JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 399–406.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.826

Research Article

Ultrasonic particle size reduction of radiolabeled pharmaceuticals

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Summary

High-intensity ultrasonic treatment of MeOH/H_2O, IPA/H_2O, EtOH or H_2O suspensions of C-14 labeled and unlabeled pharmaceuticals successfully reduced the average particle diameter from a range of 8–260 to 4–15 μm as determined by optical microscopy. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: ultrasonic comminution; particle size reduction; sonication; C-14 tracer

Introduction

The bioavailability of pharmaceuticals is dependent on several factors including particle size and surface area.[1] Generally, both the rate and the extent of availability are inversely related to particle size with particles between 3 and 6 μm considered optimal for some formulations.[1] Additional properties of the final dosage form can be affected by particle size. The segregation of a powder mix during formulation is reduced and powder flow properties are enhanced by controlling the particle size distribution (PSD) of the drug and the other components.[1] Also, tablet compaction characteristics and tablet disintegration can be affected by particle size.[1]

Jet milling is often used to reduce the particle size of a bulk solid when samples are prepared for human oral administration. While often ignored, particle size control is also important for formulation of radiolabeled tracers which are routinely used in the clinical development of new pharmaceuticals to

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Received 3 March 2004
Accepted 16 March 2004

assess human metabolism and pharmacokinetics (PK). Lack of particle size control during these early clinical studies can result in the compound exposure being lower than intended. Different exposure levels can alter metabolism and thus the results from studies using unmilled tracers may not be consistent with those from studies using solids with a controlled particle size. Oral solution dosing of tracers can generally provide good exposure levels; however, the solvents and surfactants used may affect the PK. Ideally, the tracer should match the unlabeled material as closely as possible in all ways, including particle size.

Jet milling is not a very attractive way to achieve the particle size reduction of tracers for several reasons. Typically the mass recoveries from jet milling are good, but when milling is performed near the 10 g scale the mass loss is approximately 20%. Since the synthetic scale for tracers is in this range (5–25 g), jet milling will result in an unacceptably high loss of material at the end of the preparation. More importantly, there is the concern that milling a radiolabeled solid could result in the contamination of the mill, the environment, and the researcher.

Limited literature precedence for ultrasonic particle size reduction of radiolabeled materials is available. In the one available case the particle size of [^{188}Re]rhenium sulfide suspensions was ultrasonically reduced and the effect of the resulting particle size on biodistribution after intra-tumor injection was presented.[2] In contrast to radiolabeled compounds, ultrasonic treatment of unlabeled pharmaceuticals such as zinc oxide, bismuth subcarbonate, sulfathiazole, and procaine penicillin G[3] is more common. Other examples include the sonication of progesterone[4] and salbutamol[5] that gave average particle sizes of approximately 5 μm. Size reduction of the energetic materials hexogen, octogen, 5-nitro-1,2,4-triazol-3-one and hexanitrostilbene showed that these impact sensitive compounds could all be successfully reduced to less than 60 μm without detonation or deflagration.[6] Treatment of ammonium nitrate[6,7] and NaCl[7], however, gave more limited size reduction. Additional literature precedence for immersed-probe ultrasonic treatment of alumina, silica, and titania powders[8–10] and cellulose[11] and models for the ultrasonic-induced cavitation and resulting particle size reduction have been given.[8–12] Finally, other forms of ultrasonic assisted milling have been described in the literature, such as roller,[13] wet-ball[14] and anvil milling.[15] Here we describe the use of ultrasonic treatment to reduce the particle size of C-14 labeled and unlabeled pharmaceutical powders.

Results and discussion

To conduct a relevant comparative toxicological study in rodents, the PSD of C-14 labeled **1** (The structures of 1–4 are proprietary and thus cannot be shown, but they are not necessary to understand this work.) needed to be

similar to the jet-milled material prepared by Merck Process Research. Mortar and pestle grinding of unlabeled compound **1** reduced the particle size, but the mass recovery was moderate (85%) and some larger particles escaped size reduction (Table 1). Direct probe sonic treatment of compound **1** reduced the average diameter from 18 to 4.3 μm (Table 1) with good mass recovery (>95%) and acceptable PSD data. Initially the average particle diameter sharply decreased with increasing sonication time and then was followed by a slower continued reduction in particle size (Figure 1). Similar sonication time to particle size curves were observed for SiC powders[12] and titania.[14] However, an ultrasonic power-dependent time lag between sonication initiation and size reduction, as was previously described for titania and silica,[14] was not observed for compound **1**. Therefore, it is postulated that the ultrasonic power used with **1** generated a cavitation energy large enough that particle fracture could occur without repeated cavitation related particle fatigue.[8] Subsequent treatment of C-14 labeled **1** as two nearly equal batches reduced the average particle size from 14 to 4.8 μm. A complete set of radiochemical and chemical analytical data was acquired and the material was judged acceptable for delivery.

Unlabeled and C-14 labeled **2** were required for formulation and human ADME studies, respectively. Ultrasonic treatment of C-14 labeled **2** gave a powder with an average particle size of 6.4 μm (Table 1). As has been described

Table 1. Particle size distribution by image analysis

Sample	Range (μm)	Mean diameter (max) (μm)	95% < (μm)	% < 25 μm
Crystallized **1**	1–146	18.1	ND	ND
Ground **1**	1–143	3.3	9	99.6
Sonicated **1** (1 g)	1–43	4.3	10	99.9
Jet-milled **1**	1–106	4.8	17	97.1
Crystallized [^{14}C]**1**	1–196	14.2	50	74.8
Sonicated [^{14}C]**1** (11 g)	1–32	4.8	11	99.7
Crystallized **2**	1–86	16.7	41	80.6
Sonicate **2** (1 g)	1–31	3.9	9	99.9
Sonicate **2** (8 g)	1–39	4.9	12	99.7
Jet-milled **2**	1–28	4.9	11	99.8
Sonicated [^{14}C]**2** (11 g)	1–37	6.4	18	98.6
Crystallized **3**	1–76	10.4	27	91.9
Sonicated **3** (1 g)	1–40	3.7	8	100
Cup horn sonicated **3** (1 g)	1–34	7.5	18	98.9
Crystallized **4**	1–163	8.0	30	92.9
Sonicated **4** (1 g)	1–129	4.8	12	99.6
Sonicated **4** (2 g)	1–34	5.0	11	99.8
Jet-milled **4**	1–21	4.3	9	100
Acetaminophen (**5**)	40–995	259	ND	ND
Sonicated **5** (10 g, H_2O)	1–86	14.8	40	83.3
Aspirin	1–1022	21	ND	ND
Sonicated aspirin (10 g, H_2O)	1–127	13.6	38	88.3

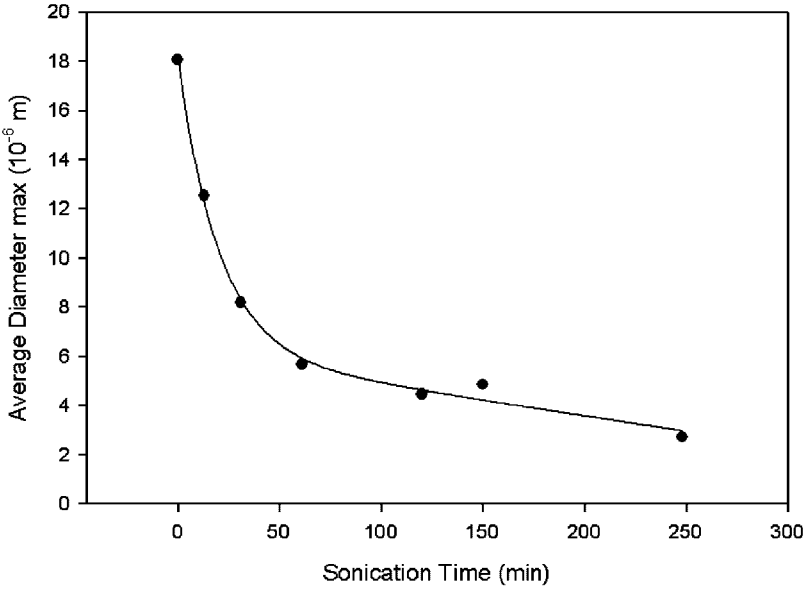

Figure 1. Particle size of 1 vs sonication time

previously,[8,10] an increase in the sonication scale (1, 8, 11 g) increased the particle size (3.9, 4.9, 6.4 μm). While the average diameter of the labeled sonicate was slightly larger than the jet-milled material, it was judged acceptable by the user.

One liability of direct probe sonication is that erosion of the probe (90% Ti, 6% Al, 4% V) results in metal contamination of the bulk drug. Approximately 30 ppm of titanium was detected in two unlabeled 1 g sonicates of **1** when using a previously used 3 mm probe. Unlabeled sonicates of **2** produced using new or used 6.5 mm probes contained 70 and 408 ppm of titanium, respectively. Aluminum and vanadium were detected at levels consistent with the presence of the probe alloy in the powders. As expected, metals were not observed in the crystallized, ground and jet-milled solids. As a result of the metal contamination issue, new probes should be used for all critical samples and the advantages of sonication vs metal contamination should be judged according to the users needs.

Indirect cup horn sonication, which is similar to a very high-intensity sonication bath, was investigated as an option to eliminate the metal contamination. Presumably because of the lower cavitation intensity[10] of cup horn sonication, the average diameter of **3** was reduced less (7.5 μm) than with direct probe sonication (3.7 μm) even when the sonication duration was longer (4 h). However, this level of particle size reduction could be useful depending on the requirements of the study at hand and will be investigated further.

Direct probe sonication of separate aqueous suspensions of acetaminophen and aspirin gave average diameters ($\sim$14 μm) considerably larger than for compounds **1–4**. It is unlikely that the 10 g batch size used to produce the acetaminophen and aspirin sonicates is the only reason for the larger observed particle size as both **1** and **2** underwent size reduction to $\sim$5–6 μm near the 10 g scale. Likely, the larger initial particle size, the higher viscosity of water and the crystal properties[6,7] of each could have contributed to the larger final particle size of the acetaminophen and aspirin sonicates. Regardless, a significant reduction in the average size of acetaminophen was achieved and the size ranges of both sonicated materials were considerably smaller than the untreated solid.

Conclusion

Direct probe ultrasonic treatment of C-14 labeled **1** and **2** and unlabeled **1–4** suspensions reduced the average diameter to a range (4–6 μm) similar to that of the unlabeled jet-milled materials. Erosion of the sonic probe during treatment, however, resulted in 30–400 ppm of Ti being present in the sonicates. Sonication of separate aqueous suspensions of aspirin and acetaminophen reduced the average particle diameter from 260 to 15 μm and from 21 to 14 μm, respectively.

Experimental

General

Methanol (Optima, Fisher Scientific), 2-propanol (IPA, HPLC Grade, Fisher Scientific), water (sterile water for injection, Abbott Labs), dimethylformamide (DMF, HPLC grade, Aldrich) and ethanol (ETOH, absolute, Pharmco Products, Inc.) were supplied by commercial sources and were used as received. Compound **1** was crystallized from 1:1 methanol/water. Jet-milled **1** and **2** were supplied by Merck Chemical Engineering R&D. Crystallized **3** was received from Merck Process Research and was used as supplied. Aspirin (99 + %) and acetaminophen (98–101%) were obtained from Aldrich. Acetaminophen was crystallized from H_2O to increase the particle size before sonication.

Direct probe sonication was accomplished with a VC-750 Ultrasonic Processor equipped with a 6.5 mm tapered microtip (Sonics and Materials, Inc.) unless otherwise noted. A ratio of $\sim$1 g of solid to $\sim$9 ml of liquid was used for the sonication experiments. The resulting suspension in an Erlenmeyer flask was mixed by swirling and placed in an ice/water bath. The probe was inserted $\sim$5 mm into the suspension and sonication was initiated. At each of three approximately equal intervals, sonication was stopped and the flask contents were mixed by swirling the flask gently. After

the desired total sonication time was reached the suspension was filtered though a medium-frit funnel and the funnel was placed in a drying pistol at $\sim 40^{\circ}C$ under high vacuum. New microtips were used for all sonicates that were delivered to an investigator.

Similarly, indirect sonication was accomplished with a VC-750 ultrasonic processor and a cup horn with a 2.5 in radiating face (Sonics and Materials, Inc.). The suspension in an Erlenmeyer flask was placed in the room-temperature water-filled cup horn and held in place with a clamp. The bottom of the flask was ~ 2.5 cm from the radiating horn.

Particle size measurement and distribution data were acquired on an optical particle size instrument consisting of a Leica® DMLB microscope ($5\times$, $10\times$ and $20\times$ objective lens), an Optronics MagnaFire® digital camera and a Microsoft® Windows NT®-based computer running Image Pro® Plus software (v 4.1, Media Cybernetics). One or two drops of refractive index liquid (1.320 or 1.452, Cargille Laboratories) were placed on the slides (FISHERfinest, $3'' \times 1'' \times 1$ mm). The solid was dispersed by gentle swirling with a pipette tip. A coverslip (Fisherbrand, 22×22-1) was gently added. Diameter (max) was selected and measured using the software. The Single Variable Class command was used to measure the $95\% < x$ and the $\% < 25\,\mu m$ values. Seven or more images were acquired encompassing > 1000 particles. The number-weighted average of the data from each image was calculated and reported. No attempt was made to remove the data corresponding to agglomerated particles. However, the gentle swirling of the powder/refractive index liquid described above was intended to breakup agglomerates before the measurement. In general, the degree of agglomeration in the non-sonicated powders was moderate and the larger particles in these powders were observed to be single particles, not agglomerates. Smaller particles, measuring a few microns, had a higher degree of agglomeration. The PSD data for some samples was verified by members of Merck Analytical Research on a Microtrac® SRA 150 particle size analyzer. ICP-AES was used to determine the extent of metal contamination.

Compound 1: Crystallized **1** was ground with a Coors porcelain mortar and pestle using an up-and-down rubbing motion with frequent rotation of the mortar (0.9 g scale, 85% yield). A second batch was ground giving similar PSD data. The unlabeled sonicate was prepared from crystallized **1** (0.984 g) by suspending in 9 ml of 1:1 $MeOH/H_2O$ and sonicating for 2 h with a VC-50 processor (3 mm stepped microtip, 40% amplitude, 91%). C-14 labeled **1** was synthesized then diluted with carrier. The crystallized C-14 labeled material (1.32 mCi/mmol) was divided into two batches (10.5 g, 12.0 g) and each batch was suspended in 90 ml of 1:1 $MeOH/H_2O$ and sonicated for 2 h with the VC-750. The suspensions were combined, filtered and dried (98.7%). Comprehensive analytical data were acquired (chemical and radiochemical) for C-14

labeled **1** including TLC, TGA, Radio-HPLC, PSD, fraction collection/scintillation counting, LC/MS, residual solvents by GC, enantiomeric purity and crystal form analysis and these data were consistent with the regulatory specifications established for **1**.

Compound 2: Two batches of crystallized **2** (1.02 g, 7.78 g) were suspended in 1:1 MeOH/H_2O (9 ml, 72 ml) and sonicated for 2 h with the VC-750 (95%, 96%). Carrier diluted C-14 labeled **2** (10.5 g, 0.407 mCi/mmol) was suspended in 92 ml of 1:1 MeOH/H_2O and sonicated for 2 h with the VC-750 (98%). Analytical data similar to that described for **1** was acquired for C-14 labeled **2** and these data were consistent with the specifications.

Compound 3: Crystallized **3** (1.08 g) was suspended in 9 ml of EtOH and was sonicated for 2 h (96%). Indirect sonication of two batches of crystallized **3** (1.02 g, 9 ml of EtOH, 4 h, 100% amplitude, 98%; 0.97 g, 62% amp, 7 h 40 min, 92%) gave similar PSD results.

Compound 4: Two batches of crystallized **4** (1.00 g, 2.02 g) were suspended (9 ml, 18 ml) in 4:1 IPA/water and sonicated for 2 h (91%, 96%). Analytical data including TGA, LC/MS, PSD, HPLC area percent and weight percent by HPLC were gathered and indicated appropriate purity.

Acetaminophen: Crystallized acetaminophen (10.0 g) was suspended in 90 ml of H_2O and sonicated for 2 h with the VC-750 (84%).

Aspirin: Aspirin (10.2 g) was suspended in 90 ml of H_2O and sonicated for 2 h with VC-750 (94%).

Acknowledgements

We would like to thank Peter E. Maligres and Steven A. Weissman from Merck Process Research and Jennifer R. Reavis from Merck Chemical Engineering Research and Development for supplying unlabeled starting materials used in this research. Also, we would like to thank Raymond Cvetovich for the use of the VC-50 ultrasonic processor and Richard J. Varsolona for particle size measurement training. In addition, we would like to thank Russell R. Ferlita and Valorie Mayo of Merck Analytical Research for acquiring supporting particle size measurements and Charles S. Elmore for assistance in preparing this manuscript.

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