Development and Characterization of a Scalable Controlled Precipitation Process to Enhance the Dissolution of Poorly Water-Soluble Drugs

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Purpose. Poorly water-soluble compounds are being found with increasing frequency among pharmacologically active new chemical entities, which is a major concern to the pharmaceutical industry. Some particle engineering technologies have been shown to enhance the dissolution of many promising new compounds that perform poorly in formulation and clinical studies (Rogers *et. al., Drug Dev Ind Pharm* **27**:1003–1015). One novel technology, controlled precipitation, shows significant potential for enhancing the dissolution of poorly soluble compounds. In this study, controlled precipitation is introduced; and process variables, such as mixing zone temperature, are investigated. Finally, scale-up of controlled precipitation from milligram or gram to kilogram quantities is demonstrated.

Methods. Dissolution enhancement capabilities were established using two poorly water-soluble model drugs, danazol and naproxen. Stabilized drug particles from controlled precipitation were compared to milled, physical blend, and bulk drug controls using particle size analysis (Coulter), X-ray powder diffraction (XRD), scanning electron microscopy (SEM), dissolution testing (USP Apparatus 2), and residual solvent analysis.

Results. Stabilized nano- and microparticles were produced from controlled precipitation. XRD and SEM analyses confirmed that the drug particles were crystalline. Furthermore, the stabilized particles from controlled precipitation exhibited significantly enhanced dissolution properties. Residual solvent levels were below FDA limits.

Conclusions. Controlled precipitation is a viable and scalable technology that can be used to enhance the dissolution of poorly water-soluble pharmaceutical compounds.

KEY WORDS: dissolution; controlled precipitation; microparticles; nanoparticles; particle engineering.

INTRODUCTION

The increasing frequency of poorly water-soluble new chemical entities exhibiting therapeutic activity is of major concern to the pharmaceutical industry (2). These promising compounds span many therapeutic classes but are often difficult to process or administer to patients due to poor dissolution properties. A major hurdle that has prevented the commercialization of many promising poorly soluble drug candidates is dissolution rate-limited bioavailability (3,4,5). Compounds exhibiting dissolution rate-limited bioavailability are considered class II (low solubility, high permeability) according to the Biopharmaceutics Classification System (BCS) (3). If dissolution of these poorly soluble compounds can be enhanced, bioavailability following oral administration may be significantly improved.

A number of efforts exist to address the issue of enhancing the dissolution of poorly soluble compounds. These methods include, but are not limited to, milling techniques (6), supercritical fluid processing (7,8,9), solid dispersion and cosolvent formulations (10,11), inclusion complexation (12,13,14,15,16,17,18,19,20,21), precipitation techniques (22–25,26,27,28–31,32,33), and cryogenic engineering (1,18,34,35,36,37,38,39). Improvement in dissolution performance varies between the aforementioned techniques, and at least 40% of all new chemical entities exhibiting poorly soluble properties still fail to reach commercialization (40).

Obviously, there remains an unmet need to equip the pharmaceutical industry with particle engineering technologies capable of enhancing the dissolution of poorly soluble compounds. One such novel particle engineering technology has been developed and tested in our laboratories using a number of poorly water-soluble model compounds (27). This technology, controlled precipitation, has been advanced and scaled-up because of the demonstrated dissolution enhancement that has been achieved with a variety of hydrophobic compounds.

The purpose of this study was to demonstrate the applicability of controlled precipitation as a viable method for enhancing the dissolution of poorly water-soluble drugs and drug candidates. Stabilized micro- and nanoparticles, which are crystalline in nature, were engineered with significantly enhanced dissolution properties compared to the unprocessed bulk drug and controls. Two poorly soluble model drugs, danazol and naproxen, were investigated by comparing drug particles, which were processed at various temperatures via batch (laboratory-scale) or continuous (kg-scale) controlled precipitation, to bulk drug, milled, and physical blend controls. Stabilized particles and controls were characterized using particle size analysis, X-ray diffraction, scanning electron microscopy, dissolution testing, and residual solvent analysis. Finally, this study demonstrates that the controlled precipitation process, when operated in continuous mode, is capable of producing kilogram quantities of stabilized drug particles exhibiting enhanced dissolution properties.

MATERIALS AND METHODS

Danazol, USP (micronized, Spectrum Chemical Manufacturing Corporation; Gardena, CA, USA; lot no. RG1316) and Naproxen, USP (Spectrum Chemical, lot no. PT0586) were the poorly water-soluble model drugs investigated. Poloxamer 407 (POL 407, Sigma-Aldrich Company; St. Louis, MO, USA; lot no. 51K0001) and polyvinylpyrrolidone 55k (PVP, Adrich Chemical Company, Inc.; Milwaukee, WI, USA; lot no. 08218DA) were used to stabilize danazol and naproxen particles, respectively.

Preparation of Stabilized Drug Particles from Controlled Precipitation

A schematic illustration of the controlled precipitation process is shown in Fig. 1. Batch (1–5 g) controlled precipi-

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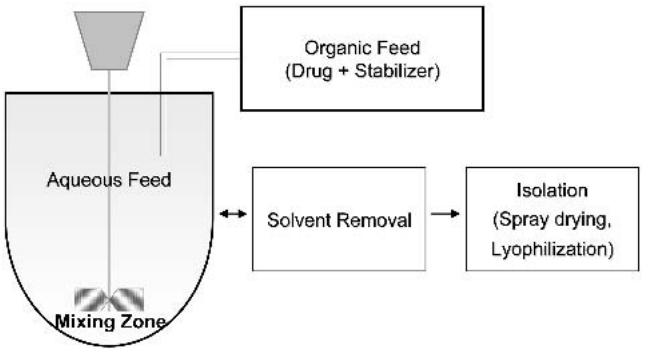

Fig. 1. Schematic illustration of the controlled precipitation process.

tation experiments were carried out with mixing zone temperatures equilibrated at 3°C, 25°C, and 50°C. The organic feeds were prepared by dissolving danazol (4.5% w/w) or naproxen (6.7% w/w) and stabilizer in methanol. The stabilizers used for danazol and naproxen were POL 407 and PVP, respectively. The organic and aqueous feeds were introduced into the mixing zone to produce stabilized active pharmaceutical ingredient (API) particles. Stabilized danazol and naproxen compositions are listed in Tables I and II. To demonstrate scalability, 1 kg of stabilized naproxen particles was produced via continuous controlled precipitation with the mixing zone temperature equilibrated at 3°C.

Following controlled precipitation, methanol was reduced to less than 1% (w/w) in the slurries precipitated in batch mode using a semicontinuous vacuum distillation process. The solvent-stripped slurries were then collected and subsequently frozen in a dry ice-acetone bath. The frozen slurries were then lyophilized using a VirTis BT4KEL manifold lyophilizer (VirTis Company, Gardiner, NY, USA). The dry stabilized particles were removed from the lyophilizer after 24–48 h.

The slurry produced in continuous (kg-scale) mode was solvent-stripped using a pilot-scale vacuum distiller. The solvent-stripped slurry was then collected and spray-dried using a Mobile Minor Spray Dryer (Niro A/S; Soeborg, Denmark) with a 0.8-meter inner diameter (I.D.) chamber. The inlet and outlet temperatures were maintained at 145°C and 65°C, respectively; and the aqueous slurry was atomized through a two-fluid nozzle at a feed rate of 45 ml/min. The dry stabilized particles were harvested from the collection chamber located beneath the cyclone portion of the spray dryer.

Table I. Compositions of Danazol Particles from Controlled Precipitation, Milling, and Physical Blending

Sample name	Precipitation temperature (°C)	Danazol (wt%)	F127 (wt%)
Danazol PPT @ 3°C	3	67	33
Danazol PPT @ 25°C	25	67	33
Danazol PPT @ 50°C	50	67	33
Danazol milled	N/A	67	33
Danazol physical blend	N/A	67	33
Bulk danazol	N/A	100	0

Note: PPT, precipitate.

Table II. Compositions of Naproxen Particles from Controlled Precipitation, Milling and Physical Blending

Sample name	Precipitation temperature (°C)	Naproxen (wt%)	PVP (wt%)
Naproxen PPT @ 3°C	3	40	60
Naproxen PPT @ 25°C	25	40	60
Naproxen PPT @ 50°C	50	40	60
Naproxen Kg-scale PPT	3	40	60
Naproxen milled	N/A	40	60
Naproxen physical blend	N/A	40	60
Bulk naproxen	N/A	100	0

Note: PPT, precipitate; PVP, polyvinylpyrrolidone; kg, kilogram

Danazol and naproxen particles produced using the controlled precipitation technology are referred to as stabilized drug particles.

Preparation of Milled Control Particles

The bulk drug and excipient powders were blended for approximately 5 min in a suitable container according to the drug-to-excipient ratios shown in Tables I and II. Next, deionized (DI) water was added, and the mixture was agitated for 10–15 min. Approximately 900 g of yttrium-stabilized zirconium oxide grinding beads (1 mm diameter, Union Process, Inc., Akron, OH, USA) were then added, and the container was subsequently placed on a roller mill set at 100 rpm for 24 to 72 h. Preliminary experiments demonstrated that a 24- to 72-h period of constant milling was sufficient in most cases to achieve steady-state particle sizes of the milled control formulations. Each milled slurry was separated from the grinding media by filtration, and subsequently frozen and lyophilized according the procedure described above. Drug-to-excipient ratios of the milled controls were identical to those of the precipitated experimental formulations.

Danazol and naproxen particles produced by milling are referred to as milled control particles.

Preparation of Physical Blend Control Particles

The bulk drug and excipient powders were blended together for approximately 2 min in a glass vial, which was at least three times the volume of the powder, using the drug-to-excipient ratios shown in Tables I and II. The powders were blended in a WAB Turbula System Schatz (Willy A. Bachofen AG Maschinenfabrik; Basel, Switzerland) for 1 min. The Turbula mixer was then stopped, and the powders were tapped and hand-shaken for 1 min. This procedure was repeated once more to ensure that the powders were uniformly blended. Drug-to-excipient ratios of the physical blend controls were identical to those of the precipitated experimental formulations.

Danazol and naproxen particles produced by physical blending are referred to as physical blend control particles.

Particle Size Analysis

Particle size distributions were measured by laser light diffraction using an LS230 Small Volume Plus Coulter Counter (Beckman Coulter Corporation; Fullerton, CA, USA). Particle size distributions (PSDs) of the slurries were mea-

sured before and after solvent stripping and after isolation to dry stabilized drug particles. Each slurry or dispersed powder was added to the sampling chamber, which was filled with DI water, under constant agitation until a polarized intensity differential scattering (PIDS) obscuration of 45–55% was achieved. The PIDS obscuration was monitored to ensure the API was not dissolving in the DI water. A reduction in PIDS obscuration indicated drug dissolution. Only naproxen required saturation of the DI water prior to analysis to maintain the PIDS obscuration between 45% and 55%. Once the desired obscuration was attained, particle size analysis was performed using a polystyrene latex optical model.

Following lyophilization or spray drying, samples from each of the stabilized drug particles were reconstituted to form a 1% (w/w) suspension in DI water, vortexed for 40 s, and sonicated for 3 min. The particle sizes of the reconstituted suspensions were measured using the procedure described above.

X-ray Diffraction (XRD) Studies

The bulk drug samples were mixed with 50% (w/w) alumina powder, and the stabilized particles, physical blends, and milled controls were blended with 10% alumina powder. The alumina powder served as an internal standard. The samples were then leveled and analyzed using the following procedure.

X-ray powder diffraction was performed using a Siemens D-500 automated diffractometer equipped with a cobalt X-ray tube and a position sensitive detector. The incident beam was collimated using a 1.0° divergence slit, and data points were collected from 5° to 55° 2θ at a rate of 0.5°/min with a step width size of 0.02° 2θ. The XRD patterns were analyzed using Jade XRD pattern processing software (Version 6; Materials Data, Inc.; Irvine, CA, USA).

Scanning Electron Microscopy (SEM)

A JEOL 6320 field emission scanning electron microscope (JEOL USA; Peabody, MA, USA) operating at 5 keV, a current setting of 3, and a working distance of 16 mm, was used to obtain SEM photomicrographs of the various samples. Prior to SEM analysis, the samples were dispersed onto a carbon-tape-coated aluminum stub, and then coated with approximately 20 nm of a gold-palladium mixture.

Dissolution Testing

Dissolution testing was performed on the samples using the USP 24 Type 2 (paddle) method (Distek Dissolution System 2100C with a TCS0200C heater/circulator; Crescent Scientific Pvt. Ltd.; Goregaon–East, Mumbai, India). Powder containing approximately 10–20 mg of active was weighed out and placed into 900 ml of dissolution media. An aqueous solution consisting of 0.3% (w/v) sodium dodecyl sulfate (SDS) was used to characterize the dissolution of danazol. An aqueous solution (nonbuffered) consisting of 5% sodium chloride (w/v) was used to characterize the dissolution of naproxen. Paddle speed and bath temperature were set at 50 rpm and 37.0 ± 0.2°C, respectively. Five-milliliter samples were collected at 2, 5, 10, 15, 20, 25, 30, and 60 min in replicates of 6 (n = 6) by a Distek Dissolution Sampling System. After the 60-min samples were collected, the paddle speed was increased to 200 rpm, and final samples were taken at 120 min to confirm complete API dissolution. Sink conditions were maintained throughout the dissolution testing period. Samples were analyzed by High-Performance Liquid Chromatography (HPLC).

HPLC Analysis

Samples for HPLC analysis were filtered through 0.45 μm Acrodisc GHP syringe filters (Pall Corporation, Ann Arbor, MI, USA), and aliquots of 20 μL were injected into a PerkinElmer liquid chromatograph (PerkinElmer, Inc.; Wellesley, MA, USA) equipped with a Zorbax Bonus–RP Rapid Resolution reverse-phase column (4.6 × 75 mm; 3.5 μm pore size; Part No. 866668–901; Agilent Technologies; Palo Alto, CA, USA) and a Zorbax Bonus–RP analytical guard column (4.6 × 12.5 mm; 5.0 μm; Part No. 820950–928). The reverse-phase and guard columns were maintained at 30°C. Danazol and naproxen were detected at UV absorbances of 288 and 230 nm, respectively. Danazol eluted at 1.3 min when running mobile phase (70% acetonitrile/30% water v/v) at 2.0 mL/min. Naproxen eluted at 1.5 min when running mobile phase (50% acetonitrile/50% trifluoroacetic acid (0.05% v/v) in deionized water) at 2.0 mL/min.

Residual Solvent Analysis

Calibration standards were prepared by weighing approximately 50–60 mg of solvent into a 25 mL volumetric flask partly filled with *N,N*-dimethyl formamide (DMF). DMF was then added to make a total volume of 25 mL. The solvent stock solution was diluted 1 mL to 25 mL with DMF. Samples to be tested were prepared by weighing approximately 100 mg of sample and dissolving each in 1.0 mL of DMF. Aliquots of 1.0 μL each of the standards and samples were injected into a 60 m × 0.32 mm × 1.0 μm film (14% cyanopropylphenyl) methyl silicone column (DB-1701; J&W Scientific, Division of Agilent Technologies). An Agilent 6890A GC chromatograph equipped with ChemStation data collection software and a 7683 Series autosampler (injector type: split, 200°C) was used to analyze the standards and samples. The flame ionization detector was set at 275°C. The oven was programmed to hold at 50°C for 1 min, then to ramp at 25°C/min to 225°C and hold for 2 min. Helium was used as the carrier gas (20 psig, 32 cm/second, split ratio: 20:1).

Statistical Analysis

One-way analysis of variance (ANOVA) was used to determine statistically significant differences between dissolution results. p values < 0.05 were considered statistically significant.

RESULTS

Particle Size Analysis

The PSDs of the stabilized precipitate and control particles of danazol are shown in Table III. Following precipitation at 3°C, the mean particle size of the stabilized danazol particles was 0.20 μm (monomodal) compared to 0.54 (bimodal) and 3.95 μm (bimodal) when precipitated at 25°C and 50°C, respectively. The mean particle sizes of the dry stabilized danazol particles were 0.46 (bimodal), 0.65 (bimodal),

Table III. Particle Size Distributions of Danazol and Naproxen Particles Produced from Controlled Precipitation, Milling, and Physical Blending

Sample	D10 (μm)	D50 (μm)	D90 (μm)	Mean (μm)	Peak shape
Danazol PPT @ 3°C					
After solvent removal	0.12	0.18	0.31	0.20	Monomodal
After isolation	0.17	0.31	1.24	0.46	Bimodal
Danazol PPT @ 25°C					
After solvent removal	0.23	0.41	1.19	0.54	Bimodal
After isolation	0.26	0.47	1.41	0.65	Bimodal
Danazol PPT @ 50°C					
After solvent removal	0.31	3.69	7.60	3.95	Bimodal
After isolation	0.33	3.70	10.8	5.00	Bimodal
Danazol milled					
After isolation	0.24	0.41	1.70	0.73	Trimodal
Danazol physical blend					
After blending	6.16	20.5	53.6	26.8	Monomodal
Bulk danazol					
Unprocessed	12.6	44.5	117	65.6	Bimodal
Naproxen PPT @ 3°C					
After solvent removal	0.094	0.21	0.41	0.27	Bimodal
After isolation	0.14	3.87	10.8	4.42	Bimodal
Naproxen PPT @ 25°C					
After solvent removal	0.16	2.40	10.5	4.15	Trimodal
After isolation	0.20	8.60	24.6	11.0	Bimodal
Naproxen PPT @ 50°C					
After solvent removal	0.20	2.11	6.09	2.54	Bimodal
After isolation	0.20	1.68	5.05	2.01	Bimodal
Naproxen Kg-scale PPT					
After solvent removal	0.10	2.26	4.57	2.15	Bimodal
After isolation	0.19	3.19	21.9	8.21	Trimodal
Naproxen milled					
After isolation	0.12	0.24	0.46	0.27	Monomodal
Naproxen physical blend					
After blending	6.01	18.5	46.0	22.6	Monomodal
Bulk naproxen					
Unprocessed	13.1	50.9	347	124	Bimodal

Note: PPT, precipitate; Kg, kilogram.

and 5.00 μm (bimodal) upon reconstitution to suspensions. The milled control particles produced a trimodal PSD with a mean particle size of 0.73 μm. The physical blend control particles produced a monomodal PSD with a mean particle size of 26.8 μm. Unprocessed bulk danazol produced a bimodal PSD with a mean particle size of 65.6 μm.

The PSDs of the stabilized precipitate and control particles of naproxen are shown in Table III. Following precipitation at 3°C, the mean particle size of the stabilized naproxen particles was 0.27 μm (bimodal) compared to 4.15 (trimodal) and 2.54 μm (bimodal) when precipitated at 25°C and 50°C, respectively. The mean particle sizes of the dry stabilized naproxen particles were 4.42 (bimodal), 11.0 (bimodal), and 2.01 μm (bimodal) upon reconstitution to suspensions.

The stabilized naproxen particles precipitated at kilogram-scale produced a bimodal distribution with a mean particle size of 2.15 μm. Following spray drying, the stabilized naproxen particles had a mean particle size of 8.21 μm with a bimodal distribution upon reconstitution. The milled and physical blend control particles yielded monomodal PSDs with mean particle sizes of 0.27 and 22.6 μm, respectively. Unprocessed bulk naproxen produced a bimodal PSD with a mean particle size of 124 μm.

X-ray Diffraction Studies

The XRD patterns of samples containing danazol are shown in Fig. 2. Bulk danazol (Fig. 2f) exhibits intense crystalline peaks between 14 and 24 2θ°. The stabilized danazol particles (Figs. 2a–2c) and control samples (Figs. 2d–2e) produced similar XRD patterns, indicating that the various samples have similar crystalline habits to that of bulk danazol.

The XRD patterns of samples containing naproxen are shown in Fig. 3. Bulk naproxen (Fig. 3f) exhibits intense crystalline peaks between 6 and 34 2θ°. The stabilized naproxen particles (Figs. 3a–3c) and control samples (Figs. 3d–3e) produced similar XRD patterns, indicating that the various samples have similar crystalline habits to that of bulk naproxen. The stabilized naproxen particles precipitated at kilogram-scale (isolated via spray drying) produced identical XRD patterns to those of the aforementioned naproxen particles that were batch-precipitated at 3°C, 25°C, and 50°C (isolated via lyophilization) (Figs. 3a–3c). With the exception of bulk naproxen, XRD analysis revealed a composite pattern

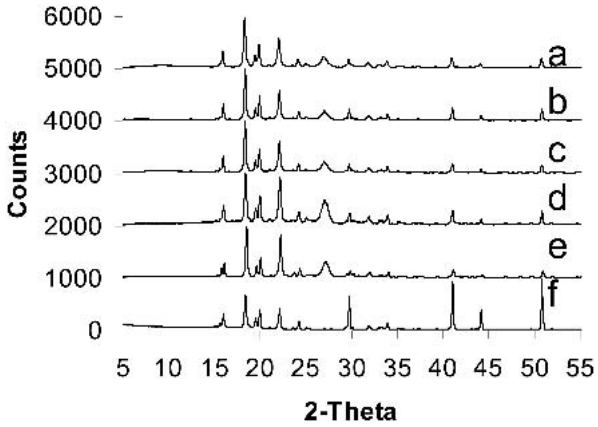

Fig. 2. XRD patterns of stabilized danazol particles precipitated at (a) 3°C, (b) 25°C, and (c) 50°C, the (d) milled and (e) physical blend controls, and (f) bulk danazol.

of crystalline naproxen and amorphous PVP for all samples investigated.

Peaks from the internal alumina standard were present in the XRD patterns at 29.8, 41, 44, and 51 2θ°.

Scanning Electron Microscopy

SEM micrographs of the various samples containing danazol are shown in Fig. 4. Figure 4a shows the surface morphology of the stabilized danazol particles precipitated at 3°C. The individual danazol crystals were submicron and rectangular, and the stabilized danazol particles were connected with POL 407 stabilizer to form loose aggregates. In Fig. 4b, it can be seen that precipitation at 25°C yielded stabilized danazol particles attached via POL 407 to form loose aggregates. In general, the individual danazol crystals precipitated at 25°C appeared to be submicrometer but were clearly larger than those precipitated at 3°C. Surface morphology differences of the stabilized danazol particles precipitated at higher temperatures are more evident in Fig. 4c. When precipitated at 50°C, the crystals were more distinctly rectangular in shape than those precipitated at lower temperatures. Stabilized danazol particles precipitated at 50°C were about 5 μm in length compared to the submicron stabilized particles precipitated at 3 and 25°C.

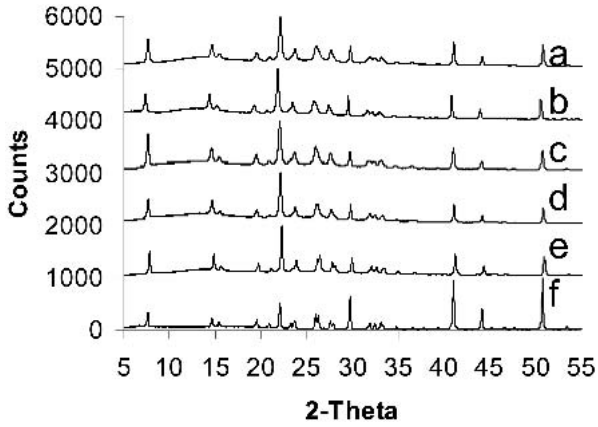

Fig. 3. XRD patterns of stabilized naproxen particles precipitated at (a) 3°C, (b) 25°C, and (c) 50°C, the (d) milled and (e) physical blend controls, and (f) bulk naproxen.

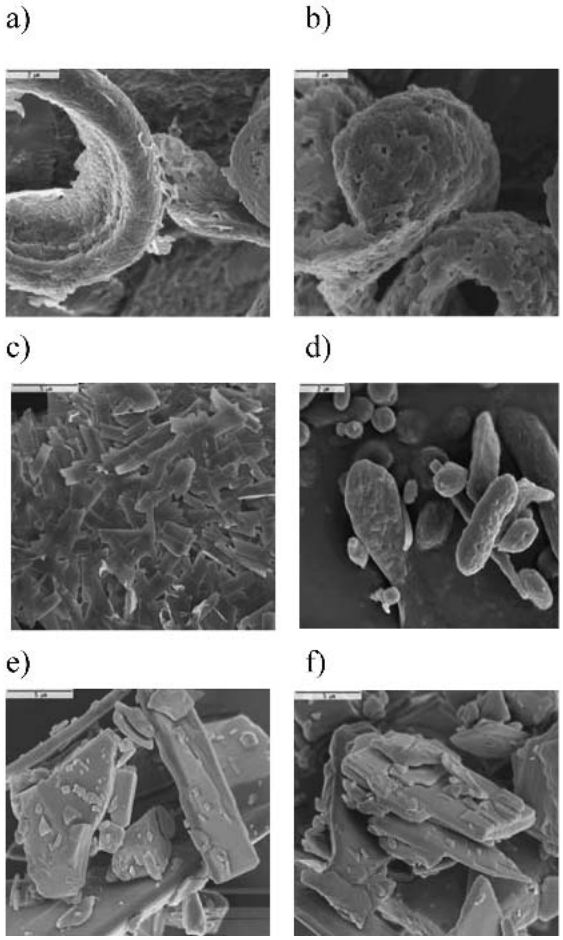

Fig. 4. SEM micrographs of stabilized danazol particles precipitated at (a) 3°C (scale bar = 2 μm), (b) 25°C (2 μm), and (c) 50°C (5 μm), the (d) milled (2 μm) and (e) physical blend (5 μm) controls, and (f) bulk danazol (5 μm).

In Fig. 4d, it is evident that the milled control particles had a different surface morphology than the stabilized danazol particles. Where the stabilized danazol particles were distinguishable and uniform in size, particles in the milled danazol control were more difficult to differentiate from POL 407 stabilizer. Upon closer investigation of Fig. 4d, small fractured crystals of danazol could be seen embedded in POL 407 stabilizer. The crystals were not as uniformly sized and shaped as the stabilized particles from precipitation, but were, however, primarily submicron. A rather large acicular crystal of danazol, about 6 μm in length, can be seen beneath the aggregates shown at the right of Fig. 4d. This crystal represents a portion of the milled control particles that was not sufficiently reduced in size during the 24- to 72-h milling period. In Fig. 4e, the physical blend control appeared to be composed of danazol crystals that were very similar in appearance to bulk danazol alone (Fig. 4f). In Fig. 4f, it is evident that bulk danazol was composed of crystalline plates with smooth surfaces and fractured edges. Bulk crystal sizes range between 1 and 20 μm in length. Smaller fractured pieces of danazol were present on the surfaces of the larger particles.

SEM micrographs of the samples containing naproxen are shown in Fig. 5. Figure 5a shows the surface morphology of the stabilized naproxen particles precipitated at 3°C. The individual naproxen crystals were submicron and cylindrical but were connected with PVP to form loose aggregates. In Fig. 5b, it can be seen that precipitation at 25°C yielded stabilized naproxen particles that were attached with PVP to form loose aggregates. In general, the individual naproxen crystals precipitated at 25°C appeared to be submicron, but were larger and more needle-like than the cylindrical particles precipitated at 3°C. Surface morphology differences of the stabilized naproxen particles precipitated at higher temperatures are more evident in Fig. 5c. When precipitated at 50°C, the crystals were more distinctly needle-like in shape than those precipitated at lower temperatures. The stabilized naproxen particles precipitated at 50°C were about 5–10 μm in length compared to the submicron stabilized particles precipitated at 3°C. These particles were also significantly larger than the particles precipitated at 25°C. The stabilized naproxen particles produced at kilogram-scale and isolated via spray drying are shown in Fig. 5d. In the SEM micrograph, naproxen crystals could not be distinguished from PVP. Hence, a homogeneous blend of naproxen crystals and PVP stabilizer comprised the smooth, granular particles, which were about 2–5 μm in diameter.

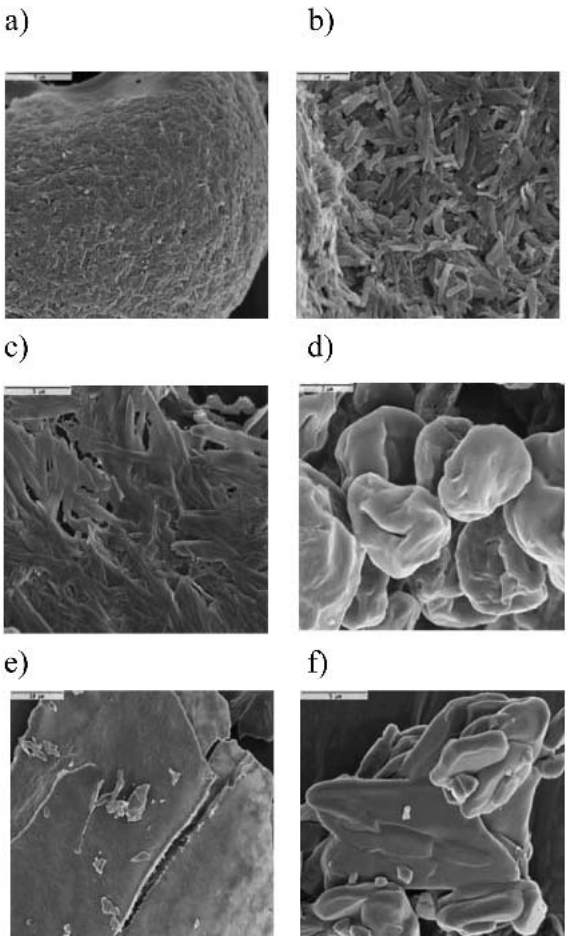

Fig. 5. SEM micrographs of stabilized naproxen particles precipitated at (a) 3°C (scale bar = 5 μm), (b) 25°C (2 μm), (c) 50°C (5 μm), (d) kilogram-scale (2 μm), and the (e) milled (10 μm) and (f) physical blend (5 μm) controls.

In Fig. 5e, it is evident that the milled control particles had a significantly different surface morphology than the stabilized naproxen particles. A single naproxen crystal, about 50 μm across, was partially fractured down the center of its plate-like face, which indicated that the 24- to 72-h milling period was not sufficient to reduce the crystal size of all naproxen particles. In Fig. 5f, the physical blend control was composed of plate-like naproxen crystals with smooth, irregular edges. The naproxen crystals in the physical blend were very similar in appearance to bulk naproxen alone (not shown). Bulk naproxen crystals were plate-like and had smooth irregular edges. Individual naproxen crystals were layered and loosely attached as stacked plates to form flat, crystalline clusters. Bulk naproxen crystals ranging between 5 and 50 μm across were observed via SEM.

Dissolution Testing

Dissolution profiles of the danazol samples are shown in Figs. 6a and 6b. The stabilized danazol particles precipitated at 50°C were completely dissolved within 2 min. The stabilized particles precipitated at 3°C and 25°C were completely dissolved within 5 and 10 min, respectively; however, the differences were not statistically significant. The milled control was completely dissolved within 5 min, but there were relatively large deviations in results between the 6 samples taken from the milled control for dissolution testing. The physical blend and bulk danazol controls were 47% and 39% dissolved within 5 min, respectively. The two controls did not dissolve completely within the 60-min testing period.

Dissolution profiles of the naproxen samples are shown in Figs. 7a and 7b. The stabilized naproxen particles precipitated at kilogram-scale dissolved significantly faster than all other naproxen samples tested except for the milled control. The stabilized naproxen particles precipitated at kilogram-scale and the milled control particles produced similar dissolution profiles. Both were 92% dissolved within 5 min and completely dissolved within 10 min. The stabilized naproxen particles precipitated at batch-scale dissolved more slowly than those precipitated at kilogram-scale, but significantly faster than the physical blend and bulk naproxen controls. The stabilized naproxen particles precipitated at 25°C dissolved slightly faster than those particles precipitated at 3°C and 50°C ($p > 0.05$); however, the stabilized particles were completely dissolved within 25 min regardless of precipitation temperature. The physical blend and bulk naproxen controls did not achieve complete dissolution during the 60-min testing period.

Residual Solvent Analysis

Residual methanol levels in the stabilized danazol and naproxen particles from controlled precipitation are shown in Table IV. Stabilized danazol particles precipitated at 3°C, 25°C, and 50°C had residual methanol levels of 70, 80, and 220 ppm, respectively. Stabilized naproxen particles precipitated at 3°C, 25°C, and 50°C had residual methanol levels of 230, 260, and 380 ppm, respectively. Stabilized naproxen particles (precipitation temperature = 3°C) precipitated at kilogram-scale had a residual methanol level of 70 ppm.

DISCUSSION

It can be construed from the significant reduction in primary particle size that both milling and controlled precipita-

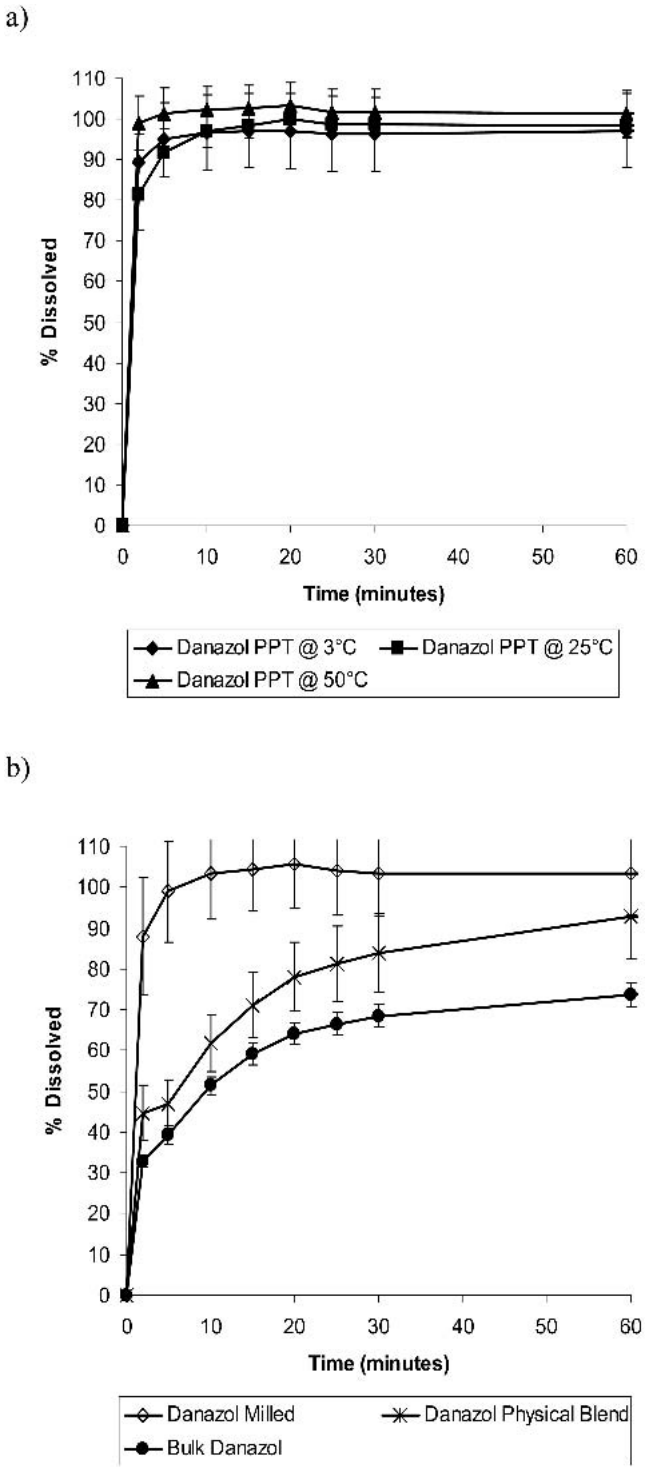

Fig. 6. Dissolution profiles of the (a) stabilized particles from controlled precipitation and (b) milled, physical blend, and bulk controls containing danazol.

tion offer great potential for enhancing the dissolution of danazol and naproxen. However, milling has several inherent disadvantages that controlled precipitation does not have. Mechanical grinding between drug particles and milling media results in localized heat generation due to friction. Subsequently, localized heating could result in drug decomposition (41). In addition, heat generated during milling could provide an environment conducive to microbial growth (42). Moreover, a portion of the particulates remains unmilled, thus resulting in a broad PSD (43). This phenomenon was observed for both model drugs investigated. Large danazol and naproxen particles that remained unmilled were not always detected in the samples taken for Coulter analysis; however, SEM analysis verified the occasional presence of large,

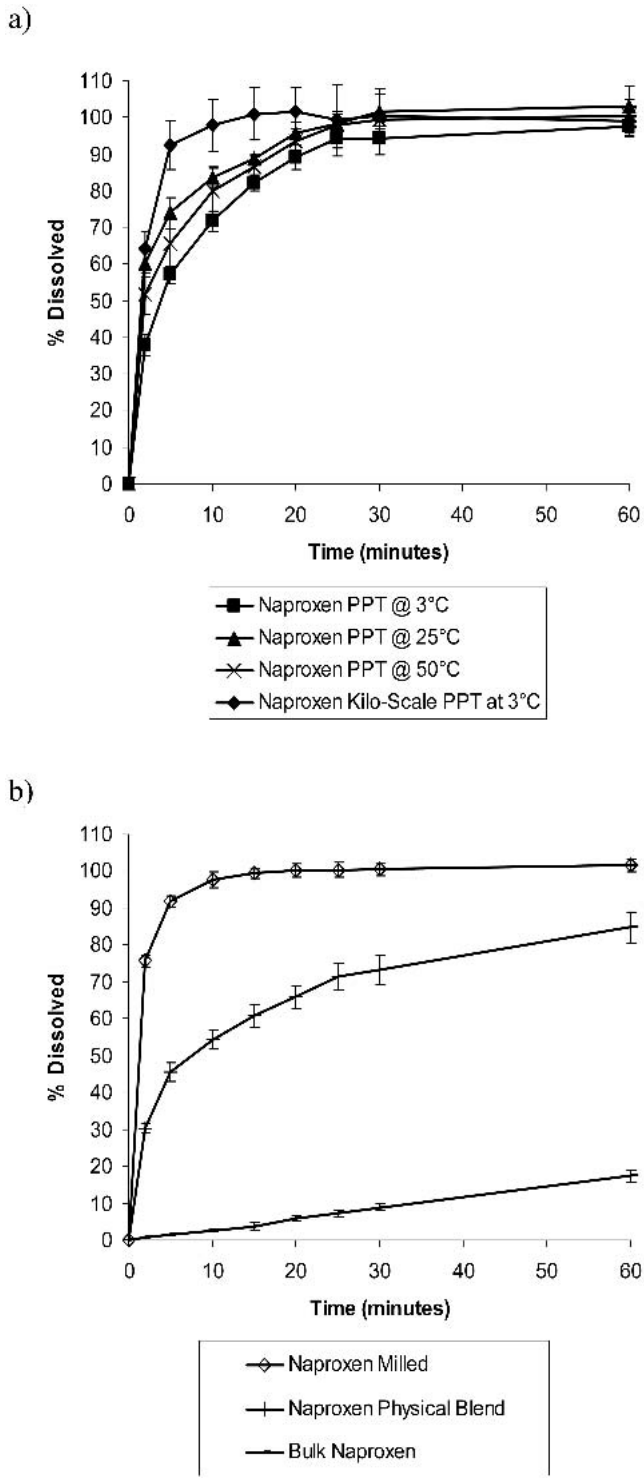

Fig. 7. Dissolution profiles of the (a) stabilized particles from controlled precipitation and (b) milled, physical blend, and bulk controls containing naproxen.

Table IV. Residual Methanol Levels in the Stabilized Danazol and Naproxen Particles Produced from Controlled Precipitation

Sample	Residual methanol % (w/w)	Residual methanol (ppm)
Danazol PPT @ 3°C	0.007	70
Danazol PPT @ 25°C	0.008	80
Danazol PPT @50°C	0.022	220
Naproxen PPT @ 3°C	0.023	230
Naproxen PPT @ 25°C	0.026	260
Naproxen PPT @ 50°C	0.038	380
Naproxen Kg-scale PPT	0.007	70

Note: PPT, precipitate; Kg, kilogram.

inadequately milled crystals (see Figs. 4d and 5e). In contrast, investigation of the stabilized particles from controlled precipitation via Coulter analysis and SEM confirmed the absence of large particles.

The grinding media used in the milling process could serve as a source of contamination. Certain types of grinding media can undergo attrition during the milling procedure. Subsequently, the resulting product is contaminated with small media fragments. This phenomenon has been observed in our laboratories. It was found that crystalline danazol particles promoted shedding from stainless steel grinding media. Upon X-ray fluorescence spectroscopy, milled particles were found to be contaminated with a silver-gray sediment comprising nickel, iron, and chromium, all of which composed the stainless steel media. Product contamination with grinding media fragments has also been documented by other research groups (22–24).

Danazol and naproxen were found via HPLC analysis to be chemically stable through controlled precipitation and isolation to dry stabilized particles. The short time period the organic solvent remained in the slurry prior to solvent removal and isolation did not affect the chemical stability of either drug. Controlled precipitation may minimize drug decomposition and contamination when compared to milling because there are minimal sources of attrition, and intense mixing facilitates the removal of heat generated within the mixing zone.

Because residence time in the mixing zone entails seconds, microbial contamination is much less of an issue in controlled precipitation. In addition, microbial growth is prevented prior to isolation of the dry stabilized particles due to the presence of organic solvent in the precipitated slurry. Milling media is not necessary in the controlled precipitation process, so this source of contamination is of no concern. Preferably, the mixing zone facilitates rapid macro- and micro-mixing of organic and aqueous phases to precipitate particles with narrow PSDs that are either nano- or microparticulate depending on a number of controllable parameters, including the drug, stabilizer(s), mixing zone temperature, organic solvent(s), or combinations thereof.

Stabilized danazol and naproxen particles were developed following preliminary investigations of the drugs in combination with numerous stabilizers and in various drug-to-stabilizer ratios. It was found that danazol particles were most effectively stabilized with POL 407 while naproxen particles were most effectively stabilized with PVP. Mean particle sizes of stabilized danazol and naproxen particles increased as precipitation temperatures were elevated from 3°C to 25°C to 50°C. Thus, it can be concluded that mixing zone temperature is a critical parameter that can be adjusted during controlled precipitation to manipulate particle size.

In contrast to many solubilization technologies, which achieve dissolution enhancement by converting drug habits from crystalline to amorphous (1,18,35–38,44), controlled precipitation significantly enhances dissolution of poorly soluble compounds by stabilizing nano- and microparticles that are crystalline. Growth of the nano- and micron-sized crystals is hindered by the rapid stabilization of the precipitated particles. The crystalline morphologies of the stabilized drug particles from controlled precipitation are similar to those of the unprocessed bulk drugs (see Figs. 2 and 3). For example, the XRD patterns of stabilized naproxen particles (Figs. 3a–3c) are very similar to the composite pattern of crystalline naproxen and amorphous PVP, that is, the physical blend control (Fig. 3e). From these findings, it can be concluded that controlled precipitation adds indispensable value to the particle engineering technology area since particle size, morphology, and crystallinity can be independently controlled. Furthermore, it is advantageous to engineer crystalline vs. amorphous particles because of the concern associated with recrystallization of amorphous forms over time.

As illustrated in Figs. 6 and 7, stabilized danazol and naproxen particles exhibited significantly enhanced dissolution properties when compared to physical blend and bulk controls. Stabilized danazol particles dissolved completely within 5 to 10 min regardless of precipitation temperature. Stabilized naproxen particles dissolved completely within 25 min regardless of precipitation temperature. The stabilized naproxen particles produced at kilogram-scale (3°C precipitation temperature) were completely dissolved within 10 min, thus dissolving significantly faster than the stabilized naproxen particles precipitated in batch mode. This was a surprising finding, and it was concluded from this study of particle performance attributes that controlled precipitation is an easily scalable process with the capability to further enhance the dissolution of some APIs upon scale-up.

Many significant milestones were achieved during scale-up efforts using naproxen as a model drug. When operated in batch mode, between 2 and 5 g of stabilized naproxen particles were isolated. When operated in continuous mode, 1.3 kg of stabilized naproxen particles were isolated. The stabilized naproxen particles produced in batch mode were lyophilized, and the stabilized naproxen particles produced in continuous mode were spray dried. The stabilized particles isolated via spray drying had a larger mean particle size, but dissolved significantly faster than the stabilized particles that were lyophilized. This was an unexpected finding, because previous experiences in our laboratories had correlated faster dissolution with stabilized particles from batch precipitation and lyophilization. However, these previous experiences were performed before the continuous controlled precipitation process was optimized to its current state. Thus, continuous controlled precipitation is advantageous to batch precipitation because production scale ranges from gram to kilogram quantities, and the stabilized particles from continuous controlled precipitation and spray drying can have superior dissolution properties to those from batch precipitation and lyophilization (Fig. 7).

In addition to exhibiting significantly enhanced dissolution properties, stabilized danazol and naproxen particles appear to be safe with respect to residual solvent levels. The Q3C Guidance for Industry document, which was drafted at the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, identifies methanol as a class 2 organic solvent (45). This guidance requires that a pharmaceutical does not contain more than 3000 ppm of methanol in order to be safe for human consumption. Residual methanol levels in the stabilized danazol and naproxen particles were well below the limit proposed by ICH guidelines (see Table IV). Thus, it can be concluded that the controlled precipitation and isolation techniques highlighted in this study can be used to produce stabilized drug particles with minimal residual solvent levels.

CONCLUSIONS

Controlled precipitation was demonstrated to enhance effectively the dissolution of two poorly soluble model compounds, danazol and naproxen. It was realized that stabilized particle size and morphology could be manipulated by altering various operating parameters during the controlled precipitation process, thus making possible the potential for particle customization. It can be concluded that controlled precipitation produces particles with minimal levels of contaminants, such as residual solvents. Thus, toxicity due to contamination should be minimal upon oral administration; however, bioavailability and toxicological studies will be highlighted in a subsequent publication. Finally, this study established the ease of scalability from milligram or gram to kilogram quantities. In conclusion, controlled precipitation offers many significant advantages and opportunities for dissolution enhancement of poorly soluble pharmaceutical compounds. Future studies include investigating the morphologies of stabilized drug particles from controlled precipitation and the mechanisms by which these novel morphologies facilitate enhanced dissolution of poorly soluble drugs.

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