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# A Combined Isolation and Formulation Approach to Convert Nanomilled Suspensions into High Drug-Loaded Composite Particles That Readily Reconstitute

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Abstract: The advantage of nanoparticles to improve bioavailability of poorly soluble drugs is well known. However, the higher-energy state of nanoparticles beneficial for bioavailability presents challenges for both the stability of nanosuspensions and preventing irreversible aggregation if isolated as dry solids. The aim of this study is to explore the feasibility of an evaporation isolation route for converting wet media milled nanosuspensions into high drug-loaded nanocomposites that exhibit fast redispersion in aqueous media, ideally fully restoring the particle size distribution of the starting suspension. Optimization of this approach is presented, starting from nanomilling conditions and formulation composition to achieve physical stability post milling, followed by novel evaporative drying conditions coupled with various dispersant types/loadings. Ultimately, isolated nanocomposite particles reaching 55–75% drug load were achieved, which delivered fast redispersion and immediate release of nanoparticles when the rotary evaporator drying approach was coupled with higher concentration of hydrophilic polymers/excipients. This bench-scale rotary evaporation approach serves to identify optimal nanoparticle compositions and has a line of sight to larger scale evaporative isolation processes for preparation of solid nanocomposites particles.

**Keywords:** dissolution enhancement; drug nanoparticles; polymers; redispersibility; rotary evaporation; co-processed active pharmaceutical ingredient; continuous manufacturing

## 1. Introduction

About 40% of marketed drugs and 90% of newly developed drugs in the pipeline of pharmaceutical companies are categorized as poorly water-soluble, which originates from their high molecular weight and hydrophobicity [1]. Due to their poor water solubility, such drugs dissolve slowly in vitro and in vivo, resulting in slow absorption and, thus, low bioavailability, which is not conducive to developing effective therapeutic medicines [2]. Widely used approaches for mitigating the slow dissolution rates of these drugs include nanoparticles–nanocomposites [3,4], amorphous solid dispersions [5], co-crystals [6], cyclodextrin complexes [1], and lipid-based drug-delivery systems [7]. These approaches enhance drug dissolution rate and bioavailability through manipulation of the drug particle size/surface area and/or kinetic solubility. Specifically, nanoparticles in the form of nanosuspensions or their dried form (nanocomposites) continue to be a proven approach considering more than a dozen nano-formulated drugs approved by Food and Drug Administration (FDA) [8]. As solid dosage forms such as tablets, capsules, etc., are preferred by patients over liquid dosage forms due to the convenience of the former [9], drug nanosuspensions are usually dried into nanocomposite powders prior to their incorporation into solid oral dosages [10]. To convert these nanosuspensions into nanocomposites, drying processes such as spray drying [11–14], fluidized bed coating [3,15], lyophilization [16,17],



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). vacuum drying [16,18], nanoextrusion [19,20], and spray-freeze drying [4] have been used. All methods aim at evaporation and removal of a solvent from the suspension, leaving behind a solid, drug-loaded nanocomposite. Readers are referred to Bhakay et al. [10] for a recent survey and a comprehensive review of the commercially relevant drying processes as well as their pros/cons.

In the pharmaceutical nanotechnology literature, drug nanosuspensions typically refer to aqueous solutions of various dispersants in which drug particles ranging 50–500 nm in size, but sometimes up to 1000 nm particles, are suspended [10,21]. Dispersants such as polymers and surfactants dissolve in water and provide physical stability to the nanoparticles by preventing their aggregation [22]. The most common and widely used method of milling a suspension, especially with the objective of producing a drug nanosuspension, is wet stirred media milling, also known as nanomilling [8,10,23]. This process has the capability of producing high drug-loaded, stable nanosuspensions and it is a robust, reproducible, scalable, solvent-free, and environmentally benign process [21,24]. It is also beneficial in its ability to have a continuous stirred process that can be cooled with the assistance of a jacket for sensitive drug compounds compared to that of other techniques such as LabRAM® vibratory milling [11,25], although a LabRAM® vibratory mill can handle much smaller sample sizes. Being versatile, the wet stirred media mill can be set up in batch mode, single-pass/multi-pass continuous mode, or in continuous recirculation mode wherein the suspension recirculates through the media chamber filled with milling beads and a holding tank [26].

Frequent collisions of drug nanoparticles stemming from turbulent shear during milling and Brownian motion during storage [27] could cause particles to aggregate through attractive interparticle forces such as van der Waals force, hydrophobic interactions, etc. [28,29]. Soluble polymers have been widely used as stabilizers to suppress aggregation, while they also serve as matrix/film formers upon drying of the nanosuspensions into drug nanocomposites [10,30]. The polymer's molecular weight, its adsorption onto drug surfaces, and viscosity could have an impact on the extent of aggregation in drug nanosuspensions [11,18]. In addition, surfactants could enhance the physical stability of the drug nanosuspensions and wettability of the nanocomposites, but their use could pose several challenges, especially during storage [7,21,30]. The latter aspect is related to the growth of the drug particles via Ostwald ripening [31,32]. Moreover, anionic surfactants are associated with additional challenges such as gastrointestinal tract irritation, incompatibilities with other ionic molecules, sensitivity to temperature, pH, or salt changes, [33,34], and even toxicity at high concentrations [34]. Hence, surfactants must be judiciously selected, and their usage should be minimized, especially if the suspension is to be used as the final product for ocular and intravenous applications.

Besides the challenges associated with the production of drug nanosuspensions, additional challenges emerge when they are converted into nanocomposites via drying [10]. Not only can drug nanoparticles aggregate in the milled suspensions during milling– storage, but they can also agglomerate, sometimes forming hard, irreversible aggregates (agglomerates) during the drying of the nanosuspensions [30]. Regardless of its mechanistic origin, formation of aggregates could effectively reduce the dissolution enhancement that is expected from drug nanoparticles. Another challenge is the slow and/or low extent of nanoparticle recovery during redispersion (reconstitution) of the nanocomposites in a relevant liquid, which occurs during in vitro and in vivo dissolution. The redispersibility of the nanocomposites is, of course, affected negatively by the presence of the aggregates. At this juncture, it should be mentioned that dispersants play a major role in mitigating the aggregation and enhancing the redispersibility of the nanocomposites, thus increasing the drug release when they are dissolved in vivo or in vitro [11,30,35,36].

The ability of nanocomposites to readily reconstitute in aqueous media to achieve the particle size of the initial suspension is critical. Redispersibility tests could be a more important indicator of in vivo performance compared with standard dissolution tests, when considering the state of undissolved drug reservoirs, and their ability to rapidly dissolve

Biopharmaceutical Classification System (BCS) Class II drugs for which solubility, not absorption, limits bioavailability [11,36]. Hence, in vitro performance of drug nanocomposites has been assessed via both redispersion and dissolution tests. Multiple groups performed dissolution testing as the method of characterizing the differences in formulations and their enhancement of the drug release [3,5,11,15,37,38]. Although dissolution testing is a staple of understanding the performance of a formulation, it is not always predictive of in vivo performance. The use of redispersion testing could complement our understanding of drug nanocomposites, which may be more discerning than traditional dissolution tests and more representative of the in vivo conditions [35,36]. Hence, it is not surprising to find that several studies made use of redispersion tests to examine the impact of various formulation–processing parameters on the nanoparticle recovery from the nanocomposites [16,18,36,39–41].

In this study, we focus on the feasibility of using a rotary evaporator, which utilizes heat and vacuum to evaporate a solvent, to generate a dry nanocomposite for downstream testing and characterization. This equipment has been reported to generate amorphous solid dispersions [42] as well as cocrystals [43] of drug formulations. Rotary evaporation did not appear in the comprehensive literature search by Bhakay et al. [10]. To the best knowledge of the authors, no comprehensive study on the production of drug nanocomposites by rotary evaporation of wet-milled drug suspensions and their characterization in terms of redispersibility exists [44].

The aim of this study is to explore the feasibility of rotary evaporation for converting wet-milled drug suspensions into high drug-loaded nanocomposites that exhibit fast redispersion and immediate drug release in aqueous media. To this end, itraconazole (model BCS Class II drug) suspensions with various stabilizers (polymers-surfactants) were wet milled in a stirred media mill. The physical stability of the suspensions after the addition of dispersants was assessed. The drying of the nanosuspensions was performed by a rotary evaporator. Evolution of this bench scale process to generate solid nanocomposites included modifying the method for feeding the nanosuspension and a process to normalize the particle size of the bulk nanocomposite particles, which varied as the formulation compositions were changed. A coarse mortar-pestle milling of the nanocomposites resulted in a dried powder with more consistent properties, and improved consistency during redispersion. The effects of drug particle size, nanocomposite particle size, and type/loading of various dispersants on aqueous redispersion and drug release from the nanocomposites during USP II dissolution tests were investigated. Besides generating fundamental insights through the analysis of the redispersion-dissolution test results, this study will establish rotary evaporation, a widely available laboratory drying method, as a potential bench-scale surrogate for commercial continuous drying processes in the production of high drug-loaded, fast redispersible drug nanocomposites. We also highlight some of the advantages of the rotary evaporation by comparing it to other widely used drying techniques such as spray drying and fluidized bed drying at the bench scale based on existing literature and our own internal knowhow.

## 2. Materials and Methods

## 2.1. Materials

Itraconazole (ITZ) was selected as a model BCS Class II drug. It was purchased from Green Chempharm Inc. (Bardonia, NY, USA). Its solubility in deionized water is  $0.002 \ \mu\text{g/mL}$  at 37 °C [45]. Hydroxypropyl cellulose SL grade (HPC SL) with an average molecular weight (MW) of 100,000 g/mol was obtained from Nisso America Inc. (New York, NY, USA). Polyvinylpyrrolidone K30 (PVP K30) with an average MW of 50,000 g/mol was purchased from AppliChem GmbHP (Darmstadt, Germany). Polyethylene Glycol 3350 (PEG 3350) with an average MW of 3350 g/mol was obtained from Spectrum Chemical MFG Corp. (Gardena, CA, USA). Kollidon VA64 (VA64) with an average MW of 57,500 g/mol was purchased from BASF (Lampertheim, Germany). Pluronic F-127 (F-127), a neutral polymeric surfactant with an average MW of 12,600 g/mol, was purchased from Sigma-

Aldrich (Milwaukee, WI, USA). In this study, HPC SL, PVP K30, PEG 3350, VA64, and F-127 were used as stabilizers in the wet-milled drug suspensions and matrix/film formers in the dried nanocomposites. Sodium dodecyl sulfate (SDS), an anionic surfactant, was purchased from Sigma-Aldrich (Milwaukee, WI, USA). Yttrium-stabilized zirconia, which is highly wear resistant, with a nominal size of 400  $\mu$ m was purchased from Norstone Inc. (Bridgeport, PA, USA).

#### 2.2. Methods

2.2.1. Formulations and Wet-Stirred Media Milling

All formulations of the precursor suspensions used in the rotary evaporation are presented in Table 1. The percentages of dispersants within each formulation are on a w/w basis with respect to the total weight of deionized water (300 g). All suspensions had identical (10%) ITZ content. The basis for all formulations stems from a known effective suspension composition of 10% ITZ–2.5% HPC-SL–0.2% SDS, which has been shown to yield stable ITZ suspensions after wet stirred media milling [11]. Hence, our baseline nanosuspension has this composition.

**Table 1.** Formulations of the precursor itraconazole (ITZ) suspensions and theoretical drug content in the nanocomposites.

Polymer Type/Grade and Mass	Dolume or MM	Suspension	Theoretical Drug	
Ratio (Polymer 1:Polymer 2)	(g/mol)	Total Polymer (% w/w) <sup>1</sup>	SDS (% w/w) <sup>1</sup>	Content (% w/w) <sup>2</sup>
HPC-SL (Baseline)	100,000	2.5	0.2	78.7
HPC-SL	100,000	5	0.2	65.8
HPC-SL	100,000	7.5	0.2	56.5
HPC-SL	100,000	10	0.2	49.5
HPC-SL/PVP K30 (1:1)	100,000/50,000	5	0.2	65.8
HPC-SL/PVP K30 (1:2)	100,000/50,000	7.5	0.2	56.5
HPC-SL/PVP K30 (1:3)	100,000/50,000	10	0.2	49.5
HPC-SL/PEG 3350 (1:1)	100,000/3350	5	0.2	65.8
HPC-SL/PEG 3350 (1:2)	100,000/3350	7.5	0.2	56.5
HPC-SL/PEG 3350 (1:3)	100,000/3350	10	0.2	49.5
HPC-SL/VA64(1:1)	100,000/57,500	5	0.2	65.8
HPC-SL/VA64 (1:2)	100,000/57,500	7.5	0.2	56.5
HPC-SL/VA64 (1:3)	100,000/57,500	10	0.2	49.5
HPC-SL/F-127 (1:0.28)	100,000/12,600	3.2	0.2	74.6
HPC-SL/F-127 (1:1)	100,000/12,600	5	0.2	65.8
HPC-SL/F-127 (1:2)	100,000/12,600	7.5	0.2	56.5

<sup>1</sup> All suspensions have 10% ITZ. % w/w is with respect to the weight of deionized water (300 g). <sup>2</sup> % w/w is with respect to the weight of the nanocomposite, assuming negligible residual moisture.

The baseline nanosuspension with 10% ITZ–2.5% HPC-SL–0.2% SDS was prepared similar to [11]. An overhead mixer (Chemglass, CG-2051-020, Vineland, NJ, USA) was setup over an 800 mL beaker to dissolve HPC-SL and SDS first, followed by dispersion of the ITZ particles. This suspension was then transferred over to a holding tank on the Netzsch wet-stirred media mill (Minicer, Netzsch, Selb, Germany). Milling conditions were adapted from a previous milling study using the Netzsch Microcer with a milling chamber volume of 80 mL, 196 g bead loading of 0.4 mm zirconia beads retained by a 0.2 mm screen, 126 mL/min recirculation rate of a 200 mL suspension, and a rotor speed of 4000 rpm [11]. Our purpose was not to perfectly scale up the Microcer process to the Minicer process, but create a similar milling environment conducive to production of ITZ nanoparticles within a time-scale of 65 min. Milling in the Minicer with the 160 mL chamber was performed using between 343–525 g of 0.4 mm zirconia beads and a screen opening size of 0.15 mm. The suspension was recirculated through the milling chamber at a rate between 189–252 mL/min with a Masterflex L/S peristaltic pump (Radnor, PA, USA) and

C-Flex L/S 17 tubing while milled at a rotor speed of 4000 rpm for 65 min. The milling chamber and holding tank were both cooled by a chiller (Huber Unistat 405w, Huber, Offenburg, Germany) to maintain the temperature of the suspension below 33 °C. Samples were taken from the outlet of the mill at various time points to track the progression of milling over time.

The baseline nanosuspension prepared above was mixed with another polymeric dispersant, or additional HPC SL, (see Table 1), to prepare precursor suspensions for drying with the ultimate objective of assessing the impact of various dispersant type/loading on the redispersion–drug-release from the nanocomposites. A stir bar within a secondary vial was used to mix the additional dispersant with the baseline nanosuspension to completely dissolve it in the nanosuspension. By doing so, we were able to test the redispersion effectiveness of a single polymer (HPC-SL) vs. binary polymers (HPC-SL–additional dispersant) at the same total polymeric dispersant concentrations of 5%, 7.5%, and 10% besides the 0.2% SDS present in all suspensions. The PSDs of the precursor suspensions were measured by laser diffraction prior to drying, ensuring no aggregation occurred during production and storage. All samples were prepared and dried within 7 days of preparing the baseline nanosuspension, which allowed us to test for short-term physical stability of the milled suspension [11,12]. All suspensions were stored in a fridge at <5 °C before drying.

## 2.2.2. Preparation of Nanocomposites via Rotary Evaporation of the Precursor Suspensions

The precursor ITZ suspensions were dried within 7 days of milling using a Rotavapor R-300 (Buchi, New Castle, DE, USA). The unit (Figure 1) was run in batch distillation mode where suspensions were placed within a round-bottom flask and "distilled" with a bath temperature of 60 °C. The vacuum was pulled down below 300 mm Hg absolute until the suspension sample was dry, evident of no condensation forming on the cold finger of the Rotavapor, followed by a 10 min hold under 10 mm Hg to ensure complete drying of the nanocomposite. Alternatively, the Rotavapor was operated in "feed and bleed" mode, where the suspension sample was fed into the round bottom via a tube with a valve while under a vacuum of 10 mm Hg absolute and a bath temperature of 90 °C. Suspension was fed intermittently, ensuring only a thin film of liquid at any given time in the round-bottom flask, until the entire precursor suspension was processed and condensation stopped, followed by additional 10 min drying to ensure complete evaporation of water.



**Figure 1.** Rotavapor setup for drying nanosuspensions using feed and bleed method: schematic of the setup (**left**) and the photograph of the actual setup (**right**).

The nanocomposite particles that adhered to the round-bottom flasks were removed using a combination of a Chem-spin scraper tool (Chemglass, Vineland, NJ, USA) and a spatula. The Chem-spin scraper was first used to collect the bulk of solids from within the round-bottom flask followed by manual removal using a spatula to collect the remaining solids. Due to variability in the state of the nanocomposite particles at this point, which changed based on the various polymer/surfactant systems, the collected nanocomposite samples were then transferred over to a mortar to be ground with medium-to-light pressure, thus improving the homogeneity of each sample and reproducibility during the redispersion tests.

#### 2.2.3. Particle Size Analysis

Particle size distribution (PSD) of all suspensions was measured by a Mastersizer 3000 laser diffraction particle size analyzer with Hydro MV cell (Malvern Panalytical, United Kingdom) using red and blue light and a detection range of 0.01  $\mu$ m to 3500  $\mu$ m. Dispersant cell was set at a stir rate of 1500 rpm and sonicated for 30 s at 60% intensity. Mie scattering theory was used to compute the volume-based PSD with a refractive index of 1.68 for ITZ and 1.33 for deionized water [11]. An alignment of the system followed by a background measurement of 10 s for red and 10 s for blue light was taken before each set of readings. Three measurements (averaged) were taken and reported following the ISO model within the Malvern Software. During measurement, the sample was added until obscuration fell between the ranges of 3 to 8%.

The particle size of the isolated nanocomposite particles was measured by HELOS/KR laser diffraction sensor in combination with the RODOS dispersion unit (Sympatec, Pennington, NJ, USA) applying Fraunhofer diffraction theory. Three measurements were averaged to obtain a stable reading. Each sample went onto the vibratory chute set at 65% intensity and a dispersion pressure of 1.0 bar was used. To capture the wide variety of PSDs, the R6 lens, which had a measurement range of 9–1750  $\mu$ m, was used for all measurements.

#### 2.2.4. SEM/EDS Imaging of the Nanocomposites

Imaging was performed using a Hitachi SU5000 (Hitachi, Japan). An aluminum stub with carbon tape was sputter coated with platinum under argon gas using a current of 30 mA for 4.75 min. The accelerating voltage was 12 kV with a 70% spot intensity for BSE detector under low vacuum. EDS was performed using an energy range of 20 KeV across 2048 channels, which was processed in 4 s. Acquisition time of the spectrum was 20 s and pulse pile-up correction was set to on. Chloride (Cl) was used to image ITZ and carbon (C) was used to image HPC, which were represented with a purple and green color, respectively, to better distinguish the two components.

## 2.2.5. Redispersion of the Nanocomposites

The redispersion of the dried nanocomposites produced by rotary evaporation was performed using the method established in [11]. A 50 mL beaker with 30 mL of 3.0 g/L aq. SDS solution was placed under an overhead mixer (Chemglass, CG-2051-020, Vineland, NJ, USA) set at a speed of 400 rpm with a 4 blade 25 mm downward-pitched impeller on a Mettler Toledo stir shaft. Nanocomposite samples containing 0.394 g ITZ equivalent were mixed with the SDS solution in the beaker at room temperature (see Table 1 for drug content in each formulation). A 0.5 mL aliquot from the mixed suspension was taken at three different time points of 2 min, 10 min, and 30 min, and the PSD was subsequently measured by laser diffraction. Two runs were performed for each sample to obtain an average and show reproducibility in the results. Considering the formulations and the aqueous redispersion medium volume, we note that (i) the dispersants could completely dissolve and release the ITZ particles with little ITZ dissolution thermodynamically, and that (ii) the redispersion medium cannot attain a F-127 concentration close to the critical micelle concentration (CMC) of 0.7% w/w F-127 for the respective nanocomposite [11,46].

## 2.2.6. ITZ Content, ITZ Solubility, and Dissolution Performance

Actual drug content of three select nanocomposite formulations, i.e., 2.5% HPC-SL (baseline), HPC-SL/F-127 (1:1) equivalent to 2.5% HPC-SL–2.5% F-127, and HPC-SL/PEG 3350 (1:1) equivalent to 2.5% HPC-SL–2.5% PEG, was measured by assay testing. First, 100 mg of the dried powders was dissolved in 20 mL dichloromethane (DCM),

sonicated for 50 min to ensure dispersion of ITZ, and then stored overnight for complete ITZ dissolution. An aliquot of 100  $\mu$ L was taken from the supernatant and diluted to 10 mL with DCM. The absorbance of all the samples was measured at 260 nm wavelength via Ultraviolet (UV)-spectrophotometer (Agilent, Santa Clara, CA, USA). Six replicates (*n* = 6) were tested for each formulation to calculate mean drug content and percent relative standard deviation (RSD).

Dissolution testing of ITZ nanocomposites was performed using a Distek 2100C dissolution tester (North Brunswick, NJ, USA) according to the USP II paddle method [11]. ITZ solubility was determined using the same dissolution test method below, except for the solubility measurement, excess drug (500 mg) was dispersed in the dissolution medium for two days at 37 °C. The ITZ solubility in the dissolution medium (1000 mL 3.0 g/L aq. SDS solution, the same medium as was used in the redispersion test) was found to be  $16.9 \pm 0.2$  mg/L. The dissolution medium was maintained at 37 °C and stirred by a paddle at 50 rpm. In the dissolution tests, nanocomposites equivalent to a dose of 20 mg of ITZ were added to the medium, and 4 mL samples were taken manually at 1, 2, 5, 10, 20, 30, and 60 min. This drug amount guaranteed non-sink dissolution conditions, which could allow for better discrimination among the nanocomposite formulations [47,48], while possibly revealing any supersaturation that can emanate from the presence of nanocrystalline or amorphous ITZ. For the fastest dissolving nanocomposite, i.e., HPC-SL/F-127 (1:1) equivalent to 2.5% HPC-SL-2.5% F-127, a 210 min time sample was taken to see if any sustained supersaturation occurred. The nanocomposite weight was determined by the theoretical drug content for each formulation. The absorbance of ITZ dissolved in the media was measured via UV spectroscopy (Agilent, Santa Clara, CA, USA) at 260 nm wavelength. Aliquots of the samples were filtered using a 0.1  $\mu$ m PVDF membrane-type syringe filter to avoid any effect of undissolved drug during UV spectroscopy. The medium solution without drug was used as the blank. The amount of drug dissolved was measured using a calibration curve generated in [11], with an  $R^2 = 0.9995$ . ITZ release was reported as a function of dissolution time for an average of six replicates. Additionally, >80% drug release within 20 min was taken as a strict criterion for immediate drug release [11,35]. Dissolution profiles of all nanocomposites were statistically compared using difference  $(f_1)$  and similarity  $(f_2)$  factors [49].  $f_1$  values up to 15 (0–15) and  $f_2$  values greater than 50 (50–100) suggest statistical similarity of two profiles [49].

#### 3. Results

## 3.1. Itraconazole Nanosuspensions Prepared via Wet Stirred Media Milling

## 3.1.1. Development of a Feasible Milling Process

As-received ITZ particles had a median size  $D_{50}$  of 15.5 µm and 90% passing size  $D_{90}$  of 45.8 µm, as measured via Rodos/HELOS laser diffraction system [11]. The suspension with the baseline formulation (10% ITZ–2.5% HPC-SL–0.2% SDS) was milled for 65 min with the goal of producing nanosuspension with all particles below 1 µm, preferably with a median size below 200 nm, to ensure that significant dissolution enhancement can be achieved. Figure 2 illustrates the time-wise evolution of characteristic particle sizes, i.e.,  $D_{50}$ ,  $D_{90}$ , and the cumulative volume percentage (passing) of colloidal/nanoparticles Q (1 µm) for multiple milling runs, which were performed with the same baseline formulation under different milling conditions. All runs exhibited a monotonic decrease in  $D_{50}$  and  $D_{90}$  and an increase in Q (1 µm), which suggests particle breakage is the dominant mechanism, and severe aggregation of the milled particles did not occur, confirming the feasibility of the baseline formulation. The sizes tended to approach a  $D_{50}$  of ~0.130 µm and a  $D_{90}$  of ~0.330 µm. This corresponds to a remarkable size reduction ratio of ~120 in 65 min based on  $D_{50}$ , which is hard to achieve with most size-reduction equipment.



**Figure 2.** Timewise evolution of characteristic drug particle sizes during the wet media milling at various bead loadings and suspension flow rates: (**a**)  $D_{50}$ , (**b**)  $D_{90}$ , and (**c**) Q (1 µm). All suspensions have 10% ITZ–2.5% HPC-SL–0.2% SDS.

Although the milling conditions did not cause drastic changes in the final milled particle sizes, the total experimental effort/time was affected due to excessive heat generation at the higher bead loading (525 g) and associated multiple shutdowns of the mill to maintain the suspension temperature below 33 °C. Hence, milling at a bead loading of 392 g and a suspension flow rate of 189 mL/min were deemed to be the best conditions overall for the Minicer equipment. These conditions in the Minicer equipment correspond to identical bead volume fraction, i.e., 0.41, and total number of batch turnovers through the mill, i.e., 41, to those of the Microcer equipment used in [11], thus ensuring a similar milling environment. It is also worth mentioning that these conditions yielded similar product particle sizes to those at the higher bead loading (525 g), but without frequent shutdowns of the mill due to high temperature rise.

## 3.1.2. Physical Stability of the Milled Drug Suspensions

The baseline nanosuspension (10% ITZ–2.5% HPC-SL–0.2% SDS) was prepared fresh for each precursor formulation in Table 1, and its PSD was measured immediately following milling (after milling samples in Figure 3). Within 7 days, an additional dispersant or additional HPC-SL was dissolved in the nanosuspension to prepare the precursor suspension, which was then dried. Since each additional dispersant selected was a water-soluble polymer that has been commonly used as stabilizers in drug nanosuspensions [21] and the baseline suspension was stable, we did not expect a complete destabilization of the milled nanosuspensions. To confirm this hypothesis, the particle sizes of each precursor suspension (see formulated stability samples in Figure 3) were measured for comparison with the particle sizes of the baseline nanosuspension.



Stabilizer and Concentration with Respect to Suspension

Figure 3. Particle size of the milled suspension and effect of the addition of polymeric dispersant: (a)  $D_{50}$  and (b)  $D_{90}$  (n = 3). All suspensions have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).

The starting particle size in the freshly prepared baseline nanosuspensions is almost identical, typically within a few percent of the average size, as they have identical baseline formulation, which demonstrates the reproducibility of the wet-stirred media milling process. Adjacent to the particle size of the baseline suspensions is that of the additional dispersant that was added after 7 days to each formulation. Aside from HPC-SL at higher concentrations, the addition of different dispersants led to a <10% increase in  $D_{50}$  and <25% increase in  $D_{90}$ . Additional HPC-SL alone exhibited the highest increase: up to 15% in  $D_{50}$  and up to 31% increase in  $D_{90}$ . The precursor suspensions remained colloidal; all final nanosuspensions had  $D_{50}$  below 150 nm and  $D_{90}$  below 400 nm, except the 10% HPC-SL nanosuspension.

With formulations containing F-127, a triblock copolymer (a polymeric surfactant), the particle size was tested 12 days after preparation with 5% concentration. This was performed to show stability and investigate Ostwald ripening which can occur at concentrations above the critical micellization concentration (CMC) of F-127 as is the case with formulations containing more than 0.7% F-127 [27,45]. Looking at the particle size of the formulated suspension in Figure 3, one can see there was no significant growth to the nanoparticles during the extended hold time that would be cause for concern with Ostwald ripening.

#### 3.2. Preparation of Nanocomposites

The precursor nanosuspensions were dried by the Rotavapor to form the nanocomposites. During initial exploratory utilization of the equipment, a bath temperature of 60 °C and the distillation approach for the suspension feed were first tested using the baseline nanosuspension with 10% ITZ–2.5% HPC-SL–0.2% SDS. This initial test yielded a nanocomposite that was very coarse when scraped off the round-bottom flask and posed numerous challenges for generating reproducible particle size readings, as well as gathering further downstream data in other analyses. Table 2 presents the particle size variation in the samples obtained from scraping the nanocomposite off the round-bottom flask.

Measurement	Formulation <sup>1</sup>	Nanocomposite Particle Size			
		D <sub>10</sub> (μm)	D <sub>50</sub> (μm)	D <sub>90</sub> (μm)	D <sub>vm</sub> (μm)
1	2.5%HPC-SL	82	240	739	336.1
2	2.5%HPC-SL	97	290.2	731.7	356.2
3	2.5%HPC-SL	150.5	457.6	1372	614.2
4	2.5%HPC-SL	161.4	612.9	1476	747.1
Aver	age (µm)	122.7	400.2	1080	513.4
SD (µm)		33.9	146.9	346.3	173.9
Relative SD (%)		27.6	36.7	32.1	33.9

**Table 2.** Characteristic sizes of the nanocomposite particles obtained from rotary evaporation of the precursor suspension with 10% ITZ–2.5% HPC-SL–0.2% SDS (distillation approach).

 $^1$  Baseline aqueous precursor nanosuspension contains 10% ITZ–2.5% HPC-SL–0.2% SDS.

To improve processability and reproducibility, we augmented the use of the Chemspin as well as mortar–pestle milling to the post processing of each rotary-evaporated sample. Two time points of 5 min and 10 min were explored. The mortar–pestle milling for 5 min returned a much more homogenous (reproducible) nanocomposite, signified by the lower SD values of the measured sizes as compared with that of the unprocessed nanocomposites (Figure 4). With the additional use of mortar and pestle up to 10 min, a slight improvement was seen over the 5 min reading with the  $D_{90}$  SD (Figure 4). In view of these data, we decided to use 5 min mortar–pestle milling because 10 min of mortar– pestle was a physically demanding task to execute for little to no improvement for most nanocomposite samples.



**Figure 4.** Characteristic sizes of the nanocomposite particles before and after 5 min and 10 min mortar–pestle (M-P) milling (n = 3). Baseline precursor nanosuspension contains 10% ITZ–2.5% HPC-SL–0.2% SDS.

#### **Evaluation of Rotary Evaporation Conditions**

While a more homogenous nanocomposite sample was generated upon 5 min mortarpestle milling, there were still challenges with downstream testing and release of nanoparticles from the HPC-based nanocomposites, as will be discussed in Section 3.3. HPC has a very low cloud point of ~40 °C despite its very good solubility at room temperature, which originates from the aggregation of the polymer chains [50]. This phenomenon could negatively affect the suspension stability and, more importantly, nanoparticle recovery during the redispersion of the nanocomposite if, upon precipitation, the HPC no longer forms a continuous phase to prevent nanoparticles from aggregating. Herein, the rotary evaporation drying method was switched from the bulk distillation mode to a feed and bleed mode, wherein a tube was passed into the round-bottom flask and using a vacuum, with suspension fed intermittently into the flask that was heated at 90  $^{\circ}$ C under 10 mmHg absolute vacuum. This process allowed for near instantaneous evaporation of the water from the suspension (fast drying) and potentially lower temperatures due to evaporative cooling. The precursor suspensions were processed utilizing this technique for minimizing hard aggregate formation, followed by mortar-pestle milling to ensure the homogeneity of the nanocomposites.

The particle sizes of the nanocomposites prepared are presented in Figure 5 (see Table S1 of the Supplementary Materials for tabulated values). Due to different types/molecular weight/loadings of the polymers and surfactants and perhaps their interactions, as well as variations in the processing–sampling, the nanocomposite particle sizes varied (Figure 5). While the median size  $D_{50}$  ranged from 62 µm to 288 µm, 11 out of 16 nanocomposites had a  $D_{50}$  within 100–150 µm. Similarly, while  $D_{90}$  ranged from 214 µm to 680 µm, 12 out of 16 nanocomposites had a  $D_{90}$  within 300–450 µm. The largest differences occurred when additional HPC was used from 2.5–7.5% in the precursor suspensions. It is likely that 5 min mortar–pestle milling was not sufficiently long for the HPC-based nanocomposites. We also note from Figure 5 that the standard deviation (SD) values were much smaller than those presented in Table 2 for the nanocomposites prepared without process optimization, i.e., feed and bleed drying followed by mortar–pestle milling. Establishing strong correlations



between the nanocomposite particle sizes and polymer properties will likely be difficult, as mortar-pestle milling had confounding effects.

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Figure 5. Characteristic sizes of the nanocomposites with different formulations prepared via rotary drying (with feed and bleed) followed by mortar-pestle milling: (a)  $D_{50}$  and (b)  $D_{90}$  (n = 3). All suspensions have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).

Using SEM and EDS techniques, we assessed the distribution of the ITZ (Cl in pink) and HPC (C in green) in the nanocomposites (Figure 6). This was in part to assess whether ITZ aggregation could be observed. A wide variety of particle sizes and shapes are visible, which resulted from the mortar-pestle milling. While the state of aggregated ITZ could not be confirmed by this approach, it did reveal features of the particles' structure and

composition. The surface of the largest particle in the center does not show a high Cl (ITZ) content, whereas the surrounding much smaller particles exhibit more uniform and notable Cl content. It is possible that the nanocomposite particles were coated by an HPC film, and mortar–pestle milling exposed the inner surfaces of the nanocomposite particles with a higher concentration of ITZ.



**Figure 6.** SEM image (**left**) and EDS overlay (**right**) of the nanocomposites with 10% ITZ–2.5% HPC-SL–0.2% SDS and  $D_{10} = 10.8 \ \mu\text{m}$ ,  $D_{50} = 66.5 \ \mu\text{m}$ , and  $D_{90} = 187 \ \mu\text{m}$ . Cl for ITZ and C for HPC in the EDS image show the distribution of both components.

## 3.3. Testing Nanoparticle Recovery via Redispersion

Initially, a feed and bleed method for feeding the precursor nanosuspension followed by mortar–pestle milling was not used. When the prepared nanocomposites were redispersed in aq. SDS medium, the size statistics of the redispersed samples exhibited high variability (Table 3). The issue can be traced back to the wide variation of the  $D_{50}$  and  $D_{90}$ of the 2.5% HPC-SL nanocomposites (see Table 2).

**Table 3.** Characteristic particle sizes upon aqueous redispersion of the nanocomposites prepared by the rotary evaporator operating with the distillation mode of suspension feeding.

Formulation <sup>1</sup> –	Redispersed Particle Size <sup>2</sup>			
	$D_{50}\pm{ m SD}$ (µm)	$D_{90}\pm{ m SD}$ (µm)	$Q$ (1 $\mu$ m) $\pm$ SD (%)	
2.5%HPC-SL	$21.2\pm1.14$	$219.0\pm38.7$	$0.05\pm0.03$	
2.5%HPC-SL	$17.9\pm3.22$	$235.5\pm 64.8$	$1.06\pm0.04$	
10%HPC-SL	$36.2\pm1.76$	$226.5\pm11.8$	$3.56\pm0.08$	
10%HPC-SL	$0.16\pm0.01$	$8.11\pm8.01$	$88.8\pm3.2$	

<sup>1</sup> All precursor suspensions of the nanocomposites have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g); <sup>2</sup> particle size was measured at the end of a 30 min aq. redispersion of the nanocomposite (n = 2).

Following the results in Table 3, a 5 min mortar–pestle step was introduced as a downstream processing step. This allowed for greater reproducibility in runs during the redispersion testing and homogenized all the following samples used throughout the rest of the study. Continuing with mortar–pestle samples and running through a few formulations demonstrated there was an issue with nanoparticle recovery that was not captured in previous runs. With the issue of reproduceable runs resolved, the impact of the rotary evaporation conditions was assessed. The feed and bleed method of introducing the precursor nanosuspension was compared with the distillation method in terms of their impact on the redispersion. After this processing change, the following formulations were explored by redispersion as a comparison to the initial runs. When coupled with mortar–pestle milling afterwards, the feed and bleed method led to smaller redispersed particle sizes and a higher fraction of particles below 1  $\mu$ m (Figure 7). The particle sizes

of the generated nanocomposites were not very different between the feed and bleed approach,  $D_{50} = 66.5 \ \mu\text{m}$  and  $D_{90} = 187 \ \mu\text{m}$ , and the distillation approach,  $D_{50} = 72.6 \ \mu\text{m}$  and  $D_{90} = 198 \ \mu\text{m}$ . Hence, there does not appear to be a nanocomposite particle size effect on the redispersion of nanoparticles from the nanocomposites on this set of data.



**Figure 7.** Impact of the suspension feeding method and the mortar–pestle (M-P) milling on the particle sizes emanating from the 30 min aqueous redispersion (n = 2). All precursor suspensions of the nanocomposites have 10% ITZ–2.5% HPC-SL–0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).

While the process optimization (feed and bleed followed by particle size normalization via mortar-pestle milling) led to improvement in redispersion from the 2.5% HPC-SL nanocomposite, only 23% of the redispersed particles had sizes below 1 µm. Two strategies were followed to enhance the redispersibility: increasing HPC-SL concentration (single polymer) and using an additional polymer/polymeric dispersant in the precursor suspension. The particle sizes from the nanocomposites with various formulations after 30 min aqueous redispersion are illustrated in Figure 8. The trend of smaller redispersed particle sizes as signified by lower  $D_{50}$  and  $D_{90}$  as well as higher Q (1 µm) at the higher dispersant concentration for almost all formulations was obvious (except for VA 64). Upon an increase in HPC concentration, the HPC-based nanocomposites released particles with ~69% and 86% below 1 µm, while the baseline formulation (10% ITZ–2.5% HPC-SL–0.2% SDS) yielded only  $\sim$ 23% particles with sizes below 1  $\mu$ m. In other words, despite the increase in the nanocomposite particle sizes upon an increase in HPC concentration in the precursor nanosuspensions (refer to Figure 5), the redispersibility improved. This finding suggests that the composition/formulation of the nanocomposite has a more dominant effect on the redispersion than the nanocomposites' particle sizes, provided  $D_{50}$  and  $D_{90}$  are well below 1000  $\mu$ m, thanks to the optimized process with the feed and bleed suspension feeding and mortar-pestle milling.



**Figure 8.** Impact of polymer type/loading on the characteristic particle sizes upon 30 min redispersion of the nanocomposites (n = 2). All precursor suspensions of the nanocomposites have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).

Comparison of the Q (1 µm) results (Figure 8) from the binary polymers vs. the single polymer (HPC-SL alone) at the same total dispersant loading could reveal the synergistic– antagonistic impact of the additional dispersant on the redispersion. In general, the addition of F-127 had a strong synergistic effect, while that of PVP K30, and especially VA-64, had an antagonistic effect. Addition of PEG 3350 is somewhat neutral or with mixed results. The molecular origin of these synergistic–antagonistic effects of binary polymers vs. single polymer and polymer–drug intermolecular interactions warrant a separate investigation, which is beyond the scope of this study.

Figure 9 presents the % redispersibility of the nanocomposites, which is defined by:

$$\%R = \frac{D_{50,p}}{D_{50,r}} \times 100,\tag{1}$$

where  $D_{50,r}$  and  $D_{50,p}$  stands for the median sizes of the particles redispersed from the nanocomposites in 30 min and the particles in the respective precursor nanosuspension, respectively. Figure 9 confirms the trends mentioned above regarding the positive impact of total dispersant concentration as well as the synergistic–antagonistic effects of various additional polymeric dispersants. The former trend can be explained as follows: an increase in the fraction of the hydrophilic polymers improved the wettability of the relatively hydrophobic drug (ITZ), which facilitated the release of the ITZ particles from the nanocomposites [11]. Moreover, a higher polymer concentration also likely mitigated or lowered the extent of hard aggregate formation as more polymer is available to cover the surfaces of the drug nanoparticles [30].



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**Figure 9.** Redispersibility of the nanocomposites back to starting nanosuspension particle size. All precursor suspensions of the nanocomposites have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).

In Figure 10, we can see the time evolution of Q (1  $\mu$ m) during the aqueous redispersion of the nanocomposites with various loadings of an additional dispersant or with additional HPC-SL. Except for VA64, which showed non-monotonic timewise variation, in general, higher dispersant concentration led to higher Q values faster, and a plateau value was approached or established within 30 min.

PEG containing formulations, PEG 3350 and F-127 (a PEG containing triblock copolymer), not only generated a nanocomposite with improved redispersibility relative to other formulations, but also contained very desirable processing enhancement due to the waxy nature of PEG. As seen in Figure 11, the final scraped round-bottom from that of F127-based composite compared to that of VA64 was evident in the clarity of the flask.

The best redispersion performance (the lowest  $D_{50}$  and  $D_{90}$  as well as the highest Q and %R) was achieved by the addition of F-127. A cursory look at F-127 redispersion data in Figures 8–10 suggests that the additional 2.5–5% F-127 was the only additional dispersant formulation that resulted in a Q (1 µm) and %R above 90% with near instantaneous redispersion. Furthermore, with additional 0.7% F-127, the formulation was able to obtain redispersion comparable to that of other polymers which needed 7.5% additional dispersant. This drastic reduction in the use of additional dispersant is very desirable in the final formulations, as it allows a higher % drug loading (drug payload) in the nanocomposites. Hence, F-127 is very desirable in the formation of ITZ nanocomposites, with the efficient processing characteristics of PEG and enhancing the wettability of the nanocomposites during the redispersion owing to its surfactant-like properties, while keeping the drug payload as high as ~75% in the final nanocomposites.

Overall, the addition of a polymeric surfactant, F-127, to HPC-SL, the baseline polymeric dispersant, appears to enhance the redispersion the most, while addition of other polymeric dispersants did not seem to have a significant positive impact (PEG), or even had antagonistic effects (PVP K30 and VA64). This finding accords well with the synergistic positive effects of hydrophilic polymer–surfactant combination in stabilizing drug nanosuspensions and improving drug nanoparticle recovery during redispersion and drug release during the dissolution [10]. Hence, we investigated the dissolution enhancement with the F-127-based nanocomposite and the baseline nanocomposite below.



**Figure 10.** Timewise evolution of particle percentage below 1  $\mu$ m during the aqueous redispersion of the nanocomposites (*n* = 2). All precursor suspensions of the nanocomposites have 10% ITZ and 0.2% SDS. % w/w is with respect to the weight of deionized water (300 g).



**Figure 11.** Round-bottom flasks at the end of scraping. Higher clarity correlated to an easily processed and isolated nanocomposite. Flask on the left formulation with additional 5% VA 64 and flask on the right contains additional 2.5% F-127.

## 3.4. Content Uniformity of the Nanocomposites and Dissolution Enhancement of ITZ

We have determined the content uniformity of the nanocomposites for three different nanocomposite formulations: 2.5% HPC-SL (baseline), HPC-SL/F-127 (1:1) equivalent to 2.5% HPC-SL–2.5% F-127, and HPC-SL/PEG 3350 (1:1) equivalent to 2.5% HPC-SL–2.5% PEG. Note that all these formulations also have a baseline 0.2% SDS in their precursor suspensions. They contained 77.7%, 64.9%, and 65.1% ITZ, respectively. These values were within 1% of the theoretical drug content values reported in Table 1. The content uniformity was acceptable, as signified by all relative standard deviation (RSD) values below 6%: 4.7%, 3.7%, and 2.4%, respectively.

Figure 12 presents the dissolution profiles of as-received ITZ (unprocessed ITZ) powder, a nanocomposite powder prepared via rotary evaporation of the baseline (2.5% HPC-SL–2.5% F-127–0.2% SDS) nanosuspension, a microparticle composite (labeled "Unmilled") prepared with the same formulation by drying of the unmilled ITZ suspension, and a physical mixture (PM) of as-received ITZ with the formulation but prepared via simple blending. The as-received ITZ particles dissolved slowly due to low solubility of the drug and their large sizes ( $D_{50} = 15.5 \mu m$  and  $D_{90} = 45.8 \mu m$ ) and perhaps presence of drug particle aggregates; only 8% of ITZ dissolved after 60 min. Presence of the hydrophilic dispersants in a PM had a slightly positive, but almost negligible effect, whereas rotary evaporation of a suspension of the unmilled ITZ with the dispersants ("Unmilled") led to a composite with a more notable increase in drug release (22% at 60 min) due to more intimate contact of the dispersants with the coarse ITZ particles. These results suggest that without some significant size reduction of the ITZ particles and/or modification of the solid state of the crystals, it would be impossible to achieve immediate ITZ release (80% release within 20 min).



**Figure 12.** ITZ dissolution from the nanocomposite (2.5% F-127) compared to that from the physical mixture (PM) and the rotary evaporated suspension without nanomilling (Unmilled) (n = 6). PM, the nanocomposite, and the composite named Unmilled have 10%ITZ– 2.5%HPC-SL–2.5%F-127– 0.2%SDS. PM refers to a physical mixture (blend) of the ITZ particles with the dispersant powders, having identical composition. Unmilled refers to the rotary evaporated suspension of the drug prepared without nanomilling. The suspension for 2.5% F-127 was milled in the media mill and dried on the rotary evaporator. As-received ITZ data was obtained from [11].

The 10% ITZ-2.5% HPC-SL-0.2% SDS suspension was wet-milled, 2.5% F-127 was dissolved in it, and the resulting precursor nanosuspension was dried by the rotary evaporator. This nanocomposite (2.5% F-127) achieved immediate release (Figure 12) and 86.4% ITZ was released after 210 min. Noting that the crystalline drug solubility corresponds to 84.5% drug release in this non-sink dissolution test, slight supersaturation above the crystalline solubility at 60 min was noted, which could be attributed to the presence of drug nanoparticles (see e.g., [9]) and/or a small fraction of amorphous drug. Extensive characterization work with XRPD/DSC has already been performed in reference [11] on spray-dried ITZ nanocomposites with various grades (including SL) of HPC, HPMC E3, and PVP K30. They concluded that the crystalline nature of ITZ was preserved after wet-stirred media milling and spray drying, despite the formation of defects during the milling and potentially small fraction of amorphous ITZ. We do not expect a stark difference in terms of ITZ solid state for the similar nanocomposites in our study. This is partly supported by the fact that if there were notable amounts of amorphous ITZ in the rotary-evaporator-dried nanocomposites, the 2.5% F127 nanocomposite would attain 20 mg ITZ dissolution (100%) after 210 min, whereas only 86.4% dissolved after 210 min. Hence, the observed remarkable increase in the dissolution rate as compared with the "PM" and "Unmilled" samples is mostly due to the large surface area of the ITZ nanoparticles in the nanocomposites. These drug nanoparticles were present in the precursor nanosuspension (refer to Figure 3):  $D_{50} = 0.132$  $\mu$ m and  $D_{90} = 0.325 \mu$ m. The ITZ nanoparticles have about a 120-times larger external surface area than the as-received, unprocessed ITZ particles. Thus, the profiles in Figure 12 signify that wet-stirred media milling is the most important processing step that enhances the ITZ dissolution.

Figure 13 shows a slightly faster drug release from the F-127 nanocomposite that was subjected to additional dry-milling (a median size of 28.4  $\mu$ m) than that obtained from the standard 5 min mortar–pestle milling (a median size of 136.4  $\mu$ m). The smaller nanocomposite particles have a higher surface area and assist with the dissolution and redispersion performance. However, based on  $f_1$  = 7.82 and  $f_2$  = 65.6 (similar dissolution profiles), the nanocomposite particle size effect in this size range was not significant. One possible explanation could be that the discrimination power of the dissolution test is low due to the use of 3.0 g/L aq. SDS solution as the dissolution medium; however, SDS was needed to obtain detectable concentrations of the poorly soluble drug. One may optimize the SDS concentration to improve the discrimination power. On the other hand, a more likely explanation is that above a certain specific surface area or below a certain nanocomposite particle size, the drug release becomes less sensitive to the nanocomposite particle size. In a recent study [51], griseofulvin nanocomposites in the form of milled extrudates were prepared by nanoextrusion followed by milling with a median size range from 50 to 720  $\mu$ m, and a highly discriminating dissolution test method was used. That study established that when the milled nanocomposite particles had a specific external surface area above 0.03 m<sup>2</sup>/cm<sup>3</sup>, (or Sauter-mean diameter below 200  $\mu$ m), the impact on dissolution became less significant. So, the nonremarkable nanocomposite particle size dependence illustrated in Figure 13 agrees well with the behavior exhibited by the nanocomposites of other poorly soluble drugs.

## 3.5. Advantages of Rotary Evaporation Process for Preparing Drug Nanocomposites

This study utilized the rotary evaporation approach as a bench-scale surrogate for commercial continuous drying processes such as spray drying and thin-film evaporator drying [23,44]. While other dryers have been used at the bench scale, the rotary evaporator is appealing because (i) it is easily accessible and available in most labs, (ii) it spares material (requiring only 18 mL of suspension), (iii) typical yield is as high as 85%–99%, (iv) simple operation of controls, and (v) fast setup to dry samples for quick design of experiments. Bench-scale fluidized bed driers, spray driers, extruders, etc., are more expensive than rotary evaporators, and each of them suffers from one or more of the following problems: low yield (for most types of spray driers) [52] and the need for large quantity of suspension

(extruders) [53] or for substrate particles for coating (fluidized bed driers) [54]. Although the flow behavior of the nanocomposite powders was not characterized here, we speculate that the nanocomposites prepared via rotary evaporation will have better flowability than the spray-dried nanocomposites because the former had larger median sizes: typically 50–150  $\mu$ m vs. 8–25  $\mu$ m [11]. This rotary evaporation process also has line of sight both in terms of nanocomposite particle size and redispersibility behavior to larger scale drying via thin film evaporation [23,55].



**Figure 13.** Dissolution of the rotary evaporated nanocomposite with different median sizes (n = 6). Nanocomposites had 10% ITZ–2.5% HPC-SL–2.5% F-127–0.2% SDS. The same nanocomposite was milled longer to obtain a smaller particle size.

## 4. Conclusions

This study established the feasibility of rotary evaporation drying of wet-milled itraconazole nanosuspensions and examined the impact of various polymers on redispersion and drug release from the nanocomposites. In rotary evaporation drying, a feed and bleed approach proved to be a superior method of preparing the nanocomposites. Overall, isolated nanocomposites with high drug loading (up to 75%), exhibited complete redispersion in 30 min, and immediate release of ITZ could be achieved upon judicious combination of HPC-SL with F-127 in the presence of SDS. Compared to the baseline formulation with HPC-SL, in general, increasing the HPC-SL concentration or using a second hydrophilic dispersant enhanced the redispersibility. However, only the addition of F-127, a polymeric surfactant, led to strong synergistic effects, while the addition of PVP and VA64 was antagonistic and that of PEG was somewhat neutral with mixed results. This rotary evaporation approach can serve as a bench-scale surrogate for a commercial continuous drying process (e.g., thin-film evaporation) for preparation of drug nanocomposites owing to its ease of use, material sparing nature, and high yield.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10 .3390/powders1020008/s1, Table S1: Particle sizes of the nanosuspensions and the nanocomposites prepared via drying (with feed and bleed) followed by mortar–pestle milling; Table S2: Particles sizes of the nanocomposites prepared via drying (with feed and bleed) followed by mortar–pestle milling and particle sizes upon aqueous redispersion.

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## References

- 1. Loftsson, T.; Brewster, M.E. Pharmaceutical applications of cyclodextrins: Basic science and product development. *J. Pharm. Pharmacol.* **2010**, *62*, 1607–1621. [CrossRef]
- 2. Fasano, A. Innovative strategies for the oral delivery of drugs and peptides. *Trends Biotechnol.* 1998, 16, 152–157.
- 3. Bhakay, A.; Azad, M.; Vizzotti, E.; Dave, R.N.; Bilgili, E. Enhanced recovery and dissolution of griseofulvin nanoparticles from surfactant-free nanocomposite microparticles incorporating wet-milled swellable dispersants. *Drug Dev. Ind. Pharm.* **2014**, 40, 1509–1522. [CrossRef]
- 4. Niwa, T.; Danjo, K. Design of self-dispersible dry nanosuspension through wet milling and spray freeze-drying for poorly water-soluble drugs. *Eur. J. Pharm. Sci.* 2013, 50, 272–281. [CrossRef]
- 5. Jackson, M.J.; Kestur, U.S.; Hussain, M.A.; Taylor, L.S. Dissolution of danazol amorphous solid dispersions: Supersaturation and phase behavior as a function of drug loading and polymer type. *Mol. Pharm.* **2016**, *13*, 223–231.
- 6. Yadav, A.V.; Shete, A.S.; Dabke, A.P.; Kulkarni, P.V.; Sakhare, S.S. Co-crystals: A novel approach to modify physicochemical properties of active pharmaceutical ingredients. *Indian J. Pharm. Sci.* **2009**, *71*, 359–370. [CrossRef]
- Müllertz, A.; Ogbonna, A.; Ren, S.; Rades, T. New perspectives on lipid and surfactant based drug delivery systems for oral delivery of poorly soluble drugs. J. Pharm. Pharmacol. 2010, 62, 1622–1636.
- Malamatari, M.; Taylor, K.M.G.; Malamataris, S.; Douroumis, D.; Kachrimanis, K. Pharmaceutical nanocrystals: Production by wet milling and applications. *Drug Discov. Today* 2018, 23, 534–547. [CrossRef]
- 9. Kesisoglou, F.; Panmai, S.; Wu, Y. Nanosizing—Oral formulation development and biopharmaceutical evaluation. *Adv. Drug Deliv. Rev.* 2007, 59, 631–644. [CrossRef]
- 10. Bhakay, A.; Rahman, M.; Dave, R.N.; Bilgili, E. Bioavailability Enhancement of Poorly Water-Soluble Drugs via Nanocomposites: Formulation–Processing Aspects and Challenges. *Pharmaceutics* **2018**, *10*, 86. [CrossRef]
- 11. Bilgili, E.; Rahman, M.; Palacios, D.; Arevalo, F. Impact of polymers on the aggregation of wet-milled itraconazole particles and their dissolution from spray-dried nanocomposites. *Adv. Powder Technol.* **2018**, *29*, 2941–2956. [CrossRef]
- 12. Rahman, M.; Arevalo, F.; Coelho, A.; Bilgili, E. Hybrid nanocrystal—Amorphous solid dispersions (HyNASDs) as alternative to ASDs for enhanced release of BCS Class II drugs. *Eur. J. Pharm. Biopharm.* **2019**, *145*, 12–26. [CrossRef]
- 13. Chaubal, M.V.; Popescu, C. Conversion of nanosuspensions into dry powders by spray drying: A case study. *Pharm. Res.* **2008**, *25*, 2302–2308. [CrossRef]
- 14. Elham, G.; Mahsa, P.; Vatanara, A.; Vahid, R. Spray drying of nanoparticles to form fast dissolving glipizide. *Asian J. Pharm.* 2015, *9*, 213–218.
- 15. Azad, M.; Moreno, J.; Bilgili, E.; Davé, R. Fast dissolution of poorly water soluble drugs from fluidized bed coated nanocomposites: Impact of carrier size. *Int. J. Pharm.* **2016**, *513*, 319–331. [CrossRef]
- Kim, S.; Lee, J. Effective polymeric dispersants for vacuum, convection and freeze drying of drug nanosuspensions. *Int. J. Pharm.* 2010, 397, 218–224. [CrossRef]
- 17. Tuomela, A.; Laaksonen, T.; Laru, J.; Antikainen, O.; Kiesvaara, J.; Ilkka, J.; Oksalae, O.; Rönkkö, S.; Järvinen, K.; Hirvonen, J.; et al. Solid formulations by a nanocrystal approach: Critical process parameters regarding scale-ability of nanocrystals for tableting applications. *Int. J. Pharm.* **2015**, *485*, 77–86. [CrossRef]
- Choi, J.-Y.; Park, C.H.; Lee, J. Effect of polymer molecular weight on nanocomminution of poorly soluble drug. *Drug Deliv.* 2008, 15, 347–353. [CrossRef]
- 19. Baumgartner, R.; Eitzlmayr, A.; Matsko, N.; Tetyczka, C.; Khinast, J.; Roblegg, E. Nano-extrusion: A promising tool for continuous manufacturing of solid nano-formulations. *Int. J. Pharm.* **2014**, 477, 1–11. [CrossRef]
- Ye, X.; Patil, H.; Feng, X.; Tiwari, R.V.; Lu, J.; Gryczke, A.; Kolter, K.; Langley, N.; Majumdar, S.; Neupane, D.; et al. Conjugation of hot-melt extrusion with high-pressure homogenization: A novel method of continuously preparing nanocrystal solid dispersions. *AAPS Pharm. Sci. Technol.* 2016, 17, 78–88. [CrossRef]
- 21. Li, M.; Azad, M.; Davé, R.; Bilgili, E. Nanomilling of drugs for bioavailability enhancement: A holistic formulation-process perspective. *Pharmaceutics* **2016**, *8*, 17. [CrossRef]
- 22. Junghanns, J.A.H.; Muller, R.H. Nanocrystal technology, drug delivery and clinical applications. Int. J. Nanomed. 2008, 3, 295–310.

- 23. Schenck, L.; Koynov, A.; Cote, A. Particle engineering at the drug substance, drug product interface: A comprehensive platform approach to enabling continuous drug substance to drug product processing with differentiated material properties. *Drug Dev. Ind. Pharm.* **2019**, *45*, 521–531. [CrossRef]
- 24. Peltonen, L. Design space and QbD approach for production of drug nanocrystals by wet media milling techniques. *Pharmaceutics* **2018**, *10*, 104. [CrossRef]
- 25. Li, M.; Zhang, L.; Davé, R.N.; Bilgili, E. An intensified vibratory milling process for enhancing the breakage kinetics during the preparation of drug nanosuspensions. *AAPS Pharm. Sci. Technol.* **2016**, *17*, 389–399. [CrossRef]
- 26. Monteiro, A.; Afolabi, A.; Bilgili, E. Continuous production of drug nanoparticle suspensions via wet stirred media milling: A fresh look at the Rehbinder effect. *Drug Dev. Ind. Pharm.* **2013**, *39*, 266–283. [CrossRef]
- 27. Knieke, C.; Azad, M.A.; Davé, R.N.; Bilgili, E. A study of the physical stability of wet media-milled fenofibrate suspensions using dynamic equilibrium curves. *Chem. Eng. Res. Des.* 2013, *91*, 1245–1258. [CrossRef]
- Lee, J. Drug nano- and microparticles processed into solid dosage forms: Physical properties. J. Pharm. Sci. 2003, 92, 2057–2068. [CrossRef]
- 29. Napper, D.H. Colloid stability. Ind. Eng. Chem. Prod. Res. Dev. 1970, 9, 467–477. [CrossRef]
- Li, M.; Lopez, N.; Bilgili, E. A study of the impact of polymer—Surfactant in drug nanoparticle coated pharmatose composites on dissolution performance. *Adv. Powder Technol.* 2016, 27, 1625–1636. [CrossRef]
- 31. Ghosh, I.; Bose, S.; Vippagunta, R.; Harmon, F. Nanosuspension for improving the bioavailability of a poorly soluble drug and screening of stabilizing agents to inhibit crystal growth. *Int. J. Pharm.* **2011**, *409*, 260–268. [CrossRef]
- 32. Verma, S.; Kumar, S.; Gokhale, R.; Burgess, D.J. Physical stability of nanosuspensions: Investigation of the role of stabilizers on Ostwald ripening. *Int. J. Pharm.* **2011**, 406, 145–152. [CrossRef]
- 33. Gupta, R.B.; Kompella, U.B. Nanoparticle Technology for Drug Delivery; Taylor & Francis: New York, NY, USA, 2006.
- 34. Liversidge, G.G.; Cundy, K.C. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int. J. Pharm.* **1995**, *125*, 91–97. [CrossRef]
- 35. Bhakay, A.; Azad, M.; Bilgili, E.; Dave, R. Redispersible fast dissolving nanocomposite microparticles of poorly water-soluble drugs. *Int. J. Pharm.* 2014, 461, 367–379. [CrossRef]
- 36. Bhakay, A.; Davé, R.; Bilgili, E. Recovery of BCS Class II drugs during aqueous redispersion of core—Shell type nanocomposite particles produced via fluidized bed coating. *Powder Technol.* **2013**, *236*, 221–234. [CrossRef]
- Norbert Rasenack, B.W.M. Dissolution rate enhancement by in situ micronization of poorly water-soluble drugs. *Pharm. Res.* 2002, 19, 1894–1900. [CrossRef]
- 38. Van Eerdenbrugh, B.; Froyen, L.; Van Humbeeck, J.; Martens, J.A.; Augustijns, P.; Van Den Mooter, G. Alternative matrix formers for nanosuspension solidification: Dissolution performance and X-ray microanalysis as an evaluation tool for powder dispersion. *Eur. J. Pharm. Sci.* **2008**, *35*, 344–353. [CrossRef]
- 39. Hu, J.; Ng, W.K.; Dong, Y.; Shen, S.; Tan, R.B.H. Continuous and scalable process for water-redispersible nanoformulation of poorly aqueous soluble APIs by antisolvent precipitation and spray-drying. *Int. J. Pharm.* **2011**, 404, 198–204. [CrossRef]
- 40. Cheow, W.S.; Ng, M.L.; Kho, K.; Hadinoto, K. Spray-freeze-drying production of thermally sensitive polymeric nanoparticle aggregates for inhaled drug delivery: Effect of freeze-drying adjuvants. *Int. J. Pharm.* **2011**, 404, 289–300. [CrossRef]
- Niels, R.; Stephen, R. Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate. U.S. Patent 6375986, 23 April 2002.
- Saboo, S.; Kestur, U.S.; Flaherty, D.P.; Taylor, L.S. Congruent release of drug and polymer from amorphous solid dispersions: Insights into the role of drug-polymer hydrogen bonding, surface crystallization, and glass transition. *Mol. Pharm.* 2020, 17, 1261–1275.
- 43. Weng, J.; Wong, S.N.; Xu, X.; Xuan, B.; Wang, C.; Chen, R.; Sun, C.C.; Lakerveld, R.; Kwok, P.C.L.; Chow, S.F. Cocrystal engineering of itraconazole with suberic acid via rotary evaporation and spray drying. *Cryst. Growth Des.* **2019**, *19*, 2736–2745. [CrossRef]
- 44. Schenck, L.R.; Lamberto, D.J.; Kukura, J.L.; Guzman, F.J.; Cote, A.; Koynov, A. Process for Preparing Pharmaceutical Compositions. WO2017106130A1, 22 June 2017.
- Ghazal, H.S.; Dyas, A.M.; Ford, J.L.; Hutcheon, G.A. In vitro evaluation of the dissolution behaviour of itraconazole in bio-relevant media. *Int. J. Pharm.* 2009, 366, 117–123. [CrossRef] [PubMed]
- Alexandridis, P.; Holzwarth, J.F.; Hatton, T.A. Micellization of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in aqueous solutions: Thermodynamics of copolymer association. *Macromolecules* 1994, 27, 2414–2425. [CrossRef]
- Liu, P.; De Wulf, O.; Laru, J.; Heikkilä, T.; van Veen, B.; Kiesvaara, J.; Hirvonen, J.; Peltonen, L.; Laaksonen, T. Dissolution studies of poorly soluble drug nanosuspensions in non-sink conditions. *AAPS Pharm. Sci. Technol.* 2013, 14, 748–756. [CrossRef] [PubMed]
- Siewert, M.; Dressman, J.; Brown, C.K.; Shah, V.P. FIP/AAPS guidelines to dissolution/in vitro release testing of novel/special dosage forms. AAPS Pharm. Sci. Technol. 2003, 4, E7. [CrossRef] [PubMed]
- 49. FDA Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms; Center for Drug Evaluation and Research: Beltsville, MD, USA, 1997.

- Khuman, P.; Singh, W.; Devi, S.; Naorem, H. Viscosity-temperature behavior of hydroxypropyl cellulose solution in presence of an electrolyte or a surfactant: A convenient method to determine the cloud point of polymer solutions. *J. Macromol. Sci.* 2014, *51*, 924–930. [CrossRef]
- 51. Li, M.; Furey, C.; Skros, J.; Xu, O.; Rahman, M.; Azad, M.; Dave, R.; Bilgili, E. Impact of matrix surface area on griseofulvin release from extrudates prepared via nanoextrusion. *Pharmaceutics* **2021**, *13*, 1036. [CrossRef]
- Malamatari, M.; Charisi, A.; Malamataris, S.; Kachrimanis, K.; Nikolakakis, I. Spray Drying for the Preparation of Nanoparticle-Based Drug Formulations as Dry Powders for Inhalation. *Processes* 2020, *8*, 788. [CrossRef]
- 53. Li, M.; Ioannidis, N.; Gogos, C.; Bilgili, E. A comparative assessment of nanocomposites vs. amorphous solid dispersions prepared via nanoextrusion for drug dissolution enhancement. *Eur. J. Pharm. Biopharm.* **2017**, *119*, 68–80. [CrossRef]
- 54. Knieke, C.; Azad, M.A.; To, D.; Bilgili, E.; Davé, R.N. Sub-100 micron fast dissolving nanocomposite drug powders. *Powder Technol.* **2015**, *271*, 49–60. [CrossRef]
- Schenck, L.; Neri, C.; Jia, X.; Schafer, W.; Axnanda, S.; Canfield, N.; Li, F.; Shah, V. A Co-Processed API Approach for a Shear Sensitive Compound Affording Improved Chemical Stability and Streamlined Drug Product Processing. *J. Pharm. Sci.* 2021, 110, 3238–3245. [CrossRef] [PubMed]