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Research paper

A novel particle engineering technology to enhance dissolution of poorly water soluble drugs: spray-freezing into liquid

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Abstract

A novel cryogenic spray-freezing into liquid (SFL) process was developed to produce microparticulate powders consisting of an active pharmaceutical ingredient (API) molecularly embedded within a pharmaceutical excipient matrix. In the SFL process, a feed solution containing the API was atomized beneath the surface of a cryogenic liquid such that the liquid-liquid impingement between the feed and cryogenic liquids resulted in intense atomization into microdroplets, which were frozen instantaneously into microparticles. The SFL micronized powder was obtained following lyophilization of the frozen microparticles. The objective of this study was to develop a particle engineering technology to produce micronized powders of the hydrophobic drug, danazol, complexed with hydroxypropyl-β-cyclodextrin (HPβCD) and to compare these SFL micronized powders to inclusion complex powders produced from other techniques, such as co-grinding of dry powder mixtures and lyophilization of bulk solutions. Danazol and HPβCD were dissolved in a water/tetrahydrofuran cosolvent mixture prior to SFL processing or slow freezing. Identical quantities of the API and HPβCD used in the solutions were co-ground in a mortar and pestle and blended to produce a co-ground physical mixture for comparison. The powder samples were characterized by differential scanning calorimetry (DSC), powder X-ray diffraction (XRD), Fourier transform infrared spectrometry (FTIR), scanning electron microscopy, surface area analysis, and dissolution testing. The results provided by DSC, XRD, and FTIR suggested the formation of inclusion complexes by both slow-freezing and SFL. However, the specific surface area was significantly higher for the latter. Dissolution results suggested that equilibration of the danazol/HPβCD solution prior to SFL processing was required to produce the most soluble conformation of the resulting inclusion complex following SFL. SFL micronized powders exhibited better dissolution profiles than the slowly frozen aggregate powder. Results indicated that micronized SFL inclusion complex powders dissolved faster in aqueous dissolution media than inclusion complexes formed by conventional techniques due to higher surface areas and stabilized inclusion complexes obtained by ultra-rapid freezing.

Keywords: Spray-freezing into liquid; Dissolution enhancement; Danazol; Hydroxypropyl-β-cyclodextrin; Micronization; Particle engineering

1. Introduction

Danazol is a corticosteroid that has low aqueous solubility (<1 μg/ml) with high permeability across biological membranes [1–3]. The bioavailability of danazol following oral administration is dissolution rate-limited since the drug can be readily absorbed across the gastrointestinal membrane following dissolution [4]. Many techniques have been utilized to enhance the dissolution of danazol, including the formation of inclusion complexes between danazol and various cyclodextrin (CD) derivatives [1,5,6].

Techniques that have been utilized with varying success to generate inclusion complexes include: (1) kneading the active pharmaceutical ingredient (API) and CD derivative together in an aqueous or cosolvent paste [7–9]; (2) co-grinding the bulk powders [10]; (3) spray drying a solution or suspension containing API and CD derivative [9]; (4) dispersion of the API within an aqueous solution containing CD derivative followed by filtration of the dispersion and lyophilization of the filtrate [7,11–13]; (5) coprecipitation of inclusion complexes from organic solution by solvent evaporation [1,5,6]; and (6) utilization of a cosolvent system to dissolve both API and CD derivative concurrently followed by solidification and lyophilization [1]. Both kneading and co-grinding have resulted in degradation of the API due to high temperatures resulting from friction

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produced during the mechanical milling processes [7,8,10]. Spray drying is not a method of choice due to only 50% dry product recovery [9]. Filtration of a dispersion to separate dissolved inclusion complex from undissolved API is not economical because only a fraction of the drug exists in the soluble complexed form, which will pass through a filter and be collected for lyophilization. The undissolved drug that is retained by the filter will be discarded. Finally, coprecipitation of inclusion complexes from organic solutions is not a method of choice due to environmental concerns with organic solvent use and disposal. A cosolvent system with minimal amounts of organic solvent present to prevent API precipitation would be less damaging to the environment.

None of the conventional complex formation techniques described above produce flowable powders except for spray drying; however, poor product yields limit the use of this technique. The powders produced from co-grinding (air jet or ball mill) adhere to the grinding media and walls of the micronization chambers and are not easily recovered. If water is used to create either a paste for kneading or dispersion for filtering, the water must be removed to dry the formulation either by evaporation or lyophilization. The resulting dried granular material must then be ground to reduce the particle size to produce a powder that flows. A dried film resulting from organic solvent evaporation must be scraped from the sides of the container and ground to produce a flowable powder. A dried cake produced from lyophilization of a solution maintains the shape of the solution as it was frozen. Therefore, the cake must be broken into pieces and forced through a sieve in order to obtain a powder with acceptable flowability.

Spray-freezing into liquid (SFL) is a novel particle engineering technology that involves the atomization of a feed liquid containing a poorly water soluble or insoluble API and solubility enhancing excipients directly into a cryogenic liquid to produce a frozen micronized powder that is subsequently dried. In situ formation of a flowable powder with the API molecularly dispersed in a micronized excipient matrix occurs during SFL processing. The advantages of the SFL process result from the ultra-rapid freezing rates achieved by atomizing the feed solution beneath the cryogenic liquid surface. Ultra-rapid freezing rates prevent phase separation of the API and excipient carrier and crystalline growth of frozen water, both of which would result in non-homogeneous powder aggregates consisting of crystalline API domains. Instead, ultra-rapid freezing rates produce a porous micronized flowable powder with a high surface area and amorphous API stabilized in an excipient matrix. Because SFL micronized powders have high surface areas and contain amorphous danazol, enhanced dissolution of poorly water soluble or insoluble APIs in aqueous media is achieved.

The objective of this study was to determine the influence of SFL processing of an aqueous/organic cosolvent solution containing danazol and hydroxypropyl-β-CD (HPβCD) on the physicochemical characteristics and dissolution enhancement of danazol.

2. Materials and methods

Micronized danazol USP, lecithin, sodium taurocholate, potassium chloride and sodium hydroxide were purchased from Spectrum Chemicals, Gardena, CA. HPβCD (Cavitron 82005) was kindly donated by Cerestar USA, Inc. (Hammond, IN). Tetrahydrofuran (THF) was purchased from Mallinckrodt (Paris, KY). Glacial acetic acid was purchased from Fisher Scientific Products (Suwanee, GA). Liquid nitrogen was obtained from Boc Gases (Murray Hill, NJ).

2.1. Preparation of SFL micronized powders

An aliquot of 0.085 g danazol was dissolved in 14.85 g THF. An aliquot of 0.365 g HPβCD was dissolved in 29.70 g purified water. The two solutions were added together to form a one-phase cosolvent solution containing danazol and HPβCD in a 1:1 molar ratio. The cosolvent solutions were equilibrated in a horizontal shaker at 25°C for 0, 12, 48, 96, 120, 144, and 168 h, then rapidly frozen by SFL to produce micronized powders. The solutions were atomized beneath the liquid nitrogen surface at 5000 psi (34.5 MPa) constant pressure through a 63.5 μm inner diameter (I.D.) polyetherether ketone nozzle. The constant pressure was supplied by an ISCO Model 100DX syringe pump (ISCO, Inc., Lincoln, NE). The powders were then collected on a 150-mesh sieve and lyophilized in a VirTis Advantage Benchtop Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY). The parameters used to dry the frozen SFL micronized powders are shown in Table 1.

2.2. Preparation of control formulations

A co-ground physical mixture consisting of 0.085 g danazol and 0.365 g HPβCD was mixed by geometric dilution and ground using a mortar and pestle for 10 min, then mixed for 30 min in a V-blender.

A solution identical to the feedstocks used for SFL processing was frozen in the VirTis Tray Lyophilizer preequilibrated at −40°C. After the solution had completely solidified, the sample was freeze-dried using the lyophilization parameters presented in Table 1. This control sample is referred to as the slowly frozen aggregate powder.

One SFL control formulation was prepared in a 2:1 molar ratio of danazol to HPβCD. An aliquot of 0.17 g danazol

Table 1
Lyophilization process parameters used to dry the SFL and slowly frozen samples

Time elapsed (min)	Shelf temperature (°C)	Vacuum (mTorr)
180	− 40	100
180	− 30	100
240	− 20	100
240	+ 10	100
360	+ 25	100

was dissolved in 14.85 g THF, and 0.365 g HPβCD was dissolved in purified water. The two solutions were added together and immediately processed by SFL.

Another SFL control formulation was prepared by spray-freezing danazol in the absence of HPβCD into liquid nitrogen. The danazol was dissolved in THF, and the organic solution was added to the same amount of water as was used in the experimental SFL formulations containing HPβCD. The control solution was then SFL processed and lyophilized.

2.3. 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 (227°C) in the various samples. Modulated DSC was used to analyze the samples from 80 to 250°C with a 10°C per minute heating rate.

2.4. 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, 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.

2.5. Fourier transform infrared (FTIR) spectrometry

The powder samples were prepared for FTIR spectrometry analysis as suggested by Ziegler and Holmes [14,15]. The samples were spread and leveled on a polytetrafluoroethylene (PTFE) FTIR card using amyl acetate to create a paste of the powder. Following evaporation of the amyl acetate, a dried film of the powder sample remained adhered to the PTFE surface. The PTFE card was then inserted into a Perkin-Elmer Spectrum 2000 FTIR spectrometer for analysis.

2.6. 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.

2.7. 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 degassed sample weight was used for surface area determination by the software.

2.8. Dissolution testing

Dissolution testing was performed on the powders using a U.S.P. Type II VanKel VK6010 Dissolution Testing Station with a Vanderkamp VK650A heater/circulator. Five milliliter samples were taken at 2, 5, 10, and 20 min by a VK8000 autosampler (VanKel Technology Group, Cary, NC). Paddle speed and bath temperature were set at 50 rpm and 37.0 ± 0.2°C, respectively. Powder containing approximately 5 mg danazol was placed into each of six dissolution vessels ($n = 6$) containing 900 ml fed-state simulated intestinal fluid (FeSSIF). FeSSIF media was prepared as reported by Dressman et al. [2,3]. The dissolution media consisted of sodium taurocholate (0.81%, w/v), lecithin (0.75%, w/v), glacial acetic acid (1.00%, w/v), and KCl (1.52%, w/v) in purified water. The pH was adjusted to 5.0 by adding 10 N NaOH solution. First, lecithin and KCl were added to approximately 5000 ml water and allowed to dissolve completely. Then the sodium taurocholate was added and thoroughly dispersed with continuous stirring until complete dissolution had occurred. After the glacial acetic acid was added, and the solution was allowed to mix thoroughly before adjusting the pH. An aqueous solution of 10 N NaOH was added drop-wise as the FeSSIF media was continuously stirred. After attaining pH 5, the volume was brought to 6000 ml by adding purified water.

Rates of dissolution were calculated using Hixson-Crowell cube root kinetics equation:

$$\left(1 - \frac{Q_d}{A}\right)^{1/3} = 1 - k_2 t$$

where Q_d is the amount of drug dissolved at time t, A is the total amount of drug present, t is time, and k_2 is the apparent rate constant for API dissolution.

2.9. Contact angle measurements

A 50 mg aliquot of the sample powder was compacted into a tablet using a Model M Carver Laboratory Press (Fred S. Carver, Inc., Menomonee Falls, WI) to apply a 500 kg compression force. A 0.03 ml drop of FeSSIF dissolution media was placed on the compact and the contact angle between the sample compact and the FeSSIF 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.

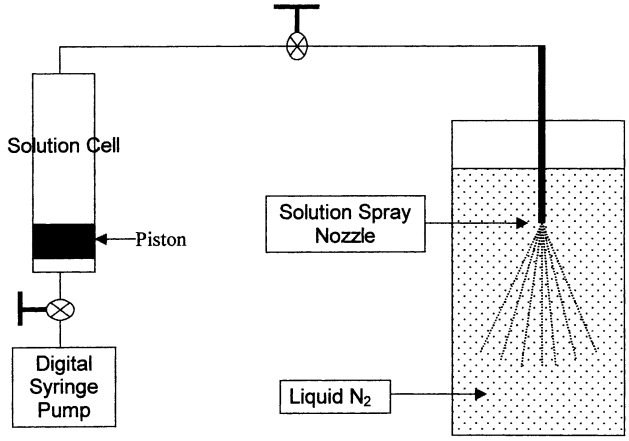

Fig. 1. Schematic representation of the novel SFL atomization process utilizing liquid nitrogen as the cryogenic medium.

3. Results and discussion

The SFL process is a novel cryogenic atomization technology in which either an aqueous or water-organic cosolvent solution containing an API and pharmaceutical excipient(s) is atomized directly into a compressed liquid, such as compressed fluid CO_2, helium, propane, ethane, or the cryogenic liquids nitrogen, argon, or hydrofluoroethers.

The novel SFL technology utilizes spraying directly into the cryogenic liquid phase to atomize the feed solution as it exits the nozzle. The liquid-liquid impingement that occurs as the feed solution impacts the cryogenic media results in intense atomization into fine microdroplets that freeze immediately upon exiting the nozzle. A schematic representation of the SFL apparatus is shown in Fig. 1. A pressurized syringe pump is used to propel the feed solution from the solution vessel through an insulated nozzle that is submerged beneath the surface of the cryogenic liquid. Nitrogen is the cryogen of choice because it is inexpensive, plentiful, and environmentally friendly. Because of the ultra-rapid freezing rates achieved by atomizing the feed solution directly into liquid nitrogen, a cryogenic suspension containing the dispersed frozen microparticles is produced. The SFL micronized powder can then be separated from the liquid nitrogen by using a fine sieve to collect the powder. The frozen powder is lyophilized to produce the dry SFL micronized powder.

As a result of the formation of high-surface area droplets by SFL processing and the low saturation temperature of liquid nitrogen, ultra-rapid freezing rates are achieved, and phase separation of solutes within the feed solution is prevented. Therefore, a solid solution is formed where the API molecules are homogeneously dispersed throughout the solidified excipient matrix of the frozen microdroplet. After lyophilization, the dried microparticle retains the shape of the microdroplet, but is highly porous due to the channels created as the solvent(s) are removed.

Because of the high surface area and porosity of the SFL microparticles, the engineered powder should wet and dissolve rapidly in aqueous dissolution media. Aqueous media will fill the pore channels and dissolve the carrier excipients, which could be utilized to enhance the aqueous dissolution of a lipophilic API. An example of a pharmaceutical excipient that is used to enhance the dissolution of a lipophilic API is HPβCD. HPβCD is composed of seven glucose molecules covalently bound into a cyclic macromolecule. The inside surface of HPβCD is hydrophobic, and the exterior surface is hydrophilic, due to the arrangement of the individual glucose units in the covalently bound macromolecule. Because of its amphiphilic characteristics, HPβCD is a suitable excipient for enhancing the aqueous dissolution of a poorly water soluble API [1,5,6,9,11,12].

Figs. 2a,b compare the macroscopic appearance of powders obtained from SFL versus slowly freezing. As shown in Fig. 2a, the representative SFL micronized powder had a superior morphology to the slowly frozen aggregate cake because the SFL sample was produced from the lyophilization of a snow-like frozen powder instead of slowly frozen solid ice. After freeze drying, the micronized

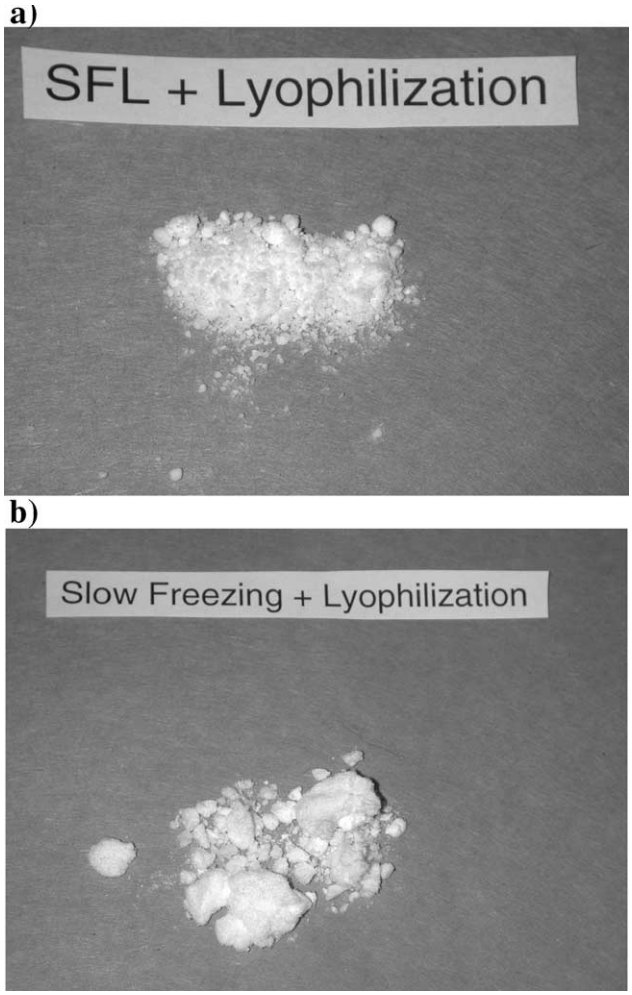

Fig. 2. Macroscopic physical appearance of SFL powder (a); and slowly frozen aggregate powder (b).

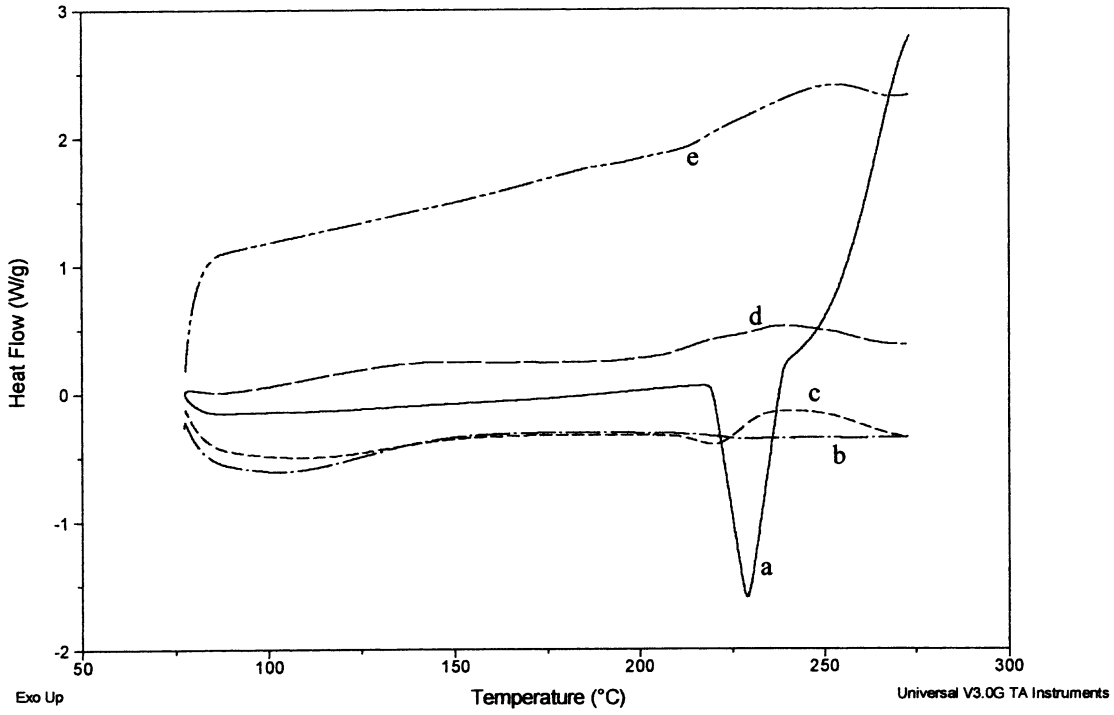

Fig. 3. Modulated DSC profiles of bulk danazol (a); bulk HPβCD (b); co-ground physical mixture (c); slowly frozen aggregate (d); and the SFL micronized powder with no equilibration (e). The DSC profile of the SFL micronized powder without equilibration is identical to the profiles obtained for all SFL micronized powders investigated regardless of equilibration time.

SFL lyophilizate was a homogeneous flowable powder consisting of discrete microparticles in contrast to the aggregates that resulted from breaking the slowly frozen freeze dried cake into smaller pieces. Following slow freezing, the solidified ice was the same shape as the solution that was frozen. Furthermore, the lyophilized cake retained the shape of the ice before freeze-drying was initiated. The dried cake was fractured into aggregates that could not be completely pulverized as shown in Fig. 2b.

To further understand the differences in the physical appearances of the various powders investigated, the samples were characterized by a number of analytical techniques. Modulated DSC has been employed to determine the formation of inclusion complexes by recording the presence or absence of an API melting endotherm in an API/CD sample [1,8,9]. Formation of an inclusion complex is confirmed when the API melting endotherm is not present. In Fig. 3, DSC profiles of bulk danazol and HPβCD, the co-ground physical mixture, slowly frozen aggregate powder, and an SFL micronized powder sample were compared. A sharp melting endotherm could be seen at 227°C in the bulk danazol sample. A slight endotherm was detected for the co-ground physical mixture, which indicated that non-complexed danazol was present. Thus, co-grinding did not produce complete inclusion of danazol into the HPβCD cavity. When the solution containing danazol and HPβCD was slowly frozen, an inclusion complex was formed as indicated by the complete disappearance of the danazol endotherm at 227°C. The endotherm disappearance was also complete for the SFL micronized powder, signifying the formation of the danazol/HPβCD inclusion complex. For clarity, only the DSC of the SFL micronized powder produced without equilibration is shown in Fig. 3. Regardless of equilibration time, all SFL sample DSC profiles were identical. In conclusion, the DSCs of the various samples proved that only slow freezing and SFL produced powders consisting of complete danazol/HPβCD inclusion complexes.

The XRD patterns of the various samples investigated are shown in Fig. 4. Bulk danazol powder is highly crystalline, as determined by intense peaks between 13 and 22 2θ degrees in the XRD pattern. These peaks were present in the co-ground physical mixture, but at a lower intensity. This reduction in peak intensity indicated that a portion of the danazol in the co-ground mixture was included into the HPβCD cavity, but a significant amount remained uncomplexed and crystalline, thus confirming the DSC findings that free danazol was present in the co-ground formulation. The high degree of crystallinity of danazol in the bulk and co-ground samples is a function of the hydrogen bonding that occurs between adjacent danazol molecules. Because this hydrogen bonding is strong, danazol naturally exists in a highly ordered crystalline habit. Grinding the bulk powder with HPβCD was not sufficient to disrupt the API intermolecular interactions to achieve complete inclusion complexation. During the production of the slowly frozen aggregate control, danazol was allowed to partition in the cosolvent solution into the HPβCD cavity prior to freezing.

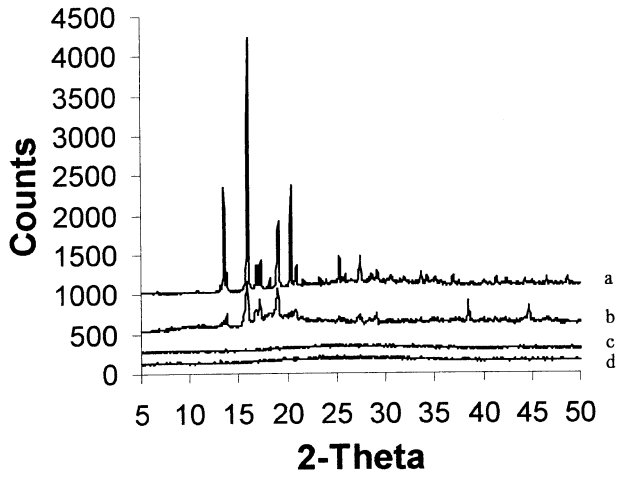

Fig. 4. XRD patterns of bulk danazol (a); co-ground physical mixture (b); SFL powder without equilibration (c); and slowly frozen aggregate (d). The XRD pattern of the SFL powder without equilibration is identical the patterns obtained for all SFL powders investigated regardless of equilibration time. The amorphous patterns c and d are identical to the XRD pattern of bulk HPβCD.

Thus, danazol crystallinity was eliminated resulting in a completely amorphous aggregate. In Fig. 4, the absence of the crystalline danazol peaks in the slowly frozen aggregate powder XRD pattern implicated inclusion complex formation. Similarly, the XRD pattern of the SFL micronized powder showed completely amorphous danazol due to inclusion within the hydrophobic HPβCD cavity. Badawy et al. have investigated danazol/HPβCD systems, concluding that the danazol molecule diffused into the cavity of an HPβCD molecule, and crystallinity was eliminated due to thermodynamically-preferred hydrogen bonding between danazol and the HPβCD hydrophobic core [1,5]. Thus, the encapsulated danazol could no longer interact with other danazol molecules as a result of its occupation in the HPβCD cavity.

FTIR spectrometry was performed on the powder samples to confirm formation of inclusion complexes by the two freezing techniques. Inclusion complex formation between danazol and HPβCD was detected by investigating the characteristic danazol hydroxyl (O-H) stretch at 3518 cm^{-1} [5]. This hydroxyl group is responsible for the hydrogen bonding that occurs between adjacent danazol molecules. When inclusion complexes between danazol and HPβCD are formed, the characteristic danazol peak would shift to a slightly higher frequency or broaden, indicating weaker, but thermodynamically favorable hydrogen bonding between danazol and the hydrophobic HPβCD core [16]. In Fig. 5a, the characteristic peak was observed at 3518 cm^{-1} for bulk danazol. No peak was present at this wave number for HPβCD (Fig. 5b). Therefore, any peak shift or broadening was a direct result of the disruption of the hydrogen bonds between danazol molecules. In Fig. 5c, the peak broadening indicated inclusion complex formation during slow freezing. A similar FTIR spectrum was obtained (Fig. 5d) for the SFL micronized powder that was processed without equilibration. Thus, both freezing techniques produced inclusion complexes. FTIR spectra of the SFL micronized powders were identical regardless of

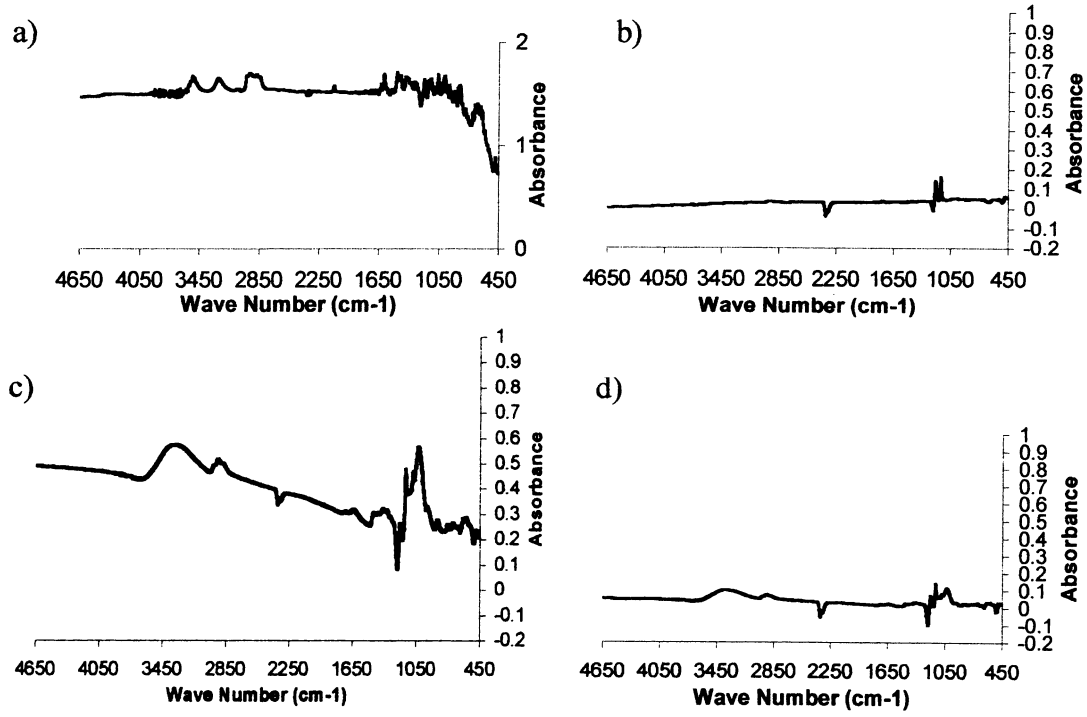

Fig. 5. FTIR spectra of bulk danazol (a); HPβCD (b); slowly frozen aggregate (c); and 96-h equilibrated SFL powder (d). The FTIR spectrum of the 96-h equilibrated SFL powder is identical to the spectra of all the SFL powders obtained regardless of equilibration time.

equilibration time, so only the spectrum of the sample produced without equilibration is shown in Fig. 5. As confirmed by the XRD results shown in Fig. 4, once a danazol molecule partitioned into the hydrophobic HPβCD core, hydrogen bonding between that molecule and other danazol molecules could no longer occur. The hydrogen bond disruption that occurred as the API molecule complexed with HPβCD produced the peak broadening at 3518 cm^{-1} in the FTIR spectra of the slowly frozen aggregate and SFL micronized samples.

To understand the physical differences between powders produced from slow freezing and SFL, the surface morphologies of the samples were observed by SEM, as shown in Fig. 6. The SEM micrograph for bulk micronized danazol,

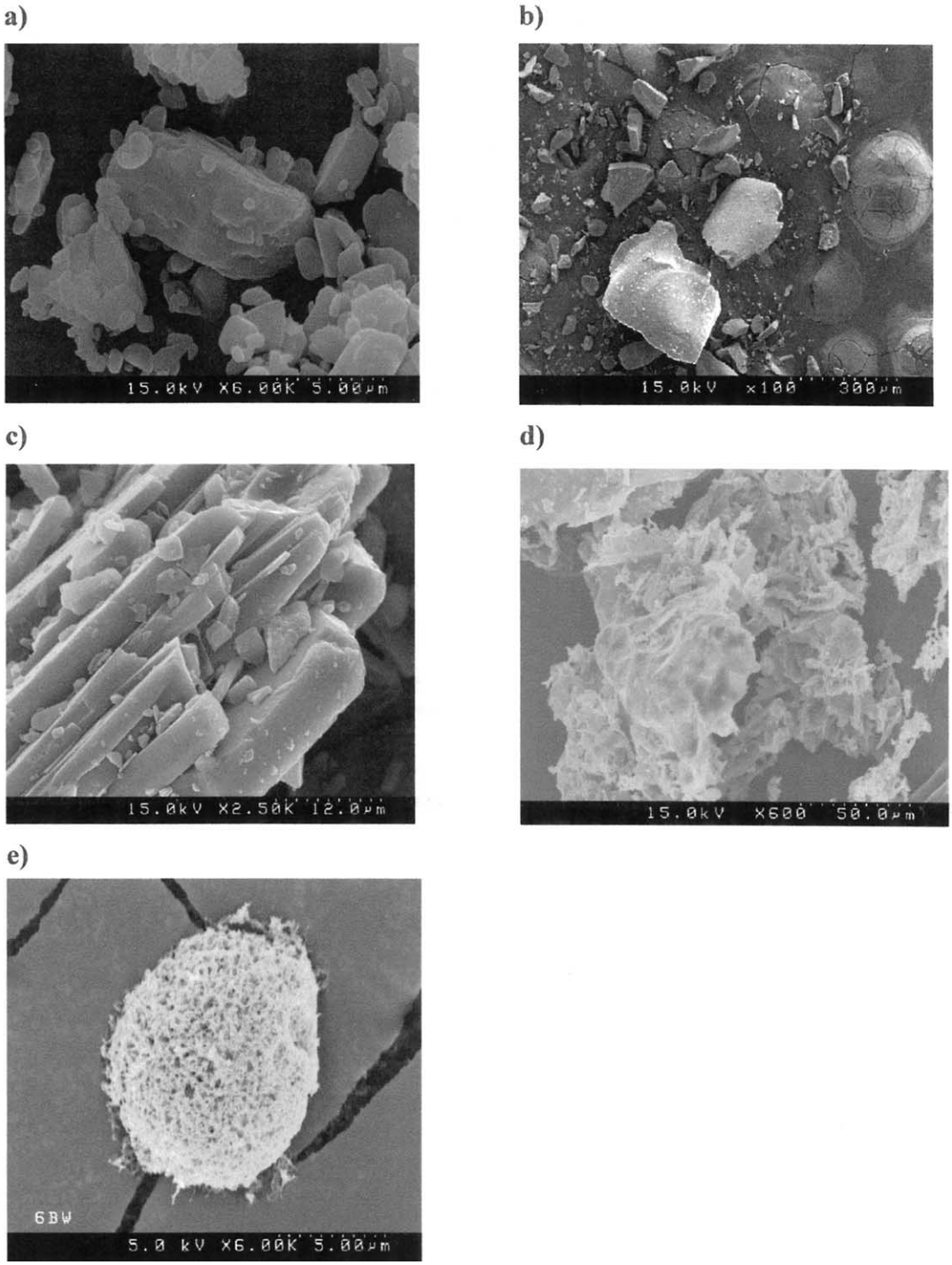

Fig. 6. SEM micrographs of bulk danazol (a); HPβCD (b); physical mixture (c); slowly frozen aggregate (d); and 96-h equilibrated SFL powder (e). The SEM micrographs for all SFL powders were identical regardless of equilibration time.

Table 2
Specific surface areas of the various powder samples investigated[a]

Sample	Surface area (m^2/g)
Bulk danazol	0.5157
Bulk HPβCD	0.3920
Physical mixture	0.4233
96-h Eq. + slow freeze	0.1664
96-h Eq. + SFL	113.4994

[a] The molar ratio of danazol to HPβCD is 1:1.

USP (Fig. 6a) confirmed the presence of micronized danazol crystals. The bulk danazol crystals varied in length from 1 to 10 μm. The large HPβCD particles (100–300 μm), shown in Fig. 6b, had no ordered structure as confirmed by the amorphous bulk HPβCD XRD pattern in Fig. 4. In Fig. 6c, the co-ground physical mixture consisted of danazol particles resting on the surface of the fragmented HPβCD particles. Although the HPβCD particle size was dramatically decreased, there remained a large size difference between the API and HPβCD particles after co-grinding the two powders. The aggregates produced from slow freezing (Fig. 6d) consisted of large continuous networks, where API and HPβCD constituents could not be distinguished. The SFL micronized powders, however, consisted of discrete porous microspheres. The diameter of the SFL particle, shown in Fig. 6e, was approximately 7 μm. No distinction could be made between danazol and HPβCD constituents in the SFL micronized powders. Because all SFL micronized powder SEM micrographs were identical regardless of equilibration time, only the SEM of the SFL micronized powder without equilibration is shown. The individual porous microparticles, shown in Fig. 6e, constituted the SFL micronized powders in contrast to the large, bulky agglomerates that constituted the slowly frozen aggregate powder (Fig. 6d). During SFL processing, the frozen microspheres were generated. From Fig. 6e, pores on the microsphere surface were produced as the solvents were evaporated.

The large differences in the morphologies of the powders produced by the various processes were further characterized by surface area measurement. Because the SFL micronized powder was composed of small porous microparticles, its surface area was significantly greater than those of the slowly frozen aggregate, co-ground physical mixture, or bulk samples. To quantify these differences, specific surface areas of the various samples were determined, and are listed in Table 2. The 96-h SFL micronized sample surface area was identical to the surface areas of the other SFL micronized powders, so only the surface area of this representative SFL micronized powder is listed in Table 2.

The SFL micronized powder had a specific surface area of 113.50 m^2/g, which was significantly greater than those of the powders and aggregates made by the other techniques investigated. Because the slowly frozen aggregate powder consisted of large aggregates of lyophilizate, its surface area was only 0.17 m^2/g. This aggregate powder had the lowest surface area investigated. The co-ground sample had a surface area of 0.42 m^2/g, which was slightly greater than that of the bulk HPβCD (0.39 m^2/g), but slightly lower than that of the bulk danazol (0.52 m^2/g). This finding was expected since the co-ground sample was a physical mixture of the API and HPβCD. A number of studies have shown that increasing the surface area of a hydrophobic API enhanced the dissolution in aqueous media [9,10,17]. Because the SFL micronized powders possessed the highest surface areas of all the samples investigated, they had the potential to exhibit the best dissolution profiles.

In Fig. 7, the dissolution profiles of the various powders in FeSSIF aqueous media are shown. The powders of bulk danazol and the physical mixture were observed to wet poorly during dissolution. Two minutes after addition to the dissolution media, only 20% of the bulk danazol had dissolved, which was identical to that found for the co-ground physical mixture and the SFL processed danazol without HPβCD. Because complete inclusion complexes were not formed between co-ground danazol and HPβCD, nor was surface area increased, danazol dissolution was not enhanced. In contrast, the slowly frozen aggregate powder was readily wetted and dissolved in the dissolution media. Although the surface area of the slowly frozen aggregate powder was low, enhanced dissolution was expected because DSC, XRD, and FTIR results suggested that a complete inclusion complex was present in the formulation.

The amount of danazol dissolved from the SFL powders within the first 10 min of introduction into the dissolution media was 100% regardless of the equilibration time.

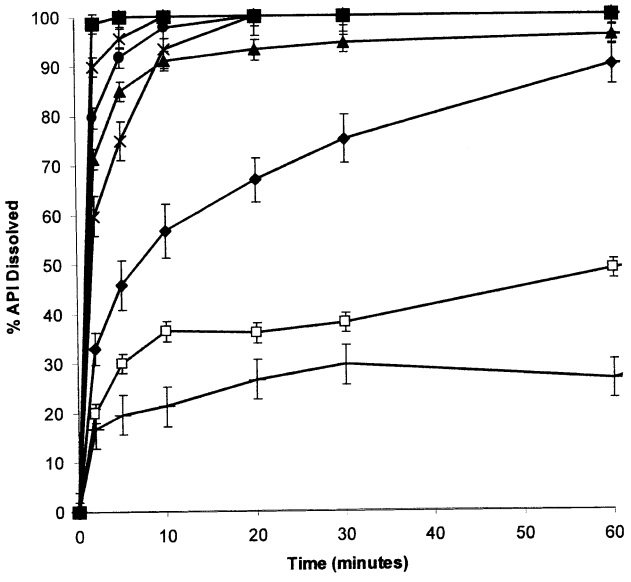

Fig. 7. Dissolution profiles of SFL micronized powders without equilibration (×), equilibrated 12 h (*), 48 h (●), 96 h (■), the slowly frozen aggregate control (▲), 2:1 SFL control (◆), co-ground physical mixture (□), and bulk danazol (+). The dissolution curve for the 96-h equilibrated SFL powder is similar to those of SFL powders that were equilibrated for 120, 144, and 168 h prior to SFL processing.

Because the presence of the THF allowed all species to be dissolved when the SFL solutions were formulated, immediate inclusion complexation occurred. Intimate contact was allowed between danazol and HPβCD in the cosolvent solutions, which eliminated the need for equilibration. There were some variations in the dissolution profiles within the first 5 min after introduction of the SFL powder into the dissolution media. Within 5 min, the SFL powder without equilibration had dissolved 75% danazol whereas the SFL powders equilibrated for 12 h or longer had dissolved at least 90% danazol. By 10 min, however, the amount of danazol dissolved from the SFL micronized powders was complete (100%) regardless of equilibration time.

The slowly frozen aggregate control was allowed to equilibrate for 96 h before it was solidified and lyophilized, as in the case of the 96-h equilibrated SFL micronized powder, however, there were significant differences between the two dissolution profiles. A total of 99% danazol was dissolved from the SFL micronized powder within 2 min compared to 70% from the slowly frozen aggregate control. Danazol (83 and 89%) was dissolved from the slowly frozen aggregated powder at 5 and 10 min, respectively. At 20 min, 90% of the danazol from the slowly frozen control was dissolved, and at 1 h, 100% (data not shown) was dissolved. In conclusion, the amount of danazol dissolved during the first 20 min was higher for all the SFL micronized powders than for the slowly frozen aggregate powder regardless of equilibration time. The amount of danazol dissolved from the SFL micronized powder equilibrated for 96 h was significantly higher than that from the slowly frozen aggregate powder equilibrated for the same amount of time until 100% of the danazol had dissolved from the slowly frozen aggregate control by 1 h. A number of studies have been done which provide insight into the mechanism of the faster dissolution rates achieved with the rapidly frozen SFL micronized powder compared to the slowly frozen aggregate sample. The primary particle size affects how rapidly a sample dissolves in aqueous media [9,17]. The smaller particle size and higher surface areas of the SFL powders contributed to better wettability and faster dissolution rates.

The Hixson-Crowell cube root kinetics equation was used to determine the rates of dissolution of the 96-h equilibrated SFL micronized powder, bulk danazol, co-ground physical mixture and slowly frozen aggregate control. It was found that the rate of dissolution of danazol from the SFL micronized powder (0.381 min^{-1}) was significantly higher than that from of bulk danazol (0.028 min^{-1}), the co-ground physical mixture (0.036 min^{-1}), or slowly frozen aggregate control (0.170 min^{-1}). Therefore, it was demonstrated that SFL processing produced micronized powders with significantly higher rates of dissolution compared to the other methods used to generate inclusion complexes.

In Table 3, the contact angle measurements of the various samples investigated are listed. The contact angle is a measurement of the relative degree of interaction between a solid surface and a liquid. A high contact angle correlates with a poor degree of interaction between the solid and liquid. The lower the contact angle, the better the wettability of the solid by the liquid [18]. The contact angle of the slowly frozen aggregate control (32.0°) was similar to those of the SFL micronized powders (27.0–32.3°), so the slower dissolution profile associated with the slowly frozen aggregate control compared to the SFL micronized powders was a result of the lower surface area of the aggregate instead of phase separation caused by freeze concentration of pure danazol from HPβCD, which would result in a powder with poor wettability. The inclusion complexes were sufficiently stable to prevent phase separation of the danazol and HPβCD during the slow freezing process as confirmed by DSC, X-ray, FTIR, and contact angle measurement.

Table 3
Contact angle measurements of the SFL and control samples investigated[a]

Sample	Average contact angle ± SD
SFL danazol alone	64.3 ± 1.1
Co-ground physical mixture	51.5 ± 0.7
Slowly frozen aggregate control	32.0 ± 0.0
No Eq. + SFL	27.0 ± 2.8
12-h Eq. + SFL	30.3 ± 2.5
48-h Eq. + SFL	32.0 ± 2.1
96-h Eq. + SFL	32.3 ± 1.1
2:1 SFL control[b]	37.5 ± 0.7

[a] FeSSIF dissolution media was used to determine the contact angle on the surface of the sample compact. The molar ratio of danazol to HPβCD was 1:1.
[b] 2:1 molar ratio of danazol to HPβCD.

In Fig. 7, the dissolution profile of a 2:1 (API:HPβCD molar ratio) SFL control formulation was observed to be intermediate between the experimental (1:1 molar ratio) SFL micronized powder dissolution profiles and the profile of the bulk danazol. It can be observed in Table 3 that the contact angle between the 2:1 SFL control formulation and FeSSIF dissolution media (37.5°) was significantly greater than those contact angles between the experimental (1:1) SFL micronized powders and FeSSIF (27.0–32.3°). Danazol and HPβCD complex in a 1:1 molar ratio. At molar ratios greater than 1:1 (e.g. 2:1), there is uncomplexed danazol present. Thus, the degree of interaction between the 2:1 SFL control powder and FeSSIF was not as high as that observed between the experimental (1:1) SFL micronized powders and FeSSIF. As a result of the presence of uncomplexed danazol, the 2:1 SFL control did not wet as readily, and dissolved more slowly in the FeSSIF media than the experimental (1:1) SFL micronized powders.

The 2:1 SFL control dissolution profile is a suitable immediate release profile compared to that observed by Badawy et al., who investigated a danazol/HPβCD coprecipitate from methanol [5]. The formulation investigated by Badawy et al. was prepared at a significantly lower ratio (1:1) of danazol to HPβCD than the 2:1 SFL control formulation, and dissolution testing was performed in 30% isopropanol in water instead of FeSSIF. However, similar

dissolution profiles were observed between the Badawy formulation and the 2:1 SFL control formulation investigated in this study. Thus, a dissolution profile similar to that of the formulation by Badawy et al. could be obtained with a significantly higher ratio of danazol to HPβCD by utilizing the SFL process to generate the micronized powder, but without the use of an aqueous-organic cosolvent dissolution media. The high surface area 2:1 SFL control micronized powder dissolved readily, even when uncomplexed danazol was present. By utilizing the SFL process, higher API loading within a formulation could be achieved while maintaining the capability of enhancing the dissolution of the API within aqueous media.

The enhancement of the danazol dissolution profile using the SFL process to form inclusion complexes consisting of a hydrophobic guest molecule within HPβCD was the result of the higher surface areas achieved by utilizing the SFL processing technique. In addition, complete danazol dissolution into FeSSIF media from the carrier CD was achieved by 10 min regardless of how long the (1:1) solutions were allowed to equilibrate prior to SFL processing. The SFL powders wetted better than the co-ground physical mixture and the 2:1 SFL control due to the presence of uncomplexed danazol in the two control formulations.

4. Conclusions

The novel particle engineering process, spray-freezing into liquid, was demonstrated to generate inclusion complexes superior to those formed by conventional techniques, such as co-grinding and slow freezing. In addition, powders with morphologically favorable characteristics were produced by SFL. Because this technique produced flowable, high-surface area powders containing API molecularly encapsulated within a macromolecular CD carrier, the SFL process may be included in the future as an intermediate step for engineering pharmaceutical powders for various routes of API delivery. This study demonstrated the usefulness of the SFL particle engineering technology as a method of enhancing the dissolution of poorly water soluble or insoluble APIs.

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