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Research paper

Micronized powders of a poorly water soluble drug produced by a spray-freezing into liquid-emulsion process

True L. Rogers[a,1], Kirk A. Overhoff[a], Parag Shah[b], Patricia Santiago[b], Miguel J. Yacaman[b], Keith P. Johnston[b,2], Robert O. Williams III[a,*]

[a]*College of Pharmacy, University of Texas at Austin, Austin, TX, USA*
[b]*Department of Chemical Engineering, University of Texas at Austin, Austin, TX, USA*

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Abstract

The purpose of this paper is to investigate the influence of the emulsion composition of the feed liquid on physicochemical characteristics of drug-loaded powders produced by spray-freezing into liquid (SFL) micronization, and to compare the SFL emulsion process to the SFL solution process. Danazol was formulated with polyvinyl alcohol (MW 22,000), poloxamer 407, and polyvinylpyrrolidone K-15 in a 2:1:1:1 weight ratio (40% active pharmaceutical ingredient (API) potency based on dry weight). Emulsions were formulated in ratios up to 20:1:1:1 (87% API potency based on dry weight). Ethyl acetate/water or dichloromethane/water mixtures were used to produce o/w emulsions for SFL micronization, and a tetrahydrofuran/water mixture was used to formulate the feed solutions. Micronized SFL powders were characterized by X-ray diffraction, surface area, scanning and transmission electron microscopy, contact angle and dissolution. Emulsions containing danazol in the internal oil phase and processed by SFL produced micronized powders containing amorphous drug. The surface area increased as drug and excipient concentrations were increased. Surface areas ranged from 8.9 m^2/g (SFL powder from solution) to 83.1 m^2/g (SFL powder from emulsion). Danazol contained in micronized SFL powders from emulsion and solution was 100% dissolved in the dissolution media within 2 min, which was significantly faster than the dissolution of non-SFL processed controls investigated (<50% in 2 min). Micronized SFL powders produced from emulsion had similar dissolution enhancement compared to those produced from solution, but higher quantities could be SFL processed from emulsions. Potencies of up to 87% yielded powders with rapid wetting and dissolution when utilizing feed emulsions instead of solutions. Large-scale SFL product batches were manufactured using lower solvent quantities and higher drug concentrations via emulsion formulations, thus demonstrating the usefulness of the SFL micronization technology in pharmaceutical development.

Keywords: Spray-freezing into liquid; Dissolution enhancement; Danazol; Emulsion; Micronization; Particle engineering

1. Introduction

Poorly water soluble compounds are common among new chemical entities being investigated for therapeutic activity as active pharmaceutical ingredients (APIs) [1,2]. Oil/water emulsions are frequently used in the pharmaceutical industry to enhance the overall concentration of poorly water soluble and insoluble drugs, due to the high solubility of the API in the dispersed oil phase [1–6]. However, emulsion stability is a concern [3,7–14]. Over time, emulsions often coalesce and settle. Additionally, the large volume of the oil and aqueous phases limits the overall drug concentration and yield. To overcome these inherent disadvantages, solvents are often removed from emulsion formulations by lyophilization [4,5]. However, it has been found that freezing emulsions has resulted in phase separation and destabilization of APIs, and that dry emulsions did not produce the same degree of dissolution enhancement as was achieved prior to lyophilization [13]. For oral delivery, it would be desirable to produce dry powders by lyophilization of emulsions that have high dissolution rates.

* Corresponding authors. College of Pharmacy (Mailstop A 1920), University of Texas at Austin, Austin, TX 78712-1074, USA. Tel.: +1-512-471-4681; fax: +1-512-471-7474.
E-mail address: williro@mail.utexas.edu (R.O. Williams).
[1] Present address: The Dow Chemical Company, Midland, MI 48674, USA.
[2] Keith P. Johnston is also a corresponding author on this paper.

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A novel particle engineering technology, spray-freezing into liquid (SFL), has been developed to produce micronized powders from solution containing amorphous drug for dissolution enhancement of poorly water soluble APIs [15–17]. The ultra-rapid freezing rates resulted in a micronized SFL powder containing amorphous API molecularly dispersed within an excipient matrix. The phase separation between API and the solvent–cosolvent mixture was sufficiently rapid during freezing that the API did not have time to crystallize or disengage from the excipients. In contrast, slowly freezing the cosolvent solution promoted API phase separation and crystallization. The resulting crystalline API dissolved significantly more slowly in aqueous media than the micronized SFL powder from an identical cosolvent solution.

The objective of this study was to utilize o/w emulsions to expand the novel SFL particle engineering technology to produce micronized powders of an insoluble API with high dissolution rates. The total concentration of API in the o/w emulsions could be much larger than in the cosolvent solutions because of the high solubility of hydrophobic API in the internal organic phase of the emulsion. Therefore, larger quantities of micronized powder could be produced per quantity of solvent, an important advantage, given the time and energy required for solvent removal during lyophilization. In addition, the proximity of the surfactant at the oil water interface to the API in the internal oil phase could offer the possibility of stabilizing API particles with much higher drug-to-surfactant ratios. High potency formulations with high drug-to-surfactant ratios and rapid dissolution rates would be advantageous in increasing dosages and in ameliorating side effects due to excipients. The results will show that micronized SFL powders from emulsion retained the high dissolution rates that have been achieved previously for SFL powders from cosolvent solutions [15–17]. The influence of the emulsion composition of the feed liquid on the surface area, crystallinity, particle size, and dissolution rate of hydrophobic drug-loaded powders was investigated to further demonstrate the utility of the SFL process.

2. Materials and methods

Micronized danazol, USP, partially hydrolyzed poly(-vinyl alcohol) (PVA, MW 22,000), poloxamer 407, 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). Tetrahydrofuran (THF, HPLC Grade) was purchased from Mallinckrodt (Paris, KY). HPLC grade acetonitrile and ethyl acetate (EA) and dichloromethane (DCM), both of reagent grade, were purchased from EM Sciences (Gibbstown, NJ). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ).

2.1. Solubility of danazol in EA and DCM

An excess of danazol was weighed into scintillation vials filled with 15 ml of EA or DCM. Separate samples were prepared for each solubility time point. The sealed vials were placed on a horizontal shaker at 25 °C and agitated. Samples were collected in replicates of three at 24, 48 and 96 h. A 5 ml aliquot was measured and filtered through a 0.45 μm Acrodisc GHP syringe filter (Pall Corporation, Ann Arbor, MI). One milliliter of filtrate was removed and diluted with 20 ml acetonitrile followed by mixing. The solution was then filtered and analyzed at 288 nm using a Shimadzu LC-10 liquid chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with an Alltech 5 μm ODS-2 reverse-phase column (Alltech Associates, Inc., Deerfield, IL). 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 $\leq 2.0\%$ 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.

2.2. Micronized SFL powder preparation from emulsion

The emulsion formulations investigated in the study are shown in Table 1. After determining the saturation solubility of danazol in both EA and DCM, predetermined amounts of danazol were weighed and dissolved in organic solvent. PVA, poloxamer, and PVP were dissolved in water. Water was heated to 80 °C to dissolve the PVA, and then cooled to room temperature to add PVP. The solution temperature was decreased to 0 °C to dissolve the poloxamer, followed by equilibration to room temperature. The oil phase was slowly poured into the aqueous phase under constant mixing, and then blended for 1 min using a Polytron rotor-and-stator homogenizer (Polytron 10/35 with TS 10 mm Generator, VWR Scientific Corporation, West Chester, PA). The emulsion was homogenized for ten cycles at 20,000 PSI (138 MPa) using an Avestin Emulsiflex-C5 (Avestin, Inc., Ottawa, ON, Canada) high-pressure homogenizer.

The emulsion formulations were processed by SFL using the apparatus shown in Fig. 1 [15–17]. Each emulsion (Fig. 1a) was atomized beneath the liquid nitrogen surface (Fig. 1b) at 5000 PSI (34.5 MPa) and a flow rate of 20 ml/min through a 127 μm I.D. polyether-ether ketone (PEEK) nozzle (Fig. 1c) of 15 cm in length using an ISCO Model 100DX syringe pump (Fig. 1d; ISCO, Inc., Lincoln, NE). The cryogenic suspension was then poured into a non-insulated beaker to allow the nitrogen to evaporate. Once the nitrogen had evaporated, the frozen micronized SFL powder was immediately vacuum freeze-dried using a VirTis Advantage Benchtop Tray Lyophilizer (The VirTis Company, Inc., Gardiner, NY) with a liquid nitrogen trap. A

Table 1
Emulsion formulations that were investigated and processed to obtain micronized SFL powders

Formulation abbreviation	Danazol (%w/w)[a]	Organic solvent (%w/w)	PVA (%w/w)	Poloxamer (%w/w)	PVP (%w/w)	Water (%w/w)	Ratio of danazol to excipients by weight (danazol/PVA/poloxamer/PVP)
Control[b]	0.22	33.11 THF	0.11	0.11	0.11	66.33	2:1:1:1
A	1.00	32.30 EA[c]	0.50	0.50	0.50	65.20	2:1:1:1
B	1.50	50.00 EA	0.75	0.75	0.75	46.25	2:1:1:1
C	3.00	30.00 DCM[d]	1.50	1.50	1.50	62.50	2:1:1:1
D	5.00	50.00 DCM	0.50	0.50	0.50	45.50	10:1:1:1
E	5.00	50.00 DCM	0.25	0.25	0.25	44.25	20:1:1:1

[a] The percentage of danazol in the total emulsion or control formulation.
[b] The control was a cosolvent solution formulated dissolving danazol in THF and PVA, poloxamer and PVP in water. The two solutions were then mixed to form the control feed solution for SFL processing.
[c] EA is ethyl acetate.
[d] DCM is dichloromethane.

vacuum of 100 mTorr was maintained throughout the lyophilization period. The shelf temperature was equilibrated to −40 °C. The frozen SFL samples were placed in the lyophilizer, and the shelf temperature was increased to +25 °C over the duration of the lyophilization cycle at a rate of 0.9 °C/min.

2.3. Preparation of control formulations

A co-ground physical mixture consisting of 1.0 g danazol, 0.5 g PVA, 0.5 g poloxamer and 0.5 g PVP (2:1:1:1 weight ratio) 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 micronized SFL powder from solution was prepared at a 2:1:1:1 (danazol/PVA/poloxamer/PVP) weight ratio and utilized as a control following an earlier procedure [15]. An aliquot of 0.11 g danazol was dissolved in 16.56 g THF.

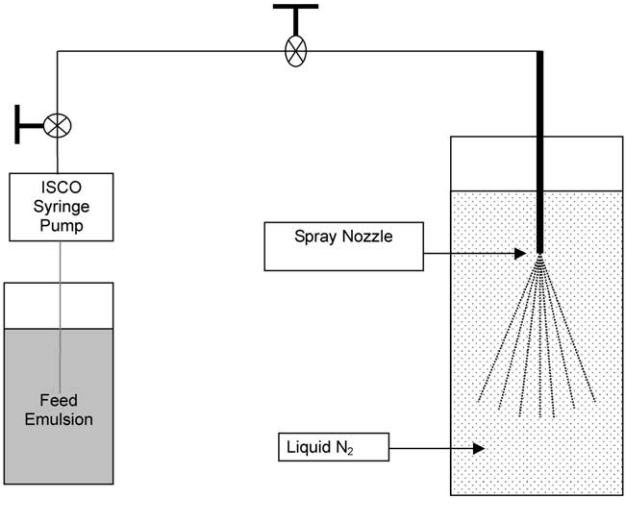

Fig. 1. Schematic illustration of the SFL process used to make the micronized SFL powders from emulsion: (a) feed emulsion, (b) liquid nitrogen, (c) 127 μm I.D. × 15 cm long insulated PEEK nozzle, (d) syringe pump, (e) high pressure valve, and (f) atomized frozen microdroplets.

Aliquots of 0.06 g PVA, 0.06 g poloxamer, and 0.06 g PVP were dissolved in 33.17 g purified water. The two solutions were added together and mixed to form a one-phase cosolvent solution. The cosolvent solution was then processed by SFL.

Immediately after the drug-loaded emulsions were prepared, half of each batch was SFL processed, and the second half was slowly frozen in a tray lyophilizer equilibrated at −40 °C, and lyophilized. These slowly frozen agglomerates served as yet another control to further evaluate the powders produced by SFL.

2.4. Particle size distribution (PSD) measurements

PSD measurements were conducted by light scattering using a Malvern Mastersizer S (Malvern Instruments Limited, Malvern, Worcestershire, UK). Micronized SFL powder PSD measurements were conducted using cold, deionized water as a dispersant. Span indexes were calculated according to the following equation:

$$\frac{(M_{90} - M_{10})}{M_{50}}$$

where M_{90}, M_{50} and M_{10} are the cumulative particle sizes less than 90%, 50% and 10%, respectively [18]. The particle diameters are reported based on volume.

2.5. 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) 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 (225 °C) in the various samples. DSC was used to analyze the samples from 25 to 300 °C with a 10 °C/min heating rate.

2.6. X-Ray powder diffraction (XRD)

A Philips 1710 X-ray diffractometer with a copper target and nickel filter (Philips Electronic Instruments, Inc., Mahwah, NJ) and Jade 5 XRD pattern processing software (Materials Data, Inc., Irvine, CA) 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 to 50 2θ degrees using a step size of 0.05 2θ degrees and a dwell time of 1 s at each step.

2.7. 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. An accelerating voltage of 15 kV was used.

2.8. Transmission electron microscopy (TEM)

Micronized SFL powder samples were secured between two 200-mesh carbon-coated copper grids (Electron Microscopy Sciences, Washington, PA). High-resolution TEM images were obtained using a Jeol 2010F microscope with 1.4 Å point-to-point resolution equipped with a GATAN digital photography system for imaging and operating with a 200 kV accelerating voltage.

2.9. 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). A known amount of powder (~200 mg) was loaded into a Quantachrome sample cell and degassed for at least 3 h prior to analysis. The model devised by Brunauer, Emmett, and Teller was utilized by the NOVA software to determine the specific surface areas of the samples investigated based upon the weights of the degassed samples [19].

2.10. Contact angle measurements

A 50 mg aliquot of powder was compacted using a Model M Carver Laboratory Press (Fred S. Carver, Inc., Menomonee Falls, WI) while incorporating a 500 kg compression force. A 0.03 ml drop of SLS/Tris dissolution media was placed on the compact, and the contact angle between the sample and media was measured using a Model 100-00-115 goniometer (Ramè-Hart Inc., Mountain Lakes, NJ). Duplicate measurements were taken for each sample contact angle analyzed.

2.11. 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 was weighed 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 six ($n = 6$) by a VK8000 autosampler (VanKel Technology Group, Cary, NC). Paddle speed and bath temperature were set at 50 rev./min and 37.0 ± 0.2 °C, respectively. Sink conditions were maintained throughout the dissolution testing period. Samples were analyzed by HPLC as discussed in Section 2.1. The dissolution media was prepared by dissolving 150 g SLS and 242 g Tris in approximately 18 l purified water followed by adjusting the pH to 9.0 with 1 N HCl and the volume to 20 l with purified water.

2.12. Statistical analysis

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

3. Results

3.1. Micronized SFL powders from emulsions

The emulsion formulations that were SFL processed to micronized powders are shown in Table 1. The equilibrium solubilities of danazol in EA and DCM were 50 and 162 mg/ml, respectively. Therefore, emulsion formulations containing 1 and 1.5% danazol (%w/w of total formulation) in EA as the internal oil phase, and emulsions containing 3 and 5% danazol in DCM as the internal oil phase were prepared. PVA in the aqueous phase enabled production of stable emulsion that did not phase separate. PVP and poloxamer were used as surfactants to enhance the wetting and dissolution of the micronized danazol particles.

The PSDs of the dry micronized SFL powders from emulsion are listed in Table 2. The M_{50} of bulk danazol was 23.10 μm. The M_{50} values were lower for the micronized SFL A (8.16 μm) and SFL D (4.15 μm) powders than the other powders investigated ($P < 0.05$). The span index ranged between 1.55 and 3.98 for the micronized SFL powders investigated, and the magnitude was influenced by composition.

The DSC studies showed that poloxamer and PVP melted at 50 °C, and PVA melted at 80 °C. Bulk danazol melted at 225 °C. All micronized SFL powders from emulsion exhibited similar DSC profiles to the co-ground physical mixture (data not shown). Poloxamer/PVP and PVA melting endotherms were observed at 50 and 80 °C,

Table 2
Particle size distribution, specific surface area and contact angle measurements of the dry micronized SFL powders and control formulations

Formulation	M_{10} (μm)	M_{50} (μm)	M_{90} (μm)	Span index[a]	Specific surface area (m^2/g)	Average contact angle (SD)
Bulk danazol	7.28	23.10	51.78	1.93	0.52	64.3 (1.1)[b]
SFL control from solution	2.55	6.52	16.28	2.10	8.90	48.9 (2.8)
SFL A	2.60	7.44	22.13	2.63	12.71	38.5 (2.1)
Slowly frozen A	N/D[c]	N/D	N/D	N/D	N/D	N/D
SFL B	3.44	16.75	70.15	3.98	41.73	36.9 (1.0)
Slowly frozen B	4.31	20.12	60.56	2.80	3.21	23.6 (9.1)
SFL C	2.98	6.07	12.42	1.55	83.06	48.0 (1.4)
Slowly frozen C	3.89	9.50	24.21	2.14	0.35	47.8 (2.1)
SFL D	5.03	12.57	30.25	2.00	20.18	35.6 (2.1)
SFL E	3.96	9.35	22.24	1.96	24.98	20.6 (11.9)

[a] The span index is $(M_{90} - M_{10})/M_{50}$ [18].
[b] It was determined that bulk danazol and danazol SFL processed in the absence of excipients gave similar contact angles and dissolution profiles.
[c] The slowly frozen A control was a solidified aggregate that could not be broken into small pieces sufficient for analysis.

respectively, and a danazol crystallization exotherm occurred at 200 °C.

XRD patterns of the micronized SFL powders from emulsion are displayed in Fig. 2. Bulk danazol (Fig. 2a-X) is highly crystalline, producing intense peaks between 14 and 25 2θ degrees. The co-ground physical mixture (Fig. 2a-P) contained crystalline danazol peaks; however, each micronized SFL powder from emulsion (Fig. 2a-A–D) contained amorphous danazol. Crystalline danazol peaks could not be detected between 14 and 25 2θ degrees for these micronized SFL powders.

SEM micrographs of the various powders are shown in Fig. 3. As shown in Fig. 3a, bulk micronized danazol was composed of smooth, crystalline plates with fractured edges that ranged in length from 1 to 15 μm. The ingredients of the co-ground physical mixture (Fig. 3b) could be identified by comparison with pure components (SEM micrographs not shown). The spherical particle is poloxamer, the smooth acicular particles are PVA, the irregular particles with rough surfaces are PVP and the smooth, crystalline plates with fractured edges are danazol particles that were adsorbed to the surface of the poloxamer particle. An SEM of a representative micronized SFL A microparticulate aggregate is shown in Fig. 3c. The SFL aggregate was spherical with a rough surface, as was also the case for the SFL D microparticulate aggregate, shown in Fig. 3d.

A TEM micrograph of the micronized SFL D powder is shown in Fig. 4a. The dark, electron-dense regions were determined by EDS spectra analysis to be danazol, and the lighter regions of lower electron density were determined to be the excipient matrix. Embedded within the excipient aggregate matrix of the dry micronized SFL D powder were small particle domains enriched in danazol that were approximately 200 nm in diameter. Towards the center of the SFL aggregate matrix, dense danazol-rich domains were observed as shown by the electron-dense center of the SFL microparticulate aggregate. These results indicated highly homogeneous mixing of the API and excipients.

Specific surface areas of the micronized SFL formulations are listed in Table 2. Whereas bulk danazol and the co-ground physical mixture had specific surface areas of 0.52 and 1.92 m^2/g, respectively, the micronized SFL powders from emulsion had significantly higher surface areas ($P < 0.05$). The specific surface areas of the micronized SFL A and SFL B powders were 12.71 and 41.73 m^2/g, respectively, while that of the micronized SFL C powder was 83.06 m^2/g. The surface areas of the micronized SFL D and SFL E powders were 20.18 and 24.98 m^2/g, respectively.

Contact angle measurements of the various powders are listed in Table 2. The contact angle between SLS/Tris media and bulk micronized danazol was 64.3°. For the co-ground physical mixture, the contact angle was 41.8°, which was lower than that produced by bulk danazol ($P < 0.05$). Representative samples of micronized SFL powders from emulsion had reduced contact angles with SLS/Tris media ($P < 0.05$) compared to bulk danazol. Contact angles between the micronized SFL powders and SLS/Tris media ranged from 20.6° (micronized SFL E powder) to 48.0° (micronized SFL C powder), and the magnitude was influenced by the composition.

The dissolution profiles of the various powders investigated are shown in Fig. 5a. Approximately 53% of bulk danazol was dissolved within 5 min, and about 88% of bulk danazol was dissolved by 60 min. The co-ground physical mixture dissolved more slowly and to a lesser extent than bulk danazol ($P < 0.05$). By 60 min, only 39% danazol had dissolved from the co-ground physical mixture. In contrast, greater than 90% danazol was dissolved from all micronized SFL powders by 5 min. The representative sample of micronized SFL powder from emulsion dissolved much more rapidly than bulk danazol or the co-ground physical mixture ($P < 0.05$).

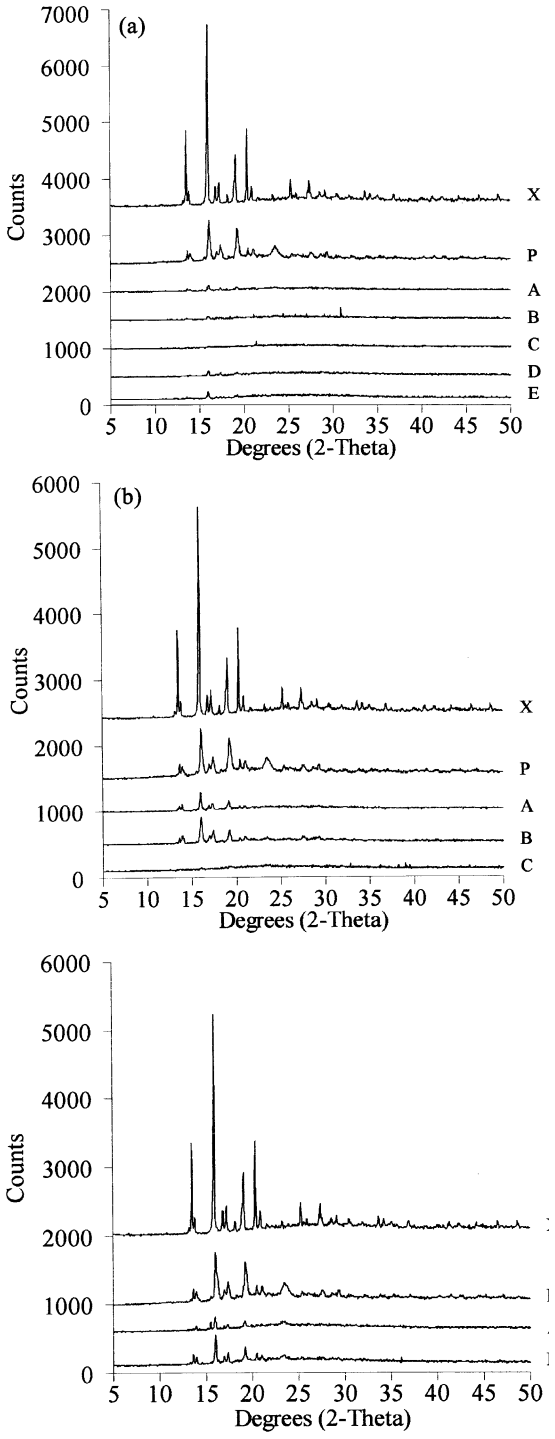

Fig. 2. (a) XRD patterns of micronized SFL powders from emulsion: bulk danazol (X), the co-ground physical mixture (P), micronized SFL A powder (A), micronized SFL B powder (B), micronized SFL C powder (C), micronized SFL D powder (D) and micronized SFL E powder (E). (b) XRD patterns of slowly frozen agglomerates from emulsion: bulk danazol (X), co-ground physical mixture (P), slowly frozen A agglomerate (A), slowly frozen B agglomerate (B) and slowly frozen C agglomerate (C). (c) XRD patterns of micronized SFL powder and controls from solution: bulk danazol (X), co-ground physical mixture (P), micronized SFL powder from solution (A) and slowly frozen control from solution (B).

3.2. Investigation of slowly frozen agglomerates from emulsion

The PSD measurements for the slowly frozen agglomerates from emulsion are listed in Table 2. The slowly frozen B agglomerate had an M_{50} of 20.12 μm, and the slowly frozen C agglomerate had an M_{50} of 9.50 μm. The M_{50} of the slowly frozen B agglomerate was higher than that of the C agglomerate ($P < 0.05$).

Slowly frozen A and B agglomerates contained crystalline danazol, as indicated by the characteristic danazol peaks between 14 and 25 2θ degrees in the XRD patterns shown in Fig. 2b-A,B, respectively. The slowly frozen C agglomerate contained amorphous danazol, as shown in Fig. 2b-C. The SEM micrograph of the slowly frozen A agglomerate is shown in Fig. 3e. SEM analysis of the slowly frozen formulations revealed large (>200 μm) agglomerates with smooth, continuous surfaces and fractured edges.

The specific surface areas and contact angles of the slowly frozen agglomerates are listed in Table 2. The slowly frozen B and C agglomerates had surface areas of 3.21 and 0.35 m^2/g, respectively. The contact angles between the slowly frozen B and C agglomerates and SLS/Tris media were 23.6 and 47.8°, respectively, but the slowly frozen aggregates dissolved slowly and incompletely in the dissolution media within the 60 min dissolution test period. The slowly frozen agglomerates dissolved more slowly and to a lesser extent than bulk danazol, but they dissolved faster and to a greater extent than the co-ground physical mixture ($P < 0.05$). By 60 min, danazol contained in the slowly frozen A and B agglomerates was 72 and 83% dissolved in the dissolution media, while danazol contained in the slowly frozen C agglomerate was only 58% dissolved.

3.3. Investigation of micronized SFL powders and slowly frozen agglomerates from solution

The PSD measurement for the micronized SFL powder from solution is listed in Table 2. The M_{50} of the powder was 6.52 μm. As indicated in Table 2, the slowly frozen control produced large agglomerates that were difficult to reduce in size. In Fig. 2c, the micronized SFL powder from solution produced an XRD pattern (Fig. 2c-A) that indicated the presence of amorphous danazol. The slowly frozen control from solution produced an XRD pattern (Fig. 2c-B) that revealed the presence of crystalline danazol as indicated by the characteristic peaks present between 14 and 25 2θ.

SEM analysis of the micronized SFL powder from solution (Fig. 3f) revealed a porous microparticulate aggregate comprised of small particle domains. SEM analysis of the slowly frozen agglomerate from solution (Fig. 3g) revealed a large (>200 μm) agglomerate with a smooth, continuous surface and fractured edges. TEM analysis of the micronized SFL powder from solution revealed small particle danazol domains that were approxi-

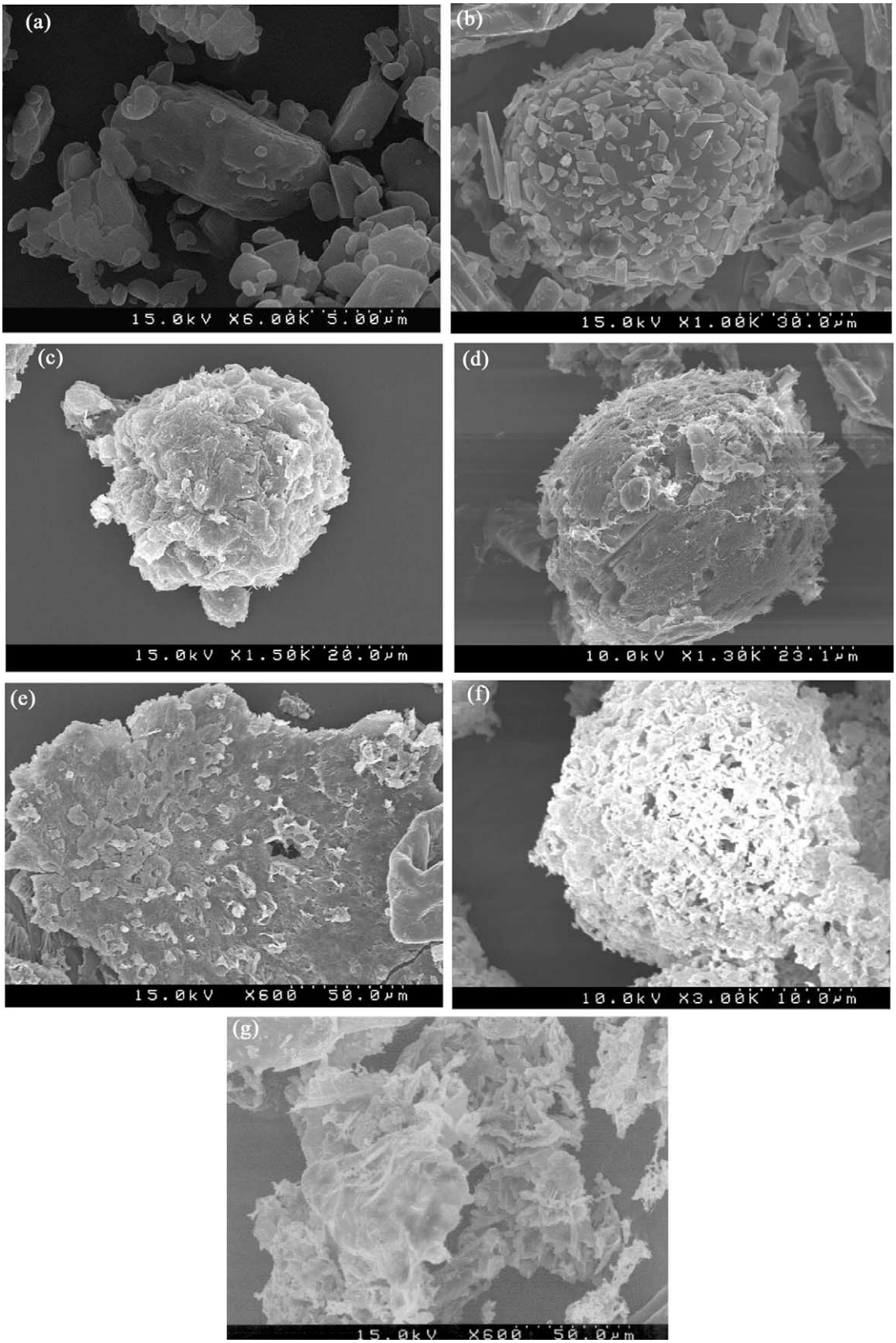

Fig. 3. SEM micrographs of (a) bulk danazol, (b) co-ground physical mixture, (c) micronized SFL A powder, (d) micronized SFL D powder, (e) slowly frozen A agglomerate, (f) micronized SFL powder from solution, and (g) slowly frozen agglomerate from solution.

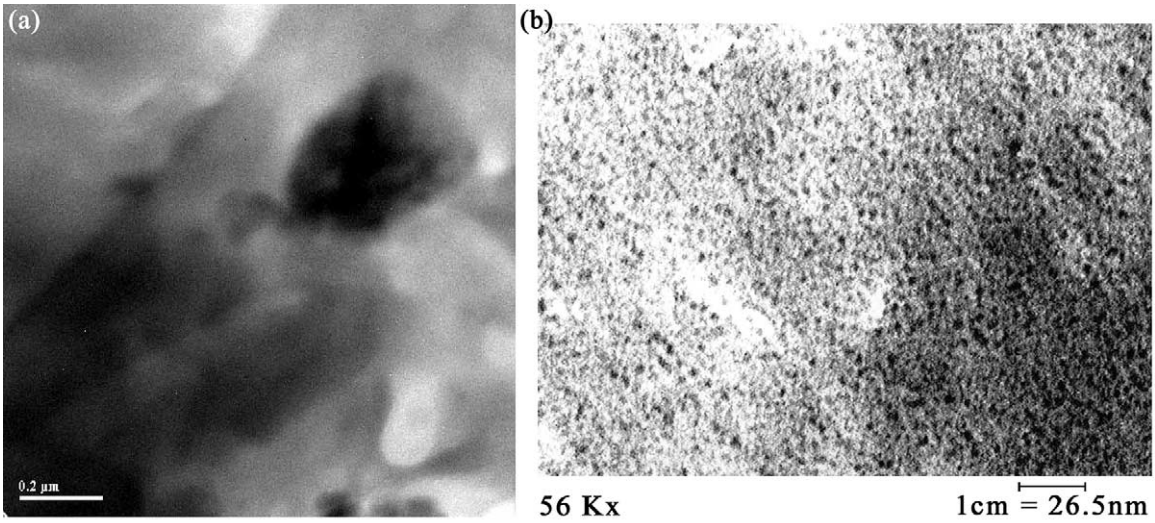

Fig. 4. TEM micrographs of (a) the micronized SFL D powder and (b) the micronized SFL powder from solution.

mately 20 nm in diameter. The electron-dense danazol domains were homogeneously dispersed throughout the porous excipient aggregate.

The specific surface area of the micronized SFL powder from solution is listed in Table 2. The micronized SFL powder from solution had a surface area of 8.90 m^2/g, and the slowly frozen agglomerate had a surface area of 3.14 m^2/g (data not shown). The difference in contact angles for these two cases was relatively minor, as listed in Table 2.

In Fig. 5c, the micronized SFL powder from solution displayed nearly complete dissolution (>90%) within 5 min, whereas the slowly frozen agglomerate did not reach 90% dissolution until 60 min. The micronized SFL powder from solution dissolved more rapidly than the slowly frozen agglomerate from solution ($P < 0.05$). Both micronized SFL powder and slowly frozen agglomerate from solution dissolved more rapidly than bulk danazol or the co-ground physical mixture ($P < 0.05$).

4. Discussion

The SFL process has been used to enhance the dissolution of hydrophobic APIs by producing microparticulate powders consisting of small drug particle domains stabilized within a hydrophilic excipient aggregate matrix [15–17]. The success of the SFL process results from intense atomization of a feed liquid directly into a cryogenic liquid to achieve ultra-rapid freezing of the atomized feed microdroplets. Once dried, the micronized SFL powders are characterized by high surface areas and contain amorphous API embedded in hydrophilic excipients that promote rapid and complete dissolution of the hydrophobic API. Previously, only cosolvent solutions were SFL processed to produce micronized powders. Because of the innate hydrophobicities of the APIs utilized in the model formulations, there was an upper limit for the API concentrations that could be dissolved in the water/THF cosolvent system. Lower API concentrations were necessary for SFL processing because higher concentrations resulted in API precipitation from the cosolvent system. To enhance product yield, o/w emulsions with higher API and excipient concentrations were formulated for SFL processing.

Emulsion destabilization in the form of coalescence during lyophilization has resulted from solvent crystal growth [8,9,14,20], which caused rupture of emulsion droplets and coalescence as well as API phase separation and crystallization. It has been reported that emulsion destabilization during lyophilization could be prevented by plunge-cooling the emulsion into organic solvent/dry ice baths or cryogenic liquids prior to lyophilization [1,7–9,14,21,22]. The solid cakes produced by lyophilization had to be ground into powders. In contrast, SFL processing offers the advantage of producing flowable micronized powders containing amorphous API in a single step [16,17,23].

From Table 1, it is evident that the utilization of emulsions increased the output of micronized SFL powder production quantities by allowing higher concentrations of API and excipients. In addition, emulsions enabled increases in API-to-surfactant ratios. The maximum ratio that could be obtained by SFL from cosolvent solutions without danazol crystallization was 2:1:1:1 (danazol/PVA/poloxamer/PVP) or 40% danazol potency based on dry weight. In contrast, ratios up to 20:1:1:1 were attainable when emulsions were employed in SFL processing. A micronized SFL powder with a 20:1:1:1 API-to-excipient ratio corresponded to an extremely large danazol potency of 87%.

Either EA or DCM internal phases were utilized in the o/w emulsions investigated in this study. Because DCM was a stronger solvent for danazol, more highly concentrated emulsions could be formulated when incorporating DCM as the internal phase. A 2:1:1:1 (danazol/PVA/poloxa-

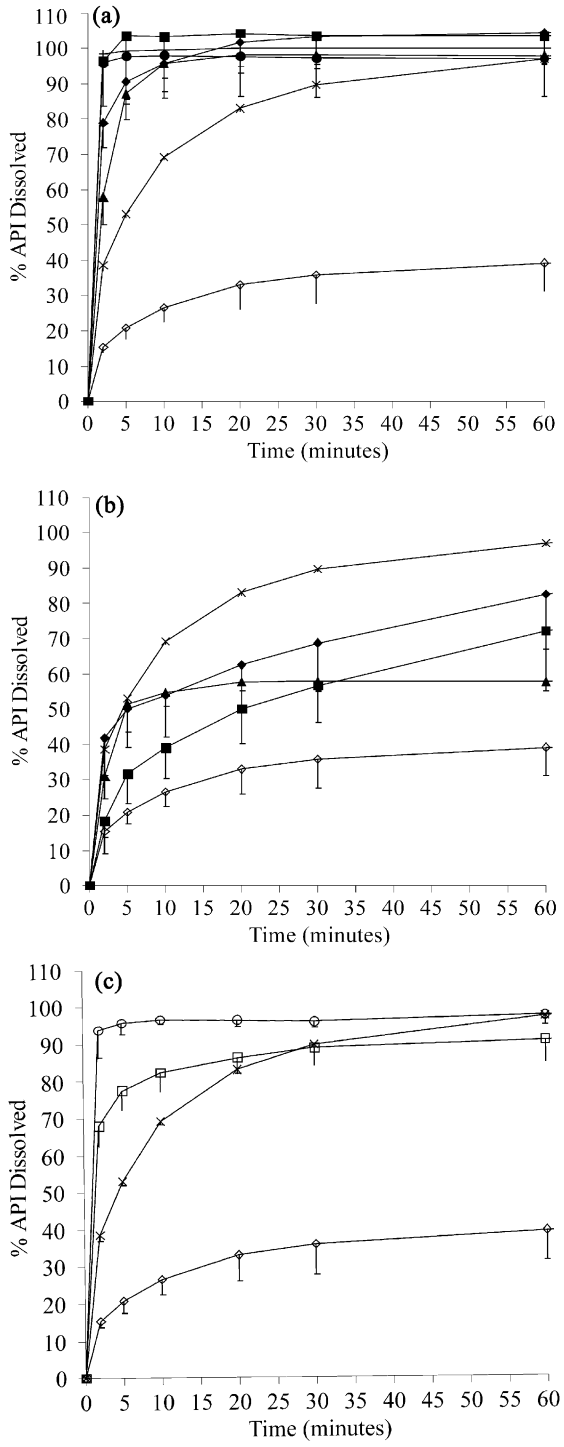

Fig. 5. (a) Dissolution profiles of micronized SFL powders from emulsion: bulk danazol (X), co-ground physical mixture (◇), micronized SFL A powder (■), micronized SFL B powder (◆), micronized SFL C powder (▲), micronized SFL D powder (•) and micronized SFL E powder (+). (b) Dissolution profiles of slowly frozen agglomerates from emulsion: bulk danazol (X), co-ground physical mixture (◇), slowly frozen A (■), slowly frozen B (◆) and slowly frozen C (▲). (c) Dissolution profiles of micronized SFL powder and controls from solution: bulk danazol (X), co-ground physical mixture (◇), micronized SFL powder from solution (○), slowly frozen agglomerate from solution (□).

mer/PVP) ratio could not be maintained at higher API concentrations in the DCM-based emulsions because the continuous aqueous phase formed a semisolid gel at higher excipient concentrations. Therefore, the micronized SFL D and SFL E powders were produced from emulsions that were highly concentrated with danazol in combination with lower concentrations of excipients. Despite the high potencies for these powders, the dissolution rates were still extremely high suggesting intimate mixing of the small amount of surfactant with the danazol. It is likely that this intimate mixing was facilitated by the close proximity of the surfactant at the interface to the concentrated danazol in the small dispersed oil droplet.

The micronized SFL powders from emulsions had M_{50} particle sizes significantly lower than that of bulk danazol ($P < 0.05$). M_{50} particle sizes from the micronized SFL A and SFL B powders increased as the API and excipient concentrations were increased in the feed emulsions used. The highly concentrated and more viscous internal phases likely atomized into larger microdroplets during SFL processing, thus producing larger particles. In addition, the quadratic increase in bimolecular collision rates with an increase in concentration led to greater particle growth. A similar phenomenon was not seen with the micronized SFL C, SFL D and SFL E powders because lower excipient concentrations produced lower continuous aqueous phase viscosities and fewer particle collisions. Therefore, the desired particle size of the micronized SFL aggregate powder was controlled by adjusting the excipient composition.

From the XRD studies, it was demonstrated that SFL processing of emulsions produced micronized powders containing amorphous danazol. In addition, amorphous danazol was present in the micronized SFL D and SFL E powders, where danazol-to-excipient ratios were 20:1:1:1 and 50:1:1:1, respectively. The ability to inhibit particle aggregation and crystallization with unusually small excipient concentrations was likely due to the proximity of the excipient on the oil surface to the API in the internal droplet.

SEM and TEM studies demonstrated that micronized porous aggregates consisting of small drug particle domains were produced by SFL processing of emulsions. Although SEM studies demonstrated that danazol, PVA, poloxamer and PVP were homogeneously blended and were not distinguishable from each other, TEM allowed the visualization of the distribution of the danazol small particle domains throughout the core of the porous aggregate structure. From the electron-dense danazol domains shown in Fig. 4a, it can be seen that the mass fraction of danazol was extremely high throughout the excipient matrix of the micronized SFL D formulation (10:1:1:1). Near the edges of the porous aggregates, it became feasible to use high magnifications to view the individual danazol domains. Small particle danazol-rich domains measuring approximately 200 nm in size were clearly visible in the porous SFL

microparticulate aggregate from emulsion. In contrast, the small particle danazol-rich domains in the porous SFL microparticulate aggregate from solution measured approximately 20 nm in diameter, and they were homogeneously dispersed throughout the porous aggregate matrix. The larger danazol domains produced from SFL processing of the emulsions was expected given the much higher feed concentrations that enhanced collisions of growing nuclei during freezing. However, the domain sizes of the SFL powders produced from either emulsions or solution were sufficiently small such that wetting and dissolution were fast in both cases. This result is remarkable given the much higher potency of the powders produced from emulsion. Again, the proximity of the surfactant excipient to the drug in the oil droplet appeared to inhibit particle growth and crystallization. It was demonstrated that the micronized SFL powders from emulsion that were highly loaded with danazol wetted and dissolved just as readily and completely as the micronized SFL powder from solution regardless of the differences in the particle domain size of danazol distributed throughout the porous aggregate matrices.

Large surface areas from 12.71 m^2/g (SFL A) up to 83.06 m^2/g (SFL C) were obtained with the micronized SFL powders, which were significantly higher than those of the slowly frozen agglomerates. The small domains of the freezing droplets in the spray led to faster freezing and greater preservation of the high surface area of the precipitated solids. The surface areas of the micronized SFL powders increased as the concentrations of danazol and excipients in the emulsions increased, which was consistent with what has been reported in the literature for samples freeze-dried or precipitated from solution or emulsions. The increase in surface area may be explained on the basis of studies of thermally induced phase separation of polymers [24–30]. For very low concentrations the API nuclei resulting from removal of the solvent during freezing grew to form small nonporous particles. For higher concentrations the mass transfer pathway on the solid–liquid equilibria phase diagram was closer to the critical point where solvent discrete phases were formed. The porosity increases from the solvent discrete domains thus elevated the surface areas of the micronized SFL powders from emulsion.

The micronized SFL powders from emulsion wetted and dissolved rapidly compared to the slowly frozen agglomerates and control formulations ($P < 0.05$). Even when higher API-to-excipient ratios were investigated, the micronized SFL D and SFL E powders dissolved greater than 90% of the danazol within 5 min in the dissolution media. Thus, it was demonstrated that micronized SFL powders with higher API-to-excipient ratios would wet and dissolve as readily as those micronized SFL powders with ten times lower (20:1:1:1 versus 2:1:1:1) API-to-excipient ratios when the SFL process was used to generate the powders. Regardless of the ratios investigated, the micronized SFL powders wetted and dissolved significantly more rapidly and completely compared to bulk danazol or the co-ground physical mixture ($P < 0.05$).

The slowly frozen agglomerates from emulsion had significantly higher M_{50} particle sizes compared to the micronized SFL powders from emulsion ($P < 0.05$). Because the agglomerates had to be mechanically broken up when dried, the primary particle sizes were larger than those sizes obtained from the SFL micronization process. Micronized powders were produced in a single step with SFL processing, so additional mechanical micronization was not necessary when micronized SFL powders were dried.

Slowly freezing the emulsions produced agglomerates that contained crystalline danazol, as demonstrated in the XRD studies. The EA-based slowly frozen agglomerates (A and B) were crystalline, but the DCM-based slowly frozen agglomerate (C) was amorphous. The differences in crystallinity could be influenced by the binary water-organic solvent phase diagrams, since the supersaturation of the API depends on the mass-transfer pathway on the phase equilibrium curve. These differences may also be related to the differences in aqueous solubilities of the EA and DCM.

From SEM analysis, it was shown that the slowly frozen agglomerates had different surface morphologies than the micronized SFL powders. Because the slowly frozen controls were composed of large agglomerates, the observed specific surface areas were significantly lower than those of the micronized SFL powders ($P < 0.05$). The slowly frozen agglomerates wetted just as readily, but dissolved slowly and incompletely in aqueous media compared to the micronized SFL powders ($P < 0.05$). Clearly, low contact angles were not enough to produce high dissolution rates, as has been observed in other studies [31,32]. This reduction in dissolution was attributed to the presence of crystalline danazol as well as reduced surface areas in the slowly frozen controls. The improved dissolution of micronized SFL powders versus slowly frozen agglomerates from emulsion was a result of large differences in morphology due to rapid freezing achieved during SFL processing, despite identical compositions.

The morphologies of the micronized SFL powders from emulsion versus those from solution were compared to understand why dissolution of danazol was rapid for both despite the large differences in potencies. The M_{50} primary particle sizes for micronized SFL powders processed from emulsions and solution were similar. The M_{50} values were most similar when lower concentrations of API and excipients in EA-based (formulation A) emulsions or more highly concentrated DCM-based emulsions (formulations C, D and E) were SFL processed. Micronized SFL powders from emulsion and solution contained amorphous danazol as demonstrated in the XRD studies; however, a much greater mass fraction of danazol was stabilized in a rigid matrix when SFL processed from emulsions with correspondingly greater excipient mass fractions, as shown

in the SEMs. Because the cosolvent solution used to produce the micronized SFL powder was much more dilute in API and excipients, the excipient matrix was not as dense and rigid as those of the micronized SFL powders from emulsion.

The importance of this rigid excipient matrix produced from emulsion was demonstrated in the TEM studies of the micronized SFL powders. The rigid, but porous, excipient aggregates from emulsion contained high mass fractions of small danazol-rich domains (~200 nm) within the interior core of the aggregates as shown in Fig. 4a. In contrast, the porous excipient aggregates from solution contained lower mass fractions of small danazol-rich domains (~20 nm) within the interior core (Fig. 4b). The higher concentrations of danazol in the internal oil phase produced ten times larger danazol domains in the interior core of the SFL processed aggregates, but with significantly higher surface areas and comparable dissolution profiles to that SFL powder obtained from a dilute cosolvent solution.

5. Conclusions

It was demonstrated in this study that emulsions could be processed by SFL to produce large batches of micronized powders with higher potencies and superior physicochemical characteristics to those of a micronized SFL powder from solution for dissolution enhancement of poorly water soluble drugs. In conclusion, the applicability of this particle engineering technology in the pharmaceutical industry for enhancing the dissolution of poorly water soluble drugs was shown.

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