recrystallization of smaller particles on bigger particles (Ostwald ripening), while the repulsive forces originating from the presence of adsorbed stabilizers mitigate the aggregation tendency.

The formulations in Runs 1–5 were not optimized to suppress the growth of the drug particles. We assessed the effect of 1-day storage of the suspensions at room temperature (23°C) on the particle growth. The particle growth in suspensions with 0.1% w/w HPMC HMW was determined by the particle size analysis, immediately after milling and after 24 h upon their storage at room temperature. The suspensions were sporadically observed to determine if settling occurred. The particle growth in suspensions with other stabilizers was not examined because other stabilizers were not as effective as HPMC HMW in stabilizing and dispersing particles of all drugs according to the overall ranking of the stabilizers, as discussed in the previous section. In view of that, no justification exists for studying 24 h stability of the suspensions containing other stabilizers within the scope of this article.

The particle growth after 24 h was observed to be more prominent for drug compounds with higher aqueous solubilities (Table 1) and with milled drugs having a significant fraction of particles smaller than 1 μm, as illustrated in Figure 4. Among the drugs considered in

this study, Sulfamethoxazole and Azodicarbonamide suspensions had a significant fraction of nanoparticles after milling and exhibited considerable growth after 24 h. The suspensions of other drugs such as Itraconazole, Ibuprofen, Fenofibrate, and Griseofulvin had small to no fraction of nanoparticles after milling. Therefore, slight aggregation occurred and the solubility effect on particle growth was expected to be negligible due to low bulk solubility of these drugs coupled with the relatively large size of the particles. The high extent of particle growth in Azodicarbonamide and Sulfamethoxazole suspensions was partly due to the higher solubility[40] of the respective drugs in water compared to the other poorly water-soluble drugs considered in this study.

The first part of this study concluded HPMC to be the most effective stabilizer for the drug compounds studied, although more HPMC with respect to the drug is clearly needed to stabilize the suspensions during storage via steric repulsion mechanism. These findings were used as guidance in the next part of this study. HPMC HMW was replaced by HPMC E15LV due to mill shutdown encountered during preliminary experiments in the second part of this study (Runs 6–12). This issue originated from the high viscosity of the HMW grade of HPMC in the media mill mode when larger quantities of HPMC were used for effective stabilization of nanoparticles. Milling of Griseofulvin particles in the attritor mode did not lead to the formation of a nanosuspension (see Table 3) and the extent of breakage was very low (2.4). This makes

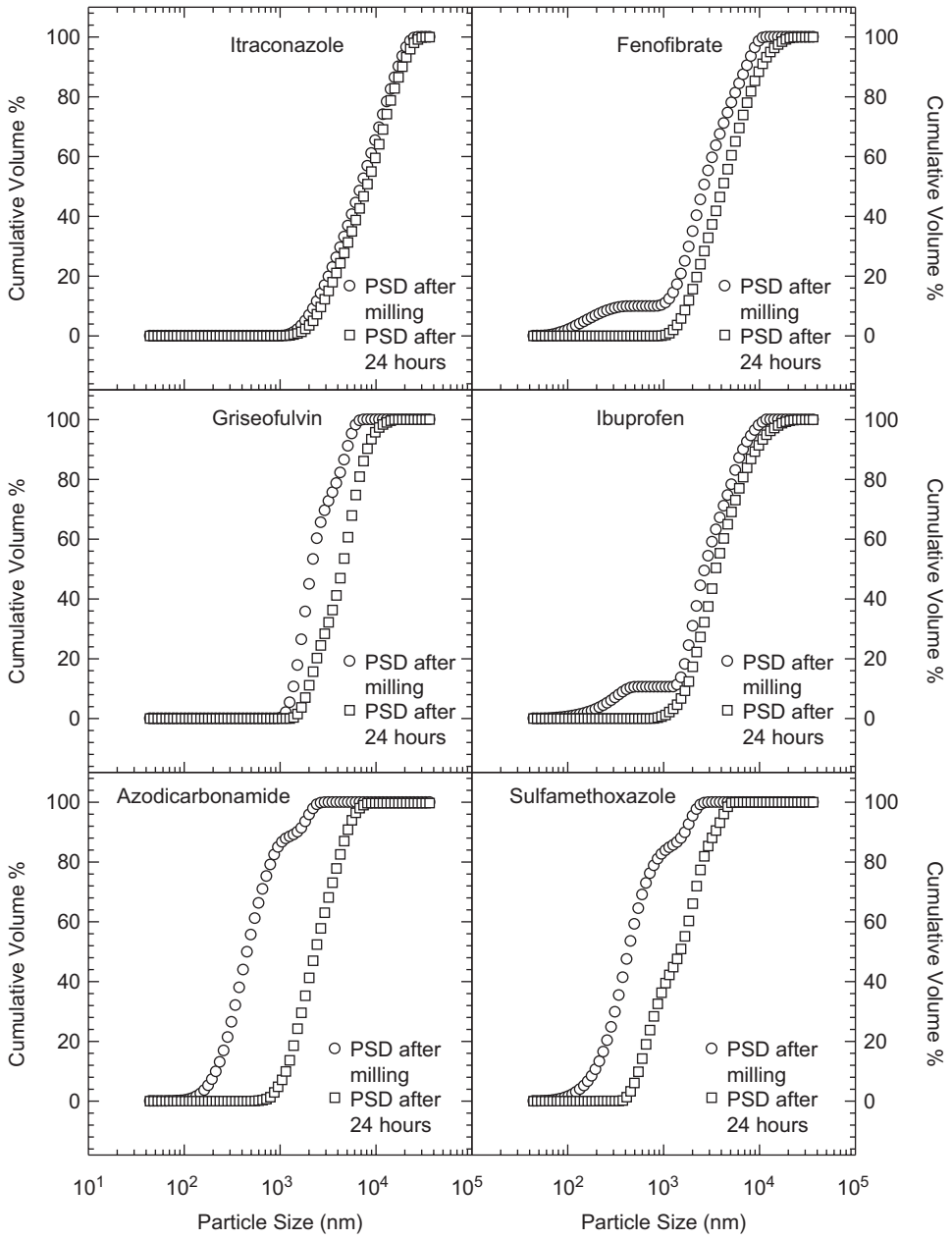

Figure 4. Effects of 24 h storage of the suspensions, which were obtained from wet milling of various drug particles with 0.1% w/w HPMC HMW in the attritor mode (Run 4) for 75 min, on the particle size distribution of produced particles.

Griseofulvin a challenging model drug for poorly water-soluble drugs from a particle breakage perspective. So, in the second part of this study, HPMC E15LV–Griseofulvin was used as a model system. Stable nanosuspensions of Itraconazole and Fenofibrate were also successfully prepared using HPMC E15LV in similar processing conditions and stirred media milling mode as for Griseofulvin (Run 9). Similar to the case with Griseofulvin, milling these two drugs in the attritor mode did not result in the formation of nanosuspensions (Table 3). The results for Itraconazole and Fenofibrate will not be presented here for the sake of brevity.

Dynamics of particle breakage and aggregation and effects of milling mode during the wet milling of Griseofulvin

Breakage of particles during wet milling generates fresh drug surfaces and increases the surface area, whereas aggregation of particles reduces the surface area[22]. Thus, there is dynamic competition between breakage and aggregation of particles. For both 1% w/w and 2% w/w HPMC E15LV concentration cases, the apparent breakage rate of the drug was observed to be greater in the stirred media mode (mill) as compared to the attritor mode (or mill), as can be seen from Figure 5A and 5B. Figure 5B shows a decrease in particle size with an increase in milling time in both modes of operation of the Micros Mill when 2% w/w HPMC E15LV was used as the stabilizer. The median particle size decreased gradually from 3.8 to 2.2 μm in 75 min in the attritor mode, whereas the stirred media mode showed a drastic decrease of median particle size from 3.8 μm to 200 nm with time. Most particle breakage occurred in the initial 20 min milling in the stirred media mode because breakage rate was high in the initial time period due to the presence of the larger particles that can be broken relatively easily. Within 20 min, the median size was reduced to ~400 nm in the stirred media mode and thereafter, the median size gradually reduced to 200 nm in the remaining time period.

The above experimental observations are in line with theoretical expectations. At a similar shaft speed and milling time, the attritor mode yields coarser suspensions as compared with the stirred media mode due to much higher stress intensity/number in the stirred media mill[41]. In the stirred media mode, particles are captured between approaching beads and are subsequently stressed depending on the bead-to-bead collision velocity. In the attritor mode, particles are stressed mainly due to shear in between the gaps of the rings, rings and sub-collars, and between the rings and wall. As a result, the particles in stirred media mode are subjected to a higher number of more intense compression events than in the attritor mode.

The PSD during milling and after milling is affected by the complex interplay between breakage and aggregation of particles. Interestingly, particles in the nanosuspension showed signs of severe aggregation even during milling if they were not stabilized properly. Figure 5A illustrates that the median particle size decreased with time in the stirred media mode in the initial 20 min milling and thereafter the so-formed primary particles began to aggregate extensively due to ineffective stabilization (insufficient coverage of freshly generated surfaces by the polymer) and the median size increased even though milling was continued. Large surface area and high surface energy of nanoparticles and associated high attractive interparticle forces lead to the loss of nanoparticles as they come together forming larger particles (aggregates) that are thermodynamically favored. However, in the attritor mode, the median particle size decreased monotonically as the milling proceeded and extent of aggregation was not significant because the fraction of fine particles, especially nanoparticles, produced by breakage was small during the milling at any time.

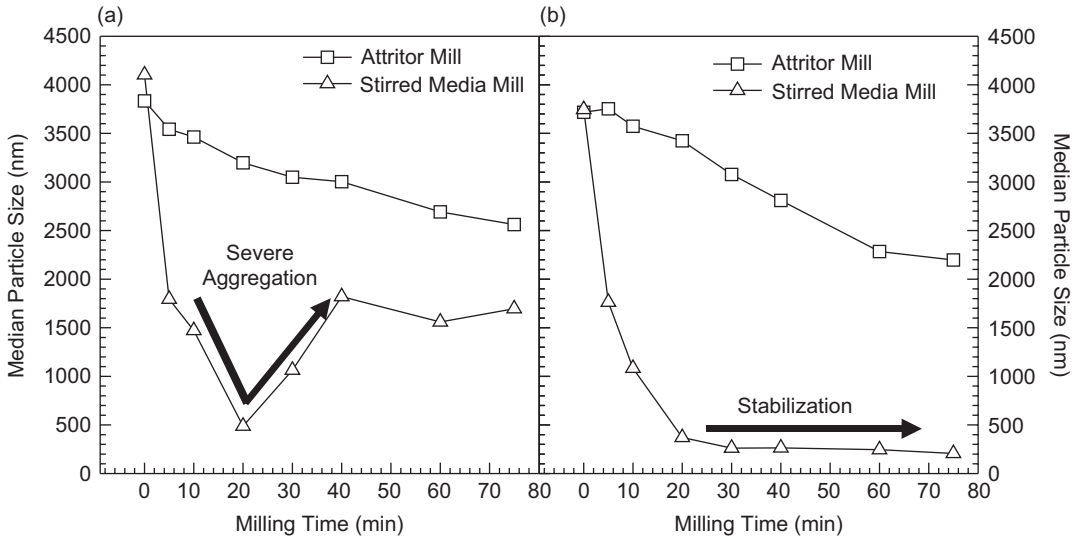

Figure 5. Timewise variation of the median particle size of Griseofulvin after milling in the attritor mode and in the stirred media mode at HPMC E15LV concentrations of (A) 1% w/w (Run 6 in attritor mode and Run 7 in stirred media mode), and (B) 2% w/w (Run 8 in attritor mode and Run 9 in stirred media mode).

The HPMC E15LV concentration of 1% w/w, which corresponds to a HPMC E15LV: Griseofulvin ratio of 0.5:1, was sufficient to stabilize the microparticles in the attritor mode. However, it led to the aggregation of nanoparticles in the stirred media mode. The amount of stabilizer required to cover the surfaces of nanoparticles is more than that for the microparticles because nanoparticles have a much larger specific surface area and total surface area for the same drug loading. The Griseofulvin particles continued to break and generate smaller particles with large total surface area as milling progressed, but the HPMC E15LV concentration was not sufficient to adsorb on all freshly generated surfaces and to stabilize the particles. These theoretical considerations explain the monotonic decrease of median size in the attritor mode and the minimum median size in the stirred media mode in Figure 5A.

It is well-known that for polymers to provide effective steric stabilization, strong adsorption at full coverage is necessary[10]. For 1% HPMC E15LV concentration in the stirred media mode, full coverage of Griseofulvin nanoparticles by HPMC did not seem to occur, which led to the aggregation of particles. As the HPMC E15LV concentration was increased to 2% w/w corresponding to HPMC:Griseofulvin ratio of 1:1, the media mill produced nanosuspensions, as shown in Figure 5B. Steric stabilization depends on concentration of polymer in the bulk solution, polymer:drug ratio, and the size and surface area of the particles. Hence, the minimal polymer concentration required for sufficient stabilization of nanoparticles was higher than that needed for the stabilization of microparticles. The HPMC:Griseofulvin ratio of 1:1 allowed more effective stabilization of the nanoparticles.

The SEM images of the microparticles and nanoparticles obtained from wet milling can be seen in Figure 6. The original Griseofulvin particles had a size range of 1.9–11 μm, and a mixture of different particle shapes can be seen in Figure 6A. The majority of the Griseofulvin particles before milling were rod-like and a smaller fraction of particles were rounded. After milling in the attritor mode, due to breakage, smoothening of the particle edges occurred and more rounded particles in the size range of 0.4–4 μm were observed in Figure 6B. Rounded nanoparticles ranging from 0.1 to 1 μm were formed in the stirred media mill due to breakage and smoothening

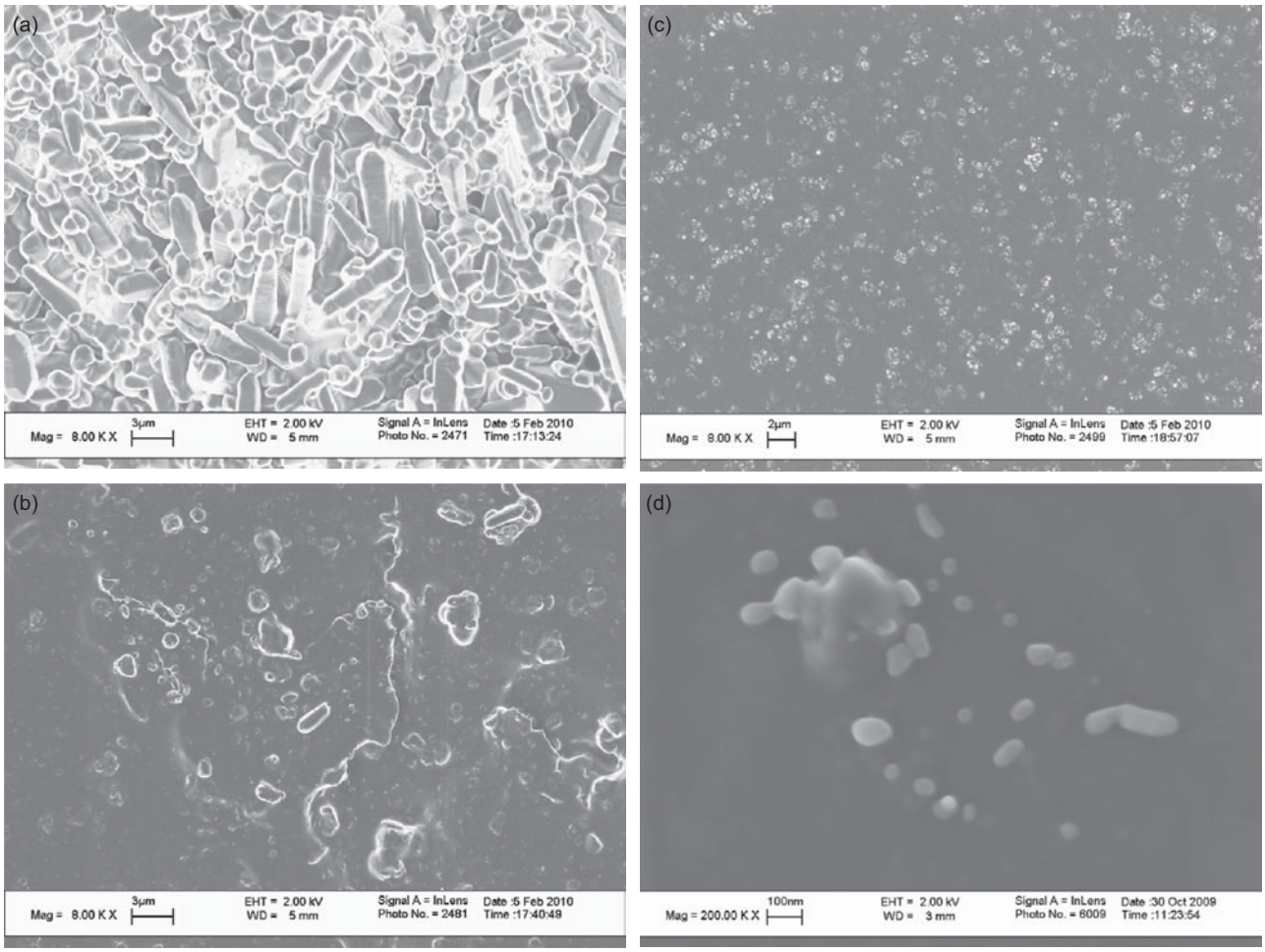

Figure 6. SEM Images of (A) as-received Griseofulvin particles, (B) Griseofulvin microparticles obtained from the attritor mode of milling (Run 8) (C) Griseofulvin nanoparticles obtained from the stirred media mode of milling (Run 9) at a magnification of 8 kx and (D) Griseofulvin nanoparticles obtained from the stirred media mode of milling (Run 9) at a magnification of 200 kx.

of the particle edges as shown in Figure 6C and 6D at different magnifications. As observed previously[5], rounding of the particles appears to be a signature of the nano-milling process.

Effects of the method of polymer addition

In the milling process for producing nanosuspensions of drugs, a pre-suspension of drug particles and stabilizer(s) in water is prepared by dissolving the stabilizer first and then adding the drug particles to this solution[13,27,42–46] or the mill is charged with beads, drug, stabilizer, and water[7,11,36,47,48] or polymer and drug slurry is prepared first and then charged to the mill[10,20–26]. These are the standard practices of wet milling in open literature with stabilizers added from the start of the processing. On the other hand, both practical and theoretical considerations suggest that initial addition of a non-ionic polymer, as a steric stabilizer, or of other stabilizers is neither required nor necessarily energy/cost-efficient. At the start of milling, particles are usually large with relatively small specific surface area that does not require much polymer for steric stabilization. In addition, adding the polymer all at once initially increases the equivalent liquid (suspension without beads) viscosity, suspension mixture (suspension with the beads) viscosity, and energy consumption during milling prematurely.

Considering the high-cost of the wet media milling process, which seems to be a barrier to extensive use of nano-milling as a platform for bioavailability enhancement, any process optimization and improvement may have a huge economic impact. Periodic polymer addition may serve such purpose among many other possibilities, which has not been considered in the open literature. Runs 10–12 were performed to elucidate various aspects of the method of polymer addition. Replicates of Runs 10 and 11 indicate that 10% and 50% (median) passing sizes of the replicate PSDs were reproduced within 5% of those of the original runs, while the 90% passing sizes were reproduced within about 28%. The relatively high variability in 90% passing size alone is due to the presence of soft aggregates in these samples, whose sizes were affected by the sampling and handling during measurements, as well as due to the measurement errors.

Effect of duration of polymer adsorption

The median size of Griseofulvin particles was reduced to 200 nm in 75 min when HPMC E15LV was added from the start of the milling process. However, in the absence of HPMC, the median particle size of 4.98 μm was reduced only to 1.66 μm after milling for 75 min (see Figure 7). The PSD was very broad and exhibited multi-modalities, indicating the presence of primary nanoparticles and their aggregates of various sizes.

It is of fundamental importance to prove that nanoparticles were actually produced in the absence of HPMC; thus supporting the conclusion that particles with 1.66 μm median size, as measured in wet laser diffraction, consist of primary nanoparticles and their aggregates. A drop of Griseofulvin suspension sample obtained from 75 min milling in the absence of HPMC was placed on a silicon chip, dried in a desiccator, and the dried sample was then used for SEM Imaging. Figure 8A shows a dense ensemble of nanoparticles and few micron-sized particles at low magnification. The primary nanoparticles of Griseofulvin in the size range of 50–400 nm can be clearly seen at high magnification in Figure 8B. Particles with a median size of 207 nm, whose sizes were measured by wet laser diffraction, were obtained upon 75 min milling followed by addition of HPMC and agitation for 2 more minutes (Run 10, Figure 7). Although the SEM image is not representative of the whole batch, one may conclude that the particles whose sizes were measured in wet laser diffraction exhibited slightly wider distribution than those observed in the SEM image (Figure 8B) probably due to the presence of aggregates and their incomplete dispersion into the nanoparticles during 2 min milling with HPMC. During the 75 min milling in the absence of HPMC, even though particle breakage occurred leading to the formation of nanoparticles, the so-formed nanoparticles aggregated very fast to a significant extent, which explains the 1.66 μm median size and the multi-modal PSD in Figure 7. The addition of HPMC E15LV followed by only 2 min additional milling resulted in the shift of the particle sizes to the nanosize domain. The aggregates that had formed in the previous 75 min milling were dispersed fast, albeit incompletely, during this period.

The so-called Rehbinder effect (see refs. 30,49) suggests that reduction in surface energy and surface structure change upon adsorption of stabilizers and other surface-active agents can lead to an increase in ductility and a decrease in hardness as well as enhanced crack propagation. Therefore, fine milling can be facilitated as a result of the adsorption of stabilizers at structural defects present or created at the particle surfaces. Rehbinder effect and steric and electrostatic stabilizing action (dispersion) of surface-active agents appear to be the two major mechanisms that drive enhanced wet breakage rate upon use of these agents[29]. On the other hand, there is still no consensus as to which mechanism is more dominant. Figure 7 shows that the PSDs were the same irrespective of HPMC E15LV addition point: at the beginning or toward the end of the milling process. Adding the polymer at the beginning or at the end did not lead to a change in the observed PSD although the surfaces of the particles were subjected to polymer adsorption much longer in the former case (75 min vs. 2 min). The data provide evidence for the main role of a non-ionic polymeric stabilizer as an agent to prevent aggregation and/or to facilitate drug aggregate dispersion via steric effects, but not as an agent that enhances particle breakage by promoting crack propagation (Rehbinder effect). For the Griseofulvin–HPMC E15LV system under consideration, we established above that the role of the polymer is mainly for proper dispersion and stabilization of the already broken Griseofulvin particles, a widely agreed-upon mechanism in wet milling by other researchers[50–53].

Griseofulvin particles in the Run 10 sample grew after 24 h because the HPMC E15LV added at the end of milling did not dissolve completely forming some small lumps, whose dissolution was extremely slow. It was concluded from Figure 5 that HPMC E15LV:Griseofulvin ratio of 1:1, i.e. a HPMC E15LV concentration of 2% w/w allowed better stabilization of the nanosuspension. A part of the HPMC E15LV that dissolved was adsorbed on the drug surface to a small extent during 2 min and it was sufficient to stabilize the particles for more than a few hours (confirmed with visual observation of the suspensions for the absence of phase separation and settling). Although some of the HPMC E15LV lumps may have dissolved during storage, the quiescent storage of the suspension with some HPMC E15LV lumps dissolving slowly did not seem to cause effective steric stabilization of the nanoparticles.

Effect of shear during periodic addition of polymer

Insufficient dissolution of HPMC E15LV and/or insufficient coverage of particle surfaces by HPMC E15LV via adsorption led to drug particle aggregation during 24 h storage that was observed in Run 10. This can be minimized by adding the polymer over a period of time to the suspensions during milling. We note from Figure 5 that size reduction of Griseofulvin was completed in about 30 min and thereafter the particle size remained approximately constant when the suspensions were sufficiently stabilized. In view of that, after milling for 30 min in the absence of HPMC, HPMC E15LV was periodically added to the suspensions at 10 min intervals in the stirred media mode (Run 11) instead of adding HPMC E15LV toward the end of milling (Run 10). Additionally, the periodic addition of HPMC E15LV was performed similarly in a low shear mixer (Run 12). The periodic addition facilitates the dispersion and dissolution of polymer particles in the suspension and helps to achieve 7-day stability of the suspensions.

Figure 5 shows that the median size was reduced to <400 nm in 30 min milling when HPMC E15LV was added from the beginning. The median size was reduced to 1.61 μm after 30 min in the absence of HPMC (Figure 9) similar to 75 min milling in absence of HPMC (Figure 7). In both cases, particle breakage had occurred while the so-formed primary particles aggregated significantly, which could only be stabilized after the addition of HPMC E15LV. Also from Figure 9, it is seen that finer and narrower PSD was obtained by periodic addition of HPMC E15LV in the stirred media mill in comparison to the low shear mixer due to high fluid shear and high stress intensity/number of bead–bead collisions in the former that led to faster breakage of aggregates and their stabilization. In the low shear mixer, no bead–bead collisions exist and dispersion of aggregates takes place mainly due to fluid shearing, which is not as effective as the turbulent shear and compression of drug particles and aggregates between the beads in the media mill.

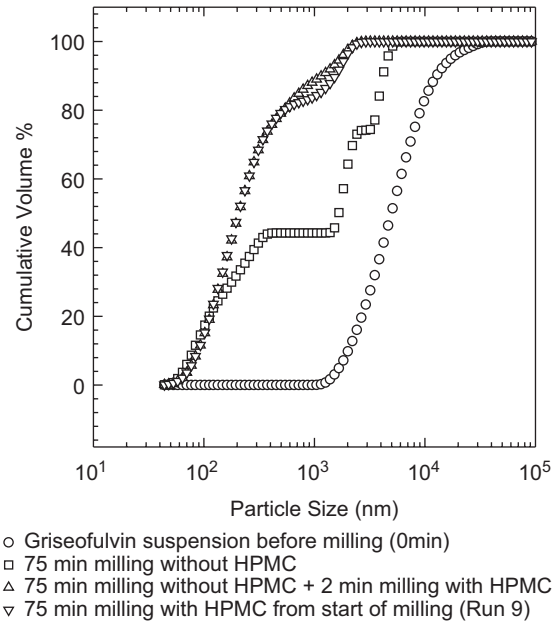

Figure 7. Effect of duration of HPMC E15LV adsorption on the particle size distribution of Griseofulvin during the wet stirred media milling (Runs 9 and 10).

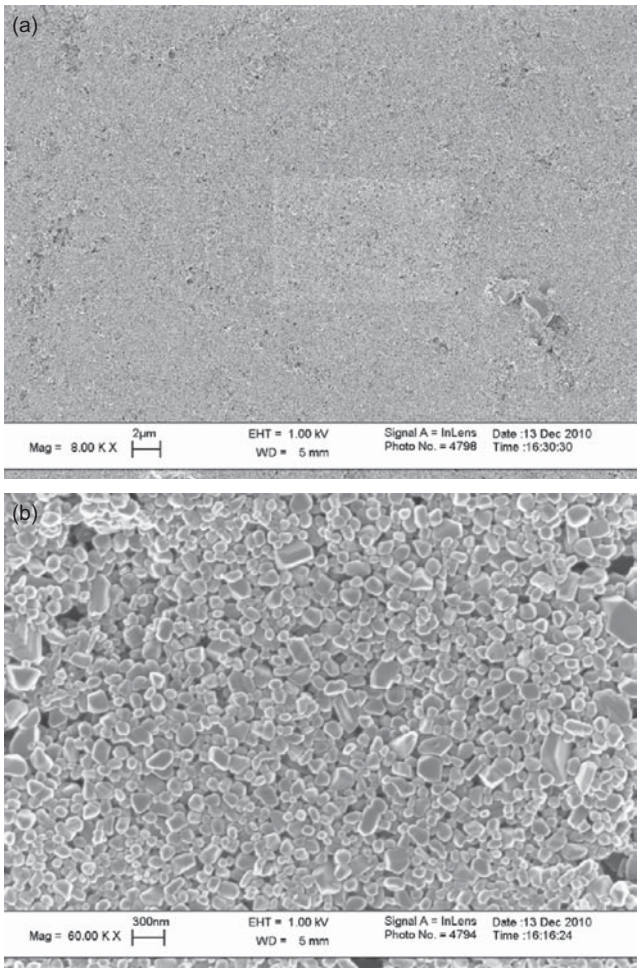

Figure 8. SEM Images of dried Griseofulvin sample obtained from stirred media milling for 75 min prior to the addition of HPMC (Run 10) at magnifications of (A) 8 kx and (B) 60 kx.

The stirred media mill and low shear mixer provide energy to disperse and dissolve HPMC E15LV particles, to break the aggregates of the drug, to wet the drug particle surfaces with HPMC E15LV solution, and to adsorb HPMC E15LV on the newly created drug surfaces. The entire process of polymer particle dispersion and dissolution, breakage and wetting of drug particles and aggregates, and HPMC E15LV adsorption for suspension stabilization requires some time and energy that is provided by periodic addition of HPMC E15LV in the stirred media mill more effectively. The suspension in Run 10, the case of polymer addition toward the end of milling, was not given sufficient time for full polymer dispersion and adsorption, thus leading to destabilization of the suspension after 24 h.

Effect of initial, final, and periodic addition of polymer

Figure 10 compares the Griseofulvin PSDs with three methods of HPMC E15LV addition in the stirred media mill: (1) HPMC E15LV addition at the beginning (initial), (2) toward the end of milling, and (3) periodic addition. A total processing time of 75 min (with 2 min deviation in Run 10) was set so that results from Run 9 and from Runs 10–12 can be compared. From a process optimization perspective, periodic addition of the polymer does not have to wait for 30 min milling time point (Runs 11 and 12). It can start immediately toward reducing the milling time for a desired particle size. A finer and narrower PSD was obtained when HPMC E15LV was periodically added to the stirred media mill. In fact, unlike all other runs, Run 11 resulted in a nanosuspension with no particles greater than about 500 nm. Method 2 (Run 10) sample was not stable after 24 h storage, while Method 1 and Method 3 samples were stable after 7 days. We add a cautionary note that these findings are based on batch wet milling of Griseofulvin with 2% w/w HPMC E15LV in the absence of a surfactant.

During wet milling, the fineness of the particles usually increases with time, which leads to an increase in the viscosity of the ground slurry. Additionally, an increase in viscosity leads to an increase in power draw to the mill. The increase in viscosity, especially above a certain viscosity level, causes the attenuation of the oscillation velocity and kinetic energy of the grinding media (dampening) and may decrease the fineness of the resulting products[54]. Hence, an increase in viscosity has two counteracting effects on the size reduction. An increase in power draw can enhance breakage rate, while the attenuation of turbulent fluctuations of the beads corresponding to a decrease in average bead oscillation velocity and frequency can reduce breakage rate. At higher viscosities, the latter dominates. A minimum amount of non-ionic polymer with respect to the drug particles is required for the stabilization of the milled particles but the higher the polymer concentration, the higher are the equivalent liquid viscosity and suspension mixture viscosity, which in turn reduces the beads oscillation velocity and frequency. By adding the polymer periodically as the surface area of the milled particles increases with milling time, one can

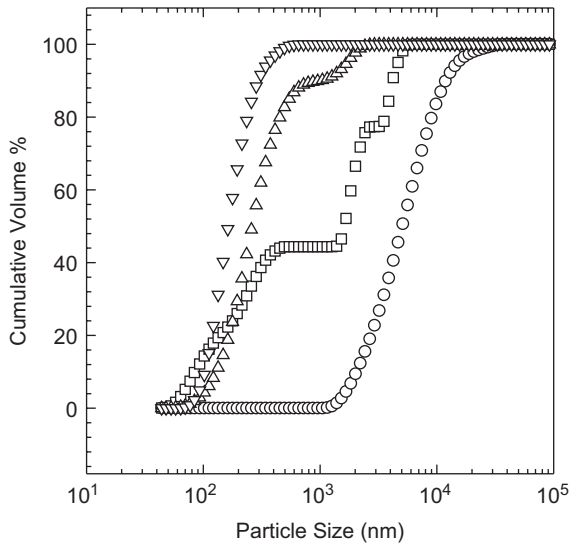

Figure 9. Effect of shear on the particle size distribution of Griseofulvin during the addition of HPMC E15LV. HPMC E15LV addition was performed either in the stirred media mill (SMM, Run 11) or in the low shear mixer (LSM, Run 12). Initial milling for 30 min was performed in the stirred media mode.

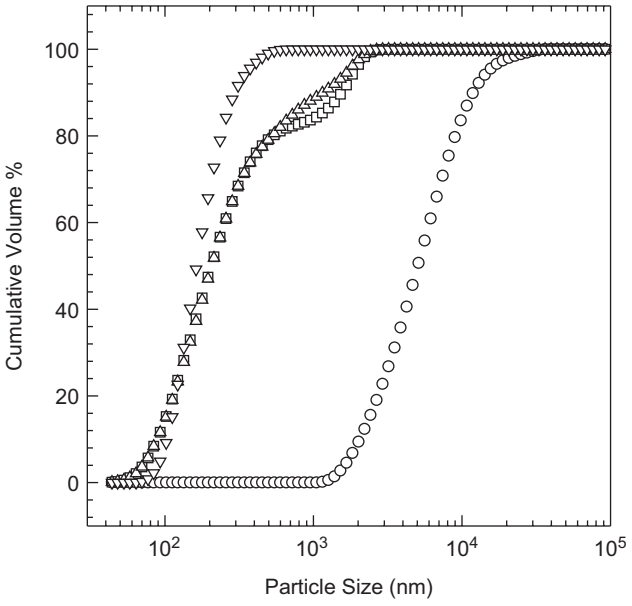

Figure 10. Effect of method of addition of HPMC E15LV on the particle size distribution of Griseofulvin. All processing was performed in the stirred media mill.

affect polymer–drug particle interactions and gradually affect the suspension rheology. The dispersion/dissolution/adsorption of the polymeric particles, stabilizing effectiveness of the polymer on the drug particles with time-dependent size and surface area, and suspension viscosity are all affected dynamically by the polymer addition method. These aspects should be extensively investigated in future studies with different drug–polymer formulations in the absence and presence of surfactants.

Conclusions

Stabilization effectiveness of four different classes of stabilizers and extent of breakage of six poorly water-soluble drugs have been assessed in the attritor mode of milling, which lead to the conclusion that steric stabilization works better than electrostatic stabilization. Extent of breakage tends to be higher for drugs with relatively high solubility, although no strong correlation or theoretical foundation exists. The concentration of polymer required for stabilization of the nanoparticles is higher than that for the microparticles due to higher specific surface area of the former. There exists a minimum stabilizer concentration or stabilizer–drug ratio to stabilize the suspensions. The major role of a non-ionic polymer during the production of fine particles is determined to be its stabilization action through steric effects, and no experimental evidence was found to support the Rehbinder effect.

Microsuspensions and nanosuspensions of poorly water-soluble drugs can be produced either in the attritor milling mode or in the stirred media milling mode. However, the stirred media mode offers significant advantages over the attritor mode as it allows a much faster breakage of particles than the attritor mode leading to shorter processing times. This study sheds light on the breakage dynamics of Griseofulvin during milling and the complex interplay between breakage and aggregation of particles. Milling mode, polymer concentration, and polymer addition method all affected the competition between breakage and aggregation. Although nanoparticles are produced even in the absence of stabilizers, they aggregate fast and extensively. Only upon proper stabilization, nanoparticles can be fully recovered.

We have demonstrated a novel processing route by adding a polymeric stabilizer to a drug suspension periodically during batch, wet stirred media milling. The periodic addition of the polymer yielded the narrowest and finest PSD. Running the mill by periodic addition of stabilizer(s) may also reduce processing time and total power consumption when a specific mean particle size is targeted. The method can be easily modified for periodic or end-point addition of surfactants either alone or in combination with polymers. Moreover, surfactants and polymers can be added sequentially: e.g. periodic polymer addition followed by surfactant addition toward the end. Depending on the presence or absence of a surfactant, polymer and surfactant synergy, and operation mode (batch vs. continuous recirculation), the effects stemming from different addition methods can be reduced or magnified. The findings from the current batch milling study can also be extended to continuous recirculation milling that is commonly used in the pharmaceutical industry at small, pilot, and large scales. In this mill, the stabilizer(s) can be added periodically or continuously to the suspension holding tank either as powder or as aqueous solution so as to implement the process improvement.

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Declaration of interest

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