Enhanced Aqueous Dissolution of a Poorly Water Soluble Drug by Novel Particle Engineering Technology: Spray-Freezing into Liquid with Atmospheric Freeze-Drying

True L. Rogers,[1,3] Andrew C. Nelsen,[1] Marazban Sarkari,[2,4] Timothy J. Young,[3] Keith P. Johnston,[2] and Robert O. Williams, III[1,5]

Received August 23, 2002; accepted November 5, 2002

Purpose. The purpose of this work was to investigate spray-freezing into liquid (SFL) and atmospheric freeze-drying (ATMFD) as industrial processes for producing micronized SFL powders with enhanced aqueous dissolution. Micronized SFL powders dried by ATMFD were compared with vacuum freeze-dried SFL powders.

Methods. Danazol was formulated with polyvinyl alcohol (MW 22,000), polyvinylpyrrolidone K-15, and poloxamer 407 to produce micronized SFL powders that were freeze-dried under vacuum or dried by ATMFD. The powders were characterized using Karl-Fischer titration, gas chromatography, differential scanning calorimetry, X-ray diffraction, scanning electron microscopy, surface area, and dissolution testing (SLS 0.75%/Tris 1.21% buffer media).

Results. Micronized SFL powders containing amorphous drug were successfully dried using the ATMFD process. Micronized SFL powders contained less than 5% w/w and 50 ppm of residual water and organic solvent, respectively, which were similar to those contents detected in a co-ground physical mixture of similar composition. Micronized SFL powders dried by ATMFD had lower surface areas than powders produced by vacuum freeze-drying (5.7 vs. 8.9 m^2/g) but significantly greater surface areas than the micronized bulk drug (0.5 m^2/g) and co-ground physical mixture (1.9 m^2/g). Rapid wetting and dissolution occurred when the SFL powders were introduced into the dissolution media. By 5 min, 100% dissolution of danazol from the ATMFD-micronized SFL powder had occurred, which was similar to the dissolution profile of the vacuum freeze-dried SFL powder.

Conclusions. Vacuum freeze-drying is not a preferred technique in the pharmaceutical industry because of scalability and high-cost concerns. The ATMFD process enables commercialization of the SFL particle-engineering technology as a micronization method to enhance dissolution of hydrophobic drugs.

KEY WORDS: spray-freezing into liquid; dissolution enhancement; danazol; atmospheric freeze-drying; micronization; particle engineering.

INTRODUCTION

Poorly water-soluble compounds are common among new chemical entities currently being investigated in the pharmaceutical industry as promising new active pharmaceutical ingredients (APIs). Several micronization techniques have been studied as methods to enhance the dissolution of hydrophobic APIs, as discussed in a recent review article (1). Compressed or supercritical CO_2 solvent and antisolvent techniques have been used with varying degrees of success to produce micronized pharmaceutical powders (2–5); however, limited CO_2 solvent capacity has been an obstacle in promoting adequate product load or recovery. Spray-freezing into vapor-over-liquid (SFV/L) processes have been used to micronize powder formulations for various purposes, including dissolution enhancement of hydrophobic APIs (6). During SFV/L, atomized droplets typically start to freeze in the vapor phase before they contact the meniscus of the cryogenic liquid. The frozen solvent is then removed in a subsequent step. As the solvent freezes, the API becomes supersaturated in the unfrozen regions of the atomized droplet, so API particles may nucleate and grow. Recently, we introduced a new technology, spray-freezing into liquid (SFL), for ultra-rapid freezing to minimize API particle growth (7). The fast freezing rates achieved with SFL have led to micron and submicron-sized amorphous particles with high surface areas depending on experimental conditions.

Because micronized SFL powders, characterized by porous aggregates composed of small particle domains and high surface areas, contain amorphous APIs nanostructurally embedded in hydrophilic excipient matrices, these powders have promoted rapid wetting and enhanced dissolution of insoluble APIs (6–8). Recently, a study showed that SFL processing of a danazol/hydroxypropyl-β-cyclodextrin inclusion complex from solution produced a micronized SFL powder that dissolved more rapidly and to a greater extent in aqueous media than inclusion complexes prepared using conventional techniques (co-grinding, slow-freezing with lyophilization; Ref. 7). Because the SFL process has been shown to produce micronized powders exhibiting faster dissolution than powders produced using conventional methods, the SFL process is a promising new technology in the pharmaceutical industry that must be investigated and optimized.

To obtain dry micronized SFL powders, the frozen solvent(s) must be sublimed. Before this study, vacuum freeze-drying (e.g., tray lyophilization) was used to remove the solvents while maintaining the structure of the microparticles. Because of difficulty with scale-up and the high expense associated with vacuum requirements, vacuum freeze-drying is not a preferred processing technique in the pharmaceutical industry. Therefore, a technique has been devised that is capable of freeze-drying at or above atmospheric pressure. This technique, atmospheric freeze-drying (ATMFD), uses cryogenic air to fluidize the powder, facilitating mass transfer rates in solvent sublimation.

The SFL and ATMFD technologies involve ultra-rapid freezing rates achieved with SFL and subsequent removal of frozen solvents at atmospheric pressure by ATMFD for the purpose of enhancing the aqueous dissolution of hydrophobic APIs. However, it has not been determined whether micronized SFL powders dried by ATMFD wet and dissolve readily in aqueous media, a goal that has been achieved with vacuum freeze-dried micronized SFL powders.

The objective of this study was to demonstrate that

[1] College of Pharmacy, University of Texas at Austin, Austin, Texas 78712.
[2] Department of Chemical Engineering, University of Texas at Austin, Austin, Texas 78712.
[3] Present address: The Dow Chemical Company, Midland, Michigan, 48674.
[4] Present address: RxKINETIX, Westminster, Colorado, 80031.
[5] To whom correspondence should be addressed. (e-mail: williro@mail.utexas.edu)

0724-8741/03/0300-0485/0 © 2003 Plenum Publishing Corporation

ATMFD is an effective solvent-removal technique that can produce micronized SFL powders with similar enhanced dissolution profiles to those micronized SFL powders dried by vacuum freeze-drying. The effect of the drying method (ATMFD vs. tray lyophilization) on the physical characteristics of the micronized SFL powders was determined.

MATERIALS AND METHODS

Micronized danazol, USP, hydrolyzed poly(vinyl alcohol) (PVA; MW 22,000), poloxamer 407 (Pol), polyvinylpyrrolidone (PVP) K-15, sodium lauryl sulfate (SLS), tris(hydroxymethyl)aminomethane (Tris) and 1 N hydrochloric acid (HCl) were purchased from Spectrum Chemicals (Gardena, CA, USA). Tetrahydrofuran (THF, high-performance liquid chromatography [HPLC] grade) was purchased from Mallinckrodt (Paris, KY, USA). Acetonitrile, *N*,*N* dimethylformamide, and 1-butanol, all of HPLC grade, were purchased from EM Sciences (Gibbstown, NJ, USA). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ, USA).

Preparation of Micronized SFL Powders

Micronized SFL powders were prepared as described in a previous study (10). An aliquot of 2.5 g of danazol was dissolved in 372.5 g of THF. Aliquots of 1.25 g PVA, 1.25 g poloxamer, and 1.25 g PVP were dissolved in 746.25 g of purified water. The two solutions were added together and mixed to form a one-phase cosolvent solution. The cosolvent solution was then rapidly frozen by SFL to produce the micronized SFL powder. A schematic illustration of the SFL process is shown in Fig. 1. Each solution (Fig. 1a) was atomized beneath the liquid nitrogen (Fig. 1b) surface at 5000 psi (34.5 MPa) constant pressure and a flow rate of 20 mL/min through a 63.5-μm I.D. polyether-ether ketone nozzle (Fig. 1c) 15 cm in length using a Jasco Model PU-1586 HPLC pump (Fig. 1d; Jasco, Inc., Easton, MD, USA). The cryogenic suspension was then poured into a noninsulated beaker to allow the nitrogen to evaporate. Once the nitrogen had evaporated, the frozen micronized SFL powder was immediately dried by either vacuum freeze-drying or ATMFD.

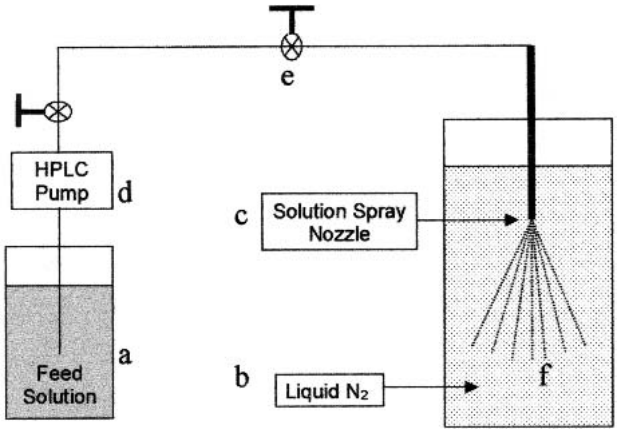

Fig. 1. Schematic illustration of the SFL process used to make the micronized powder samples. (a) Cosolvent feed solution, (b) liquid nitrogen, (c) 127-μm I.D. × 15-cm long insulated polyether-ether ketone nozzle, (d) high-performance liquid chromatography pump, (e) high-pressure valve, and (f) atomized frozen microdroplets.

Vacuum Freeze-Drying by Tray Lyophilization

Vacuum freeze-drying was performed in a VirTis Advantage Benchtop Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY, USA). The lyophilizer tray was equilibrated to −40°C before vacuum freeze-drying. The tray lyophilization parameters used to dry the micronized SFL powders and slowly frozen control (described below) are listed in Table I.

ATMFD

ATMFD was used to dry the micronized SFL powders using an apparatus devised in this laboratory. A schematic illustration of the ATMFD apparatus is shown in Fig. 2. The major functional components of the ATMFD apparatus consisted of an external compressed air source (Fig. 2a; in-house compressed air), compressed air dryer (Fig. 2b; Model HR2-12FM-000, Twin Tower Engineering, Broomfield, CO, USA), 14-plate brazed heat exchanger (Fig. 2c; Model CB14-14H-T06, Thermal Transfer Systems, Inc., Dallas, TX, USA), refrigerated circulating cooler (Fig. 2d; VWR Scientific Corporation, West Chester, PA, USA), and an ATMFD chamber constructed from a 300-mL stainless-steel Whitey (5.08 cm I.D. × 22.7 cm long) gas vessel (Fig. 2e; Arthur Valve and Fitting, Austin, TX, USA). The ATMFD chamber was rounded on the bottom to a quarter-inch opening. A fine wire mesh (Fig. 2f) was placed at the bottom of the ATMFD chamber to prevent powder loss. A cotton cloth filter (Fig. 2g) was used to prevent powder loss from the top of the ATMFD chamber. All air and coolant lines, as well as the 14-plate brazed heat exchanger and ATMFD chamber, were covered with two layers of insulation tape. Before ATMFD, the system was precooled to −30°C. A 50-g aliquot of frozen micronized SFL powder was charged into the ATMFD chamber for each run. The ATMFD parameters used to dry the micronized SFL powders are listed in Table II.

First, moisture and impurities were removed from the air by the compressed air dryer (Fig. 2b); then, the air was circulated through the 14-plate brazed heat exchanger (Fig. 2c) and cooled to the desired temperature using the refrigerated circulating coolant (Fig. 2d). After circulating through the heat exchanger, the cold dry air was routed into the ATMFD chamber (Fig. 2e) through the quarter-inch opening fitted to fluidize and dry the micronized SFL powder. A cotton cloth filter retained the fluidized powder inside the ATMFD chamber. Once dried, the micronized SFL powder was removed from the inside of the ATMFD chamber and collected from the cotton filter.

Preparation of Control Formulations

A co-ground physical mixture consisting of 1.0 g of danazol, 0.5 g of PVA, 0.5 g of poloxamer, and 0.5 g of PVP

Table I. Vacuum Freeze-Drying Parameters Used to Dry the Micronized SFL Powders

Time (h)	Temperature (°C)	Vacuum (mTorr)
24	−40	500
3	−40	100
3	−30	100
4	−20	100
4	+10	100
34	+25	100

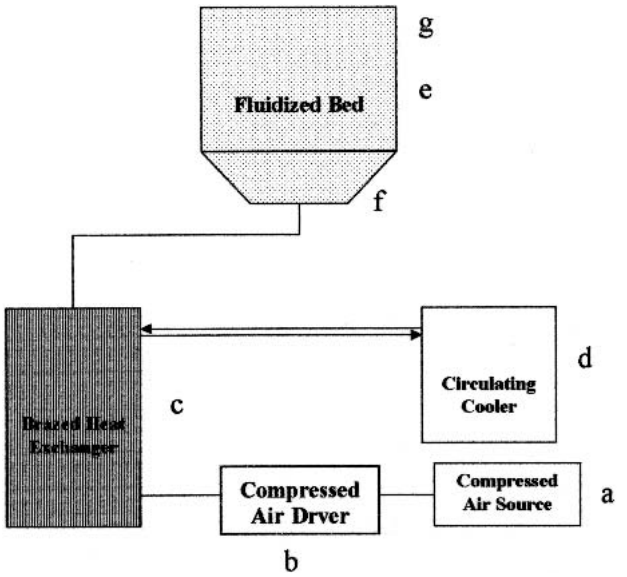

Fig. 2. Schematic illustration of the ATMFD process designed in this study to dry the frozen micronized SFL powders. (a) Compressed air source, (b) compressed air dryer, (c) 14-plate brazed heat exchanger, (d) circulating cooler, (e) ATMFD chamber, (f) wire mesh to prevent powder escape from bottom of ATMFD chamber, and (g) cotton filter cloth to prevent SFL powder escape from top of ATMFD chamber.

(identical to API:excipients ratio composing the micronized SFL formulations) was mixed by geometric dilution and ground using a mortar and pestle for 10 min and then mixed for 30 min in a V-blender.

A solution identical to the feed solutions used for SFL processing was frozen in the VirTis Tray Lyophilizer pre-equilibrated at –40°C. After the solution had completely solidified, the sample was vacuum freeze-dried using the lyophilization parameters listed in Table I. This sample is referred to as the slowly frozen control.

Karl-Fischer Titration

Residual water levels were determined using an Aquatest 8 Karl-Fischer Titrator (Photovolt Instruments, Indianapolis, IN, USA). Ten milligrams of powder were weighed and transferred into the vessel opening of the titrator following equilibration and calibration of the machine. Each sample was measured in replicates of three (n = 3).

Gas Chromatography (GC) Analysis

GC measurements of residual THF levels in the formulations were determined using a Hewlett-Packard 5890A gas chromatograph. Samples were dissolved in dimethylformamide, and 1-butanol was incorporated as an internal standard. Each sample was then filtered through a 0.45-μm Acrodisc GHP membrane filter (Pall Corporation, Ann Arbor, MI, USA) into an HPLC vial (Wheaton, Millville, NJ, USA) and capped. An aliquot of 1 μL from each sample was then injected into the GC instrument. Eleven calibration standards were made ranging from a THF concentration of 50 ppm to 50,000 ppm. The calibration standards were used to quantitate the residual THF levels present within the micronized SFL powder samples.

Table II. Atmospheric Freeze-Drying Parameters Used to Dry the Micronized Spray-Freezing into Liquid Powders

Time (h)	Temperature (°C)	Compressed air flow
12	–30	28
12	–20	28
12	–10	28
12	0	28
12	+10	28
12	+25	28

Differential Scanning Calorimetry (DSC)

Aliquots weighing between 1 and 20 mg were leveled in an aluminum pan (Kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT, USA) and crimped with an aluminum lid. A DSC 2920 TA Instruments Thermal Advantage Instrument Control and Universal Analysis 2000 software were used to measure the presence or absence of the danazol melting endotherm (227°C) in the various samples. DSC was used to analyze the samples from 25–300°C with a 10°C per minute heating rate.

X-Ray Powder Diffraction (XRD)

A Philips 1710 X-ray diffractometer with a copper target and nickel filter (Philips Electronic Instruments, Inc., Mahwah, NJ, USA) and Jade 5 XRD pattern processing software (Materials Data, Inc., Irvine, CA, USA) were used to obtain the XRD patterns of the samples. Approximately 10 mg of powder was dispersed in two drops of amyl acetate, and the paste was spread and leveled onto a glass microscope slide. After the amyl acetate had evaporated under vacuum, the XRD pattern of the leveled powder was measured from 5–50 2θ degrees using a step size of 0.05 2θ degrees and a dwell time of 1 s at each step.

Scanning Electron Microscopy (SEM)

A Hitachi S-4500 field emission scanning electron microscope was used to obtain SEM micrographs of the powder samples, which had been gold-palladium sputter coated prior to analysis.

Surface Area Analysis

Specific surface area was measured using a NOVA-2000 Version 6.11 instrument with NOVA Enhanced Data Reduction Software Version 2.13 (Quantachrome Corporation, Boynton Beach, FL, USA). A known amount of powder (~200 mg) was loaded into a Quantachrome sample cell and degassed for at least 3 h before analysis.

Dissolution Testing

Dissolution testing was performed on the powders using a United States Pharmacopeia 24 Type 2 apparatus (VanKel VK6010 Dissolution Testing Station with a Vanderkamp VK650A heater/circulator). Approximately 10 mg of powder containing about 4 mg of danazol was weighed out and placed into 900 mL of SLS/Tris dissolution media. Five-milliliter samples were collected at 2, 5, 10, 20, 30, and 60 min in replicates of 6 (n = 6) by a VK8000 autosampler (VanKel Tech-

nology Group, Cary, NC, USA) and analyzed by HPLC. Paddle speed and bath temperature were set at 50 rpm and 37.0 ± 0.2°C, respectively. Sink conditions were maintained throughout the dissolution testing period. The dissolution media was prepared by dissolving 150 g of SLS and 242 g of Tris in approximately 18 L of purified water followed by adjusting the pH to 9.0 with 1 N HCl and the volume to 20 L with purified water while continuously stirring.

HPLC Analysis

Samples for HPLC analysis were filtered through 0.45-μm Acrodisc GHP syringe filters (Pall Corporation, Ann Arbor, MI, USA) and analyzed at 288 nm using a Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an Alltech 5 μm ODS-2 (C-18) reverse-phase column (Alltech Associates, Inc., Deerfield, IL, USA). The danazol peak eluted at 5 min when running mobile phase (70% acetonitrile/30% water, v/v) at 1 mL/min. System suitability requirements were met (correlation coefficient (r^2) ≥ 0.998, precision of five replicate injections ≤ 2.0% RSD, theoretical plates > 500 plates/column and peak asymmetry ≤ 1.5). A check standard was injected after each five unknown samples throughout the HPLC batch run.

Statistical Analysis

One-way analysis of variance was used to determine statistically significant differences between results. Results with p values < 0.05 were considered statistically significant.

RESULTS

The micronized SFL powders investigated in this study were investigated by varying the drying step using either vacuum freeze-drying or ATMFD, and a number of analytical techniques were used to characterize and compare the powders. KF titration was used to determine the residual water content of each of the various samples investigated, and the results are listed in Table III. Because bulk danazol is hydrophobic, residual water was not detected in this compound. However, co-grinding the bulk danazol together with PVA, poloxamer, and PVP produced a powder with a residual water content of 4.1% (w/w). PVP is a hygroscopic material that was determined to have 5.1% residual water, but PVA and poloxamer had lower residual moisture levels. Hence, the residual moisture of the co-ground physical mixture resulted from the incorporation of PVP into the formulation. Residual moisture in the slowly frozen control (7.6%) was significantly higher than that in the co-ground physical mixture ($p < 0.05$). SFL processing produced micronized powders with similar residual moisture levels to that of the co-ground physical mixture. The vacuum freeze-dried and ATMFD-micronized SFL powders had comparable residual moisture contents of 4.7% and 5.4%, respectively. Therefore, solvent sublimation via either vacuum freeze-drying or ATMFD produced dry micronized SFL powders with significantly lower residual moisture levels than that in the slowly frozen control ($p < 0.05$).

Table III. Residual Water Content of the Various Powders Investigated in This Study

Sample	Residual water content (w/w%)
Bulk danazol	None
Dan/PVA/Pol/PVP physical mixture	4.1 ± 0.3
Slowly frozen Dan/PVA/Pol/PVP	7.6 ± 1.0
Lyophilized SFL Dan/PVA/Pol/PVP	4.7 ± 0.3
ATMFD SFL Dan/PVA/Pol/PVP	5.4 ± 0.4

Note: PVA, poly (vinyl alcohol); Dan, danazol; Pol, poloxaner 407; PVP, polyvinylpyrrolidine; ATFMD, atmospheric freeze-drying; SFL, spray-freezing into liquid.

GC was used to determine the residual organic solvent levels within the micronized SFL powders after processing. When both vacuum freeze-dried and ATMFD micronized SFL powders were analyzed by GC, residual THF was not detected within either formulation. Because the lowest calibration standard was 50-ppm THF concentration, the results are stated as < 50 ppm.

The DSC profiles of the various samples investigated are shown in Fig. 3. From the bulk danazol DSC profile (Fig. 3a), it was determined that the melting point of danazol occurred at 225°C, as was evident from the sharp endotherm at that temperature. When investigating the DSC profile of the co-ground physical mixture (Fig. 3b), broad melting endotherms for poloxamer (50°C), PVP (75°C), and PVA (100°C) were detected; however, the danazol melting endotherm at 225°C was absent. When the excipients formed a molten solution between 50 and 100°C, danazol dissolved in the molten excipient mixture, thus eliminating the melting endotherm at 225°C. It can be seen when investigating the DSC profiles of the slowly frozen control (Fig. 3c), vacuum freeze-dried (Fig. 3d), and ATMFD (Fig. 3e)-micronized SFL powders that the melting endotherm of danazol was not present at 225°C. Danazol recrystallization exotherms occurred between 185 and 200°C for the various formulations investigated due to excipient burn-off.

DSC was of limited value to study crystallinity of danazol in the presence of the excipients; therefore, XRD was used to verify the presence or absence of crystalline danazol within the various dry powder formulations investigated. In Fig. 4, it can be seen from the presence of intense peaks between 10 and 25 2θ degrees that danazol in the bulk form is highly crystalline (Fig. 4a). Furthermore, it was observed that characteristic crystalline danazol peaks were found in the XRD patterns of the co-ground physical mixture (Fig. 4e) and the slowly frozen control (Fig. 4f), respectively, thus indicating the presence of crystalline API in these two formulations. The vacuum freeze-dried micronized SFL powder exhibited an amorphous XRD pattern (Fig. 4g). The ATMFD micronized SFL powder exhibited an amorphous XRD pattern identical to that of the vacuum freeze-dried micronized SFL powder (Fig. 4h). Specific surface areas of the powders investigated in this study were measured to determine differences in surface area as a function of the freezing process and the sublimation technique used. As can be seen from Table IV, bulk danazol had the lowest specific surface area of 0.52 m^2/g. The co-ground physical mixture had a higher specific surface area (1.92 m^2/g) than that of the bulk API, but slow freezing and subsequent vacuum freeze-drying of the solution produced a powder with a significantly higher specific surface area (3.14 m^2/g) than the other two control formulations ($p < 0.05$). The vacuum freeze-dried micronized SFL powder had a significantly higher specific surface area (8.90 m^2/g) than that of the

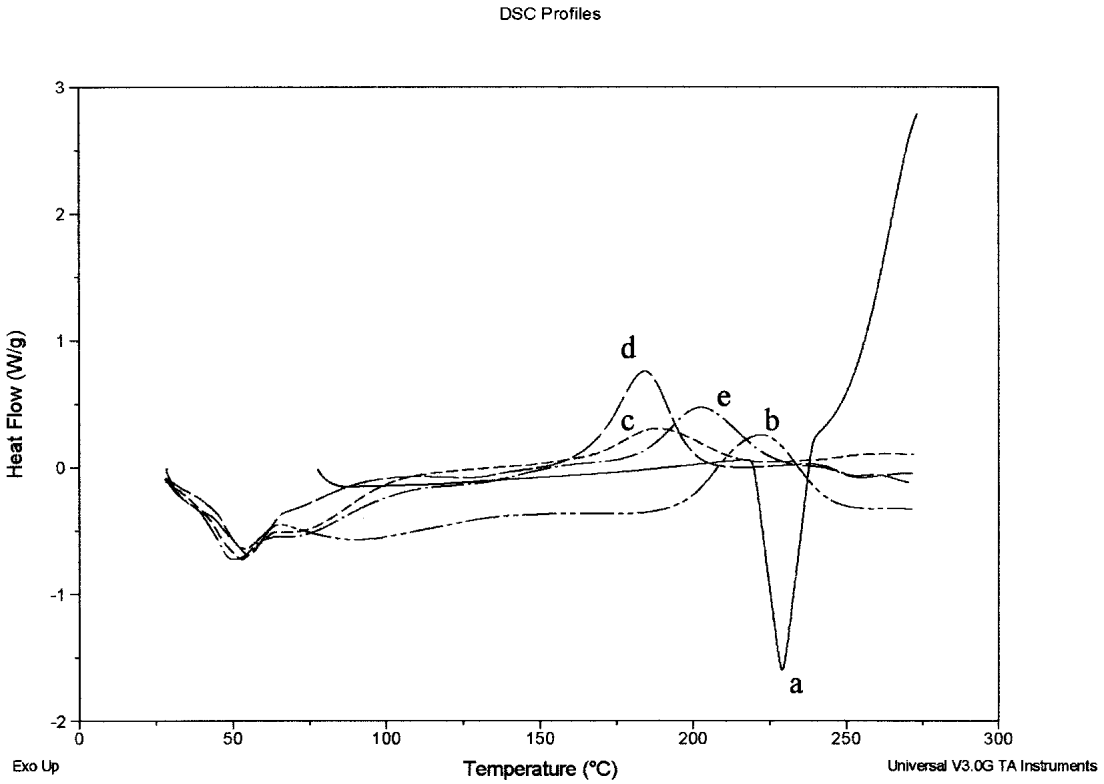

Fig. 3. DSC profiles of bulk danazol (a), physical mixture (b), slowly frozen control (c), vacuum freeze-dried micronized SFL powder (d), and ATMFD micronized SFL powder (e).

ATMFD micronized SFL powder (5.72 m^2/g; $p < 0.05$). However, the ATMFD micronized SFL powder had a significantly higher surface area than those of the control formulations ($p < 0.05$).

SEM was used to investigate surface morphologies of the samples. SEM micrographs of the samples are shown in Fig. 5. It can be seen in Fig. 5a that bulk micronized danazol consisted of crystalline plates with smooth surfaces and fractured edges. The particle sizes ranged from 1 to 15 μm in length. In Fig. 5b, the SEM micrograph of the co-ground physical mixture revealed a heterogeneous blend of API and excipients. The spherical poloxamer particle had danazol plates adsorbed to its surface. In Fig. 5c, the SEM micrograph of the slowly frozen control displayed large (≥100 μm) continuous aggregates with smooth surfaces, and API and excipients were homogeneously blended together as a result of slowly freezing the cosolvent solution. The SEM micrograph of the vacuum freeze-dried micronized SFL powder is shown in Fig. 5d. The vacuum freeze-dried micronized SFL powder consisted of porous aggregates comprised of small drug particle domains. The porous microparticulate aggregates were approximately 20 μm in diameter and were loosely connected to form a network with adjacent aggregates. SEM micrographs of the ATMFD micronized SFL powder are shown in Figs. 5e and 5f. Fluidization of the frozen micronized SFL powder produced individual porous aggregates composed of small drug particle domains. The ATMFD SFL porous aggregate microparticles were approximately 10 μm in diameter, so these particles were smaller than the vacuum freeze-dried SFL microparticles.

The dissolution profiles of the various samples investi-

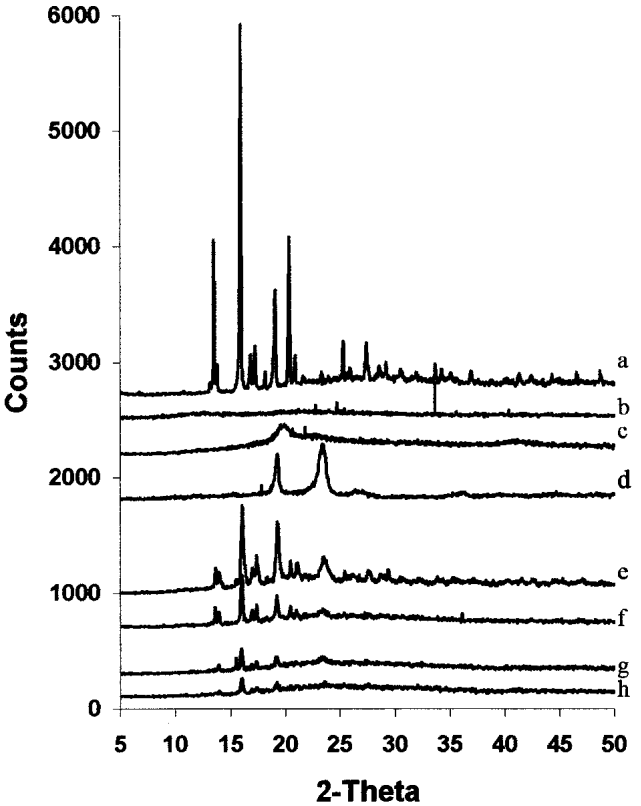

Fig. 4. XRD patterns of bulk danazol (a), bulk PVP (b), bulk PVA (c), bulk poloxamer (d), co-ground physical mixture (e), slowly frozen control (f), vacuum freeze-dried micronized SFL powder (g), and ATMFD micronized SFL powder (h).

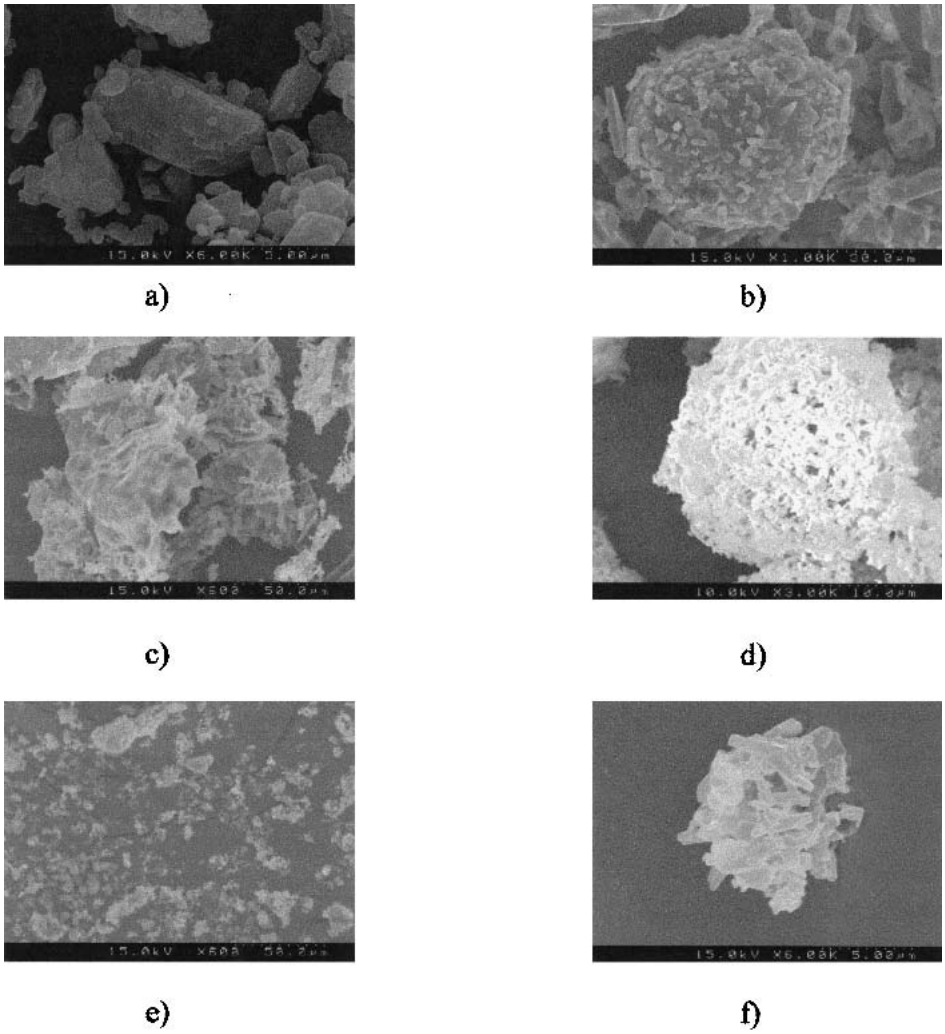

Fig. 5. SEM micrographs of bulk danazol (a), co-ground physical mixture (b), slowly frozen control (c), vacuum freeze-dried micronized SFL powder (d), and ATMFD micronized SFL powder (e, f).

gated are shown in Fig. 6. It can be seen that the co-ground physical mixture wetted and dissolved significantly more slowly and to a lesser extent than bulk danazol ($p < 0.05$). In the absence of excipients, bulk danazol dissolved significantly faster in the dissolution media than the co-ground physical mixture ($p < 0.05$). The slowly frozen control wetted and dissolved significantly faster in aqueous media than bulk danazol or the co-ground physical mixture ($p < 0.05$).

It can be seen in Fig. 6 that complete (100%) dissolution of danazol occurred from both micronized SFL powders

Table IV. Specific Surface Areas of the Various Powders Investigated

Sample	Specific surface area (m^2/g)
Bulk danazol	0.52
Dan/PVA/Pol/PVP physical mixture	1.92
Slowly frozen Dan/PVA/Pol/PVP	3.14
Lyophilized SFL Dan/PVA/Pol/PVP	8.90
ATMFD SFL Dan/PVA/Pol/PVP	5.72

Dan, danazol; PVA, poly (vinyl alcohol); Pol, poloxamer 407; PVP, polyvinylpyrrolidine; ATFMD, atmospheric freezing-drying; SFL, spray-freezing into liquid.

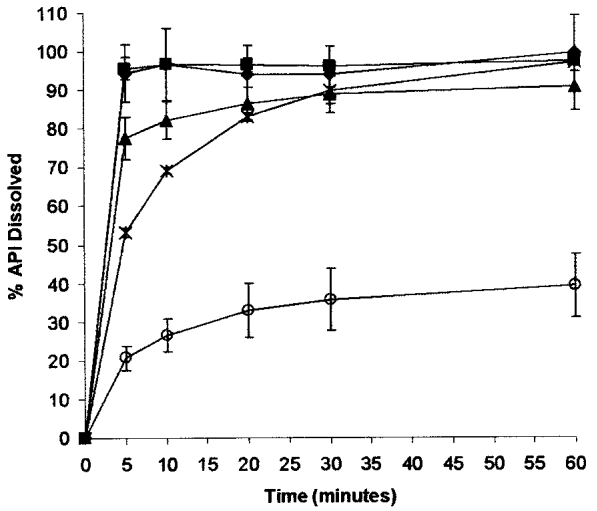

Fig. 6. Aqueous dissolution profiles of the co-ground physical mixture (o), bulk danazol (*), slowly frozen control (▲), ATMFD micronized SFL powder (♦), and vacuum freeze-dried micronized SFL powder (■) (n = 6; mean ± SE).

within 5 min, which was significantly higher than the amount dissolved from the slowly frozen control (78%) within the same time period ($p < 0.05$). Furthermore, the amounts of danazol dissolved from the two micronized SFL powders were significantly higher for the first 20 min of the dissolution study than that dissolved from the slowly frozen control, co-ground physical mixture or bulk danazol ($p < 0.05$).

DISCUSSION

Traditional SFV/L processing followed by freeze-drying has been investigated in the ceramics and semiconductor industries (9–12), in rapid freezing of biologic samples (13), and in the pharmaceutical industry for manufacture of API powders (14–20). In contrast with the SFV/L technologies, the SFL process was developed to incorporate direct liquid–liquid impingement between the atomized feed solution and cryogenic liquid to provide more intense atomization into microdroplets and consequently significantly faster freezing rates. The SFL particle engineering technology has been used to produce micronized powders, which contain an amorphous API molecularly dispersed within an excipient matrix, for the purpose of enhancing the aqueous dissolution of insoluble or poorly water soluble compounds (6). A schematic illustration of the SFL process is shown in Fig. 1 and was described earlier in "Preparation of the Micronized SFL Powders" section.

Numerous studies have demonstrated that significantly faster freezing rates could be achieved by plunging a liquid sample directly into a cryogenic liquid rather than solidifying the liquid in the corresponding vapor phase (13,17,21–24). However, Heller et al. (17) found that even faster freezing rates than those achieved by plunge-cooling could be accomplished by atomizing a feed solution into small, high surface area droplets that settled through the vapor phase onto the liquid nitrogen meniscus and solidified.

The SFL technique developed in this study used the direct impingement between the feed and cryogenic liquids to atomize the feed solution into microdroplets that have been initially plunged beneath the surface of the cryogenic liquid because of the placement of the nozzle directly into the cryogen. As a result, the microdroplets were frozen immediately after atomization. Therefore, the SFL process was advantageous compared with the SFV/L processes because faster freezing rates are achieved, thus resulting in reduction of the primary particle size of the powder, generation of the amorphous form of the API, and enhancement of the surface area of the micronized powder, all of which contribute to enhanced dissolution of poorly water-soluble APIs.

Frozen solvents must be sublimed to obtain dry micronized SFL powder. Until recently, vacuum freeze-drying was the only method to dry the frozen SFL powder while maintaining a temperature beneath its freezing point, which is necessary to retain the structural integrity of the engineered microparticles. Tray lyophilization is a freeze-drying technique, where the shelf temperature can be adjusted to optimize the solvent sublimation rate while maintaining a temperature that does not allow melt-back of a frozen sample (17,22–28). Although solvent(s) can be removed from a frozen sample to obtain dry product using vacuum freeze-drying, this technique has significant disadvantages. The batch size of the sample is limited by the capacity of the vacuum pump used. Hence, tray lyophilization of a large batch requires the use of a higher capacity vacuum pump. A second drawback of vacuum freeze-drying occurs when organic solvents are present in the formulation. Organic solvents are necessary in SFL feed solutions to dissolve the hydrophobic API. Because many of the hydrophilic polymers used to enhance the aqueous dissolution of an API are not soluble in organic solvents, water is necessary as a cosolvent. Thus, a cosolvent solution containing both the API and the hydrophilic polymers is formed to allow intricate mixing between all pharmaceutical ingredients. Because the organic solvents typically have higher vapor pressures than water, a large capacity pump, capable of attaining a sufficient vacuum in the presence of sublimed organic solvent, is required. In addition, an organic solvent trap must be used to remove the solvent as it sublimes; however, a suitable vacuum will not be attained within the system until the trap has completely captured the solvent vapor. Organic solvent removal using a liquid nitrogen trap is a time consuming, but necessary, step in vacuum freeze-drying. Because of the drawbacks associated with batch size scale-up and high cost with tray lyophilization, vacuum freeze-drying is not a preferred technique for solvent removal in the pharmaceutical industry. Hence, an acceptable alternative to vacuum freeze-drying is desirable to effectively remove solvents from frozen micronized SFL powders without allowing product melt-back.

ATMFD is a process where a frozen micronized SFL powder is fluidized by dry air, which is maintained at a temperature beneath that of the melting point of the frozen powder. As the air fluidizes the powder, frozen solvents are sublimed, and the solvent vapors are removed from the fluidized bed. The dry micronized SFL powder is retained within the ATMFD fluidized bed and can be collected following solvent removal. A schematic drawing of the ATMFD apparatus is shown in Fig. 2 and was described earlier in the "AFMFD" section. Drying rates are higher with ATMFD compared with vacuum freeze-drying because of more efficient solvent removal resulting from constant powder fluidization and high air throughput.

ATMFD derives it name from the fact that frozen solvents, both organic and aqueous, are sublimed from the micronized SFL powder at or above atmospheric pressure rather than under a vacuum. Thus, scalability issues due to vacuum limitations are eliminated with ATMFD. Powder batch sizes can range from milligram to kilogram quantities, and the fluidized bed system can be altered to operate at cryogenic temperatures, regardless of bed size, by incorporating air coolers and using a well-insulated system. ATMFD has been developed based upon principles from conventional fluidized bed technology (14). Fluidized beds are used in spray-drying apparatuses to evaporate liquid solvents under elevated temperatures, thus producing dry powders. However, a high-temperature fluidized bed drying process cannot be used to remove liquid solvent(s) from thermally labile compounds or excipients with low melting points, such as surfactants or low molecular weight polymers, nor can a fluidized bed be used to remove solvents from a frozen powder without allowing sample melt-back to occur. Mumenthaler and Leuenberger adapted a Wurster-type fluidized bed apparatus to allow the use of cryogenic fluidizing air rather than high temperature air (14). The objective of their study was to design a spray-freeze drying apparatus that produced particles by atomizing a feed solution into the vapor above a fluidized bed where the

vapor was air cooled by dry ice. The powder was fluidized, and the frozen solvent was sublimed by the low temperature air to produce dry powder.

In the present study, atomization into vapor was not favored because faster freezing rates were obtained by atomizing directly into the cryogenic liquid. Therefore, the SFL and ATMFD technologies combine the advantages of ultra-rapid freezing with solvent removal at atmospheric conditions. The ATMFD process used in this study was developed by adapting the cryogenic fluidized bed concept to dry the frozen micronized SFL powders. Micronized SFL powders were dried by both vacuum freeze-drying and ATMFD, and compared to determine any physical differences as a function of the drying technique used. As can be seen from Table I, up to 24 h at –40°C were required to remove THF from the frozen micronized SFL powders to obtain a suitable vacuum during vacuum freeze-drying. From Table II, it can be seen that only 12 h at –30°C were necessary to remove THF from the frozen micronized SFL powders when using the ATMFD process. The organic solvent removal rates were higher due to fluidization and agitation that occurred when cryogenic air was passed through the ATMFD chamber. In addition, a liquid nitrogen trap was not required to remove THF from the ATMFD system, because the fluidization media removed the solvent quickly and efficiently with positive air pressure.

Whereas 34 h were necessary to sublime secondarily bound water at 25°C with vacuum freeze-drying, only 12 h were needed to obtain a dry flowable powder with ATMFD. Hence, fluidization of the powder at or greater than atmospheric pressure significantly increased both organic and aqueous solvent removal rates. Optimization of the ATMFD process is ongoing, and overall drying times will be significantly shorter with a fully optimized system because enhanced mass transfer rates of the solvents from the solid phase to the vapor phase during sublimation.

It was concluded that ATMFD was as effective as vacuum freeze-drying at subliming water from frozen micronized SFL powders. It was also concluded that ATMFD was as effective as vacuum freeze-drying at reducing residual organic solvent in micronized SFL formulations. The International Conference on Harmonization (ICH) classifies THF as a class 3 solvent with low toxic potential (29). Amounts of THF less than 5000 parts per million (ppm) per day are acceptable. From Table IV, it was determined that the residual THF present in the micronized SFL powders (< 50 ppm) were well below the limits set by the ICH (29).

Because the micronized SFL powders dried by vacuum freeze-drying and ATMFD contained completely amorphous danazol, they both exhibited increased specific surface areas compared to the bulk API, co-ground physical mixture and slowly frozen control, and amorphous danazol was homogeneously dispersed within the hydrophilic excipient matrices of the SFL powders; rapid and complete aqueous dissolution of the vacuum freeze-dried and ATMFD micronized SFL powders was achieved.

It was determined from the DSC studies (Fig. 3) that danazol readily interacted with the pharmaceutical excipient mixture. SEM studies (Fig. 5) verified the interactions between API and excipients, especially that between danazol and poloxamer (Fig. 5b). The hydrophobic portions of the poloxamer particle adsorbed the danazol plates to its surface. Because poloxamer is amphiphilic, it was capable of simultaneously interacting with the hydrophobic API and aqueous dissolution media. Therefore, this interaction proved useful to enhance the dissolution of danazol.

From the XRD studies, it is concluded that SFL processing was the critical step in generating a micronized powder containing amorphous API. Micronized SFL powders dried by both vacuum freeze-drying and ATMFD contained amorphous danazol. It was demonstrated that the amorphous nature of the API could be preserved by incorporating the ATMFD process to dry the micronized SFL powders.

Specific surface areas were decreased (Table IV) and surface porosities less remarkable (Fig. 5) when ATMFD was used to dry the micronized SFL powders ($p < 0.05$). These changes were due to the collisions that occurred between microparticles during fluidization in the ATMFD process. However, it was demonstrated that these changes did not slow the dissolution of danazol into aqueous media from the engineered powders (Fig. 6). In addition, the surface areas of the ATMFD micronized SFL powders were significantly higher than those of the bulk products, co-ground physical mixture or slowly frozen control ($p < 0.05$). Therefore, surface area was reduced when powders were dried by ATMFD, but not to the magnitude of the controls.

Surprisingly, the co-ground physical mixture dissolved more slowly and to a lesser extent than any other control formulation. This slow dissolution occurred because in the bulk form, the excipients initially hydrated and swelled when introduced into the aqueous media. This phenomenon was encountered when preparing the aqueous aliquots containing the excipients for the SFL cosolvent feed solution formulations. After the hydrated polymeric gels were agitated for several minutes, they dissolved completely during SFL feed solution formulation. In the case of aqueous dissolution of the co-ground physical mixture, the excipients hydrated and swelled when introduced to the dissolution media, subsequently trapping the danazol crystals within the gel layer and not allowing complete dissolution to occur for the duration of the dissolution testing period.

Because there were crystalline domains of danazol present within the slowly frozen control powder, it did not wet and dissolve as rapidly as the vacuum freeze-dried and ATMFD micronized SFL powders, which both contained amorphous danazol. It was demonstrated that micronized SFL powders, dried by vacuum freeze-drying or ATMFD, promoted rapid and complete dissolution of the hydrophobic API, danazol.

CONCLUSIONS

This study demonstrated the use of the ATMFD process to effectively dry micronized powders produced by the SFL process. Because of the numerous disadvantages associated with vacuum freeze-drying, ATMFD enables the SFL particle-engineering technology to be commercially used as a viable method in the pharmaceutical industry for enhancing the aqueous dissolution of insoluble drugs.

ACKNOWLEDGMENTS

The authors kindly acknowledge the financial support from The Dow Chemical Company. This material is based upon work supported by the STC Program of the National

Science Foundation under Agreement No. CHE-9876674. True L. Rogers is a continuing American Fellowship for Pharmaceutical Education (AFPE) Fellow from 2000-2002 and a 2001-2002 University Continuing Fellowship recipient. Andrew C. Nelsen is a 2000-2001 University of Texas Undergraduate Research Fellowship recipient and a 2001-2002 AFPE Undergraduate Research Fellow.

REFERENCES

1. T. L. Rogers, K. P. Johnston, and R. O. Williams III. Solution-based particle formation of pharmaceutical powders by supercritical or compressed fluid CO_2 and cryogenic spray-freezing technologies. *Drug Dev. Ind. Pharm.* **27**:1003–1015 (2001).
2. B. Subramaniam, R. A. Rajewski, and K. Snavely. Pharmaceutical processing with supercritical carbon dioxide. *J. Pharm. Sci.* **86**:885–890 (1997).
3. R. Bodmeier, J. Wang, D. J. Dixon, S. Mawson, and K. P. Johnston. Polymeric microspheres prepared by spraying into compressed carbon dioxide. *Pharm. Res.* **12**:1211–1217 (1995).
4. T. W. Randolph, A. D. Randolph, M. Mebes, and S. Yeung. Submicrometer sized biodegradable particles of poly (lactic acid) via the gas antisolvent spray precipitation process. *Biotechnol. Prog.* **9**:429–435 (1993).
5. S. Palakodaty and P. York. Phase behavioral effects on particle formation processes using supercritical fluids. *Pharm. Res.* **16**: 976–985 (1999).
6. T. L. Rogers, J. Hu, Z. Yu, K. P. Johnston, and R. O. Williams III. A novel particle engineering technology: spray-freezing into liquid. *Int. J. Pharm.* **242**:93–100 (2002).
7. R. L. Rogers, A. C. Nelsen, J. Hu, J. N. Brown, M. Sarkari, T. J. Young, K. P. Johnston, and R. O. Williams III. A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid. *Eur. J. Pharm. Biopharm.* (2002).
8. J. Hu, T. L. Rogers, J. N. Brown, T. J. Young, K. P. Johnston, and R. O. Williams III. Improvement of dissolution rates of poorly water soluble APIs using the novel spray freezing into liquid technology. *Pharm. Res.* **19**:1278–1284 (2002).
9. T. Yokota, Y. Takahata, T. Katsuyama, and Y. Matsuda. A new technique for preparing ceramics for catalyst support exhibiting high porosity and high heat resistance. *Catal. Today* **69**:11–15 (2001).
10. J. Kirchnerova and D. Klvana. Synthesis and characterization of perovskite catalysts. *Solid State Ionics* **123**:307–317 (1999).
11. D. Klvana, J. Vaillancourt, and J. Kirchnerova. and J. Chaouki. Combustion of methane over $La_{0.66}Sr_{0.34}Co_{0.7}O_3$ and $La_{0.4}Sr_{0.6}Fe_{0.4}Co_{0.6}O_3$ prepared by freeze-drying. *APCAD A: General* **109**:181–193 (1994).
12. W. Zipprich, S. Waschilewski, F. Rocholl, and H.-D. Wiemhofer. Improved preparation of $La_{1-\chi}Me_{\chi}CoO_{3-\delta}$ (Me = Sr, Ca) and analysis of oxide ion conductivity with ion conducting microcontacts. *Solid State Ionics* **101-103**:1015–1023 (1997).
13. J. A. N. Zasadzinski. A new heat transfer model to predict cooling rates for rapid freezing fixation. *J. Microsc.* **150**:137–149 (1988).
14. M. Mumenthaler and H. Leuenberger. Atmospheric spray-freeze drying: a suitable alternative in freeze-drying technology. *Int. J. Pharm.* **72**:97–110 (1991).
15. H. R. Costantino, L. Firouzabadian, K. Hogeland, C. Wu, C. Beganski, K. G. Carrasquillo, M. Cordova, K. Griebenow, S. E. Zale, and M. Tracy. Protein spray-freeze drying. Effect of atomization conditions on particle size and stability. *Pharm. Res.* **17**: 1374–1383 (2000).
16. Y.-F. Maa, P.-A. Nguyen, T. Sweeney, S. J. Shire, and C. C. Hsu. Protein inhalation powders: spray drying vs. spray freeze drying. *Pharm. Res.* **16**:249–254 (1999).
17. M. C. Heller, J. F. Carpenter, and T. W. Randolph. Protein formulation and lyophilization cycle design: prevention of damage due to freeze-concentration induced phase separation. *Biotechnol. Bioeng.* **63**:166–174 (1999).
18. Y.-F. Maa and S. J. Prestrelski. Biopharmaceutical powders: particle formation and formulation considerations. *Curr. Pharm. Biotechnol.* **1**:283–302 (2000).
19. J. L. Cleland, O. L. Johnson, S. Putney, and A. J. S. Jones. Recombinant human growth hormone poly(lactic-co-glycolic acid) microsphere formulation development. *Adv. Drug Deliv. Rev.* **28**:71–84 (1997).
20. K. G. Carrasquillo, A. M. Stanley, J. C. Aponte-Carro, P. D. Jesus, H. R. Costantino, C. J. Bosques, and K. Griebenow. Non-aqueous encapsulation of excipient-stabilized spray-freeze dried BSA into poly(lactide-co-glycolide) microspheres results in release of native protein. *J. Control. Release* **76**:199–208 (2001).
21. G. Ji, G. Xue, J. Ma, and C. Dong. Concentration dependence of crystallinity of polycarbonate by shock-cooling and subsequent freeze-drying of its various solutions. *Polymer* **37**:3255–3258 (1996).
22. M. C. Heller, J. F. Carpenter, and T. W. Randolph. Manipulation of lyophilization-induced phase separation: implications for pharmaceutical proteins. *Biotechnol. Prog.* **13**:590–596 (1997).
23. J. M. Sarciaux, S. Mansour, M. J. Hageman, and S. L. Nail. Effects of buffer composition and processing conditions on aggregation of bovine IgG during freeze-drying. *J. Pharm. Sci.* **88**: 1354–1361 (1999).
24. J. A. Searles, J. F. Carpenter, and T. W. Randolph. Annealing to optimize the primary drying rate, reduce freezing-induced drying rate heterogeneity, and determine T_g in pharmaceutical lyophilization. *J. Pharm. Sci.* **90**:872–887 (2001).
25. S.-L. Wu, D. Leung, L. Tretyakov, J. Hu, A. Guzzetta, and Y. J. Wang. The formation and mechanism of multimerization in a freeze-dried peptide. *Int. J. Pharm.* **200**:1–16 (2000).
26. K. Ozaki and M. Hayashi. The effects of glucose oligomers (maltodextrins) on freeze-drying liposomes. *Chem. Pharm. Bull.* **45**:165–170 (1997).
27. B. Leuckel, D. Bodmer, B. Helk, and H. Leuenberger. Formulations of sugars with amino acids or mannitol—influence of concentration ratio on the properties of the freeze-concentrate and the lyophilizate. *Pharm. Dev. Technol.* **3**:325–336 (1998).
28. B. Lueckel, B. Helk, D. Bodmer, and H. Leuenberger. Effects of formulation and process variables on the aggregation of freeze-dried interleukin-6 (IL-6) after lyophilization and on storage. *Pharm. Dev. Technol.* **3**:337–346 (1998).
29. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Impurities: Guideline for Residual Solvents, 1997.