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**A NANOPARTICLE ENGINEERING PROCESS: SPRAY-FREEZING INTO
LIQUID TO ENHANCE THE DISSOLUTION OF POORLY WATER
SOLUBLE DRUGS**

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LIQUID TO ENHANCE THE DISSOLUTION OF POORLY WATER
SOLUBLE DRUGS**

by

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Dedication

To my dedicated Husband, Zhongshui and my loving Son, Austin.

To my Father and Mother, Mr. Yitang Hu and Mrs. Lanqing Kong

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**A NANOPARTICLE ENGINEERING PROCESS: SPRAY-FREEZING INTO
LIQUID TO ENHANCE THE DISSOLUTION OF POORLY WATER
SOLUBLE DRUGS**

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It is estimated that about 40% of compounds being developed by the pharmaceutical industry are poorly water soluble. A limiting factor in the oral bioavailability of poorly water soluble compounds is their inadequate dissolution rates. Increasing the dissolution rate of poorly water soluble active pharmaceutical ingredients (APIs) has become a major challenge in pharmaceutical formulation development. The spray freezing into liquid (SFL) particle engineering process was developed to enhance the wetting and dissolution properties of poorly water soluble APIs.

The SFL process was developed and optimized in order to achieve broad applications in drug delivery systems. Firstly the use of the SFL process to enhance the dissolution of poorly water soluble APIs was investigated and the influence of the SFL process on the physicochemical properties of poorly water soluble API was determined and compared to the current particle formation techniques including milling, co-grinding, and freeze-drying.

The SFL process was further enhanced for preparation of nanoparticles of poorly water soluble APIs, using organic solvents like acetonitrile, as the solution source solvent. Using acetonitrile as the solution source solvent increased drug loading in the feed solution and reduced the drying time in the SFL process. In addition, the influence of the solution type (organic vs aqueous/organic) on physicochemical properties of SFL micronized powders was determined.

The SFL process was then extended to produce rapidly dissolving high potency powders with high surface areas and dissolution rates. The potencies ranged from 50% to 90%, in contrast with typical values of only 33% in previous studies. In order to achieve these high potencies, high concentrations of APIs were dissolved in pure or mixed organic solvents to prepare the feed solutions. This study tested the hypothesis that only small amounts of surfactant or polymer were sufficient to form SFL nanostructured aggregates with amorphous API, high surface areas, and enhanced wettability, properties which enhance dissolution.

Furthermore, the ability of stabilization of amorphous SFL micronized powders was investigated. The influence of excipient type and glass transition temperature (T_g) on the stability of amorphous SFL danazol powders was determined. The influence of moisture content, danazol potency, and excipient type on T_g of SFL micronized powders was determined.

Lastly, the incorporation of SFL micronized powder into rapid release tablet formulations by direct compression was studied. The hypothesis of this study was that the high dissolution properties of SFL micronized powder would be maintained during the blending and direct compression processes by optimizing the SFL powder composition and tablet excipient type. Influence of SFL powder composition and tableting excipient composition on the rapid release of poorly water soluble API from tablets was determined.

The results of this research demonstrated that the SFL process offers a highly effective approach to produce nanoparticles of poorly water soluble drug contained in larger structured aggregates with high potency, high surface area, amorphous API, enhanced wettability, and rapid dissolution rates. Therefore, the SFL process is an effective particle engineering process for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs.

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CHAPTER 1: Nanoparticle Engineering Processes for Enhancing the Dissolution Rates of Poorly Water Soluble Drugs –A Comprehensive Review

1.1 INTRODUCTION

It has been reported that about 40% of the compounds being developed by the pharmaceutical industry are poorly water soluble (1, 2). A limiting factor to the *in vivo* performance of poorly water soluble drugs after oral administration is their inadequate ability to be wetted by and dissolved into the fluid in the gastrointestinal (GI) tract. Therefore, increasing the dissolution rate of poorly water soluble drugs is an important and significant challenge to pharmaceutical scientists in order to maximize absorption. According to the Noyes-Whitney equation, reducing the particle size and thus increasing the surface area will increase the dissolution rate of poorly water soluble drugs (3). Commercially used processes for micronization include mechanical milling, recrystallization, solid dispersion, freeze drying, and spray drying (4-8). However these processes have limitations that include organic solvent use, thermal degradation, large residual solvent content, and difficulties in controlling particle size and size distribution during processing. These limitations will affect drug particle

stability, powder flow properties, and efficiency of delivery system. In particular, further reduction of particle size of poorly water soluble drugs into the nanoparticle range remains challenging (5, 9, 10).

Over the last 10 years, nanoparticle engineering processes have been developed and reported for pharmaceutical applications. Purposefully increasing solubility and dissolution rate for these poorly soluble drugs by increasing surface area is a very promising way to improve drug bioavailability (5). Several reviews discussing particle engineering processes (4-6, 9-20) have been written. The present review will focus on several commercially or potentially commercially available nanoparticle engineering processes recently reported in the literature for enhancement of dissolution of poorly water soluble drugs. These nanoparticle engineering processes involve the use of mechanical micronization techniques, supercritical fluid processes, cryogenic spray processes, and solvent evaporation processes. This review will discuss each process and the specific characteristics of nanoparticles reported from each process.

1.2 MECHANICAL MICRONIZATION PROCESSES

A common approach used for many years in the pharmaceutical industry is micronization of poorly soluble drugs by milling (11, 12). The standard microparticles produced by milling have particle size about 5 μm . The formation of particles below 1 μm is a challenge due to aggregation of high surface area materials. Until now, two mechanical processes were commercialized for

nanoparticle preparation. First, the wet milling technique, NanoCrystals[®] technology, was developed and patented in 1992 by Liversidge *et al* and formerly owned by Sterling Drug Inc., later acquired by Elan Corporation (9, 12, 21). The second process is a high pressure homogenization process, DissoCubes[®] technology, which was developed and patented by Müller *et al* in 1994 and formerly owned by the Drug Delivery Services GmbH in Germany and now owned by SkyePharma PLC (11, 12, 22).

1.2.1 Wet Milling

Wet milling is an attrition process in which large micron size drug crystals are wet milled in the presence of grinding media and a surface modifier (21). The rigid grinding media is typically spherical in form, having an average size less than about 3 mm. The grinding media used in the process include zirconium oxide, such as 95% ZrO stabilized with magnesia, zirconium silicate, and other media, such as glass grinding media stainless steel, titania, alumina, and 95% ZrO stabilized with yttrium (21). The surface modifiers include various polymers, low molecular weight oligomers, natural products and surfactants, such as polyvinyl pyrrolidone, pluronic F68, pluronic F108, and lecithin. The particle size of the starting materials is typically less than 100 μm and was micronized by a jet-milling process. The particle size of the final products is less than 400 nm (9, 23).

In the wet milling process, the poorly water soluble drug is first dispersed in an aqueous-based surfactant solution, and then the resulting suspension is wet milled with the grinding media. High-energy-generated shear forces and the forces generated during impaction of the milling media with the solid drug provide the energy to fracture drug crystals into nanometer-sized particles (9, 24). Processing temperatures commonly are less than 40°C (21) and processing pressures are up to about 20 PSI. Milling efficiency is dependent on the properties of the poorly water soluble drug, medium and stabilizer. Liversidge (23, 25) reported that a significant reduction particle size was observed within 24 hours wet milling. Routinely, the drug/surfactant slurry was milled to a final size of less than 400 nm and generally this could be achieved over a four-day period.

Poorly water soluble drugs in the nanoparticle suspension are reported to be in a crystalline state due to a low energy used in the milling process (9). The nanoparticles are typically less than 400 nm and are physically stabilized with a polymeric excipient (2, 9). A physically stable nanosuspension is obtained when the weight ratio of drug to stabilizer was 20:1 to 2:1 (9). Liversidge *et al* reported that poorly water soluble drug, naproxen, was reduced average particle size from 20-30 μm to 270 nm over 5 days wet milling. The PVP K-15 was used as a stabilizer in the suspension and the ratio between naproxen and PVP K-15 in the nanosuspension was 5:3. The naproxen nanosuspension did not aggregate and remained physically and chemically stable for up to 4 weeks at 4°C (25). The

nanosuspension containing 2% paclitaxel and 1% pluronic F127 was prepared by wet milling for 7 days and had average particle size about 280 nm. It was found that the higher molecular weight polymeric stabilizer was optimal for effective particle size reduction and shelf stability (9, 24, 25).

NanoCrystals[®] particles can be used for oral delivery. In the study of preparing a nanosuspension of naproxen, the bioavailability of NanoCrystals[®] naproxen was compared to that of the marketed products: Naprosyn[®] (suspension) and Anaprox[®] (tablet) (9, 24). The time to reach maximal drug concentrations is approximately 50% less for the NanoCrystals[®] dispersion, while maintaining a 2.5–4.5-fold increase in the AUCs during the first hour of the study (9, 24). NanoCrystals[®] suspensions are also a suitable dosage form for poorly-water-soluble injectable products. The nanoparticles produced by wet milling process provided a significantly higher level dosing than using a traditional approach. Harsh solvent or co-solvent used in the formulation of poorly water soluble drugs are dose limiting due to the toxicity of solvent or excipients (9). In the comparison of the performance of paclitaxel in the marketed product (Taxol[®]) using Cremophor EL/ethanol mixture and NanoCrystals[®] nanosuspension, the maximum tolerated dose of the nanoparticle paclitaxel formulation is greater than that of the commercial product. This advantage could improve the delivery efficacy of the poorly water soluble drugs (9, 21, 26).

A limitation of the wet milling process is that the contamination of the product by grinding material has been reported (12). During the wet milling process, erosion of grinding materials occurs and leads to contamination of the product and the grinding media, such as glass and zirconium oxide are insoluble in the fluid of the GI tract. In addition, wet milling is a batch process. There is batch-to-batch variation detected in the quality of dispersion, processing time, crystallinity degree of drugs, and particle size distribution. These variations will affect drug particle stability, powder flow properties, and efficiency of delivery system. Milling over a few days also brings the risk of microbiological problems, especially when performing the milling at 30°C or having dispersion media providing nutrition to bacteria.

1.2.2 High Pressure Homogenization

High pressure homogenization is another mechanical process to prepare nanometer size particle in suspension containing poorly water soluble drugs. The principle of forming nanosuspensions is the cavitation forces created in high pressure homogenizer like piston-gap homogenizer (12). In the process, the poorly water soluble drug is first dispersed in an aqueous surfactant solution by high speed stirring and the suspension is then passed through a high pressure homogenizer applying a typical pressure of 1500 bar and 3 to 20 homogenization cycles (12). The suspension passes a very small homogenization gap in the homogenizer, typically having a width of 25 μm at 1500 bar. Due to the

narrowness of the gap, the streaming velocity of the suspension increases and the dynamic fluid pressure also increases. In addition, the static pressure on the fluid decreases below the boiling point of water at room temperature. In consequence, water starts boiling at room temperature and gas bubbles are formed which implode (=cavitation) when the fluid leaves the homogenization gap (12). These cavitation forces are strong enough to break the drug microparticles into drug nanoparticles (12, 22).

Nanosuspension particles have an average particle size ranging from 40 nm to 500 nm, the proportion of particles larger than 5 μm in the total population being less than 0.1% (12, 22). The particle size of nanosuspension depends on the hardness of the drug substance, processing pressure and number of cycles applied. For the poorly water soluble drug, budesonide, a pressure of 1500 bar and 10 cycles led to a mean diameter of 511 nm, increasing the cycle numbers to 15 reduced the size to 462 nm, and increasing the pressure to 2500 bar and 10 cycles led to particles with a mean diameter of 363 nm (11, 12, 22). The particle size of poorly water soluble drug can be produced in a controlled way by adjusting the production parameters pressure and cycle accordingly. Changes in drug crystallinity have been reported for high pressure homogenization process. A fraction of poorly water soluble drugs in the particles was amorphous in some cases, while the drugs were found to be completely amorphous in other cases (12). The application of high pressures in the process was found to promote the

amorphous structure. The variation in crystallinity level might be a challenge for quantity control and long term stability.

The stabilization against aggregation and coalescence is a main challenge for forming nanosuspensions. The stability of nanosuspensions can be determined by the zeta potential. For a physically stable nanosuspension solely stabilized by electrostatic repulsion, a zeta potential of ± 30 mV is required as a minimum (12, 22). In addition, Ostwald ripening is also used to determine the stability of highly dispersed systems (12). The absence of Ostwald ripening indicates long-term physical stability as an aqueous suspension (27). It was observed that there was no Ostwald ripening in the nanosuspensions produced by high pressure homogenization process. This was attributed to the uniform particle size created by the homogenization process (12, 22). The concentration differences in the nanosuspension system were sufficiently low to avoid the ripening effect.

The ability for large-scale production of a drug delivery system is the essential prerequisite for the current nanoparticle engineering processes to be introduced into the pharmaceutical market. High pressure homogenizers are available with different capacities from a few hundreds to a few thousands liters per hour (12, 22). In addition, instead of passing a few times through one homogenizer, several homogenizers can be placed in series to produce the product in a continuous mode. In addition, there are also homogenizers used on a

laboratory scale, which can have a minimum batch volume of 20 ml and a maximum of 40 ml, thus allowing the cost-effective processing of expensive drug materials or small amount of experimental compounds.

The disadvantage of high pressure homogenization process is that the high pressures used can cause changes in the crystal structure, and as a result, the amorphous fraction in the particle increases in some cases. The batch to batch variation in crystallinity level might be an issue for quantity control. The stability of partially amorphous nanosuspensions will also present a challenge in pharmaceutical industry applications.

1.3 SUPERCRITICAL FLUID TECHNOLOGY

Since high energy milling and long processing times can lead to contamination, batch variation, downstream processing difficulties and compromised stability, a single-step supercritical fluid process would be an excellent alternative, where microparticles and nanoparticles can be formed directly from drug solution. Two processes that use supercritical fluids for particle formation have been developed to improve solubility and dissolution of poorly water soluble drugs (4). They are rapid expansion of supercritical solutions (RESS) and precipitation with a compressed antisolvent (PCA), which is also referred to as the supercritical antisolvent (SAS) method or the solution enhanced dispersion by the supercritical fluids (SEDS) process (13, 28-33). In the PCA process, the CO₂ expands the solvent and the solvent dissolves in CO₂, thus

lowering the solvent strength, resulting in precipitation of poorly water soluble drug into a micronized powder (34). In the RESS process, the solubility of the solute in the CO₂ must be sufficient at a given temperature and pressure to produce adequate yields of the micronized powder. The drug solubility in supercritical CO₂ can be altered by manipulating the temperature and pressure, and hence, the density of the compressed gas or by adding a co-solvent.

1.3.1 Precipitation with Compressed Fluid Antisolvent (PCA)

The PCA/SAS/SEDS technique applies a similar concept to the use of an antisolvent in solvent-based recrystallization process. In the PCA process, CO₂ is used as an antisolvent. Poorly water soluble drug and/or polymer solutions are atomized into a chamber containing compressed CO₂. As the two liquids collide, intense atomization into micronized droplets occurs. Because the solvent(s) must be miscible with the compressed fluid CO₂, subsequent drying of the microdroplets occurs as the solvent(s) and CO₂ mix. Microparticles and nanoparticles are formed after drug precipitation caused by two way mass transfers: extraction of the organic solvent into CO₂ and CO₂ diffusion into the droplets (35). The mass transfer is dependent upon atomization efficiency and the dispersing and mixing phenomena between the solution droplet and the compressed fluid CO₂. Thus, to minimize particle agglomeration frequently observed and to reduce or eliminate drying times, high mass-transfer rates are desired (5).

High mass transfer rates have been successfully achieved in SEDS process, which uses a coaxial nozzle design with a mixing chamber (36). The SEDS process, which is equivalent to the PCA or SAS process, was developed and patented by Hanna and York and owned by University of Bradford. The use of a coaxial nozzle (28, 36) provides a means whereby the drug in the organic solvent solution interacts and mixes with the compressed fluid CO₂ antisolvent in the mixing chamber of the nozzle prior to dispersion, and flows into a particle-formation vessel via a restricted orifice. Such nozzles achieve solution breakup through the impaction of the solution by a relatively higher velocity fluid. The high velocity fluid creates high frictional surface forces causing solution disintegration into droplets. A wide range of materials have been prepared as microparticles and nanoparticles using the SEDS process (10), such as salmeterol xinafoate. By controlling and changing the working conditions of the particle formation process, salmeterol xinafoate products composed of particles with different particle sizes and particle shapes were produced. The mean particle size of salmeterol xinafoate particles ranged from 1 µm to 20 µm (36). It was reported that salmeterol xinafoate particles obtained at 50°C had a blade-like shape with reduced elongation compared with the acicular, needle shaped particles produced at 60°C (36). By processing in different regions of the supercritical phase with the same organic solvent, two salmeterol xinafoate polymorphs have been prepared by the SEDS process. Samples of the two polymorphs exhibited

physicochemical stability and no measurable interconversion after five years. A new polymorph of fluticasone propionate has been produced by the same process, which also enabled control over the particle size and shape of formed particles. The new polymorph of fluticasone propionate exhibited improved drug delivery characteristics in a metered dose inhaler (MDI) formulation compared with conventionally crystallized and micronized drug (37).

Subramaniam *et al* developed a PCA process which involved deliberate generation of high energy sonic waves and substantially independent of any impaction and frictional forces typical of nozzles used in PCA process. The process was patented (38) by the University of Kansas. The sonic waves are generated in the energizing gas stream or in the dispersion itself. The ultrasonic nozzle-based process is capable of producing discrete nanoparticles in a narrow size range. As discussed above, a key step in the formation of nanoparticles is to enhance the mass transfer rate between the droplets and the antisolvent before the droplets coalesce to form bigger droplets (13, 38). With the ultrasonic nozzle, the sound waves assist the antisolvent's break up into small droplets increasing the interfacial area. The turbulence created by the focused sound waves enhances the mass transfer rate between the droplets and the CO₂ (13, 38). A significant decrease in the average particle size is achieved with the use of the ultrasonic nozzle-based SAS process. It is reported that the particles were discrete, nearly spherical, and appeared to be narrowly distributed around 500 nm. In contrast,

the 1 μm wide and nearly 1 mm long fibers were produced by using the conventional SAS process with the 100 μm capillary nozzle (38). Sound waves, rather than inertial or frictional forces, are exploited for droplet formation. Hence, the diameter of the line into which the spray solution is introduced can be larger than that of either the capillary or micro-orifice nozzles. This larger diameter allows for higher solution throughput and reduces the probability of nozzle plugging, dramatically increasing manufacturing efficiency.

1.3.2 Rapid Expansion from Liquefied-Gas Solution and Homogenization (RELGS/RELGS-H/RESAS)

Johnston *et al* developed a process based on supercritical fluid, rapid expansion from supercritical to aqueous solution (RESAS) which produced stable nanosuspensions of poorly water soluble drugs (39-41). The principle of this process is to induce rapid nucleation of the supercritical fluid dissolved drugs in the presence of surface modifying agents resulting in particle formation with a desirable size distribution in a very short time (6). Phospholipids or other suitable surfactants are integrated into the process as a solution or dispersion in the supercritical fluid (6, 42). The surface modifying agents serve to stabilize the newly formed small particles and suppress any tendency of particle agglomeration or particle growth while they are being formed (42). While very rapid precipitation is a characteristic of precipitation of solutes from supercritical fluids, the rapid intimate contact with the surface modifier is achieved by having the

surface modifiers dissolved in the supercritical fluid containing the dissolved drug (42). A rapid intimate contact between the surface modifier and the newly formed particle substantially inhibits the crystal growth of the newly formed particle.

The RESAS process successfully incorporates aqueous stabilizing solutions into the RESS process to trap 500 nm particles of poorly water soluble drugs, such as cyclosporine (40). In RESAS, the surfactant diffuses rapidly to the particle surface to impede particle agglomeration and growth. Young *et al* reported that cyclosporine nanoparticles were formed by spraying a solution (in CO₂) into a Tween-80 solution to prevent nanoparticle flocculation and agglomeration. Cyclosporine particles by RESAS had a size of 500-700 nm and could be stabilized for drug concentrations as high as 6.20 and 37.5 mg/mL in 1.0% and 5.0% (w/w) Tween-80 solutions, respectively (40). At a drug/surfactant ratio above 0.6-0.7 the surfactant can no longer stabilize the particles, resulting in broader size distributions (40). RESAS produced smaller particle sizes of organic and inorganic water-insoluble drug than in the case of RESS into air (40).

Pace *et al* combined the RESAS process and high pressure homogenization process to provide a stable and high payload drug delivery system (6, 42). The microparticle or nanoparticle suspensions were formed by rapid expansion of a liquefied-gas dissolved drug substance and surface modifiers into an aqueous medium to form a aqueous suspension and then homogenizing the suspension with a high pressure homogenizer (Rapid Expansion of Liquefied-Gas

Solution and Homogenization, RELGS-H) (6, 42). This process is patented (42) and Baxter Healthcare Corporation includes this process in the Nanoedge™ technology.

In this process (6), a poorly water soluble drug and a surface modifier are first dissolved in a liquefied compressed gas solvent. A range of compressed gases in the supercritical or sub-critical fluid include but are not limited to gaseous oxides such as carbon dioxide and nitrous oxide; alkanes; alkenes alcohols and isopropanol; ketones; ethers; esters; halogenated compounds *et al* (42). The compressed fluid solution prepared in step one is then expanded into an aqueous solution containing a second surface modifier and water-soluble agents thereby producing a suspension (42). The last step is to homogenize the suspension into nanosuspensions (42). The process produces surface modified particles in a range of 5 to 100 nm in size. The particle size of nanosuspensions is controlled by parameters of the rapid expansion and homogenization process. For example, a solution containing fenofibrate (2 g), Lipoid E-80 (0.2 g), Tween-80 (0.2 g) in the liquefied carbon dioxide pressurized to 3000 PSI was expanded through a 50 mm orifice plate into water held at atmospheric pressure and room temperature (22°C) (42). A fine suspension of fenofibrate was obtained with a mean particle size of about 200 nm.

The process provides drug payloads up to 200 mg/mL. The ratio between the drug and lipid was reported as high as 5:1 (w/w) in the formulation of poorly

water soluble drug, itraconazole (6, 42). As it is well known, there are very serious destabilizing problems of nanosuspensions like aggregation and sedimentation because of physical force. This process circumvents these problems. It was reported that formulations of propofol showed stability at storage temperature between 2-8°C and 40°C over a period of more than one year.

The success of the above supercritical fluid techniques depends heavily, however, on the efficiency of atomization of the solution into the supercritical fluid. The formation of a particle containing a poorly water soluble drug and a water soluble excipient is limited by the lack of solvent systems that will dissolve both hydrophilic and hydrophobic substances. The low solubility of most poorly water soluble drugs and surfactants in supercritical CO₂, results in low production rates of powders. The typical approaches to enhance drug loading in CO₂ are to increase the process temperature, but the elevated temperature may accelerate degradation drugs. Another limitation is the high pressure required for these processes. Such operating pressures are not commonly used in pharmaceutical industry.

1.4 CRYOGENIC SPRAY PROCESS

The cryogenic spray process is an attractive alternative to form microparticles and nanoparticles of poorly water soluble drugs. Spray-freezing into vapor processes have been developed. Both halocarbon refrigerants and liquid nitrogen have been used as cryogenic media in conventional spray-freezing

into vapor processes (43-54). In these procedures, the feed solution is atomized through a nozzle positioned at a distance above the boiling refrigerant. The droplets gradually solidify while passing through the cold halocarbon vapor, and freeze completely as contact is made with the boiling refrigerant liquid. Gombotz *et al* and Gusman and Johnson have developed spray-freezing into nitrogen vapor processes for the purpose of using an inert cryogen to capture frozen drug particles following atomization (55-58). Because atomization occurs in the nitrogen vapor above the liquid gas, the solution droplets gradually agglomerate and solidify while passing through the vapor phase then settle onto the surface of the cryogenic liquid (5). As a result of droplet agglomeration, broad particle size distributions and non-micronized dry powders may result. Recently, a new cryogenic spray process, spray freezing into liquid (SFL) process was developed to overcome such problems and this process has been patented by The University of Texas at Austin in 2001 (59) and commercialized by The Dow Chemical Company.

Spray Freezing into Liquid (SFL) Process

SFL is a cryogenic atomization process in which either an aqueous, organic or aqueous-organic co-solvent solution, aqueous-organic emulsion, or suspension containing an drug and pharmaceutical excipient(s) is atomized directly into a compressed liquid, such as compressed fluid CO₂, helium, propane, ethane, or the cryogenic liquids including nitrogen, argon, or hydrofluoroethers

(59). The SFL process utilizes the atomization of a feed solution drugs and/or excipient(s) directly into a cryogenic liquid to produce frozen nanostructured particles. The frozen particles are then lyophilized to obtain dry, free flowing micronized powders. The advantages of the SFL process result from intense atomization and rapid freezing rates. Because liquid-liquid impingement occurs between the pressurized feed solution exiting the nozzle and cryogenic liquid, a high degree of atomization is achieved by spraying directly into the cryogenic liquid as opposed to spraying into the vapor phase above the cryogenic liquid. Ultra-rapid freezing rates are achieved because of the low temperature of liquid nitrogen and the formation of high-surface area droplets. The ultra-rapid freezing rates prevent the phase separation of solutes within the feed solution and induce formation of amorphous structures. The high degree of atomization and ultra-rapid freezing rate led to amorphous nanostructured particles with high surface areas, enhanced wetting and significantly enhanced dissolution rates (5, 35, 60-66).

Hu *et al* reported that the SFL process may use organic solvents such as acetonitrile, as the solution source solvent in addition to solution containing water or water/organic co-solvent (61). The SFL powders from organic system and aqueous-organic co-solvent system exhibited similar and significantly enhanced dissolution rates compared to the micronized bulk drugs. The SFL acetonitrile system offers several advantages including increasing the drug loading in the SFL

feed solution and decreasing the drying time for lyophilization following the SFL process. All of these attributes have a positive effect on the scale-up of the SFL process in the product manufacture.

Recent study also demonstrated that rapidly dissolving SFL micronized powders with high potency (up to 91%) have been produced by SFL with an organic solvent mixture (62). The high potency SFL powders contained amorphous nanostructured aggregates with high surface area and excellent wettability. For example, SEM micrographs of the SFL danazol/PVP K-15 with 50% potency revealed small porous aggregates with an average geometric diameter of about 1.5 μm . Higher magnification showed that the aggregate was also composed of many smooth primary nanoparticles with a geometric diameter of about 100 nm. In only 2 minutes, 99% of the danazol dissolved from the SFL danazol/PVP K-15 powders. The composition of surfactant in the product could be controlled simply by the feed composition, as all of the surfactant precipitated with the drug. In SFL, solvent mixtures may be formulated to achieve high solubility of drug in order to produce high potency powders. Rogers *et. al* reported that highly concentrated emulsions could also be processed by SFL (67). The large quantities of poorly water soluble drugs and excipients could be processed with lower quantities of solvents when emulsion formulations were SFL processed. SFL micronized powders from emulsions wetted and dissolved as fast as the SFL micronized powders from solutions.

SFL processed frozen powders can be dried by lyophilization or the atmospheric freeze-drying (ATMFD) technique, which uses cryogenic air to fluidize the powder and facilitating mass transfer rates in solvent sublimation (64). ATMFD is a scalable freeze-drying process that does not require the use of vacuum to sublime solvents. Thus, scalability issues due to vacuum limitation in lyophilization are eliminated with ATMFD. Therefore, large micronized SFL powder batches can be produced. The engineered SFL micronized powders can be used for different delivery systems. If the dissolution enhancement of poorly water soluble drug is desired, the poorly water soluble drug could be engineered within an excipient matrix for immediate release. SFL micronized powders can also be used for the respiratory delivery. SFL micronized powders have been compressed into a tablet for oral delivery. SFL process offers a highly effective approach to produce high potency nanoparticles contained in larger structured aggregates with rapid dissolution rates for poorly water soluble drugs. The ability to stabilize nanostructured high surface area drug powders in high glass transition temperature (T_g) formulations and to maintain rapid dissolution rate of SFL micronized powders in the tablet formulation offers great promise for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble drugs.

1.5 SOLVENT EVAPORATION PROCESS-EVAPORATIVE PRECIPITATION INTO AQUEOUS SOLUTION (EPAS) PROCESS

Another new nanoparticle formation process, evaporative precipitation into aqueous solution (EPAS) was developed and patented by The University of Texas at Austin and licensed to The Dow Chemical Company in 2001.

EPAS process utilizes rapid phase separation to nucleate and grow nanoparticles and microparticles of poorly water soluble drugs. Poorly water soluble drug is first dissolved in a low boiling liquid organic solvent. This solution is pumped through a tube where it is heated under pressure to a temperature above the solvent's normal boiling point and then sprayed through a fine atomizing nozzle into a heated aqueous solution. A stabilizing surfactant is added to the organic solution, the aqueous solution, or both to optimize the particle formation and stabilization. The nozzle is immersed into the aqueous solution to ensure that the nucleating drug particles contact the hydrophilic stabilizing surfactant rapidly, inhibiting crystallization and growth of the drug particles. The stable aqueous drug suspension is dried by a variety of techniques including ultra rapid freezing in conjunction with lyophilization, or spray drying. The stabilization of the drug particles with water soluble stabilizers in the aqueous suspensions facilitates dissolution rates of the final powder after drying. EPAS process offers several advantages versus spray drying process. The rapid evaporation of the heated organic solution in EPAS results in fast nucleation

leading to amorphous nanoparticle suspensions. A variety of hydrophilic stabilizers were found to diffuse to the surface of the growing particles rapidly enough to prevent growth of the nanoparticles. The Johnston's lab has produced the nanoparticle suspensions of cyclosporine A (68) with a particle size ranging from 130 nm to 460 nm using the EPAS process.

The EPAS process produces surfactant stabilized aqueous suspensions that are dried to form micronized powder with rapid dissolution rates. Sarkari *et al* reported that the dissolution rates of PVP K15 stabilized powders produced by EPAS are much faster than those produced earlier by solvent evaporation without an aqueous phase (68, 69). In EPAS, the surfactant migrates to the drug-water interface during particle formation, and the hydrophilic segment is oriented outwards towards the aqueous continuous phase. In spray drying process, which does not use water, this driving force for forming a hydrophilic coating on the particle is not present. In EPAS, the rapid nucleation of the drug followed by adsorption of surfactant at the drug-aqueous solution interface leads to colloidal suspensions of micron-sized drug particles coated by a hydrophilic stabilizer. The stabilizer inhibits crystallization of the growing particles (68). After drying to form a powder, a hydrophilic surface is available on the drug particle surface to facilitate rapid dissolution rates. These conditions could be achieved with a variety of ionic and nonionic low molar mass and polymeric stabilizers present originally in the organic phase, aqueous phase or both. Hydrogen bonding and/or

hydrophobic interactions between poorly water soluble drugs and stabilizers provide intimate contact and much higher dissolution rates than obtained from a simple physical mixture of the two.

Chen *et al* reported that high dissolution rates were obtained for danazol produced by EPAS with PVP 40T, PVP K-15 or PVP 40T together with SLS as stabilizers (70). The high dissolution rates, about 90% in two minutes with a drug/surfactant ratio of 9:1, are remarkable given the high potencies and very low amount of excipients. Since only about 5% of the surfactant required for forming suspensions is adsorbed onto the particles, the rest could be removed after centrifugation. After removal of the free surfactant and drying, the particles were re-dispersed in pure water without any growth in particle size or loss in surface area. The dissolution rates were high only for surfactants with an adsorption of 10 % (w/w) or more during the EPAS spray (70). The high surfactant adsorption levels were required to prevent particle agglomeration and growth. These results indicate that the small particle size and large surface area of the surfactant-coated particles, with reduction in crystallinity less than 20%, was enough to produce high dissolution rates. The EPAS suspensions may be used in parenteral formulations to enhance bioavailability or may be dried to produce oral dosage forms. The ability to engineer stable particles with high potencies and high dissolution rates with EPAS presents new opportunities in the development of commercial formulations for poorly water soluble drugs.

In summary, recent advances in commercially or potentially commercially available nanoparticle engineering processes have been discussed. These nanoparticle engineering processes including wet milling, high pressure homogenization, PCA/ SEDS/SAS, RESAS/ RELGS/RELGS-H, SFL, and EPAS techniques have successfully incorporated poorly water soluble drugs alone, or with excipients into the microparticles or nanoparticles with significantly improved *in vitro* dissolution rate and *in vivo* bioavailability. As the percentage of poorly water soluble experimental compounds is increasing, nanoparticle engineering processes for enhancement of dissolution rates of poorly water soluble drugs offer great promise for pharmaceutical development and manufacturing to bring these experimental compounds into the market.

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1.7 RESEARCH OBJECTIVES AND DISSERTATION OUTLINE

The spray freezing into liquid (SFL) particle engineering technology was developed to micronize powders for the purpose of enhancing the wetting and dissolution properties of poorly water soluble APIs. The amorphous structure, high surface area and enhanced wettability of the SFL nanostructured particles were the predominant characteristics of the poorly water soluble APIs that enhanced dissolution. SFL technology is a cryogenic particle engineering process that utilizes the atomization of a feed solution containing APIs and/or excipient(s) directly into a cryogenic liquid to produce frozen nanostructured particles. The frozen particles are then lyophilized to obtain dry, free flowing micronized powders. Advantages of the SFL process result from intense atomization in conjunction with rapid freezing rates. Because liquid-liquid impingement occurs between the pressurized feed solution exiting the nozzle and the cryogenic liquid such as liquid nitrogen, the highest degree of atomization is achieved by spraying directly into the cryogenic liquid as opposed to spraying into the vapor phase above the cryogenic liquid. Ultra-rapid freezing rates are achieved because of the low intrinsic temperature of liquid nitrogen and the formation of high-surface area droplets. The ultra-rapid freezing rates prevent the phase separation of solutes within the feed solution and induce formation of amorphous structures. The intense atomization in conjunction with rapid freezing rates have led to

nanostructured aggregates composed of amorphous API nanoparticles with high surface areas and enhanced wettability.

The following studies were designed to further develop and optimize SFL process in order to achieve broad application in drug delivery system.

In the study of Chapter 2, SFL process was demonstrated to produce free flowing powder of nanostructured particles containing poorly water soluble APIs, danazol or carbamazepine. The objective of this study was to develop and demonstrate the use of the novel SFL particle engineering technology to enhance the dissolution rate of two poorly water soluble APIs, danazol and carbamazepine, and to determine the physicochemical stability of SFL powders.

In the study of Chapter 3, the SFL acetonitrile system was demonstrated to produce nanostructured particles, which have similar characteristics to the particles prepared by the SFL THF/water co-solvent system. The objective of this study was to enhance SFL technology for preparation of nanoparticles of CBZ, using organic solvents like acetonitrile as the solution source for the SFL process. Using acetonitrile as a solution source could increase CBZ loading in the feed solution and reduce the drying time in the SFL process. In addition, the influence of the solution type (organic vs aqueous/organic) on physicochemical properties of SFL micronized powders was determined in this study.

The study in Chapter 4 demonstrated that rapid dissolving SFL danazol/PVP K-15 powders with high potency (up to 91%) have been produced

by SFL with an organic solvent mixture. The objective of this study was to extend the SFL process to produce rapid dissolving high potency powders with high surface areas and dissolution rates. The potencies ranged from 50% to 90% in contrast with typical values of only 33% in a previous study (13, 17). In order to achieve these high potencies, high concentrations of APIs were dissolved in pure or mixed organic solvents to prepare the feed solutions.

The study in Chapter 5 was to stabilize the amorphous solid to prevent crystallization during storage. The first objective of this study was to investigate the influence of excipient type on the particle size, surface area, glass transition temperature (T_g), and dissolution rate of amorphous danazol powders produced by the SFL process. All of the excipients led to powders with high danazol dissolution rates. Therefore, we chose to focus additional experiments on the excipient that led to the highest T_g , PVP K-15. The effect of drug potency (or drug/polymer ratio) and moisture on T_g was determined. The stability of danazol/PVP K-15 powders was studied after six months to examine whether any crystallization occurred and if the dissolution rate changed.

The study in Chapter 6 demonstrated that a rapid release tablet formulation containing SFL micronized powder was successfully developed. The objective of this study was to incorporate SFL micronized powder into tablet formulations by direct compression and achieve rapid release and acceptable mechanical properties. The hypothesis of this study was that the high dissolution

rates of SFL micronized powder may be maintained after the blending and compression processes by optimizing the SFL powder composition and tablet excipient composition.

In Chapter 7, the overall conclusions and significant observations are presented.

CHAPTER 2: Improvement of Dissolution Rates of Poorly Water Soluble APIs Using the Novel Spray Freezing into Liquid Technology

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The objective of this study was to develop and demonstrate a novel particle engineering technology, spray freezing into liquid (SFL) to enhance the dissolution rates of poorly water soluble active pharmaceutical ingredients (APIs).

2.1 INTRODUCTION

Many potentially bioactive molecules have been rejected during the early stages of development because they are poorly water soluble and difficult to wet. Active pharmaceutical ingredients (APIs) with poor aqueous solubility often demonstrate low bioavailability when administered orally due to the dissolution rate-limiting absorption in the gastrointestinal (GI) tract. Of particular interest are the poorly water soluble, highly permeable APIs discussed in the Biopharmaceutics Classification System (BCS Class II), (1) such as danazol and

carbamazepine. The irregular and delayed absorption of danazol and carbamazepine has been attributed to slow dissolution rates (2-3). The physicochemical properties of the APIs play a significant role in controlling their dissolution from a dosage form. According to the Noyes-Whitney equation, the aqueous solubility of an API is a factor determining dissolution rate. Other factors that influence the dissolution rate include particle size distribution, degree of crystallinity, as well as the presence of surfactants and other additives. Other physical properties such as wettability, density and viscosity contribute to particle flocculation, flotation and agglomeration, which further influence dissolution rates.

Increasing the dissolution rate of poorly water soluble APIs is a significant challenge to pharmaceutical scientists. Technologies that have been commonly used to improve the dissolution rate of poorly water soluble APIs include mechanical milling, spray drying, precipitation and freeze-drying. Spray drying is a widely used technology (4). However, because the spray drying process uses the elevated temperatures, it is not always appropriate for use with thermolabile compounds. The technology of precipitation with a compressed fluid antisolvent (PCA) has been used to produce particles containing poorly water-soluble API and water-soluble excipients (5). This process can be limited by the lack of solvent systems that dissolve both hydrophilic and hydrophobic substances simultaneously. Lyophilization or freeze-drying (6) is a promising technique for

producing pharmaceutical powders. However, the freezing rate in some cases is too slow so that the solvent crystallizes as it is frozen.

Spray-Freezing into Liquid (SFL) is a novel cryogenic atomization technology in which either an aqueous or an aqueous-organic cosolvent solution containing an API and pharmaceutical excipient(s) is atomized directly into a compressed liquid, such as compressed fluid CO₂, helium, propane, ethane, or the cryogenic liquids nitrogen, argon, or hydrofluoroethers.

SFL is a technology that has been created by the adaptation of several atomization processes. SFL is derived directly from the precipitation with compressed antisolvent (PCA) technology, which utilizes liquid-liquid impingement between an organic or organic/aqueous feed solution through a nozzle that is submerged into compressed CO₂ fluid. As the two liquids collide, intense atomization into micronized droplets occurs. Because the solvent(s) must be miscible with the compressed fluid CO₂, subsequent drying of the microdroplets occurs as the solvent(s) and CO₂ mix. Typically, elevated temperatures and pressures are required to promote miscibility between feed solvents and compressed fluid CO₂, and dry product recovery is low due to adherence of the powder to the surfaces of the pressurized PCA vessel and high-pressure tubing (5). With the novel SFL technology, the solvents are not required to be miscible with the cryogenic liquid; so, in contrast to PCA, this is not a limiting factor with SFL. Liquid nitrogen is commonly used in the SFL process

instead of CO₂ because of the ultra-rapid freezing rates obtained using a cryogen with an intrinsic temperature at −196°C. The liquid nitrogen is held static in an insulated retaining vessel at atmospheric conditions, and the feed solution is atomized directly into the cryogen. The suspended frozen powder can then be separated from the cryogenic continuous phase by sieve filtration or other means.

In addition to PCA, there are spray-freezing into vapor processes that have influenced the evolution of the novel SFL technology. Both halocarbon refrigerants and liquid nitrogen have been used as cryogenic media in conventional spray-freezing into vapor processes (7-11). Spray-freezing into halocarbon vapor has been performed by Briggs and Maxwell and Adams *et al.* (7- 8). In these procedures, the feed solution is atomized through a nozzle positioned at a distance above the boiling refrigerant. The droplets gradually solidify while passing through the halocarbon vapor, and freeze completely as contact is made with the boiling refrigerant liquid. Particles formed using these spray-freezing into halocarbon vapor techniques are reported to range in diameter from 0.84 mm to 1.68 mm (7).

Significant problems arise when using halocarbon refrigerants as cryogenic media. Chlorofluorocarbons (CFCs) are deleterious to the ozone layer, and the replacement hydrofluoroalkane (HFA) refrigerants are expensive (12). In addition, both CFCs and HFAs are suitable solvents for lipophilic compounds, so API extraction into the halocarbon cryogen can result in low product yield (13).

Gombotz *et al.* and Gusman and Johnson have developed spray-freezing into nitrogen vapor technologies for the purpose of using an inert cryogen to capture frozen API particles following atomization (14-15). Because atomization occurs into the nitrogen vapor above the liquid gas, the solution droplets gradually agglomerate and solidify while passing through the vapor phase then settle onto the surface of the cryogenic liquid. Because droplet agglomeration is allowed to occur while passing through the nitrogen vapor, broad particle size distributions and non-micronized dry powders may result.

The novel SFL technology utilizes spraying directly into the cryogenic liquid phase to atomize the feed solution as it exits the nozzle. As with PCA, the liquid-liquid impingement that occurs as the feed solution impacts the cryogenic media results in intense atomization into fine microdroplets that freeze instantaneously. In contrast to PCA, however, mixing between solvent(s) and cryogen is not a limiting factor with SFL. A schematic representation of the SFL apparatus is shown in Figure 2.1. 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 then lyophilized to produce the dry SFL micronized powder.

The SFL process is superior to the competing technologies mentioned above for a number of reasons. The highest degree of atomization is achieved by spraying directly into the cryogenic liquid because of the liquid-liquid impingement that occurs between the pressurized feed solution exiting the nozzle and the static liquid nitrogen. Because of the formation of high-surface area droplets and the low intrinsic temperature of liquid nitrogen, ultra-rapid freezing rates are achieved. As a result of the ultra-rapid freezing rates, 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 microparticle. 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. The API is molecularly dispersed within a homogeneous excipient matrix that composes the porous microparticle.

Aqueous media should immediately wet and dissolve the SFL microparticles due to the high surface area of the porous microparticles. The dissolution media will fill the pore channels and dissolve the hydrophilic excipients, which are utilized to enhance the aqueous dissolution of a lipophilic API.

The objective of this study was to develop and demonstrate the use of the novel SFL particle engineering technology to enhance the dissolution rate of two poorly water soluble APIs, danazol and carbamazepine, and to determine the physicochemical stability of SFL powders.

2.2 MATERIALS AND METHODS

2.2.1 Chemicals

Danazol USP and carbamazepine USP were obtained as micronized powders. A polyoxyethylene-polyoxypropylene copolymer (pluronic F127; poloxamer 407), polyvinylpyrrolidone (PVP) K-15, sodium lauryl sulfate(SLS) NF, sodium taurocholate, lecithin NF, potassium chloride NF, and sodium hydroxide NF were purchased from Spectrum Quality Products Inc. (Gardena, CA). Tetrahydrofuran (THF), methanol, acetonitrile and acetic acid were obtained from EM Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

2.2.2 SFL Processing

SFL feed solutions were prepared according to the following procedure. Danazol or carbamazepine was dissolved in THF. Poloxamer 407, PVP K-15, and/or SLS were dissolved in water. The aqueous and organic solutions were

then mixed to obtain danazol/excipient blend and carbamazepine/excipient blend SFL feed solutions.

A schematic diagram of the SFL apparatus is shown in Fig. 2.1. The SFL feed solution was placed into the solution cell (A). A constant pressure of 4000 PSI from the ISCO syringe pump (B) (Model 100 DX, ISCO Inc., Lincoln, NE) provided a flow rate of 11 mL/min for the SFL feed solution. The solution cell was connected to an insulated nozzle (C), which was positioned to atomize the SFL feed solution beneath the surface of the cryogenic liquid (D). In these experiments, liquid N₂ was used as cryogenic liquid. The atomizing nozzle used was composed of polyetheretherketone (PEEK) tubing with an inner diameter of 63.5 µm. The SFL feed solutions were then sprayed through the nozzle and atomized directly into the liquid N₂ phase. Frozen particles formed instantaneously. The frozen particles were collected and lyophilized in a Labconco freeze dryer (Freeze dryer 5, Labconco Corporation, Kansas City, MO) for 48 hours.

To investigate the influence of the ultra rapid freezing rate in conjunction with atomization in the SFL technology, a lyophilized mixture formed directly by conventional lyophilization process was used as a control (LM control). The lyophilized mixture is made of a mixture of API and excipients frozen at -78°C and lyophilized for 48 hours. The compositions of the lyophilized mixtures were identical to the SFL powders. The influence of the excipients on the

physicochemical properties of the SFL powders was further studied by comparison with a physical mixture of API and excipients (PM control). The physical mixtures were of the same composition as the SFL API powders. The micronized APIs were also used as a control. The SFL powders and control samples were stored in glass vials over desiccant in a vacuum desiccator at room temperature before the characterization measurement.

2.2.3 Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using the $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powders were placed in a glass sample holder. Samples were scanned from 5° to 45° (2 θ) at a rate 0.05°/sec. For comparative purposes, the three highest values for relative line intensity and their corresponding line position 2 θ were compared for the API samples (16).

2.2.4 Scanning Electron Microscopy (SEM)

A HITACHI S-4500 field emission scanning electron microscope (Hitachi Instruments Inc., Irvine, CA) was used to examine the surface morphology of each sample powder. The sample was fixed to a SEM stage with double-sided adhesive tape and gold sputter coated.

2.2.5 Particle Size Distribution

The particle size distribution of the sample powders was determined using the method based on a time-of-flight measurement (Aero-Disperser[®], Amherst Process Instrument Inc., Amherst, MA). The mean particle diameter and span index were determined for the SFL powers and controls.

2.2.6 Surface Area Measurement

A Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL) was used to determine N₂ sorption at 77.40 deg K. The surface area per unit powder mass was calculated from the fit of adsorption data to the Brunauer, Emmett, and Teller (BET) equation (17).

2.2.7 Contact Angle Measurement

Compacts of sample powders were prepared at a 500 kg compression force using a Carver Laboratory Press (Model M, ISI Inc., Round Rock, TX) with flat-faced 6mm diameter punches. A droplet of purified water or Fed state simulated intestinal fluid Fed state simulated intestinal fluid fed state simulated intestinal fluid (FeSSIF) media (3 μ l) was placed onto the surface of compact and observed using a low power microscope. The contact angle was determined by measuring the tangent to the curve of the droplet on the surface of the compact using a Goniometer (Model No.100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ).

2.2.8 Dissolution Studies

The amount of API dissolved, as a function of time, was determined using USP Apparatus 2 method (Vankel 7000, Vankel Technology Group, Cary, NC). All dissolution studies were conducted at sink conditions. The FeSSIF media was prepared according to Dressman *et al.* (18). SFL danazol/poloxamer 407 powders or control samples containing approximately 5mg danazol were added to 900 mL of FeSSIF media (37°C). The paddle speed was 50 rpm. 5 mL aliquot was taken at each time point and filtered through a 0.45µm filter then diluted with acetonitrile, filtered through a 0.45µm filter again, and analyzed by HPLC (19-20). Purified water (900mL) was used for the carbamazepine as the dissolution media and the same operating parameters discussed for danazol. Dissolution profile for SFL carbamazepine/excipients powders were studied as well as their controls.

2.2.9 Stability study

Stability studies were conducted at 25°C/60% RH. Sample powders were stored in the capped glass vials (20 mL) and characterized as a function of exposed time.

2.2.10 Statistical analysis

The data were compared using a student's t-test of the two samples assuming equal variances to evaluate the differences. The significance level ($\alpha = 0.05$) was based on the 95% probability value ($p < 0.05$).

2.3 RESULTS AND DISCUSSION

2.3.1 Crystallinity

The crystallinity greatly impacts the solubility and dissolution rate of poorly water soluble APIs (21). The chemical potential of a metastable high-energy amorphous state can be markedly larger than that of an equilibrium crystal. This higher chemical potential can lead to a substantially greater local solubility of the API near the interface and thus a faster mass transfer rate into the dissolution media. Therefore, the crystallinity of an API can be reduced in order to enhance the dissolution rate. Powder XRD patterns of SFL powders and control samples are presented in Figures 2.2 and 2.3. Micronized danazol had a similar X-ray diffraction pattern to that reported by Liversidge and Cundy (22). The high peak intensities of danazol indicated a high degree of crystallinity. The physical mixture and lyophilized mixture showed the characteristic diffraction peaks (16) of danazol at 15.8, 17.2 and 19.0 (20). The X-ray pattern of the SFL danazol/poloxamer 407 powder exhibited a significant reduction in peak intensity for danazol. The diffraction peaks of danazol were not evident whereas those of

poloxamer 407 at 19.3 and 23.4 (2θ) were present. This indicated that danazol contained in SFL danazol/poloxamer 407 powder was in an amorphous state. Similarly, lack of crystallinity was also found for the SFL carbamazepine/SLS powder (Fig. 2.3). The absence of characteristic peaks of carbamazepine (23) at 15.3, 25.0 and 27.6 (2θ) indicated an amorphous state. The controls (either physical mixture or lyophilized mixture) exhibited diffraction peaks of carbamazepine suggesting that it was not entirely amorphous. For both danazol and carbamazepine, the powders produced by SFL technique exhibited very little crystallinity, in contrast with the significant crystallinity for powders made by the conventional lyophilization process. Apparently, the freezing rates in the SFL technique were fast enough to trap the API in an amorphous state without allowing time for crystallization. Ultra-rapid freezing resulted from the high surface area of the atomized droplets and the rapid heat transfer from the droplets to the liquid nitrogen.

2.3.2 Particle Size Distribution and Morphology

The particle size of an API is another important parameter controlling the dissolution rate. Decreasing the particle size increases the surface area, which enhances the dissolution rate (24). The particle size distribution for the SFL powders and controls (Table 2.1) were determined in an Aero-Disperser[®] based on time-of-flight measurements. The mean particle diameter of the SFL

danazol/poloxamer 407 powder was 7.10 μm . In contrast, the mean particle diameters of the danazol and lyophilized mixture were 18.27 μm and 18.86 μm , respectively. Similar results were found for the SFL carbamazepine/SLS powder. The mean particle diameter of the SFL carbamazepine/SLS powder was 7.11 μm , which was smaller than that of the controls. The span index is used to describe the polydispersity in a given particle size distribution and is defined as $(D_{90}-D_{10})/D_{50}$, where D_{10} , D_{50} , and D_{90} are the respective particle size at 10, 50, 90% cumulative percent undersize (25). The span index of carbamazepine was 2.94 indicating high polydispersity. For the SFL powder the polydispersity was decreased markedly down to 1.31. The conventional lyophilization process reduced the mean particle diameter only slightly compared to the micronized APIs. However, the size reduction was significant after SFL processing. For both danazol and carbamazepine, the SFL technique produced nanostructured particles with narrow particle size distributions. Various factors contributed to the limited particle growth during SFL processing. The atomized droplets were frozen immediately upon contact with the liquid N_2 at a rapid freezing rate. The small droplet size and the rapid freezing rate limited the propensity for particle growth. Also, the surfactant in the unfrozen domains in the sprayed droplets inhibited particle coalescence and crystal growth.

To further investigate the effect of the SFL technique on the morphology of APIs, sample powders were examined by SEM. The SEM micrograph of the

micronized danazol (Fig. 2.4a) illustrates large crystalline particles. The SEM micrograph of the lyophilized mixture particles (Fig.2.4b) indicates a smooth surface when compared to the micronized danazol. In contrast, the SFL danazol/poloxamer 407 particles (Fig.2.4c) had a highly porous morphology. The surface area of the SFL danazol/poloxamer 407 powder was $11.04 \text{ m}^2/\text{g}$, which was a significant increase over the lyophilized mixture ($1.27 \text{ m}^2/\text{g}$). This result confirmed the highly porous structure of SFL powders observed by SEM. Such high surface area indicated that the SFL powder particles were highly porous, similar to the spray-freeze dried protein particles reported by Maa *et al.* (4) and Costantino *et al.* (26). Similar results for SFL carbamazepine/excipients powders were observed in the SEM analysis and surface area measurement. For example, the SEM micrograph of the SFL carbamazepine/SLS demonstrated a fine powder consisting of porous microparticles. Powder made by the conventional lyophilization process was larger and less porous relative to the SFL powder. The surface area of SFL carbamazepine/SLS powder was $12.81 \text{ m}^2/\text{g}$, which was greater than that of the lyophilized mixture ($2.33 \text{ m}^2/\text{g}$).

2.3.3 Wettability

The importance of wettability on dissolution rate, that is the area of contact between a powder and dissolution media, has been well studied (27). To characterize wettability, the contact angle values for the SFL powders and controls are reported in Table 2.2. The limits in the contact angle are 0° for complete

wetting and 180° for no wetting (28). The mean value of contact angle for the SFL danazol/poloxamer 407 powder was only 25° against the FeSSIF media, which was significantly lower than that of the SFL danazol (55°), lyophilized mixture (34°) and physical mixture (58°) ($P < 0.05$). The mean value for SFL carbamazepine/SLS powder was 24° against purified water, which was significantly lower than for the physical mixture (52°) and SFL carbamazepine ($P < 0.05$). The significant reduction of contact angle for the SFL powders compared to the controls indicated the presence of a hydrophilic solid surface. Specifically, the contact angle is described by the equation: $\cos \theta = f_1 \cos \theta_1 + f_2 \cos \theta_2$ (29). For the physical mixture, θ_1 is for the hydrophilic excipient and θ_2 is for the hydrophobic API, and the ratio of f_1 and f_2 is related to the bulk volume fractions of the API and excipient. However, the contact angle of SFL powders was well below that of the physical mixture indicating that the solid surface of SFL powder was enriched by the hydrophilic excipient. This enrichment may be formed during the SFL process. As the API and excipient precipitated from the concentrated unfrozen aqueous phase, the precipitate was surrounded by hydrophilic solvent. The hydrophilic solvent attracts the hydrophilic excipient molecules preferentially to the surface of the particles. The lower contact angle of the SFL powders compared to the lyophilized mixture may be due to the surface roughness. The rougher surface for the SFL powders relative to the lyophilized mixture as depicted in the SEMs and the higher surface areas may be expected to

decrease the contact angle as observed. So, both preferential enrichment of the powder surface with the hydrophilic excipient and the surface roughness lower the contact angle and favor wetting of the SFL micronized powder relative to the controls.

2.3.4 Dissolution

The profiles presented in Figures 2.5 and 2.6 illustrate the dissolution of the SFL powders and controls for danazol and carbamazepine, respectively. The FeSSIF media used for the danazol dissolution studies reportedly simulates *in vivo* gastrointestinal fluid; low levels of surfactants are recommended to be included in the dissolution media to give a better correlation between *in vitro* and *in vivo* data (18). The rate of dissolution of micronized danazol (Fig. 2.5) was slow; only 21% of the danazol dissolved in 60 minutes. The dissolution of the physical mixture was also quite slow, only about 48% in 60 minutes. The conventional lyophilization process increased the dissolution rate considerably, with 80% danazol dissolved in 60 minutes. However, the amount dissolved reached 99% in only 10 minutes for the SFL danazol/poloxamer 407 formulation. Major improvements in the dissolution rates were also found for SFL carbamazepine/excipient powders relative to the controls (Fig.2.6). For example, the dissolved carbamazepine from SFL carbamazepine/SLS powder in 10 minutes was 98%, a profound enhancement when compared to 4% for the micronized carbamazepine. The difference in the dissolution rate of SFL carbamazepine

powders prepared by various excipients was negligible in 60 minutes and only slightly different in 20 minutes. Each of the excipients was successful in producing extremely rapid dissolution.

The increased dissolution rate of the SFL powders was mainly attributed to the amorphous nature of the API, the reduction in particle size and consequent enhanced surface area. The rapid freezing rate resulting from the intense atomization led to porous nanostructured particles with high amorphous API fraction and large surface area. This morphology is quite different than the low porosity, low surface area semi-crystalline cakes produced by the conventional lyophilization process. At the same time, the enhanced wettability or better contact between the surface of the solid and the liquid increased the dissolution of the SFL powder. Also, the porous channels of the API/excipient matrix created in the SFL process allowed the dissolution media to easily penetrate into the particles and facilitate dissolution.

2.3.5 Stability

A stability study was conducted for the SFL danazol/poloxamer 407 at 25°C/60%RH for two months to examine any changes in crystallinity or other properties of the SFL powders. The X-ray diffraction pattern (Fig.2.2) of SFL danazol/poloxamer 407 powder exhibited no change in peak intensity of danazol, but a slightly increased peak intensity of poloxamer 407. Despite the high humidity, the danazol crystallinity was unchanged over two months. The

dissolution results (Fig.2.5) demonstrated that there was only a 5% difference in the dissolution profiles between the initial and two-month samples, which was calculated by the similarity factor f_2 for the dissolution profile comparison using the method reported by Shah (30). The slightly decreased initial release of danazol may be due to the change in crystallinity of poloxamer 407.

2.4 CONCLUSIONS

The novel SFL technology was demonstrated to produce free flowing powder of nanostructured particles containing danazol or carbamazepine. The SFL powders exhibited significantly enhanced dissolution rates compared to the powders formed by the conventional lyophilization process. In addition, an amorphous structure, high surface area and increased wettability of the flowable SFL powders are predominant characteristics, so the novel SFL technology is an effective particle engineering process for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs for oral delivery systems.

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CHAPTER 3: Spray Freezing into Liquid (SFL) Particle Engineering Technology to Enhance Dissolution of Poorly Water Soluble Drugs: Organic Solvent vs Aqueous-organic Co-solvent Systems

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A spray freezing into liquid (SFL) particle engineering technology has been developed to produce micronized powders to enhance the dissolution of poorly water soluble active pharmaceutical ingredients (APIs). Previously, a THF/water co-solvent was used as the solution source in the SFL process. In the present study, an organic system was developed to further enhance the properties of particles produced by SFL. The influence of solution type (e.g. organic vs organic/water) on the physicochemical properties of SFL powders was investigated and compared.

3.1. INTRODUCTION

It is estimated that about 40% of compounds being developed by the pharmaceutical industry are poorly water soluble (1). Although several routes of administration can be considered for these compounds, the most popular remains

the oral route. A limiting factor to the *in vivo* performance of active pharmaceutical ingredients (APIs) with poor aqueous solubility after oral administration is their inadequate dissolution rates (2). Carbamazepine (CBZ) is classified by the Biopharmaceutics Classification System (BCS) as a class II API (3, 4). CBZ has a very low aqueous solubility but high permeability. The irregular and delayed absorption of CBZ has been attributed to its slow dissolution rate (5, 6). Since dissolution of poorly water soluble APIs like CBZ is the rate-limiting step to absorption, it is important to improve their dissolution rate and thus enhance absorption and bioavailability.

The spray freezing into liquid (SFL) particle engineering technology was developed to micronize powders for the purpose of enhancing the wetting and dissolution properties of poorly water soluble APIs (7-11). The amorphous structure, high surface area and enhanced wettability of the SFL nanostructured particles were the predominant characteristics of the poorly water soluble APIs that enhanced dissolution. SFL technology is a cryogenic particle engineering process that utilizes the atomization of a feed solution containing APIs and/or excipient(s) directly into a cryogenic liquid to produce frozen nanostructured particles. The frozen particles are then lyophilized to obtain dry, free flowing micronized powders. Advantages of the SFL process result from intense atomization in conjunction with rapid freezing rates. Because liquid-liquid impingement occurs between the pressurized feed solution exiting the nozzle and

the cryogenic liquid such as liquid nitrogen, the highest degree of atomization is achieved by spraying directly into the cryogenic liquid as opposed to spraying into the vapor phase above the cryogenic liquid. Ultra-rapid freezing rates are achieved because of the low intrinsic temperature of liquid nitrogen and the formation of high-surface area droplets. The ultra-rapid freezing rates prevent the phase separation of solutes within the feed solution and induce formation of amorphous structures (8, 10). The high degree of atomization and ultra-rapid freezing rate achieved with the SFL technology have led to micronized amorphous particles with high surface areas.

The first step in the SFL process is to dissolve hydrophobic API and/or hydrophilic excipients in a feed solution. Prior to this study, a tetrahydrofuran (THF)/water co-solvent was used to form the SFL feed solution (8, 10). Some poorly water soluble APIs, such as CBZ, have relatively low solubility in the THF/water co-solvent resulting in low solution loading. However, the use of organic solvents, such as acetonitrile, ethanol, acetone, methanol, methylene chloride, tert-butyl alcohol can greatly increase the solubility of hydrophobic APIs like CBZ resulting in enhanced CBZ loading in the SFL feed solution. In addition, organic solvents with higher vapor pressures than water have faster sublimation rates. Therefore the drying time will be decreased.

The objective of this study was to enhance SFL technology for preparation of nanoparticles of CBZ, using organic solvents like acetonitrile as the solution

source for the SFL process. Using acetonitrile as a solution source could increase CBZ loading in the feed solution and reduce the drying time in the SFL process. In addition, the influence of the solution type (organic vs aqueous/organic) on physicochemical properties of SFL micronized powders was determined in this study. The dissolutions of micronized bulk CBZ and slowly frozen control samples were compared with those of the SFL micronized powders.

3.2. MATERIALS

Carbamazepine (CBZ) USP was obtained as micronized powder from Spectrum Quality Products Inc. (Gardena, CA). The M50 (50% cumulative percent undersize) of bulk CBZ was 40 μm . The A polyoxyethylene-*b*-polyoxypropylene-*b*-polyoxyethylene triblock copolymer (Poloxamer 407), and polyvinylpyrrolidone (PVP) K-15 were purchased from Spectrum Quality Products Inc. (Gardena, CA). THF, methanol, and acetonitrile were obtained from EM Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

3.3. METHODS

3.3.1. Preparation of SFL micronized powders

CBZ, poloxamer 407 and PVP K15 (2:1:1) were dissolved in either acetonitrile or THF/water co-solvent (33% w/w) and processed by the SFL (Fig.3.1) technology (10). The SFL feed solution was placed into the solution cell (A). A constant pressure of 4000 PSI from the ISCO syringe pump (B) (Model 100 DX, ISCO Inc., Lincoln, NE) provided a flow rate of 11 mL/min (Table 3.1) for the SFL feed solution composed of THF/water co-solvent. A constant pressure of 2000 PSI provided a flow rate of 20 mL/min for the SFL feed solution composed of acetonitrile. The solution cell was connected to an insulated nozzle (C), which was positioned to atomize the SFL feed solution beneath the surface of the cryogenic liquid (D). In these experiments, liquid N₂ was used as the cryogenic liquid. The atomizing nozzle used was composed of polyetheretherketone (PEEK) tubing with an inner diameter of 63.5 µm. The SFL feed solutions were then sprayed through the nozzle and atomized directly into the liquid N₂ phase. Frozen particles formed instantaneously and were collected and dried by a VirTis Advantage Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY).

Different CBZ loadings in the feed solutions were investigated. The slowly frozen control was prepared by a conventional freeze drying method as previously described (10). It was composed of CBZ/poloxamer 407/PVP K15

(2:1:1) dissolved in acetonitrile, frozen at -78°C and then lyophilized. The micronized CBZ (as received) was also used as a control.

3.3.2. Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powders were placed in a glass sample holder. Samples were scanned from 5° to 45° (2 θ) at a rate of 0.05°/sec. For comparative purposes, the three highest values for relative peak intensity and their corresponding 2 θ positions were compared using the bulk CBZ as reference (12).

3.3.3. Scanning Electron Microscopy (SEM)

A Hitachi S-4500 field emission scanning electron microscope (Hitachi Instruments Inc., Irvine, CA) was used to examine the surface morphology of sample powders. The samples were gold sputter coated prior to analysis.

3.3.4. Particle Size Measurement

The particle size distribution of the sample powders was determined by laser light diffraction using a Malvern Mastersizer S (Malvern Instruments Limited, Malvern, Worcestershire, UK). M90, M50 and M10, which were the respective particle size at 10, 50, 90% cumulative percent undersize, were determined for the SFL powders and controls (13).

3.3.5. Karl-Fisher (KF) Titration

Residual water content in the SFL powders was measured by an Aquatest 8 Karl-Fisher Titrator (Photovolt Instrument, St. Louis Park, MN). Aliquot of 10 mg sample powders were added into the titrator vessel following equilibration of the instrument. Each sample was measured in replicates of three ($n = 3$).

3.3.6. Gas Chromatography (GC) Analysis

Residual organic solvent content in the SFL powders was determined using a Hewlett-Packard 5890A gas chromatograph. Samples were dissolved in dimethylformamide. 1-Butanol was used as an internal standard. The calibration standard ranged from 50 ppm to 50,000 ppm for THF and from 20 ppm to 50,000 ppm for acetonitrile. The calibration standards were used to calibrate the residual organic solvent level in the SFL powders produced from acetonitrile or THF/water co-solvent.

3.3.7. Surface Area Measurement

Surface area was measured using a Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL). Each sample was degassed for at least 5 hours prior to analysis. The surface area per unit powder mass was calculated from the fit of adsorption data to the Brunauer, Emmett, and Teller (BET) equation (14).

3.3.8. Contact Angle Measurement

Compacts of sample powders were prepared at a 500 kg compression force using a Carver Laboratory Press (Model M, ISI Inc., Round Rock, TX) with flat-faced 6 mm diameter punches. A droplet of purified water (3 μ l) was placed onto the surface of the compact and observed using a low power microscope. The contact angle was determined by measuring the tangent to the curve of the droplet on the surface of the compact using a Goniometer (Model No.100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ).

3.3.9. Solubility

The equilibrium solubility of CBZ in the purified water, THF/water co-solvent (33% w/w) and acetonitrile was measured. An excess of CBZ was added into each of three scintillation vials (n=3) containing 15 ml of the purified water, THF/water co-solvent, or acetonitrile. The sealed vials were equilibrated in a horizontal shaker at 37°C for 72 hours. The known amount of sample was filtered through a 0.45 μ m syringe filter, diluted with acetonitrile and analyzed by HPLC.

3.3.10. Dissolution Studies

The amount of API dissolved, as a function of time, was determined using USP Apparatus 2 method (Vankel 7000, Vankel Technology Group, Cary, NC). All dissolution studies were conducted at sink conditions. SFL CBZ/poloxamer 407/PVP K15 powders or control samples containing approximately 12 mg CBZ

were added to 900 mL of purified water (37°C). The paddle speed was 50 rpm. Five-milliliter samples in replicates of 3 (n=3) were taken at each time point, filtered through a 0.45µm filter, diluted with acetonitrile, filtered through a 0.45µm filter again, and then analyzed by HPLC (10, 15).

3.3.11. HPLC Analysis

The concentration of CBZ in the samples was assayed by HPLC. Each sample was filtered through 0.45 µm Acrodisc GHP syringe filter (Pall Corporation, Ann Arbor, MI) and analyzed at 288 nm using a Shimadzu LC-10 chromatograph (Shimadzu Corporation, Kyoto, Japan). An Alltech 150 mm X 4.6 mm Intersil 5 µm ODS-2 reverse-phase column (Alltech Associates, Inc., Deerfield, IL) was used for the HPLC analysis. The CBZ peak eluted at 5 minutes when running mobile phase (water/methanol/acetonitrile at 10/7/3 ratio, v/v) at 1.5 mL/min. A check standard was injected after each 6 unknown samples through HPLC batch run. System suitability requirements were met (correlation coefficient (r^2) $\geq$ 0.998, precision of 5 replicate injection $\leq$ 2.0% RSD, theoretical plates $>$ 500 plates/column and peak asymmetry $\leq$ 1.5).

3.3.12. Statistical analysis

The data were compared using a student's t-test of the two samples assuming equal variances to evaluate the differences. The significance level (α = 0.05) was based on the 95% probability value ($p < 0.05$).

3.4. RESULTS AND DISCUSSION

3.4.1. Solubility and feed solution loading

The SFL CBZ/poloxamer 407 /PVP K15 (2:1:1) powders investigated in this study were prepared from both the acetonitrile and THF/water co-solvent systems. The equilibrium solubilities of CBZ in purified water, THF/water co-solvent (33% w/w) and acetonitrile were 57 µg/mL, 3.7 mg/mL and 32.1 mg/mL, respectively. Therefore, the maximum CBZ loading in the SFL feed solution was 0.22% (w/w) for THF/water co-solvent and 2.2% (w/w) for acetonitrile.

3.4.2. Crystallinity

The magnitude of the peak intensities of bulk CBZ indicated a high degree of crystallinity (Fig. 3.2). The slowly frozen control exhibited the characteristic diffraction peaks of CBZ (16) at 15.3, 25.0 and 27.6 (2 θ) suggesting that CBZ was crystalline. The X-ray pattern of the SFL CBZ/poloxamer 407 /PVP K15 powder sprayed from the THF/water feed solution exhibited a significant reduction in peak intensity of CBZ. The large reduction in the characteristic peaks of CBZ indicated an amorphous state. The SFL powder sprayed from the acetonitrile feed solution exhibited an amorphous XRD pattern, which was similar to that of the SFL powders from the THF/water co-solvent. The SFL CBZ/poloxamer 407 /PVP K15 with high feed solution loading (2.2%) contained amorphous CBZ, as indicated in its X-ray pattern (Fig. 3.3).

3.4.3. Surface morphology

SEM was used to determine the surface morphologies of SFL micronized powders produced by the acetonitrile and THF/water co-solvent systems. The SEM micrograph of the micronized bulk CBZ (Fig. 3.4a) revealed large crystalline plates with fractured edges. The slowly frozen control (Fig. 3.4b) contained relatively large particles ($>50\text{ }\mu\text{m}$) with smooth surfaces. In Figure 3.4c, the SEM micrograph indicated the SFL CBZ/poloxamer 407/PVP K15 particles prepared from THF/water co-solvent system had a highly porous morphology with a geometric diameter of about $5\mu\text{m}$. The SEM micrographs of the SFL micronized powders from acetonitrile with 0.22% CBZ loading are shown in Figures 3.4d and 3.4e. It can be seen in Figure 3.4d that there are many small porous aggregates. Higher magnification revealed nanoparticles with a geometric diameter of about 500 nm (Fig. 3.4e). This nanoparticle is comprised of many small subunits. The SFL microparticle (Fig. 3.4f) produced at 2.2 % feed solution loading was spherical with a rough surface about $10\text{ }\mu\text{m}$ in diameter, which was larger and less porous compared to the SFL particles produced at 0.22% feed solution loading.

3.4.4. Residual solvent

Karl-Fisher titration was used to determine the residual water content of SFL CBZ/poloxamer 407/PVP K15 micronized powders from the THF/water co-

solvent system. The SFL micronized powders prepared from the THF/water co-solvent system had a moisture content of 5.8%. There was no water detected in the SFL micronized powders from acetonitrile.

The residual organic solvent levels in the SFL micronized powders were measured by GC analysis. No organic solvents were detected in SFL CBZ/poloxamer 407/PVP K15 powders prepared from feed solutions composing either THF/water or acetonitrile. Since the calibration standard started from 50 ppm for THF and 20 ppm for acetonitrile, the residual organic solvent was less than 50 ppm THF in the powders made from the THF/water co-solvent system and less than 20 ppm acetonitrile in the powders prepared from the SFL acetonitrile system.

3.4.5. Particle size measurement

The particle size measurement of the SFL micronized powders and controls are listed in Table 3.2. The M50 of slowly frozen control was 22.05 μm . In contrast, the M50 of the micronized powder produced from SFL THF/water feed solution was 7.06 μm . The M50 of the micronized powder prepared from the acetonitrile system with 0.22% CBZ loading was 680 nm. When the CBZ loading in the feed solution was increased to 2.2%, the M50 of micronized powder increased to 14.11 μm .

3.4.6. Surface area

The surface area of the powders is listed in Table 3.2. The surface area of the SFL micronized powders from THF/water co-solvent system was 13.31 m²/g and the surface area of the SFL micronized powders from acetonitrile was 12.89 m²/g, both of which were a great increase over the slowly frozen control (1.84 m²/g). The SFL micronized powder produced from acetonitrile feed solution with 2.2% CBZ had a surface area of 3.88 m²/g, which is still greater than the slowly frozen control.

3.4.7. Wettability

The contact angle was used as an indicator of the wettability of SFL micronized powders by purified water, which was the dissolution media for CBZ. The contact angles for the SFL micronized powders and controls are reported in Table 3.3. The limits of contact angle are 0° for complete wetting and 180° for no wetting (17). The mean value of contact angle for the SFL CBZ/poloxamer 407/PVP K15 powder from THF/water co-solvent was 29° (±1.5°). The SFL micronized powders from acetonitrile had contact angles of 34° (±1.5°) and 38° (±2.5°) for 0.22% loading and 2.2 % loading, which were significantly lower than that of the SFL CBZ (45° ± 3.4°) and slowly frozen control (43°±2.6°) (P< 0.05).

3.4.8. Dissolution

The dissolution profiles presented in Figure 3.5 illustrate the dissolution of the SFL powders sprayed from acetonitrile and THF/water feed solution (n=3). The rate of dissolution of micronized CBZ (Fig. 3.5) was slow; only 5% of CBZ was dissolved in 20 minutes. The dissolution of CBZ from the slowly frozen control was faster than bulk CBZ, with 60% CBZ dissolved in 10 minutes. However, SFL micronized powders displayed very rapid release profiles where greater than 90% CBZ was dissolved in only 10 minutes for the SFL CBZ/poloxamer 407/PVP K15 powders produced from either acetonitrile or THF/water feed solution. Major improvements in the dissolution rates were also found for SFL micronized powders with high solution loading relative to the controls. The dissolved CBZ from SFL micronized powder was 87% in 10 minutes, a significant enhancement when compared to only 5% of CBZ dissolved in 20 minutes. The SFL powders from both systems exhibited significantly enhanced dissolution rates compared to the bulk CBZ and slowly frozen control.

3.4.9. Discussion

Prior to this study, feed solutions composed of THF/water co-solvent were utilized to make SFL micronized powders. It was found that CBZ and other poorly water soluble APIs had low solubilities in the THF/water co-solvent and thus resulted in a very low loading of API in the feed solution. The CBZ loading

in the feed solution for the THF/water co-solvent was only 0.22%. The limited solution loading lowers the production rate of frozen powders, which also leads to a long drying time. These problems are detrimental factors to the scale-up the SFL process. To overcome these problems, an organic solvent without water was utilized to further optimize SFL technology. The utilization of acetonitrile increased the CBZ loading in the feed solution to 2.2%, which was 10-fold greater than that of the SFL THF/water co-solvent system. For organic solvent, this increased the production rate of API by a loading factor of ten (18, 19).

In the SFL process, the solvents must be sublimed from the frozen particles in order to obtain dry SFL micronized powders. Using the SFL THF/water co-solvent system, the drying time was 72 hours. Studies reported in the literature have demonstrated that increasing sublimation rate will decrease the drying time (20, 21). Specifically, the sublimation rate is described by Equation:

$$\text{Sublimation rate} = \text{pressure difference/resistance}$$

About 90% of the resistance is typically from the product. Acetonitrile has a higher vapor pressure (73 kPa) than THF (21.6 kPa)/water (3.2 kPa) at 20°C. Therefore, there is a greater driving force for sublimation when acetonitrile is used in the feed solution because the vapor pressure difference is a dominant factor controlling the sublimation rate (22). This study demonstrated that the drying rate was significantly increased when using acetonitrile as the SFL feed

solution source and the drying time was decreased to approximately 20% of the time that the THF/water co-solvent system required.

In the use of the SFL organic systems, it was also important to be aware of the toxicity from the organic solvents. The International Conference on Harmonization guidance classified various solvents based on a safety assessment (23). Acetonitrile and THF are Class II solvents, meaning that their concentration in pharmaceutical formulations should be limited to a range from 100-1000 ppm (24). The results showed that residual THF or acetonitrile concentrations in the SFL micronized powders were below the limit.

The SFL micronized powders from either solvent had M50 particle sizes less than that of the control samples. Several factors contributed to the size of the microparticles produced by the SFL process (8, 10, 24). The atomized droplets of the drug solution were frozen immediately upon contact with the liquid nitrogen. The small droplet size and rapid freezing rate limited the propensity for particle growth. However, the SFL micronized powders processed from the acetonitrile system were comprised of nanostructured particles and each nanoparticle was an aggregate of many small subunits. The freezing rate of the acetonitrile solution was much faster than that of the THF/water solution because acetonitrile has a higher freezing point (-48°C) compared to THF (-108°C). The formation of ice nuclei and their growth rate depends on the freezing rate of solution. The faster the freezing rate, the smaller the size of the ice particles (25). M50 particle size

from the SFL acetonitrile system increased as the CBZ concentration in SFL feed solution was increased. The higher the concentration of the SFL feed solution, the more viscous it was. As a result, it likely atomized into larger microdroplets in the SFL process and thus produced relatively larger microparticles.

The XRD results indicated that CBZ/poloxamer 407/PVP K15 powders processed by either SFL acetonitrile or THF/water co-solvent system were amorphous. However, the characteristic diffraction peaks of CBZ were found in the slowly frozen control. The freezing rate in the conventional freezing drying process was slowed sufficiently so that the solute crystallized as it was frozen. In the SFL process, rapid freezing rates were achieved by atomizing the feed solution directly into liquid nitrogen. These freezing rates were fast enough to trap the API in an amorphous state without allowing time for crystallization. Regardless of the solvent types or feed solution loading, the CBZ contained in the SFL micronized powders was amorphous. The high internal energy of the amorphous state relative to the crystalline state led to faster dissolution rates.

The surface areas of SFL powders from both systems were similar and greater than that of slowly frozen control. Such high surface area indicated that the SFL powder particles were highly porous, similar to the spray-freeze dried protein particles reported by Maa *et al.* (26) and Costantino *et al.* (27, 28). The highest degree of atomization was achieved in the SFL process and formed high-surface area microdroplets. After lyophilization, the dried microparticles retained

the shape of the microdroplets, but their highly porous structures were due to the channels created as the solvent(s) were removed during the sublimation process. As a result, the large surface areas were obtained with SFL micronized powders. It was also confirmed that the highly porous structure of SFL micronized powders produced from both systems by SEM. It is quite different from the low surface area crystalline cakes produced by the conventional lyophilization process. The surface area of SFL micronized powders was decreased as the CBZ loading in the feed solution increased, which was due to the increased particle size of SFL micronized powders from acetonitrile with high CBZ loading in the feed solution.

The SFL powders from both systems wetted and dissolved rapidly compared to slowly frozen control and bulk CBZ ($p < 0.05$). When increasing CBZ loading in the feed solution from SFL acetonitrile system, the rapid dissolution of CBZ from SFL micronized powders was not affected. The dissolved CBZ from SFL micronized powders in 10 minutes was over 90%, a significant enhancement when compared to 5% for the micronized CBZ and 60% for the slowly frozen control. The increased dissolution rate of the SFL micronized powders was mainly attributed to the amorphous state of the API, the reduction in particle size and consequent enhanced surface area. At the same time, the enhanced wettability increased the dissolution of the SFL micronized powder. All of these physicochemical properties were attributed to the SFL particle engineering process.

3.5. CONCLUSIONS

The SFL acetonitrile system was demonstrated to produce nanostructured particles, which have similar characteristics to the particles prepared by the SFL THF/water co-solvent system. The SFL acetonitrile system offers several advantages versus SFL THF/water co-solvent system. Using acetonitrile greatly increased CBZ loading in the SFL feed solution and decreased drying time in the SFL process. All of these attributes have a positive effect on the scale-up of the SFL process in the product manufacture. In conclusion, the SFL technology using acetonitrile system is an effective particle engineering process for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs for oral delivery systems.

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CHAPTER 4: Rapid Dissolving High Potency Danazol Powders Produced by Spray Freezing into Liquid (SFL) Process with Organic Solvents

The objectives of this study were to investigate the use of organic solvents in the spray freezing into liquid (SFL) particle engineering process to make rapid dissolving high potency danazol powders and to examine their particle size, surface area and dissolution rate.

4.1. INTRODUCTION

It is estimated that about 40% of compounds being developed by the pharmaceutical industry are poorly water soluble (1, 2). A limiting factor in the oral bioavailability of poorly water soluble compounds is an inadequate dissolution rate. Increasing the dissolution rate of poorly water soluble active pharmaceutical ingredients (APIs) has become a major challenge in pharmaceutical formulation development. A promising approach is to use a particle engineering technology to overcome poor wetting and low dissolution rate of an API. Techniques that have been commonly used include mechanical milling, spray drying, solid dispersion, supercritical CO₂ precipitation techniques

like RESS and PCA/SAS/SEDS (3-6), and solvent evaporation techniques including evaporative precipitation into aqueous solution (EPAS) (7). These particle formation techniques have been used to micronize poorly water soluble API alone or in the presence of a polymer and/or surfactant(s). As a result, the decreased particle size and increased surface area lead to greatly enhanced dissolution rates (3, 5-7).

Pharmaceutical powders produced by the current particle engineering technologies, however, often have very low drug potency or drug/surfactant ratio. This is due to difficulty of the stabilization of small particles because of the thermodynamic driving force to lower the interfacial area. Therefore, the final product often contains large amounts of stabilizing excipients resulting in very low drug potency. For example, the API/surfactant(s) ratio in a solid dispersion must be below about 1:2 to keep the drug molecularly dispersed, otherwise, it may form small crystals that lower the dissolution rates (5). The application of supercritical CO₂ rapid expansion techniques was limited by the low solubility of API in the CO₂. The high percentages of surfactants were often used to enhance solubility of API in the CO₂ and to stabilize the system as well (3, 4). Typically, API/surfactant ratios ranging from 1:40 to 3:1 are used in the current particle formation techniques (8-12). However, solid oral dosage forms often require high potency or high API/surfactant ratios in order to achieve a therapeutic effect with tolerability to the API and minimal side effects from the excipients. However, it

is highly challenging in current particle engineering technologies to achieve high dissolution rates for poorly water soluble APIs with high potency because only a small amount of stabilizing excipients can be used in the process.

The spray freezing into liquid (SFL) particle engineering process was developed to improve the wetting and enhance the dissolution rate of poorly water soluble APIs (13-16). In the SFL particle engineering process, a feed solution containing poorly water soluble API and excipient(s) is atomized directly into a cryogenic liquid to produce frozen particles. The frozen particles are then collected and lyophilized to obtain dry powders. The intense atomization in conjunction with rapid freezing rates have led to nanostructured aggregates composed of amorphous API nanoparticles with high surface areas and enhanced wettability. Recently, a study (13) showed that SFL carbamazepine/PVP K-15/poloxamer 407 powders prepared by SFL exhibited significantly enhanced dissolution rates (> 92% dissolved in 10 minutes in the purified water). In contrast, only 5% of bulk carbamazepine was dissolved in 20 minutes. The SFL powders wetted and dissolved instantaneously upon contact with the dissolution media because of an amorphous structure, high surface area and increased wettability. However, these SFL formulations had relatively low drug potency typically 33%.

The objective of this study was to extend the SFL process to produce rapid dissolving high potency powders with high surface areas and dissolution rates.

The potencies ranged from 50% to 90% in contrast with the typically obtained 33% in previous studies (13, 17). In order to achieve these high potencies, high concentrations of APIs were dissolved in pure or mixed organic solvents to prepare the feed solutions. The hypothesis of this study was that only small amounts of surfactant or polymer are sufficient to form SFL nanostructured aggregates with amorphous API, high surface areas, and enhanced wettability properties which enhance dissolution rates. Danazol was used as a model API in this study. The dissolution rate was determined as a function of the danazol potency.

4.2. CHEMICALS

Danazol USP was obtained as micronized powder from Spectrum Quality Products Inc. (Gardena, CA). Polyvinylpyrrolidone (PVP) K-15, sodium lauryl sulfate (SLS), tris(hydroxymethyl)aminomethane (Tris), and hydrochloric acid (HCl) were purchased from Spectrum Quality Products Inc. (Gardena, CA). Acetonitrile and methylene chloride were obtained from EM Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

4.3. METHODS

4.3.1 High Potency Danazol/PVP K-15 Powders by SFL

Danazol and PVP K-15 were dissolved in acetonitrile or acetonitrile/methylene chloride mixtures and processed using SFL technique (13, 15, 17). The formulations of SFL feed solutions are listed in Table 4.1. The SFL feed solution was placed into the solution cell. A constant pressure (2000 PSI) from the ISCO syringe pump provided a flow rate of 50 ml/min for the SFL feed solution. The atomizing nozzle was composed of polyetheretherketone (PEEK) tubing with an inner diameter of 127 μm . The SFL feed solutions were sprayed through the nozzle and atomized into small droplets directly into the liquid N_2 phase. Frozen particles formed instantaneously and were collected and dried by a VirTis Advantage Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY). The SFL powders and control samples were stored in glass vials over desiccant in a vacuum desiccator at room temperature before characterization measurements. The micronized bulk danazol was used as a control.

4.3.2 Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 $\text{\AA}$ at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powders were placed in a glass sample holder. Samples were scanned from 5° to 45° (2θ) at a

rate 0.05°/sec. For comparative purposes, the three highest values for relative line intensity and their corresponding line position 2θ were compared for the micronized bulk danazol (18).

4.3.3 Scanning Electron Microscopy (SEM)

A HITACHI S-4500 field emission scanning electron microscope (Hitachi Instruments Inc., Irvine, CA) was used to examine the surface morphology of each sample powder. The sample was fixed to a SEM stage with double-sided adhesive tape and gold sputter coated.

4.3.4 Surface Area Measurement

A Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL) was used to determine N₂ sorption at 77.40 deg K. The surface area per unit powder mass was calculated from the fit of adsorption data to the Brunauer, Emmett, and Teller (BET) equation (19).

4.3.5 Contact Angle Measurement

Compacts of sample powders were prepared at a 500 kg compression force using a Carver Laboratory Press (Model M, ISI Inc., Round Rock, TX) with flat-faced 6mm diameter punches. A droplet of SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%, 3 μ l) was placed onto the surface of the compact and observed using a low power microscope. The contact angle (20) was determined by

measuring the tangent to the curve of the droplet on the surface of the compact using a Goniometer (Model No.100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ).

4.3.6 Solubility Study

The equilibrium solubility of danazol in purified water, THF/water co-solvent (33% w/w), acetonitrile, methylene chloride, and in the SLS/Tris dissolution media was measured. An excess of danazol was added into each of three glass vials (n=3) containing 15 ml of the solvent or dissolution media. The sealed vials were equilibrated in a horizontal shaker at 37°C for 72 hours. The known amount of sample was filtered through a 0.45 µm syringe filter, diluted with acetonitrile and analyzed by HPLC.

4.3.7 Dissolution Studies

The amount of danazol dissolved, as a function of time, was determined using USP Apparatus 2 (paddles) at 50 rpm (Vankel 7000, Vankel Technology Group, Cary, NC). All dissolution tests (n=3) were conducted under sink conditions. SFL danazol/PVP K-15 powders with different danazol potencies or micronized bulk danazol containing approximately 12 mg danazol were added to 900 mL of SLS/Tris dissolution media (37°C). The paddle speed was 50. A 5 mL aliquot was taken at each time point and filtered through a 0.45µm filter then

diluted with acetonitrile, filtered through a 0.45 μ m filter again, and analyzed by HPLC (13, 14).

4.3.8. HPLC Analysis

Each sample was filtered through 0.45 μ m Acrodisc GHP syringe filter (Pall Corporation, Ann Arbor, MI) and analyzed at 288 nm using a Shimadzu LC-10 chromatograph (Shimadzu Corporation, Kyoto, Japan). An Alltech 150 mm X 4.6 mm Intersil 5 μ m ODS-2 reverse-phase column (Alltech Associates, Inc., Deerfield, IL) was used for the HPLC analysis. The danazol peak eluted at 5 minutes when running mobile phase (acetonitrile/water at 7/3 ratio, v/v) at 1 mL/min. A standard was injected after each 6 unknown samples through HPLC batch run. System suitability requirements were met (correlation coefficient (r^2) $\geq$ 0.998, precision of 5 replicate injection $\leq$ 2.0% RSD, theoretical plates $>$ 500 plates/column and peak asymmetry $\leq$ 1.5).

4.3.9. Stressed cycle stability study

SFL danazol/PVP K-15 (3:1) powders were sealed in 20 ml glass vials containing desiccant and placed in a temperature controlled oven. The temperature cycle was to increase temperature from -5°C to 40°C over 30 minutes, hold at 40°C for 2.5 hours and then decrease temperature from 40°C to -5°C over 30 minutes. The cycle was repeated 6 times per day. The duration of the stability study was one month.

4.3.10 Statistical analysis

The data were compared using a student's t-test of the two samples assuming equal variances to evaluate the differences. The significance level ($\alpha = 0.05$) was based on the 95% probability value ($p < 0.05$).

4.4. RESULTS AND DISCUSSION

4.4.1. Solubility and Potency

Recently, the SFL process was extended to use organic solvents for the feed solutions(17). The main advantage of the use of organic solvents was to significantly increase the solubility of hydrophobic API. In this study, organic solvents were used in the feed solutions to make high potency danazol powders. Firstly, the equilibrium solubility of danazol in each solvent was measured. The equilibrium solubilities of danazol (4. 2) in purified water, THF/water co-solvent (33% w/w), acetonitrile, methylene chloride, and SLS/Tris dissolution media were 0.97 $\mu\text{g/mL}$, 0.71 mg/mL , 21.36 mg/mL , 92.18 mg/mL , and 0.15 mg/mL , respectively. High potency danazol/PVP K-15 powders were produced by the SFL process using acetonitrile or acetonitrile/methylene chloride mixture. The potency of the SFL Danazol/PVP K-15 powders ranged from 33% to 91% (Table 4.1). The sample with 91% (w/w) potency was prepared from an acetonitrile/methylene chloride mixture. The solubility of danazol was higher in this mixed solvent than in pure acetonitrile. The results below demonstrate that a

relatively small amount of PVP K-15 was needed to form SFL nanostructured aggregates with amorphous structure, high surface area, and enhanced wettability. These properties of the SFL powders led to rapid dissolution rates as described by the Noyes-Whitney equation(21).

4.4.2. Influence of High Potency on Danazol

4.4.2.1. Crystallinity

Crystallinity greatly impacts the solubility and dissolution rate of poorly water soluble APIs (22). Micronized bulk danazol (Fig. 4.1) had a similar X-ray diffraction pattern to that reported by Liversidge and Cundy (23). The peak intensities indicated a high degree of crystallinity. The X-ray pattern of the SFL danazol/PVP K-15 powders with 33% danazol potency exhibited the opposite behavior. The characteristic diffraction peaks of danazol (18) at 15.8, 17.2 and 19.0 (2 θ) degrees were not evident in SFL danazol/PVP K-15 indicating that the danazol was in an amorphous state. Similarly, a lack of crystallinity was also found for the SFL danazol/PVP K-15 powders with higher potencies (50% to 91%). XRD indicated that bulk micronized danazol was in the crystalline form, but danazol in high potency SFL danazol/PVP K-15 powders was amorphous. This is because rapid freezing rates achieved by atomizing the feed solution directly into liquid nitrogen trapped the danazol in an amorphous state without allowing time for crystallization. The high chemical potential of the amorphous

form relative to the crystalline state is a factor that may be expected to lead to faster dissolution rates. Several studies demonstrated that amorphous APIs dramatically enhanced bioavailability of poorly water soluble APIs (24, 25). Therefore, the amorphous SFL danazol powders might have better bioavailability compared to control.

4.4.2.2. Surface Area and Surface Morphology

Another important factor that influences the dissolution rate is the available surface area of the API. The surface areas of the high potency powders are listed in Table 4.3. The surface areas of the SFL danazol/PVP K-15 powders ranged from 115.52 m²/g to 28.50 m²/g. All surface areas of SFL danazol/PVP K-15 powders were increased markedly over the micronized bulk danazol (0.52 m²/g). To further investigate the effect of high potency on the morphology of SFL danazol powders, the powders were examined by SEM. The SEM micrograph of bulk danazol (Fig. 4.2J) revealed a large crystalline plate with a fractured edge. In Figure 4.2A, the SEM micrograph indicated that the SFL danazol/PVP K-15 particles with 33% potency were porous aggregates with a geometric diameter of about 700 nm. A higher magnification SEM micrograph (Fig. 4.2B) revealed the aggregates were composed of many nanoparticles with a geometric diameter of about 50 nm. The surface of all nanoparticles was very smooth. The SEM micrographs of the SFL danazol/PVP K-15 with 50% potency revealed (Figure 4.2C) that there was a small porous aggregate with a geometric

diameter of about 1.5 μm . Higher magnification showed that the aggregate was also composed of many smooth nanoparticles with a geometric diameter of about 100 nm (Fig. 4.2D). Both the SFL Danazol/PVP K-15 particles with 66% potency (4.2E and 4.2F) and 75% (4.2G and 4.2H) potency had a porous morphology with a geometric diameter ranging from 1.5 μm to 750 nm. SEM micrographs showed that these particles were also comprised of many small subunits. The SFL particles (Fig. 4.2I) with 91% potency had a different surface morphology. They were microparticles about 3 μm in diameter, which were larger and less porous compared to the SFL particles with lower potency.

The dissolution rate is directly proportional to the wetted surface area of the API, which in turn increases with decreasing particle size. SEM micrographs revealed that SFL danazol/PVP K-15 nanostructured aggregates had a porous morphology and were composed of many nanoparticles with a geometric diameter of about 50 nm-100 nm. Surface areas of SFL danazol/PVP K-15 high potency powders were in the range of 28 to 115 m^2/g . Several factors contributed to the large surface area and the high porosity of nanostructured aggregates produced by the SFL process (3, 13, 15). An unusually high degree of atomization was achieved in the SFL process leading to the formation of high-surface area microdroplets. The freezing rate of the acetonitrile solution was very rapid. The formation of nuclei and their growth rate depends on the freezing rate of solution. The rapid freezing rate resulting from the small droplet size limited the propensity

for particle growth. Also, PVP K-15, which was in the unfrozen domains in the sprayed droplets, inhibited particle coalescence and crystal growth. In addition, the dried particles retained the shape of the micronized droplets after lyophilization. Their porous structures were due to the channels created as the solvent(s) were removed during the sublimation process. As a result, large surface areas and porous SFL powders composed of nanostructured aggregates were obtained by the SFL process. The surface area of SFL danazol/PVP K-15 powders decreased as the danazol potency increased. Here, the higher danazol loading in the feed solution led to faster growth rates during atomization and freezing and thus larger particles with lower porosity.

4.4.2.3. Wettability

The effective surface area also depends on the ability of the dissolution media to wet the particle surface. The wettability of SFL powders can be determined from the contact angle at the liquid/solid interface. For high contact angles, danazol is not very well wetted by the dissolution media. The contact angles for the SFL micronized powders and controls against SLS /Tris dissolution media are reported in Table 4.4. The mean contact angle for the SFL danazol/PVP K-15 powder was 22° ($\pm 0.5^\circ$), 24° ($\pm 0.4^\circ$), 27° ($\pm 1.0^\circ$), and 35° ($\pm 2.0^\circ$) for 50% potency, 66% potency, 75% potency, and 91% potency, respectively, which were significantly lower than that of the SFL danazol (57° $\pm$

3.4°) ($P < 0.05$). The contact angle was an indicator of the wettability of SFL powders by the dissolution media. The significant reduction of contact angle for the high potency SFL powders compared to the controls indicated the presence of a hydrophilic solid surface (13, 28). This enrichment may be formed during the SFL process. As the danazol and PVP K-15 precipitated from the concentrated unfrozen acetonitrile, the precipitate was surrounded by hydrophilic solvent. The hydrophilic acetonitrile attracts the hydrophilic PVP K-15 molecules preferentially to the surface of the particles. In addition, the rougher surface for the SFL powders as indicated in the SEM micrographs and the higher surface areas may be expected to decrease the contact angle as observed (13). So, both preferential enrichment of the powder surface with the hydrophilic excipient and the surface roughness lower the contact angle and favor wetting of the high potency SFL powder relative to the controls. The contact angle of SFL danazol/PVP K-15 powders increased as the danazol potency increased, which was due to the decreased preferential enrichment of the powder surface with the hydrophilic excipient. Less enrichment may be expected as the danazol/PVP K-15 ratio increases.

The morphology of the API-surfactant composite may be expected to be quite different in particles produced by spray drying. In the spray drying process, the continuous phase is vapor with an extremely low cohesive energy density. Therefore, a polar surfactant would not be expected to migrate preferentially to

the surface of the particles. In contrast, the high polarity of the continuous phase (acetonitrile based) in SFL favors preferential adsorption of the polar surfactant on the surface of the particles.

4.4.2.4. Dissolution

The dissolution rate of SFL powders with different potencies is significantly greater than micronized bulk danazol (Figure 4.3). The rate of dissolution of micronized bulk danazol was slow; only 30% of the danazol was dissolved in 2 minutes. However, the amount dissolved reached 95% in only 2 minutes for the SFL danazol/PVP K-15 powders (33% to 75% potencies). At 5 minutes, 99% of the danazol dissolved in the dissolution media. However, about 87% of the danazol in the SFL powder with 91% potency ratio dissolved in 2 minutes and 100% danazol dissolved in 10 minutes. Each of the SFL powders with high potencies exhibited a significantly enhanced dissolution rates. The SFL powders wetted and dissolved immediately upon contact with the dissolution media. The increased dissolution rate of the SFL powders may be attributed to the amorphous nature of danazol, the reduction in particle size, the enhanced surface area, and increased wettability. However, when danazol potency reached at 91%, about 87 % of danazol dissolved in the first 2 minutes, which is lower than that of the SFL particles with lower potency. This may be caused by the lower surface area, and larger particles size.

4.4.3. Stability Assessment

The limitation with the use of the amorphous pharmaceutical powders is the eventual conversion of the high energy, high soluble form to the lower energy crystalline form. The stress imposed in stability testing is an important factor influencing the solid-state properties of amorphous powders. A stability study was conducted for the SFL danazol/PVP K-15 high potency (3:1) at cycle stability conditions (-5°C to 40°C , 6cycles/day) for one month to examine any changes in crystallinity and dissolution. Sample powders were stored in glass vials (20 mL) with aluminum-lined caps over a desiccant. The X-ray diffraction pattern (Fig.4.4) of the SFL danazol/PVP K-15 (3:1) powder exhibited no change in peak intensity for danazol, suggesting that amorphous SFL powders were physically stable. The dissolution results (Fig.4.5) demonstrated that there was no significant difference in the dissolution profiles between the initial and one month samples, which were calculated by the similarity factor f_2 for the dissolution profile comparison according to the method reported by Shah (26). The dissolution rate results further confirmed the high stability of amorphous SFL powders observed by XRD. The high stability of amorphous SFL powders was partially attributed to PVP K-15 used in the SFL formulations. The inhibition of crystal growth by PVP is a well-known phenomenon (27-29). The amorphous forms can be maintained with a stabilizing agent like PVP in the formulation.

The inhibitory effect of PVP on crystallization may be due to the interaction of the API with PVP resulting in a reduction in the molecular mobility of the API.

4.5. CONCLUSIONS

Rapid dissolving SFL danazol/PVP K-15 powders with high potency (up to 91%) have been produced by SFL with an organic solvent mixture. The high potency SFL powders contained amorphous nanostructured aggregates with high surface area and excellent wettability. In only 5 minutes, 99% of the danazol dissolved. The production of surface areas on the order of 100 m²/g required small amounts of surfactant stabilizer due to the rapid freezing rate, which allowed little time for particle growth. The composition of surfactant in the product could be controlled simply by the feed composition, as all of the surfactant precipitated with the API. Thus, the formulation is simpler to prepare than techniques that require separation of free surfactant from adsorbed surfactant in an aqueous suspension to achieve high potency (30). In SFL, solvent mixtures may be formulated to achieve high solubility of API in order to produce high potency powders. Furthermore, the polar solvent mixture in the continuous phase favors migration of the polar surfactant to the surface of the particles during particle formation. The coating with polar surfactant favors wetting of the high surface area during dissolution. The stability study with desiccant demonstrated that high potency amorphous SFL powders were physical stable without crystallization under a high stress cycle. The SFL process offers a highly

effective approach to produce high potency nanoparticles contained in larger structured aggregates with rapid dissolution rates, and is especially applicable to delivery systems containing poorly water soluble drugs.

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CHAPTER 5: Stable Amorphous Danazol Nanostructured Powders with Rapid Dissolution Rates Produced by Spray Freezing into Liquid

The objectives of this study were to produce by the spray freezing into liquid (SFL) process high potency, high glass transition temperature (T_g) danazol/polymer powders that remain amorphous and exhibit high dissolution rates after six months.

5.1. INTRODUCTION

The spray freezing into liquid (SFL) particle engineering technology was developed to enhance the wetting and dissolution properties of poorly water soluble active pharmaceutical ingredients (APIs) (1-4). In the SFL process, a feed solution containing poorly water soluble API and/or excipient(s) is atomized directly into a cryogenic liquid to produce frozen particles. The frozen particles are then collected and lyophilized to obtain dry SFL powders. The intense atomization in conjunction with rapid freezing rates have led to nanostructured aggregates composed of amorphous API nanoparticles with high surface areas and enhanced wettability. Recent studies demonstrated that SFL micronized powders

containing danazol were amorphous as indicated by X-ray diffraction (1, 5, 6). SEM micrographs indicated that SFL nanostructured aggregates were highly porous; surface areas ranged from 11 to 115 m²/g. The dissolution of amorphous SFL danazol powders at 2 minutes was about 95%, a profound enhancement when compared to 30% for the crystalline micronized bulk danazol (1, 6).

Because the amorphous form of a pharmaceutical solid has a higher chemical potential than the thermodynamically stable crystalline form, it can exhibit enhanced dissolution rates and bioavailability. A key challenge is to stabilize the amorphous solid to prevent crystallization during storage (5, 7). The glass transition temperature (T_g) of an amorphous pharmaceutical solid can have a large influence on its physicochemical stability (8). The molecular mobility of a high energy amorphous API in a solid mixture with an excipient decreases when the storage temperature is reduced below T_g . This loss in molecular mobility and diffusion retards nucleation and growth of crystalline domains. Many studies reported that the viscosity of amorphous material was more than 10^{12} P (9, 10). However, when the temperature rises above T_g , the increased molecular mobility of the API may lead to crystallization. It has been reported that certain amorphous pharmaceutical solids stored at 20°C to 50°C below T_g were stable against crystallization (11, 12). For high surface area nanostructured amorphous materials, the driving force for crystallization is large, thus it will be important to form a very rigid blend with a high T_g . There are several factors that influence

the T_g of amorphous pharmaceutical solid mixtures. A key factor is the T_g of the excipient and its compatibility with the API. Miscible API -polymer blends exhibit a single T_g , which is intermediate to the T_g values of the API and polymer (13-15). In addition, the API/polymer ratio has a large effect on the T_g value of mixture. Therefore, the choice of excipient type and API/polymer ratio will greatly impact the stability of amorphous pharmaceutical solids. Moisture is another major factor that causes crystallization of the API. Water can act as a plasticizer to depress the T_g and enhance crystallization(8, 16).

The first objective of this study was to investigate the influence of excipient type on the particle size, surface area, glass transition temperature (T_g), and dissolution rate of amorphous danazol powders produced by the SFL process. All of the excipients led to powders with high danazol dissolution rates. Therefore, we chose to focus additional experiments on the excipient that led to the highest T_g , PVP K-15. The effect of drug potency (or drug/polymer ratio) and moisture on T_g was determined. The stability of danazol/PVP K-15 powders was studied after six months to examine whether any crystallization occurred and if the dissolution rate changed.

5.2. MATERIALS AND METHODS

5.2.1. Chemicals

Danazol USP was obtained as a micronized powder from Spectrum Quality Products Inc. (Gardena, CA). Polyvinylpyrrolidone (PVP) K-15, PEO-*b*-PPO-*b*-PEO (Pluronic F127; poloxamer 407), polyethylene glycols 8000 (PEG 8000), sodium lauryl sulfate (SLS), and tris(hydroxymethyl)aminomethane (Tris) were purchased from Spectrum Quality Products Inc. (Gardena, CA). Acetonitrile and methylene chloride were obtained from EM Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore S. A., Molsheim Cedex, France).

5.2.2. Preparation of SFL Danazol Powders

Danazol/PEG 8000, or danazol/poloxamer 407 mixtures were dissolved in acetonitrile, and danazol/PVP K-15 were dissolved in acetonitrile or acetonitrile/methylene chloride mixtures and processed by SFL (1, 6). A constant pressure (2000 PSI) from the ISCO syringe pump provided a spray flow rate of 50 ml/min for the SFL feed solution. The atomizing nozzle consisted of polyetheretherketone (PEEK) tubing with an inner diameter of 127 μm and length of 15 cm. The SFL feed solutions were atomized into small droplets directly in the liquid N_2 phase. The rapidly frozen particles were collected and dried by a VirTis Advantage Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY).

The SFL powders and control samples were stored in glass vials over a sodium calcium aluminosilicate hydrate desiccant in a vacuum desiccator at room temperature before. The micronized bulk danazol were used as a control.

5.2.3. Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted with $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA utilizing a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powders were placed in a glass sample holder and scanned from 5° to 45° (2 θ) at a rate 0.05°/sec. The peak intensities for the three largest peaks were compared with those of the micronized bulk danazol (17).

5.2.4. Scanning Electron Microscopy (SEM)

A HITACHI S-4500 field emission scanning electron microscope (Hitachi Instruments Inc., Irvine, CA) was used to examine the surface morphology of each sample powder. The sample was fixed to a SEM stage with double-sided adhesive tape and gold sputter coated.

5.2.5. Particle Size Measurement

The particle size distribution of the SFL powders and their controls were determined by laser light diffraction using a Malvern Mastersizer S (Malvern Instruments Limited, Malvern, Worcestershire, UK). M90, M50 and M10, the

respective particle size at 10, 50, 90% cumulative percent undersize, were determined for the SFL powers and controls (18).

5.2.6. Surface Area Analysis

A Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL) was used to determine N₂ sorption at 77.40 deg K. The surface area per unit powder mass was calculated from the fit of adsorption data to the Brunauer, Emmett, and Teller (BET) equation (19).

5.2.7. Contact Angle Measurement

Compact discs of sample powders were prepared at a 500 kg compression force using a Carver Laboratory Press (model M, ISI Inc., Round Rock, TX) with flat-faced 6 mm diameter punches. A droplet of SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%, 3 µl) was placed onto the surface of the compact disc and observed using a low power microscope. The contact angle was determined by measuring the tangent of the droplet on the surface with a Goniometer (Model No. 100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ).

5.2.8. Karl-Fisher (KF) Titration

Residual water content in the SFL powders was measured by using an Aquatest 2010a Karl-Fisher Titrator (Photovolt Instrument, St. Louis Park, MN). Aliquots of 10 mg sample powders were added into the titrator vessel following

equilibration of the instrument. Each sample was measured in replicates of three ($n = 3$).

5.2.9. Modulated Differential Scanning Calorimetry (mDSC)

The glass transition temperature was determined by mDSC analysis using a TA Instruments DSC 2920 with mDSC capability. Aliquots weighing between 2 to 10 mg were placed in an aluminum pan (kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT) and crimped with an aluminum lid. The samples were heated from 25°C to 275°C to encompass the danazol melting point (225 °C) at a heating rate 10°C/min under a dry nitrogen gas purge. In the case of SFL poloxamer 407 and SFL danazol/poloxamer 407 powders, the sample was cooled to -70°C and then heated to 275°C at heating rate 3°C/min. All samples were initially heated to 275°C at 10°C/min to erase the effect of previous thermal history (20-22).

5.2.10. Dissolution Studies

The amount of danazol dissolved, as a function of time, was determined using USP Apparatus 2 method (Vankel 7000, Vankel Technology Group, Cary, NC). All dissolution testing ($n=3$) was conducted under sink conditions. SFL micronized powders and control sample containing approximately 12 mg danazol were added to 900 mL of SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%) at 37°C. The paddle speed was 50 rpm. 5 mL aliquot was taken at each time point

and filtered through a 0.45µm filter then diluted with acetonitrile, filtered through a 0.45µm filter again, and analyzed by HPLC (1).

5.2.11. HPLC Analysis

Each sample was filtered through 0.45 µm Acrodisc GHP syringe filter (Pall Corporation, Ann Arbor, MI) and analyzed at 288 nm using a Shimadzu LC-10 chromatograph (Shimadzu Corporation, Kyoto, Japan) and an Alltech 150 mm X 4.6 mm Intersil 5 µm ODS-2 reverse-phase column (Alltech Associates, Inc., Deerfield, IL). The danazol peak eluted at 5 minutes when flowing mobile phase (acetonitrile/water at 7/3 ratio, v/v) at 1 mL/min. A standard was injected after each 6 unknown samples through HPLC batch run. System suitability requirements were met (correlation coefficient (r^2) $\geq$ 0.998, precision of 5 replicate injection $\leq$ 2.0% RSD, theoretical plates $>$ 500 plates/column and peak asymmetry $\leq$ 1.5).

5.2.12. Stability Study

Sample powders were stored in type I glass vials (30 mL) and an aluminum-lined caps over a desiccant. The stability testing was conducted under the International Conference on Harmonization (ICH) stability conditions (23). The SFL powders were stored at 25°C/60%RH for 6 months and characterized as a function of exposed time.

5.2.13. Statistical Analysis

The data were compared using a student's t-test of the two samples assuming equal variances to evaluate the differences. The significance level ($\alpha = 0.05$) was based on the 95% probability value ($p < 0.05$).

5.3. RESULTS AND DISCUSSION

5.3.1 Influence of Excipient Type on SFL Powders

5.3.1.1. Crystallinity

The degree of crystallinity of danazol in the SFL powders was determined by powder X-ray diffraction (XRD). Micronized bulk danazol (Fig. 5.1) had a similar X-ray diffraction pattern to that reported by Liversidge and Cundy indicating a high degree of crystallinity. However, for the SFL danazol/PVPk15, danazol/poloxamer 407 and danazol/PEG 800 powders the absence of characteristic peaks at 15.8, 17.2 and 19.0 (2θ) indicated an amorphous morphology for danazol (1, 24). The diffraction peaks of poloxamer 407 at 19.3 and 23.7 (2θ) and PEG 8000 at 19.5 and 23.4 (2θ) were present. XRD results showed that danazol was amorphous regardless of excipient type used in the SFL composition. These freezing rates were fast enough to trap the danazol in an

amorphous state without allowing time for crystallization. The rapid freezing rates result from atomizing the feed solution directly into liquid nitrogen.

5.3.1.2. Particle Size Distribution and Morphology

The particle size distribution for the SFL powders and controls (Table 5.1) were determined by laser light diffraction using a Malvern Mastersizer. The mean particle diameter (M50) of the SFL danazol/PVP powders was 1.18 μm . In contrast, M50 of the danazol/PEG 8000 and danazol/poloxamer 407 were 6.52 μm and 8.68 μm , respectively. The size reduction was significant after SFL processing compared to the starting material, micronized bulk danazol, which had an M50 of 23.42 μm . Various factors contributed to the limited particle growth during SFL process. The liquid-liquid impingement that occurs as the feed solution impacts the liquid nitrogen resulted in intense atomization into fine microdroplets. The atomized droplets were frozen upon contact with the liquid N_2 very rapidly. The small droplet size and the rapid freezing rate limited the propensity for particle growth. However, the SFL danazol/PVP K-15 powders had M50 particle sizes less than those of danazol/PEG 8000 and danazol/poloxamer 407 powders.

The effect of excipients on the morphology of SFL powders was examined further by using SEM. The SEM micrograph of bulk danazol (Fig. 5.2E) revealed a large crystalline plate with a fractured edge. In contrast, the morphology of SFL danazol/PVP K-15 was a porous aggregate with a geometric diameter of about

700 nm. A higher magnification SEM micrograph (Fig. 5.2B) revealed an aggregate composed of many smooth nanoparticles with a geometric diameter of about 50 nm. The porous structures of SFL particles were due to the channels created as the solvent(s) were removed during the sublimation process. The SEM micrograph of the SFL danazol/PEG 8000 (Fig. 5.2C) powder revealed porous microparticles with a geometric diameter of about 6 μm . SFL danazol /poloxamer 407 particles (Fig.5.2D) were larger and less porous relative to the danazol/PEG 8000 and danazol/PVP K-15 particles. The SEM result confirmed the nanostructure of SFL particles observed by the particle size analysis.

5.3.1.3. Surface Area and Wettability

The surface area (Table 5.2) of SFL danazol/PVP K-15, danazol/PEG 8000 and danazol/poloxamer 407 were 89.8 m^2/g , 12.0 m^2/g and 5.49 m^2/g , respectively. All surface areas of SFL powders were more than an order of magnitude greater than that of the micronized bulk danazol (0.52 m^2/g). The large surface areas of SFL powders reflect the small particle size and porous morphology observed in the SEM micrographs. The surface areas of danazol/PEG 8000 and danazol/poloxamer 407 were lower than that of danazol/PVP K-15, which was consistent with the latter's larger particle size and less porous morphology.

The effective surface area also depends on the ability of the dissolution media to wet the particle surface. The wettability of SFL powders was determined from the contact angle at the SLS /Tris dissolution media/compact of SFL powders. The contact angles (Table 5.2) of SFL danazol/PVP K-15, danazol/PEG 8000 and danazol/poloxamer 407 were 22, 41, 39, degrees, respectively. The great reduction of contact angle for SFL powders compared to the control (57 degrees) indicated the presence of a more hydrophilic surface due to the polar excipient (1, 6). In addition, the rougher surface for the SFL powders as indicated in the SEM micrographs may be expected to decrease the contact angle as observed (1). So, both preferential enrichment of the powder surface with the hydrophilic excipient and the surface roughness lower the contact angle and favor wetting of the SFL powder relative to the control.

5.3.1.4. Dissolution Rate

The profiles presented in Figure 5.3 illustrate the dissolution rates of the SFL powders and micronized bulk danazol (n=3). Only 58 % of the micronized bulk danazol control dissolved in 5 minutes. However, profound improvements in the dissolution rates were found for SFL powders relative to the control (Fig.5.3). For example, the amount of dissolved danazol from SFL danazol/PVP K-15 powders in 5 minutes was 99%. The difference in the dissolution rates of SFL powders prepared with the various polymer excipients was negligible in 5 minutes and only slight in the first 2 minutes. Each of the excipients was

successful in producing extremely rapid dissolution. The increased dissolution rate of the SFL powders may be attributed to the amorphous nature of danazol, reduced particle size, and enhanced surface area. Furthermore, the hydrophobic polymer excipient aids the wettability of the large surface area.

5.3.2. Influence of Moisture, Potency, and Excipient type on T_g of SFL

Powders

The T_g of the sample powders was determined by mDSC. The T_g was defined as the point on the curve with the steepest slope in the heat capacity increment during the second heating cycle (21, 22). T_g was used as an indicator to predict stability of amorphous pharmaceutical solids in many studies. The hypothesis of this study was that the physicochemical properties of amorphous SFL powders could be predicted and maintained by controlling the T_g of the SFL powder composition. Therefore, the influence of moisture content, danazol potency, and excipient types on T_g of amorphous SFL powders was investigated in the study.

5.3.2.1. T_g and Excipient Type

The mDSC results (Table 5.3) showed that the T_g of SFL danazol, PVP K-15, and poloxamer 407 were 88°C, 146°C, and -21°C, respectively. The T_g of SFL danazol/PVP K-15 powder was 126°C, which was much higher than that of SFL danazol/poloxamer 407 (-5°C) powder. The different excipients greatly

impacted the T_g value of SFL powders. In addition, the mDSC results showed that both SFL danazol/PVP K-15 and danazol/poloxamer 407 had a single T_g intermediate to that of the pure components indicating that danazol was in a miscible state with these polymers (21, 25). Either the two components are thermodynamically miscible or the rapid freezing trapped the blend in a single T_g metastable state.

5.3.2.2. T_g and Danazol Potency

A recent study demonstrated that the SFL process produced rapid release high potency (33% to 91% w/w) SFL danazol powders for a danazol/PVP K-15 formulation (6). In the present study, the influence of danazol potency or likewise, the danazol/PVP K15 ratio, on T_g of SFL powders was investigated. The mDSC results demonstrated that as the danazol potency was increased from 33% to 91%, the T_g of SFL danazol/PVP K-15 powders decreased from 126 °C to 104 °C (Fig. 5.4), still 16 °C above that of pure danazol. These results indicated a concentration dependent T_g lying between the T_g of the danazol and PVP K-15. The Gordon Taylor equation (26) may be used to predict the dependence of T_g on the weight fractions, w_1 and w_2 , in a mixture

$$T_{g12} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \quad (1)$$

where the subscripts 1 and 2 refer to the API and polymer, respectively. The constant K , which is a measure of interaction between the API and polymer can be estimated using the Simha-Boyer equation (27),

$$K \approx \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \quad (2)$$

where ρ_1 and ρ_2 are the true densities of two components. Figure 5.4 shows that the measured T_g values of SFL danazol/PVP K-15 were higher than the T_g values predicted by Gordon Taylor equation, suggesting a positive deviation from ideal behavior. This positive deviation suggests that the API and polymer bind more strongly to each other than would be expected from the average of the pure component interactions. This positive deviation was present for all of potencies investigated.

5.3.2.3. T_g and Water Content

The residual water associated with amorphous pharmaceutical solids can have significant effects on their T_g (8, 16, 28). Water has a very low T_g of approximately -134°C (13). Uptake of water can plasticize the drug or polymer and greatly increase the free volume of the solid, leading to a decrease in T_g . The SFL danazol/PVP K-15 powders were exposed to $40^\circ\text{C}/75\%\text{RH}$, and the water content was determined as a function of exposed time. Table 5.4 shows that the T_g decreased from 127°C to 64°C as the moisture content increased from 1.48% to 11.38%. The initial moisture content was due to absorption of water vapor

from the surrounding atmosphere during the packing step and as a result of water vapor in the atmosphere in the lyophilization process. This large reduction in T_g indicates that these amorphous pharmaceutical solids should be stored with a desiccant.

5.3.3. Stability of SFL Powders

The current ICH guideline recommends long term stability testing will be conducted at 25°C/60%RH (ICH Guideline Q1A(R) condition. A stability study was conducted for the SFL danazol/PVP K-15 at these conditions for six months. Sample powders were stored in glass vials (30 mL) with aluminum-lined caps over a desiccant. The XRD, DSC, and *in vitro* dissolution rates for the amorphous pharmaceutical solids were used to check their stability. The X-ray diffraction pattern (Fig.5.5) of SFL danazol/PVP K-15 powders exhibited no change in peak intensity for danazol, suggesting that the danazol crystallinity was unchanged for six months. In addition, the DSC results showed there were no endotherms at 225°C, which was melting point of danazol. Furthermore, the T_g of six month sample was 117.41°C, which was similar to the T_g of initial SFL danazol/PVP K-15 powders (118.76°C). If the danazol crystallized, the mixture T_g would not have stayed constant. Thus, the DSC results indicated that danazol was amorphous after six months storage. Figure 5.6 demonstrates that the difference in the dissolution profiles between the initial and six-month samples was insignificant. The profiles were compared in terms of the similarity factor f_2

according to the method reported by Shah (29). The results of the XRD patterns, DSC, and dissolution rates indicate excellent stability for the amorphous SFL danazol/PVP K-15 powders at 25°C/60%RH for six months.

5.4. CONCLUSIONS

For the three polymeric excipients, the SFL process produced rapid dissolving amorphous danazol powders with small particle size, high surface area and enhanced wettability. A single T_g peak was observed for each system indicating a miscible blend. The danazol/PVP K-15 powders had the highest T_g values and were thus utilized for long term stability studies. The T_g of the SFL danazol/PVP K-15 powders remained high decreasing from 126 °C to 104 °C, as the danazol potency increased from 33% to 91%. The danazol/PVP K-15 powders stored with the desiccant at 25°C did not crystallize even after six months as demonstrated with XRD and mDSC measurements, and the dissolution rate remained high. The ability to stabilize nanostructured high surface area API powders in high T_g formulations offers great promise for the development of poorly water soluble drugs with high dissolution rates.

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CHAPTER 6: Rapid Release Tablet Formulation of Micronized Danazol Powder Produced by Spray Freezing into Liquid (SFL)

The objectives of this study were to prepare a rapid release tablet formulation containing SFL micronized powders by direct compression and to investigate the effects of SFL powder compositions and tableting excipient compositions on drug release.

6.1. INTRODUCTION

It is estimated that about 40% of compounds being developed by the pharmaceutical industry are poorly water soluble (1, 2). One limiting factor to *in vivo* performance of active pharmaceutical ingredients (APIs) with poor aqueous solubility after oral administration is slow dissolution rates (3). Danazol is classified by the Biopharmaceutics Classification System (BCS) as a class II API (4-6). It has a very low aqueous solubility but high permeability. The irregular and delayed absorption of danazol has been attributed to its slow dissolution rate (7, 8). Increasing the dissolution rate of poorly water soluble APIs is a major challenge in pharmaceutical development.

The spray freezing into liquid (SFL) particle engineering process was shown to be highly promising for enhancing the wetting and dissolution properties of poorly water soluble APIs (9-12). In the SFL process, a feed solution containing poorly water soluble API and/or excipient(s) is atomized directly into a cryogenic liquid to produce frozen particles. The frozen particles are then collected and lyophilized to obtain dry SFL micronized powder. The intense atomization and rapid freezing rate enable formation of highly porous nanostructured aggregates composed of amorphous API nanoparticles with very high surface areas (11 to 115 m²/g) and enhanced wettability (9, 13, 14). Greater than 95% of SFL micronized danazol powder dissolved in 2 minutes, a significant enhancement when compared to only 30% dissolved in 2 minutes for the micronized crystalline danazol (9, 14).

Although multiple routes of administration are available for poorly water soluble APIs, the most popular is the oral dosage form because of ease of manufacture, convenience of dosing, and high stability compared to liquid and semisolid dosage forms. Properties of SFL micronized danazol, such as high surface area, porosity and amorphous structure contribute significantly to enhanced dissolution. These properties will influence the process of incorporating the SFL powder into a tablet formulation as well as the tablet composition. Amorphous drugs can crystallize when exposed to heat and/or moisture during processing and storage. Manufacture of tablets by direct

compression is advantageous over wet granulation because of reduced processing steps and lack of exposure to heat and moisture (15). However, it has been reported that recrystallization of amorphous materials in a pharmaceutical operation like direct compression may occur due to mechanical stresses (16, 17). Amorphous solids must be stabilized to prevent crystallization during the tableting process.

Recently, Hu *et al.* reported that the stability of amorphous SFL micronized powder may be maintained by controlling the glass transition temperature (T_g) of the SFL composition (18). During direct compression (15), the particles first move to occupy the void space between each other after the tableting blends are filled into a die. When particles can no longer rearrange themselves, they will start to deform elastically (15). However, when the elastic limit of the excipients is exceeded, the particles will deform plastically or destructively. SFL micronized powder with such high surface area is very brittle. Directly compressible excipients will compress by plastic deformation. Therefore, successful development of rapid release tablets containing SFL micronized powders depends on achieving the right balance between brittle fracture from the SFL powders and plastic properties from the compressible excipients (15, 19).

The objective of this study was to incorporate SFL micronized powder into tablet formulations by direct compression and achieve rapid release and

acceptable mechanical properties. The hypothesis of this study was that the high dissolution rates of SFL micronized powder may be maintained after the blending and compression processes by optimizing the SFL powder composition and tablet excipient composition. A tablet formulation containing micronized crystalline danazol was used as the control.

6.2. MATERIALS AND METHODS

6.2.1. Chemicals

Danazol was obtained as micronized powder from Spectrum Quality Products Inc. (Gardena, CA). Polyvinylpyrrolidone (PVP) K-15, polyethylene-polypropylene glycol (Pluronic F127; poloxamer 407), sodium lauryl sulfate (SLS), tris(hydroxymethyl)aminomethane (Tris), and magnesium stearate were purchased from Spectrum Quality Products Inc. (Gardena, CA). Microcrystalline cellulose (Avicel[®] PH112) and croscarmellose sodium (Ac-Di-Sol[®]) were obtained from FMC BioPolymer (Newark, DE). Silicified microcrystalline cellulose (Prosolv[®] HD 90) and sodium starch glycolate (Explotab[®]) were purchased from Penwest Pharmaceuticals Co. (Patterson, NY). Lactose Monohydrate, spray dried was from Foremost (Baraboo, WI). Ludipress[®] was from BASF (Ludwigshafen, Germany). Hydroxypropyl cellulose (L-HPC 11) with a low degree of substitution was obtained from Shin-Etsu Chemical Co. Ltd (Niigata, Japan). Colloidal silicon dioxide (Cab-o-sil M5P) was obtained from

Cabot Corporation (Tuscola, IL). Acetonitrile and methylene chloride were obtained from EM Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore, Molsheim Cedex, France).

6.2.2. Preparation of SFL Danazol powder

Danazol and PVP K-15 with or without poloxamer 407 were dissolved in acetonitrile, and danazol/PVP K-15/SLS was dissolved in acetonitrile/water mixtures. These solutions were processed using the SFL process (9, 14). The SFL feed solution was placed into the solution cell. A constant pressure 2000 PSI from the ISCO syringe pump provided a spray flow rate (50 ml/min) for the SFL feed solution. The atomizing nozzle used was composed of polyetheretherketone (PEEK) tubing with an inner diameter of 127 μm . The SFL feed solutions were then sprayed through the nozzle and atomized into small droplets directly into the liquid N_2 phase. Frozen particles formed instantaneously and were collected and dried by a VirTis Advantage Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY). The SFL micronized powders were stored in glass vials over desiccant in a vacuum desiccator at room temperature before the characterization analysis.

6.2.3. Characterization of SFL Micronized Powders

6.2.3.1. Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using the $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powder was placed in a glass sample holder. Samples were scanned from 5° to 45° (2 θ) at a rate 0.05°/sec. For comparative purposes, the three highest values for relative line intensity and their corresponding line position 2 θ were compared for the micronized crystalline danazol (20).

6.2.3.2. Scanning Electron Microscopy (SEM)

A HITACHI S-4500 field emission scanning electron microscope (Hitachi Instruments Inc., Irvine, CA) was used to examine the surface morphology of each sample powder. The sample was fixed to a SEM stage with double-sided adhesive tape and gold sputter coated.

6.2.3.3. Surface Area Analysis

A Nova 3000 surface area analyzer (Quantachrome Corporation, Boynton Beach, FL) was used to determine N₂ sorption at 77.40 deg K. The surface area per unit powder mass was calculated from the fit of adsorption data to Brunauer, Emmett, and Teller (BET) equation (21).

6.2.3.4. Contact Angle Measurement

Compact disks of sample powder were prepared at a 500 kg compression force using a Carver Laboratory Press (model M, ISI Inc., Round Rock, TX) with flat-faced 6 mm diameter punches. A droplet of SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%, 3 μ l) was placed onto the surface of the disk and observed using a low power microscope. The contact angle was determined by measuring the tangent to the curve of the droplet on the surface of the disk using a Goniometer (Model No. 100-00-115, Ramè-Hart Inc., Mountain Lakes, NJ).

6.2.4 Direct Compression and Tablet Characterization

Tablets containing SFL micronized powder were prepared by direct compression. Tablets containing micronized crystalline danazol were used as the control. Five tablet formulations containing SFL micronized powder were investigated as described in Table 1. First, all excipients were sieved through a 60 mesh screen. SFL micronized powder was then mixed with excipients (except for magnesium stearate) for 15 min. Magnesium stearate 0.5% (w/w) was added and blended for an additional one minute and 30 seconds. All mixtures were prepared by geometric dilution in a V-blender (Patterson Kelley, east Stroudsburg, PA). The tablet blends were compressed on a single-punch tableting machine (Steker-F, FJStrokes, Philadelphia, PA), fitted with 9 mm shallow concave punches. The total weight of the tablet was 200 mg, which contained 8% SFL micronized

powder. The average tablet weight and hardness were determined from 10 individually weighed tablets.

Tablet friability was calculated as the percentage weight loss of 30 tablets after 100 rotations in a friabilator (Vanderkamp, Van-kel Industrial Inc., Chatham, NJ). The disintegration time ($n=6$) was determined (Vanderkamp, Van-kel Industrial Inc., Chatham, NJ) in SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%) at 37°C using disks. The crystallinity of danazol in the tablets was determined by differential scanning calorimetry (DSC). Aliquots of grinded tablet powder weighing between 5 to 20 mg were placed in an aluminum pan (Kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT) and crimped with an aluminum lid. The samples were heated from 20°C to 300°C at a heating rate 10°C/min under dry nitrogen gas purge (22-24).

6.2.5. Dissolution Studies

The amount of danazol dissolved as a function of time was determined using USP Apparatus 2 (Vankel 7000, Vankel Technology Group, Cary, NC). All dissolution testing ($n=3$) was conducted at sink conditions. SFL micronized powder and tablets containing approximately 12 mg danazol were added to 900 mL of SLS/Tris dissolution media (SLS 0.75%/Tris 1.21%) at 37°C. The paddle speed was 50 rpm. A 5 mL aliquot was taken at each time point without volume replacement and filtered through a 0.45 μ m filter, then diluted with acetonitrile, filtered again, and analyzed by HPLC (9).

6.2.6. Statistical Analysis

The data were compared using a one way analysis of variance (anova). The significance level ($\alpha = 0.05$) was based on the 95% probability value ($p < 0.05$).

6.3. RESULTS AND DISCUSSION

6.3.1 Physicochemical Properties of SFL micronized powder

6.3.1.1. Crystallinity

The crystallinity of danazol in the SFL micronized powder was determined by powder X-ray diffraction. Micronized crystalline danazol (Fig. 1) had a similar X-ray diffraction pattern to that reported by Liversidge and Cundy (25). The high peak intensities of danazol indicated a high degree of crystallinity. However, the characteristic diffraction peaks of danazol at 15.8, 17.2 and 19.0 (2 θ) degrees were not evident in the SFL micronized powders, indicating that danazol contained in SFL danazol/PVP K-15 powder was amorphous. Similarly, a lack of crystallinity was also found for the SFL danazol/PVP K-15/poloxamer 407 and danazol/PVP K-15/SLS powders. XRD results showed that danazol was amorphous regardless of excipient type used in the SFL composition. The rapid freezing rates, achieved by atomizing the feed solution directly into liquid

nitrogen during the SFL process trapped the danazol in an amorphous state without allowing time for crystallization.

6.3.1.2. Surface Area and Surface Morphology

The surface area of the SFL micronized powders is listed in Table 2. The surface areas of the SFL micronized powder were 79.9 m²/g, 48.0 m²/g, and 30.0 m²/g for danazol/PVP K-15, danazol/PVP K-15/SLS, and danazol/PVP K-15/poloxamer 407, respectively. All surface areas of SFL danazol/PVP K-15 powder were higher than that of the micronized crystalline danazol (0.52 m²/g) (14). SEM was used to further investigate the influence of excipients on the morphology of SFL micronized powders. The SEM micrograph of bulk danazol (Fig. 2A) revealed large crystalline plates with fractured edges. In contrast, the SEM micrograph (Fig. 2B) indicated that the SFL danazol/PVP K-15 particle was an aggregate with a geometric diameter of about 1.5 μm. This aggregate had a highly porous morphology. The SEM micrograph of the SFL danazol/PVP K-15/SLS (Fig. 2C) fine powder indicated a porous aggregate of microparticles with a geometric diameter of about 5 μm. SFL danazol /PVP K-15/poloxamer 407 particles (Fig.2D) were larger and less porous relative to danazol/PVP K-15 and danazol/PVP K-15/SLS particles.

Several factors contributed to the high surface areas and porosities of the nanostructured aggregates produced by the SFL process (9, 26, 27). An high

degree of atomization is achieved in the SFL process leading to the formation of high-surface area microdroplets. The freezing rate of the acetonitrile solution was very rapid due to the low temperature of liquid N₂ (-196°C) and the temperature difference between the liquid N₂ and SFL feed solution (25°C). The formation of nuclei and their growth rate depend on the freezing rate of the solution. The rapid freezing rate resulting from the small droplet size limited the propensity for particle growth. In addition, the dried particles retained the shape of the small droplets after lyophilization. Their porous structures were due to the channels created as the solvent(s) was removed during sublimation. As a result, high surface areas and porosities of SFL micronized powder were obtained by the SFL process.

6.3.1.3. Wettability

The available surface area also depends on the ability of the dissolution media to wet the particle surface. The wettability of SFL micronized powder was determined from the contact angle of dissolution media on the compacted surface of SFL micronized powders. The contact angles (Table 2) of SFL danazol/PVP K-15, danazol/PVP K-15/SLS, and danazol/PVP K-15/poloxamer 407 were 24, 23, 29 degrees, respectively. The significant ($P < 0.05$) reduction of contact angle for SFL micronized powder compared to the danazol control (57 degrees) indicated a more hydrophilic solid surface (14), due to the blending with hydrophilic excipient during the SFL process (18). The formation of hydrophilic

surfaces occurs during the SFL process. In addition, the rough surface for the SFL micronized powder, as indicated in the SEM micrographs will decrease the contact angle (9). In summary, the hydrophilic surface and surface roughness lowered the contact angle and enhanced wettability of the SFL micronized powder relative to the control.

6.3.1.4. SFL Micronized Powder Dissolution

The profiles presented in Figure 3 illustrate the dissolution of the SFL micronized powder and micronized crystalline danazol (n=3). The rate of dissolution of micronized crystalline danazol was slow; only 55% of the danazol dissolved in 5 minutes. However, significant ($P<0.05$) improvements in the dissolution rates were found for SFL micronized powder compared to the controls (Fig.3). For example, 99% danazol was dissolved in 5 minutes from the SFL danazol/PVP K-15 powder, a significant enhancement when compared to only 55% for the micronized crystalline danazol in the same time period ($P<0.05$). The dissolution rates of SFL micronized powders prepared with different excipients were similar in 10 minutes. Each of the excipient combinations produced extremely rapid dissolution rates for SFL micronized powders. The increased dissolution rate of the SFL micronized powder may be attributed to the amorphous danazol morphology, reduced particle size and increased surface area, wettability and porosity.

6.3.2. Rapid Release Tablet Formulation Containing SFL Micronized Powder

6.3.2.1. Influence of Excipient Type on Dissolution of Tablet

The literature reported that high porosity fast wetting powders are ideal for the rapid release oral tablets (28, 29). Therefore, the first aim of this study was to identify tablet excipient compositions with acceptable strength that would enable rapid tablet disintegration and dissolve of danazol. Five tablet formulations containing SFL danazol/PVP K-15 (2:1) powders were investigated (Table 1). The physical properties of the tablets containing SFL danazol/PVP K-15 powder are listed in Table 3. All tablet formulations had disintegration times between 20 to 40 seconds, indicating that the tablets disintegrated rapidly after they contacted the dissolution media. The hardness of the tablets ranged from 3.78 to 5.58 Kg. All tablets showed a low friability as a result of their high compressibility.

The profiles presented in Figure 4 illustrate the dissolution of tablets containing SFL danazol/PVP K-15 with different direct compression excipients. The dissolution rates of tablet formulations A and B were slow; only 55% of the danazol was dissolved in 5 minutes. The use of Ludipress[®] in formulation C increased the dissolution rate considerably, with 67% danazol dissolved in 5 minutes. However, the amount dissolved reached 75% in only 5 minutes for formulation D. The improvement in the dissolution rate for tablet formulation D

was attributed to using microcrystalline cellulose as a main filler-binder compared to formulations A, B and C, which used lactose monohydrate (30-32). Microcrystalline cellulose deforms plastically during compaction as demonstrated by excellent compatibility at low compression pressures (15). In contrast, lactose monohydrate is brittle and the particles are fragmented during compaction (33). High compression pressure is required to produce hard tablets. Therefore, microcrystalline cellulose was a better carrier for high surface SFL powders compared to lactose. In addition, because microcrystalline cellulose is composed of porous particles (15), the SFL particles may be trapped inside of the void spaces during the mixing step further preventing SFL particle deformation during the compression step (34). In formulation E, silicified microcrystalline cellulose was used as the filler-binder and the dissolution rate of tablet was similar to the tablet formulation D. Silicified microcrystalline cellulose is composed of 98% microcrystalline cellulose that is silicified with 2% colloidal silicone dioxide. The rapid release of danazol from tablet formulation E confirmed the result in formulation D that microcrystalline cellulose was an excellent carrier for high surface SFL micronized powders.

6.3.2.2. Influence of SFL Powder Composition on Dissolution of Tablet

Danazol contained in tablet formulation E dissolved the fastest in the five tablet formulations investigated. Therefore, this formulation was chosen for additional experiments to identify the most rapidly dissolving SFL powder

composition. From Table 3, it can be seen that desirable mechanical properties were attained for each tablet formulation containing the SFL micronized powders investigated. Among the three SFL powder compositions, tablet formulation E (SFL danazol/PVP K-15/SLS powder) displayed the most rapid dissolution rate, (Figure 5), 94% of the danazol was dissolved in only 10 minutes. This dissolution profile was similar to the one observed for the corresponding SFL micronized powder prior to tableting (Figure 6). In contrast, the dissolution rate of danazol from tablet formulation E containing SFL danazol/PVP K-15 powder was lower than that of SFL danazol/PVP K-15 powder (Figure 4). In this case, it is likely that the surface area of SFL danazol/PVP K-15 powder available for wetting was reduced during the compression process thus lowering the dissolution rate. With the addition of a wetting agent, SLS, into the SFL powder composition, the dissolution of danazol from the tablet was similar to the SFL powder. The enhanced wettability of SFL danazol/PVP K-15/SLS powder appeared to compensate for any loss of surface area on compression. Therefore, after tablets disintegrated, SFL particles were rapidly wetted and the danazol dissolved rapidly in the dissolution media.

In order to further attempt to enhance wetting, another surfactant, poloxamer 407, was added into the SFL powder formulation. Although SFL danazol/PVP K-15/poloxamer 407 powders displayed rapid dissolution profiles compared to SFL danazol/PVP K15/SLS powders (Fig. 3), the dissolution rate of

the tablet containing the SFL danazol/PVP K-15/poloxamer 407 powders was much lower compared to the SFL powder prior to tableting (Fig. 5). Whereas the addition of SLS enhanced dissolution of danazol from the tablets, poloxamer 407 did not. Further investigation was conducted to explain this difference. The DSC results (Fig. 7) indicated that the danazol crystallized in the tablet containing SFL danazol/PVP K-15/poloxamer 407 powders. However, the danazol in the tablet containing SFL danazol/PVP K-15/SLS powder was still amorphous.

Because the amorphous form of a pharmaceutical solid has a higher chemical potential than the thermodynamically stable crystalline form, it can exhibit enhanced dissolution rates and bioavailability. A key challenge is to stabilize the amorphous solid to prevent crystallization during process. T_g of an amorphous pharmaceutical solid can have a large influence on its physicochemical stability. The molecular mobility of a high energy amorphous API in a solid mixture with an excipient decreases when the storage or processing temperature is reduced below T_g . This loss in molecular mobility and diffusion retards nucleation and growth of crystalline domains. It has been reported that certain amorphous pharmaceutical solids stored or processed at 20°C to 50°C below T_g were stable against crystallization (35, 36). The increased crystallinity of danazol in the tablet was attributed to the relatively low T_g (48°C) of SFL danazol/PVP K-15/poloxamer 407 powder, which was much lower than the T_g of SFL danazol/PVP K-15 /SLS powder (142°C). The danazol crystallized due to

the mechanical stress during the tableting process (35, 37) and this decreased the dissolution rate of danazol from tablet. Therefore, addition of a high T_g surfactant to the SFL powder composition can prevent recrystallization of danazol during processing and enhance the release of danazol from tablets. A high T_g excipient in SFL powder compositions is another critical parameter for preventing crystallization in formulating rapid release tablets containing SFL micronized powders.

6.4. CONCLUSIONS

A rapid release tablet formulation containing SFL danazol/PVP K-15/SLS powder was developed successfully. The dissolution rate of 94% danazol in only 10 minutes from the tablets approached the rate for the raw powder. Direct compression tablet formulations with desirable mechanical properties and rapid release profiles were achieved by formulating the SFL powder composition to include a high T_g excipient to prevent crystallization and also optimizing tablet excipient composition. The ability to maintain rapid dissolution rates of SFL micronized powders in tablet formulation offers great promise for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs for oral delivery systems.

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CHAPTER 7: Summary and Conclusion

This dissertation presented five studies to develop and optimize the SFL process in order to achieve application in drug delivery system. In the studies, it is observed that the SFL process offers advantages over current particle formation techniques. The advantages of the SFL process result from intense atomization in conjunction with rapid freezing rates. Because liquid-liquid impingement occurs between the pressurized feed solution exiting the nozzle and liquid nitrogen, the highest degree of atomization is achieved by spraying directly into the cryogenic liquid as opposed to spraying into the vapor phase above the cryogenic liquid. Ultra-rapid freezing rates are achieved because of the low intrinsic temperature of liquid nitrogen and the formation of high-surface area droplets. The ultra-rapid freezing rates prevent the phase separation of solutes within the feed solution and induce formation of amorphous structures. The high degree of atomization and ultra-rapid freezing rate achieved with the SFL process led to amorphous nanostructured particles with high surface areas and enhanced wetting.

In the study of Chapter 2, the SFL process was demonstrated to produce free flowing powder of nanostructured particles containing poorly water soluble APIs, danazol or carbamazepine. The X-ray diffraction pattern indicated that SFL powders were amorphous. SEM micrographs indicated that SFL particles were

highly porous. The mean particle diameter of SFL carbamazepine/SLS powder was about 7 μm . The dissolution of SFL danazol/poloxamer 407 powder at 10 minutes was about 99%. The SFL powders exhibited significantly enhanced dissolution rates compared to the powders formed by the conventional lyophilization process. In addition, an amorphous structure, high surface area and increased wettability of the flowable SFL powders are predominant characteristics to enhance the dissolution of poorly water soluble APIs.

In the study of Chapter 3, the SFL acetonitrile system was demonstrated to produce nanostructured particles, which have similar characteristics to the particles prepared by the SFL THF/water co-solvent system. The CBZ loading in the feed solution of the SFL acetonitrile system was 2.2% (w/w), which was greater than 0.22% (w/w) loading of the THF/water co-solvent system. The SFL powders from both systems exhibited similar and significantly enhanced dissolution rates compared to the bulk CBZ. The SFL acetonitrile system offers several advantages versus SFL THF/water co-solvent system. Using acetonitrile greatly increased CBZ loading in the SFL feed solution and decreased drying time in the SFL process. All of these attributes have a positive effect on the scale-up of the SFL process in the product manufacture.

The study in Chapter 4 demonstrated that rapid dissolving SFL danazol/PVP K-15 powders with high potency (up to 91%) have been produced by SFL with an organic solvent mixture. The high potency SFL powders

contained amorphous nanostructured aggregates with high surface area and excellent wettability. In only 5 minutes, 99% of the danazol dissolved. The composition of surfactant in the product could be controlled simply by the feed composition, as all of the surfactant precipitated with the API. In SFL, solvent mixtures may be formulated to achieve high solubility of API in order to produce high potency powders. Furthermore, the polar solvent mixture in the continuous phase favors migration of the polar surfactant to the surface of the particles during particle formation. The coating with polar surfactant favors wetting of the high surface area during dissolution.

The study in Chapter 5 was to stabilize the amorphous solid to prevent crystallization during storage. A single T_g peak was observed for each system indicating a miscible blend. The danazol/PVP K-15 powders had the highest T_g values and were thus utilized for long term stability studies. The T_g of the SFL danazol/PVP K-15 powders remained high decreasing from 126 °C to 104 °C, as the danazol potency increased from 33% to 91%. The danazol/PVP K-15 powders stored with the desiccant at 25°C did not crystallize even after six months as demonstrated with XRD and mDSC measurements, and the dissolution rate remained high. The ability to stabilize nanostructured high surface area API powders in high T_g formulations offers great promise for the development of poorly water soluble drugs with high dissolution rates.

The study in Chapter 6 demonstrated that a rapid release tablet formulation containing SFL micronized powder was successfully developed. About 94% danazol was dissolved in only 10 minutes from tablets containing SFL micronized danazol/PVP K-15/SLS powder, which was similar to the dissolution rate of SFL danazol/PVP K-15/SLS powder. Direct compression tablet formulation with desirable mechanical properties and rapid release profile was achieved by optimizing the SFL powder composition to include a high T_g excipient and tablet excipient type.

In summary, these studies have extended and enhanced the SFL process and its application. These studies have demonstrated that the SFL process offers a highly effective approach to produce high potency nanoparticles contained in larger structured aggregates with rapid dissolution rates for poorly water soluble APIs. The ability to stabilize nanostructured high surface area API powders in high T_g formulations and the ability to maintain rapid dissolution rate of SFL micronized powders in the tablet formulation offers great promise for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs. In conclusion, the SFL process is an effective particle engineering process for pharmaceutical development and manufacturing to improve dissolution rates of poorly water soluble APIs.

Table 2.1. Effect of composition and process on the particle size distribution.

Sample	D10	D50	D90	Span Index
Bulk Danazol	7.14	18.27	29.56	1.23
Slowly Frozen Control	7.27	18.86	31.13	1.27
SFL Danazol/Poloxamer 407	1.54	7.10	10.20	1.22
Bulk Carbamazepine	15.56	39.70	132.33	2.94
Slowly Frozen Control	4.58	26.71	46.74	1.58
SFL Carbamazepine/SLS	1.33	7.11	10.61	1.31

Table 2.2. Effect of composition and process on the contact angle of danazol and carbamazepine powders.

Samples	Contact Angle Degrees (FeSSIF Media)
PM Danazol/Poloxamer 407	58
LM Danazol/Poloxamer 407	34
SFL Danazol	55
SFL Danazol/Poloxamer 407	25
	Contact Angle Degrees (Purified Water)
PM Carbamazepine/SLS	52
LM Carbamazepine/SLS	29
SFL Carbamazepine	45
SFL Carbamazepine/SLS	24

Table 3.1 Comparison of the processing parameters of two SFL systems.

Parameters	SFL Acetonitrile Solution Containing CBZ	SFL THF/Water Solution Containing CBZ
Nozzle diameter	63.5 μm	63.5 μm
Processing pressure	2000 PSI	4000PSI
Spray flow rate	20 mL/min	11 mL/min
Drying time	15 hours	72 hours
CBZ loading in feed solution	2.2% w/w	0.22% w/w

Table 3.2 Effect of loading degree and process on the particles size and surface area.

Samples	M10 (μm)	M50 (μm)	M90 (μm)	Surface area (m^2/g)
SFL THF/water Solution Containing 0.22% CBZ	0.24	7.06	57.46	13.31
SFL Acetonitrile Solution Containing 0.22% CBZ	0.14	0.68	15.89	12.89
SFL Acetonitrile Solution Containing 2.2% CBZ	4.09	14.11	25.12	3.88
Slowly Frozen Control	6.71	22.05	48.12	1.84

Table 3.3 Effect of loading degree and process on the contact angle.

Sample	Contact Angle (Degrees)
SFL CBZ	45 ± 3.4
Slowly Frozen Control-Acetonitrile	43 ± 2.6
SFL Acetonitrile Solution Containing 0.22% CBZ	34 ± 1.5
SFL Acetonitrile Solution Containing 2.2% CBZ	38 ± 2.5
SFL THF/water Solution Containing 0.22% CBZ	29 ± 1.5

Table 4.1. SFL Danazol/PVP K-15 concentrations in the feed solution to produce powders with differing danazol potency.

Danazol/PVP K-15 Ratio (w/w)	Danazol	PVP K-15	Acetonitrile	Methylenechloride	Potency	% Danazol Loading in Feed Solution (w/w)
1:2	0.2g	0.4 g	70 ml	—	33%	0.36
1:1	0.2g	0.2 g	70 ml	—	50%	0.36
2:1	0.4 g	0.2 g	70 ml	—	66%	0.72
3:1	0.6g	0.2 g	65 ml	5 ml	75%	1.03
10:1	1 g	0.1 g	55 ml	15 ml	91%	1.63

Table 4.2. Solubility of danazol in different solvents at 37°C.

Solvent	Solubility of danazol
Purified water	0.97 ug/ml
THF/Water (33%w/w)	0.71 mg/ml
Acetonitrile	21.36 mg/ml
Methylene chloride	92.18 mg/ml
SLS/Tris Dissolution Media	0.15 mg/mL

Table 4.3. Effect of potency on surface area of SFL danazol/PVP K-15.

Samples	Surface Area (m ² /g)
Danazol/PVP K15 (50% Potency)	115.52
Danazol/PVP K15 (66% Potency)	79.88
Danazol/PVP K15 (75% Potency)	68.88
Danazol/PVP K15 (91% Potency)	28.50
Bulk Danazol	0.52

Table 4.4. Effect of potency on the contact angle of SFL danazol/PVP K-15.

Samples	Contact Angle (degrees)
Danazol/PVP K-15 (50% Potency)	22 (0.5)
Danazol/PVP K-15 (66% Potency)	24 (0.4)
Danazol/PVP K-15 (75% Potency)	27(1.0)
Danazol/PVP K-15 (91% Potency)	35 (2.0)
SFL Danazol	57 (2.2)

Table 5.1. Effect of excipients on particle size distribution of SFL powders.

Samples	M10 (μm)	M50 (μm)	M90 (μm)
Bulk Danazol	7.42	23.42	51.7
SFL Danazol/PVP K-15	0.15	1.18	22.88
SFL Danazol/PEG 8000	1.08	6.52	26.46
SFL Danazol/Poloxamer 407	1.7	8.68	20.35

Table 5 2. Effect of excipients on surface area and wettability of SFL powders.

Samples	Surface Area (m²/g)	Contact Angle (Degrees)
Bulk Danazol	0.5	57.0
Danazol/Poloxamer 407	5.5	39.3
Danazol/PEG 8000	12.0	41.0
Danazol/PVP K-15	89.8	21.7

Table 5.3. Effect of excipients on T_g of SFL powders.

Samples	T_g ($^{\circ}\text{C}$)
SFL Danazol	88.34
SFL PVP	146.13
SFL Danazol/PVP (1:2)	126.87
SFL Poloxamer 407	-21.21
SFL Danazol/Poloxamer 407 (1:2)	-5.71

Table5. 4. Effect of moisture content on T_g of SFL danazol/PVP K-15.

Moisture (w/w %)	T_g (°C)
1.48	126.87
5.21	108.68
8.13	96.58
11.38	64.00

Table 6.1 The formulations of tablet containing SFL micronized powder.

Tablet Formulation	A	B	C	D	E
Avicel (PH112)	31.0%	31.0%	31.0%	55.0%	---
Prosolv (HD 90)	---	---	---	---	86.0%
Lactose Monohydrate	55.0%	55.0%	---	31.0%	---
Ludipress	---	---	55.0%	---	---
L-HPC	5.0%	---	5.0%	---	---
Ac-Di-Sol	---	5.0%	---	---	---
Explotab	---	---	---	4.5%	5.0%
Sodium Laury Sulfate	---	---	---	0.5%	0.5%
Cab-o-sil (M5P)	0.5%	0.5%	0.5%	0.5%	---
Magnesium Stearate	0.5%	0.5%	0.5%	0.5%	0.5%
SFL Micronized Powder	8.0%	8.0%	8.0%	8.0%	8.0%

Table 6.2. Effect of excipients on surface area and wettability of SFL micronized powder.

Samples	Surface Area m²/g	Contact Angle (Degrees)
Danazol/PVP K-15 (2:1)	79.9	24
Danazol/PVP K-15/SLS (4:1:1)	48.0	23
Danazol/PVP K-15/Poloxamer 407 (4:1:1)	30.0	29

Table 6.3. Physical properties of tablet containing SFL micronized powders.

Formulations	Weight (mg)	Hardness (Kg)	Disintegration Time (sec)	Friability
SFL Danazol/PVP K-15 (2:1) Tablet-A	200.11	5.58	31	0.03
SFL Danazol/PVP K-15 (2:1) Tablet B	199.74	4.51	23	0.21
SFL Danazol/PVP K-15 (2/1) Tablet C	199.86	4.65	27	0.26
SFL Danazol/PVP K-15 (2:1) Tablet D	200.75	4.99	25	0.15
SFL Danazol/PVP K-15 (2:1) Tablet E	199.86	4.72	30	0.1
SFL Danazol/PVP K-15/SLS (4:1:1) Tablet-E	199.80	4.15	23	0.26
SFL Danazol/PVP K-15 /Poloxamer 407 (4:1:1) Tablet-E	199.92	4.13	30	0.33
Bulk Danazol Tablet-E	200.10	4.30	33	0.14

Table A1 The formulations of capsule containing SFL micronized powder

Capsule Composition	w/w
Lactose Monohydrate	45.0%
Sodium starch glycolate	4.0%
Car-o-sil (M5P)	1.00%
SFL CBZ/PVP K-15/poloxamer 407 Powder	50%

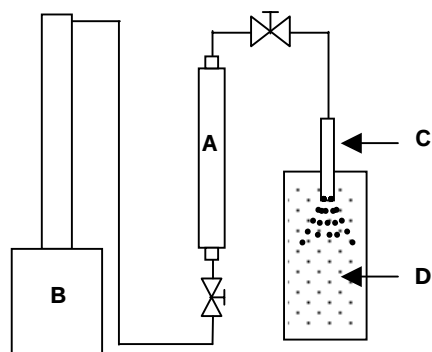


Fig. 2.1. Schematic diagram of the SFL apparatus illustrating the solution cell (A), high pressure pump (B), atomizing nozzle (C) and the cryogenic liquid cell (D).

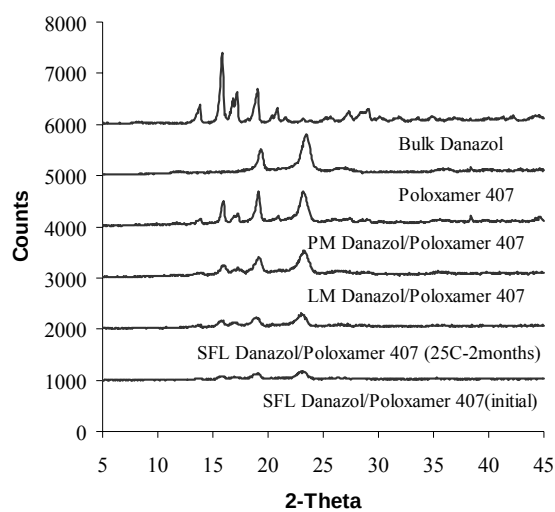


Fig. 2.2 Powder X-ray diffraction patterns of micronized danazol, PM danazol/poloxamer 407, LM danazol/Poloxamer 407 and SFL danazol/poloxamer 407.

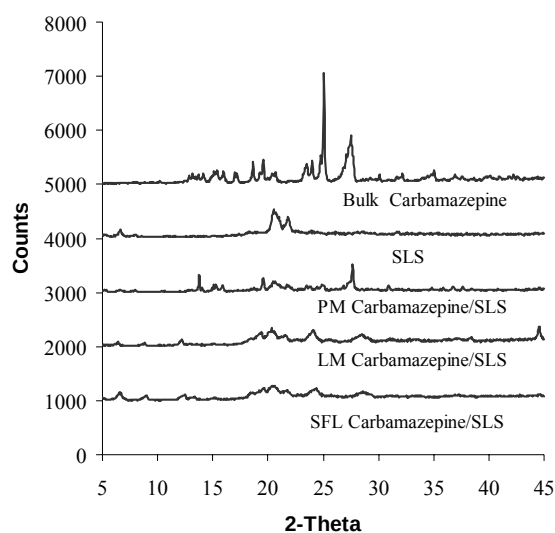


Fig. 2.3. Powder X-ray diffraction patterns of micronized carbamazepine, PM carbamazepine/SLS, LM carbamazepine/SLS and SFL carbamazepine/SLS.

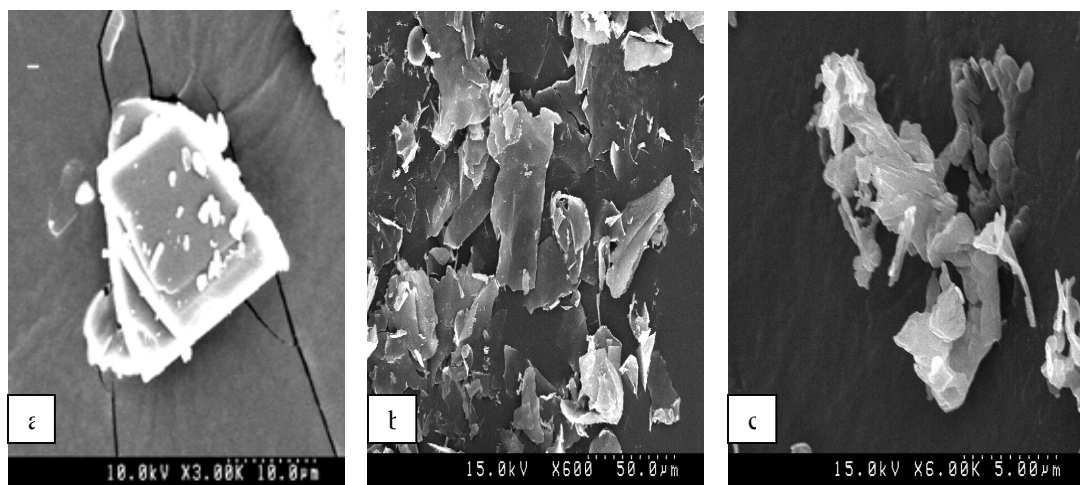


Fig. 2.4. SEM micrographs of micronized danazol control (Fig.4a), LM danazol/poloxamer 407 (Fig. 4b) and SFL danazol/poloxamer 407 (Fig.4c).

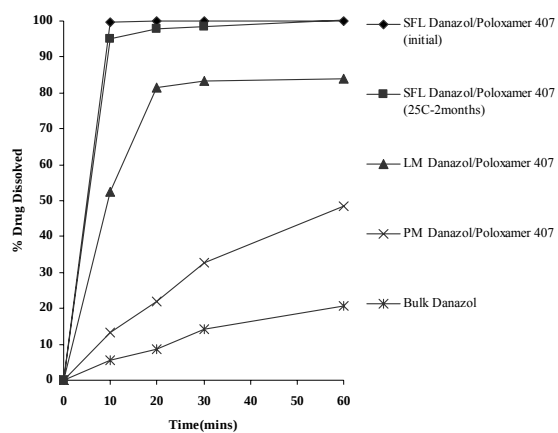


Fig. 2.5. Dissolution profiles of micronized danazol, PM danazol/poloxamer 407, LM danazol/poloxamer 407 and SFL danazol/poloxamer 407.

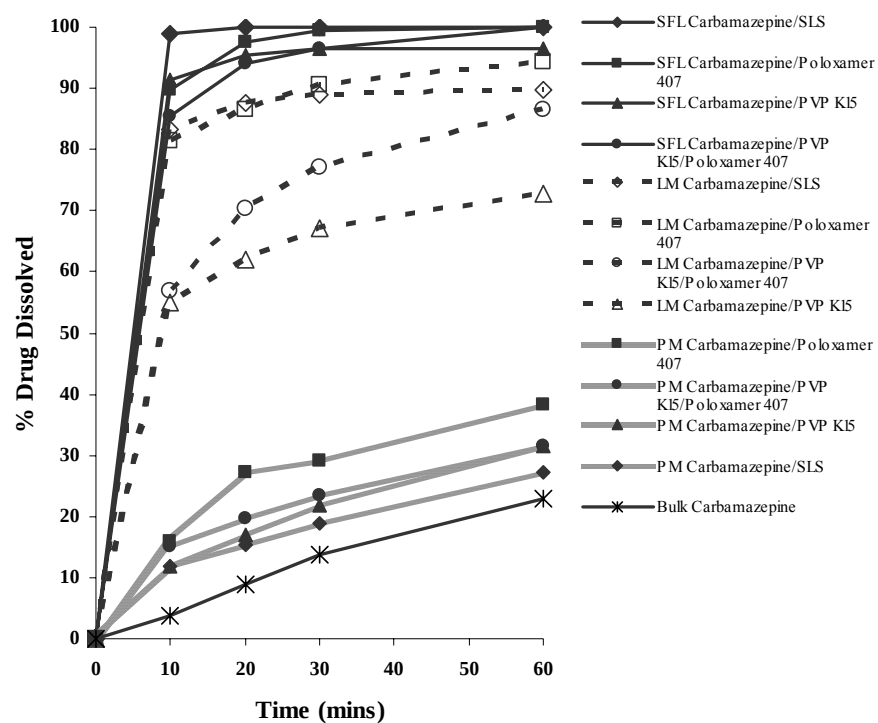


Fig. 2.6. Dissolution profiles of SFL carbamazepine/SLS, SFL carbamazepine/poloxamer 407, SFL carbamazepine/PVP K15, SFL carbamazepine/poloxamer 407/PVP K15 and their respective controls.

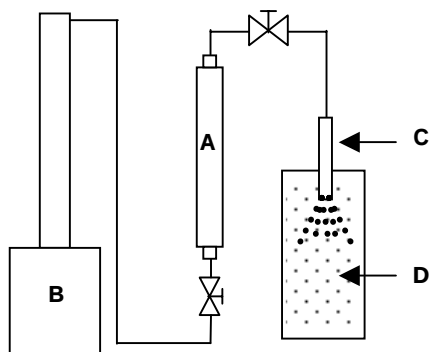


Fig. 3.1. Schematic diagram of the SFL apparatus illustrating the solution cell (A), high pressure pump (B), atomizing nozzle (C) and the cryogenic liquid cell (D).

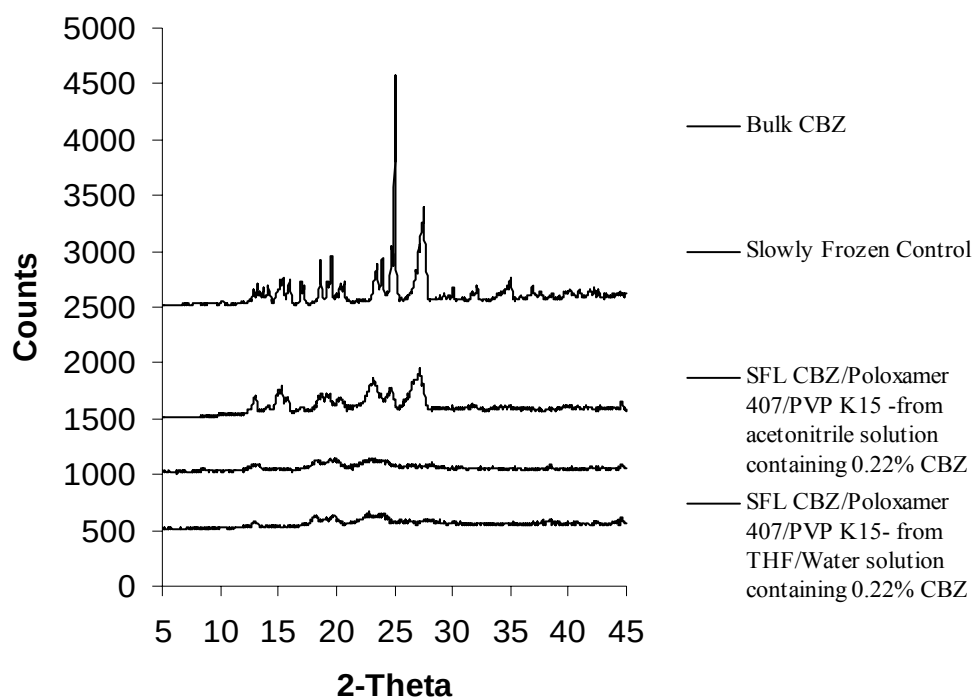


Fig. 3.2. Powder X-ray diffraction patterns of SFL CBZ/Poloxamer 407/PVP K15 powders from two system and their controls.

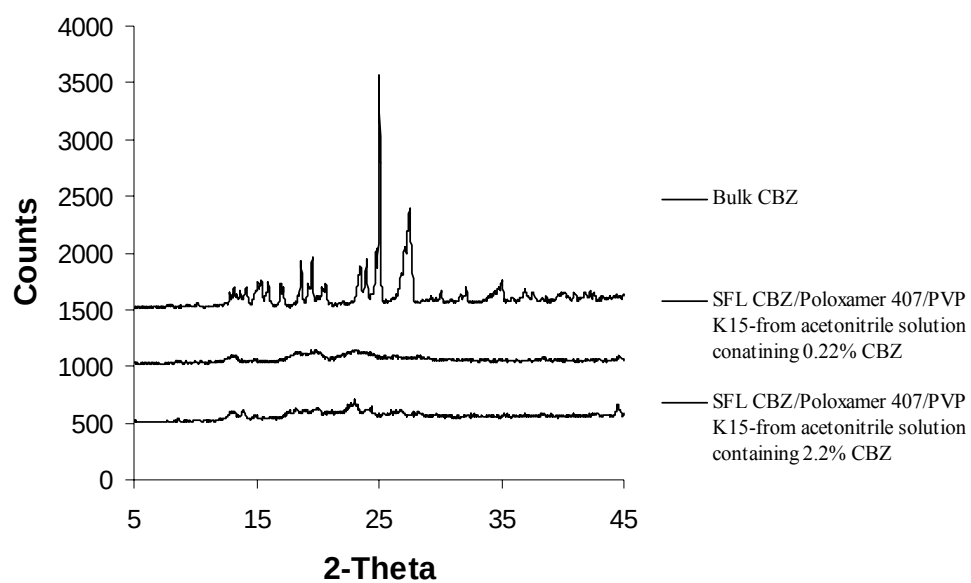


Fig. 3.3. Powder X-ray diffraction patterns of SFL CBZ powders with different loading degree and bulk CBZ.

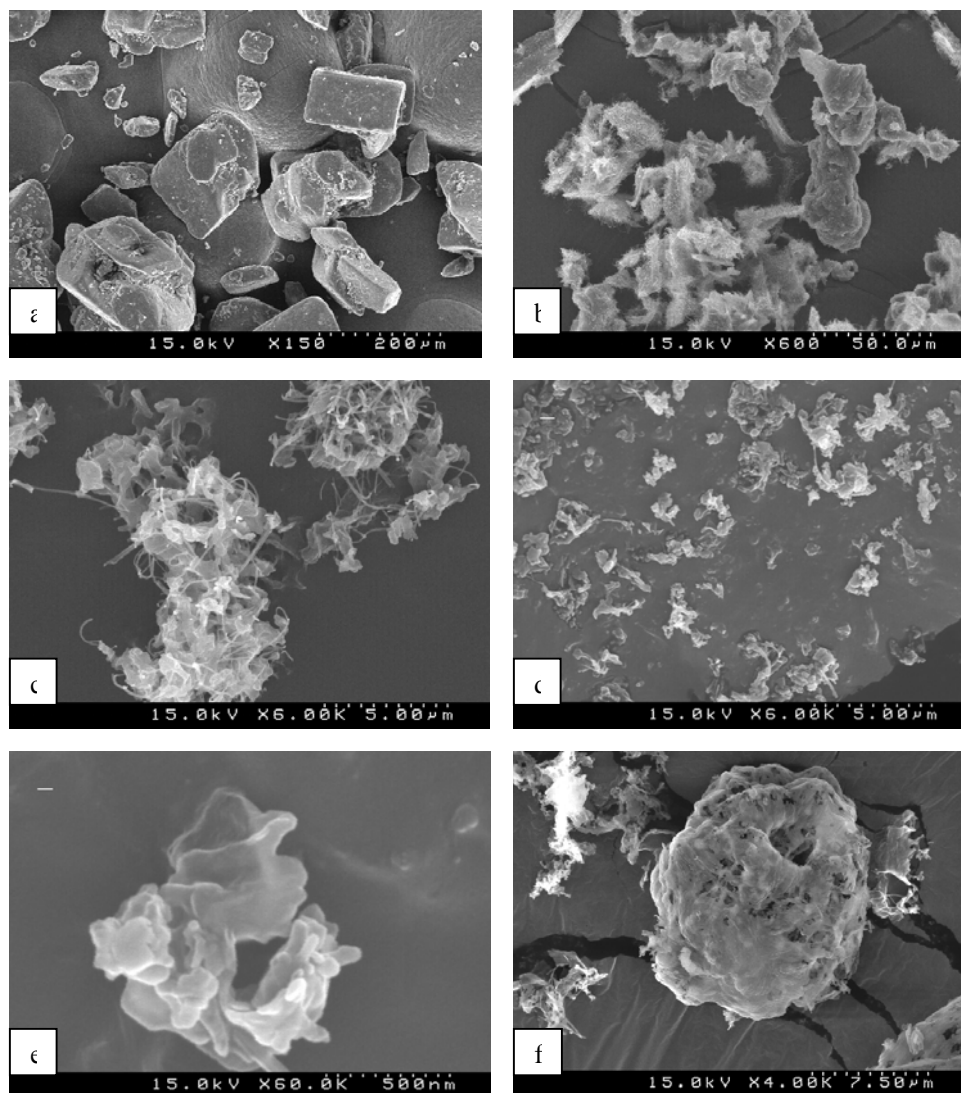


Fig. 3.4. SEM micrographs of bulk CBZ (Fig.3.4a), slowly frozen control CBZ/poloxamer 407/PVP K15 (Fig. 3.4b), SFL CBZ/poloxamer 407/PVP K15 from THF/water solution containing 0.22% CBZ (Fig.3.4c), SFL CBZ/poloxamer 407/PVP K15 from acetonitrile solution containing 0.22% CBZ (Fig.3.4d), SFL CBZ/poloxamer 407/PVP K15 from acetonitrile solution containing 0.22% CBZ (Fig.3.4e), SFL CBZ/poloxamer 407/PVP K15 from acetonitrile solution containing 2.2% CBZ (Fig.3.4f).

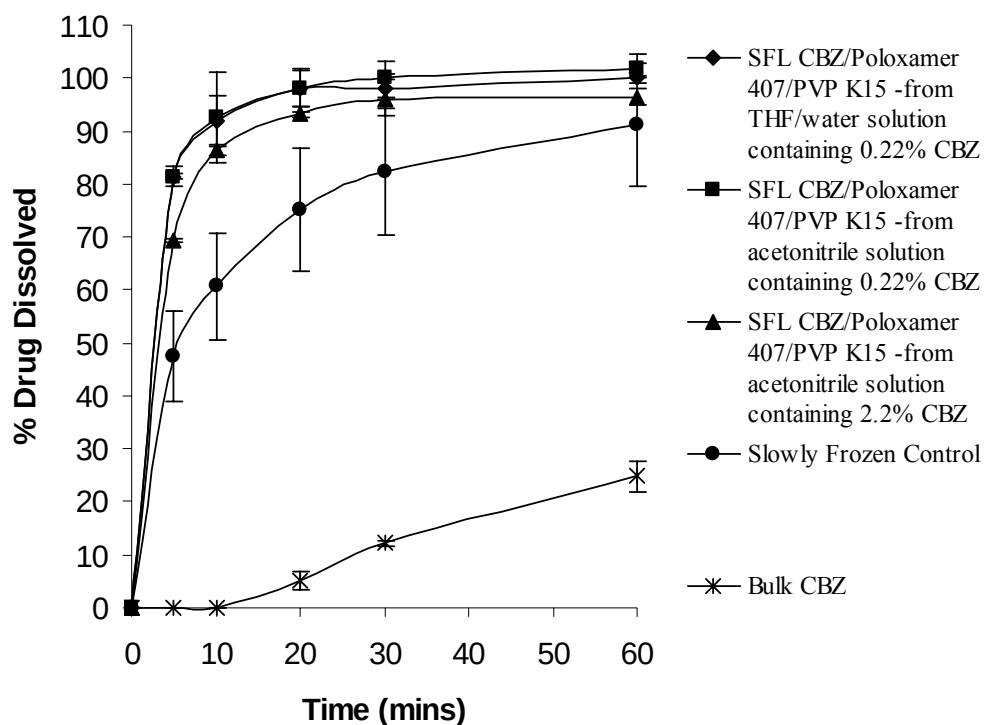


Fig. 3.5. Dissolution profiles of bulk CBZ, slowly frozen control, SFL CBZ/poloxamer 407/PVP K15 from THF/water solution containing 0.22% CBZ, SFL CBZ/poloxamer 407/PVP K15 from acetonitrile solution containing 0.22% CBZ, and SFL CBZ/poloxamer 407/PVP K15 from acetonitrile solution containing 2.2% CBZ.

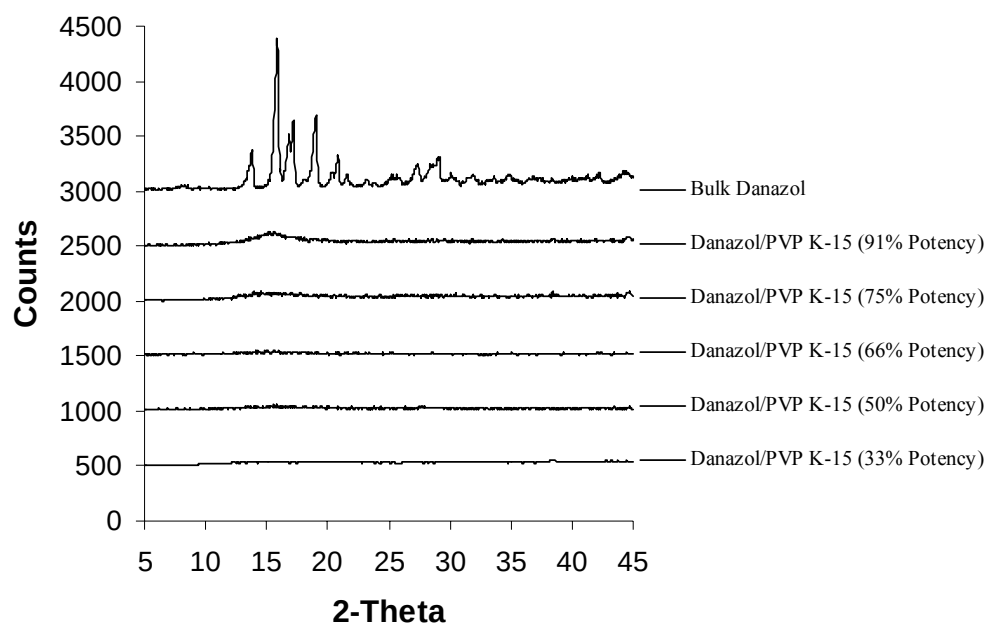


Fig. 4.1. Effect of potency on the crystallinity of danazol in SFL danazol/PVP K-15 powder as determined by X-ray diffraction.

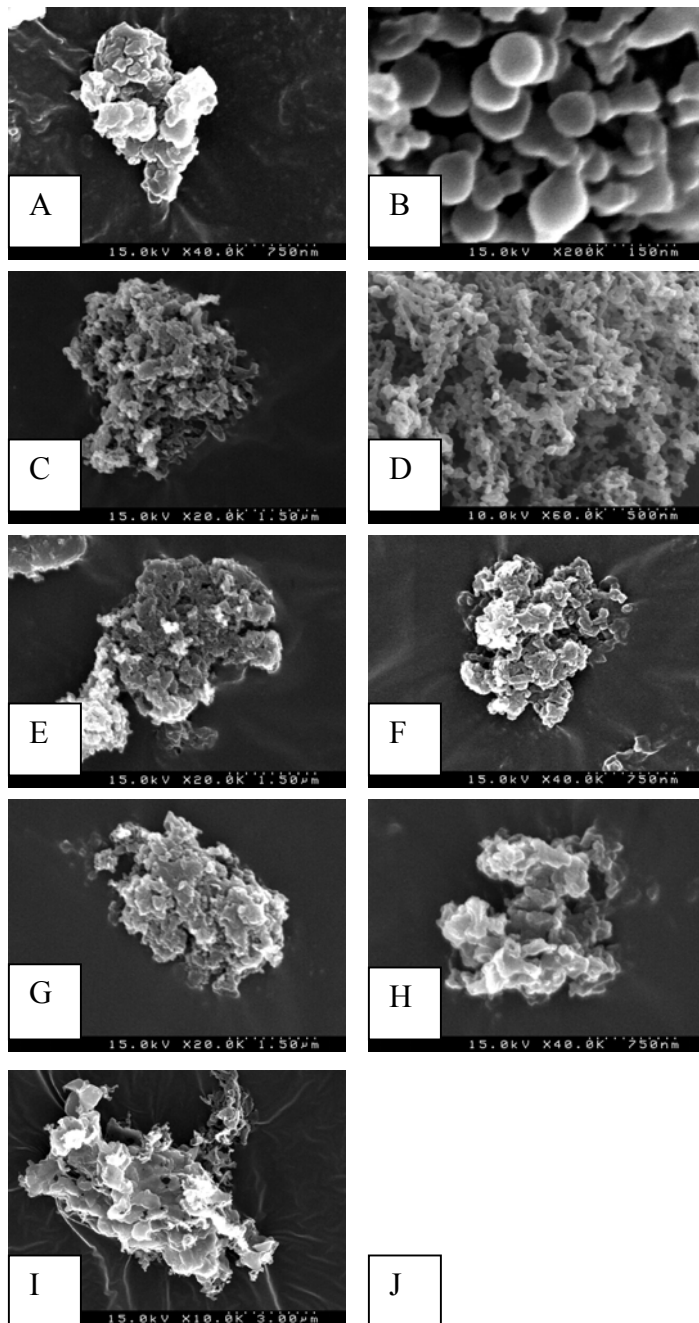


Fig. 4 2. SEM micrographs of SFL danazol/PVP K-15 (33% potency) (Fig.2A and 2B), SFL danazol/PVP K-15 (50% potency) (Fig.2C and 2D), SFL danazol/PVP K-15 (66% potency) (Fig.2E and 2F), SFL danazol/PVP K-15 (75% potency) (Fig.2G and 2H), SFL danazol/PVP K-15 (91% potency) (Fig.2I), and bulk micronized danazol (Fig.2J).

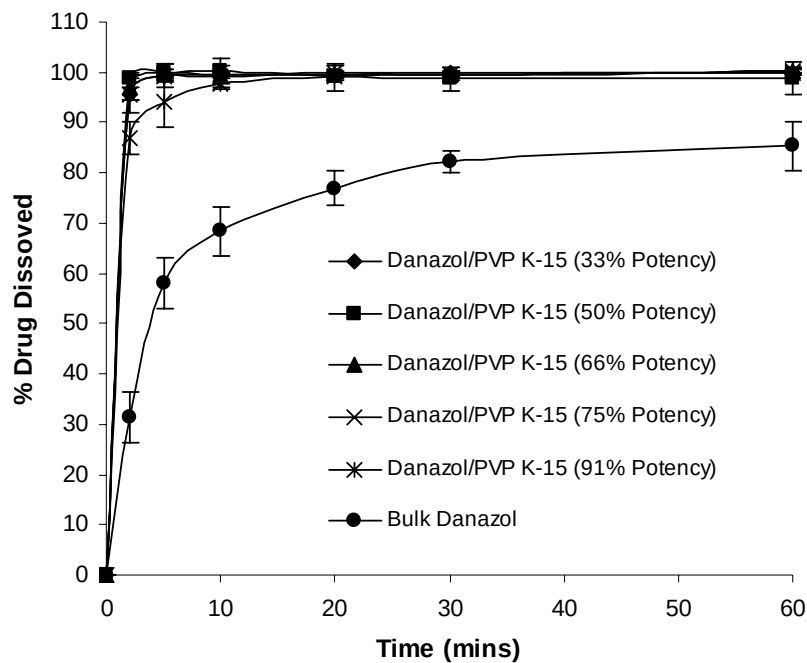


Fig. 4.3. Dissolution profiles (SLS 0.75%/Tris 1.21% buffer media) of SFL danazol/PVP K-15 powders with different potencies versus micronized bulk danazol.

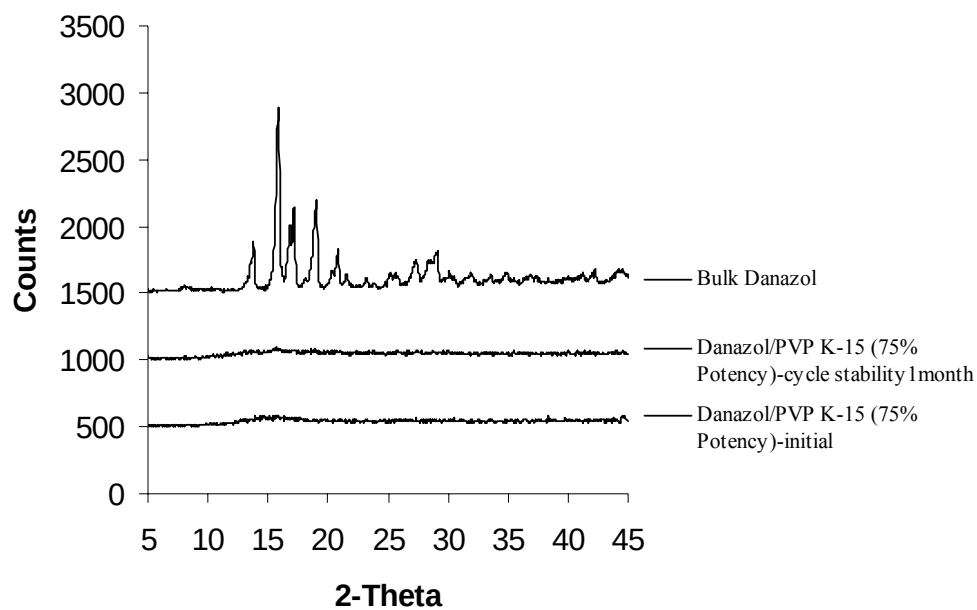


Fig. 4.4. Powder X-ray diffraction patterns of micronized bulk danazol, SFL danazol/PVP K-15 (75% potency) initial, and SFL danazol/PVP K-15 after one month cycle stability.

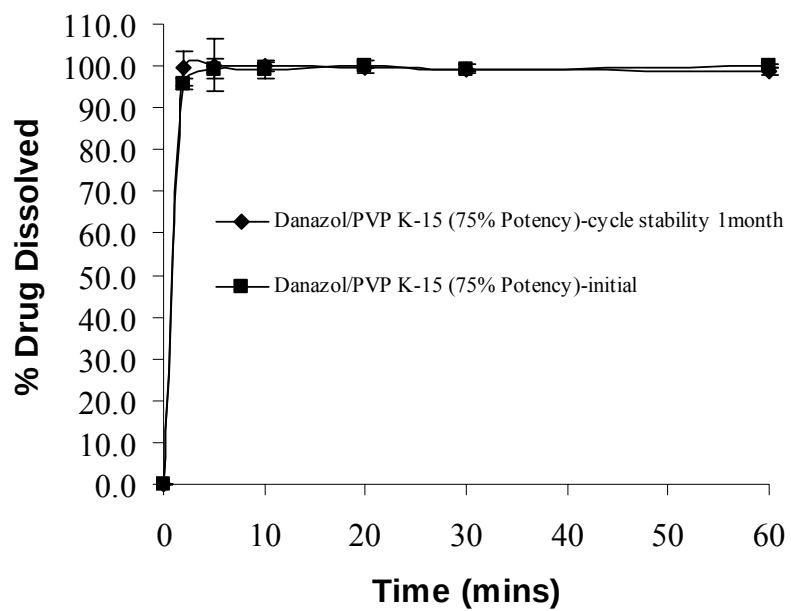


Fig. 4.5. Dissolution profiles (SLS 0.75%/Tris 1.21% buffer media, n=3) of SFL danazol/PVP K-15 (75% potency) initial and SFL danazol/PVP K-15 after one month cycle stability.

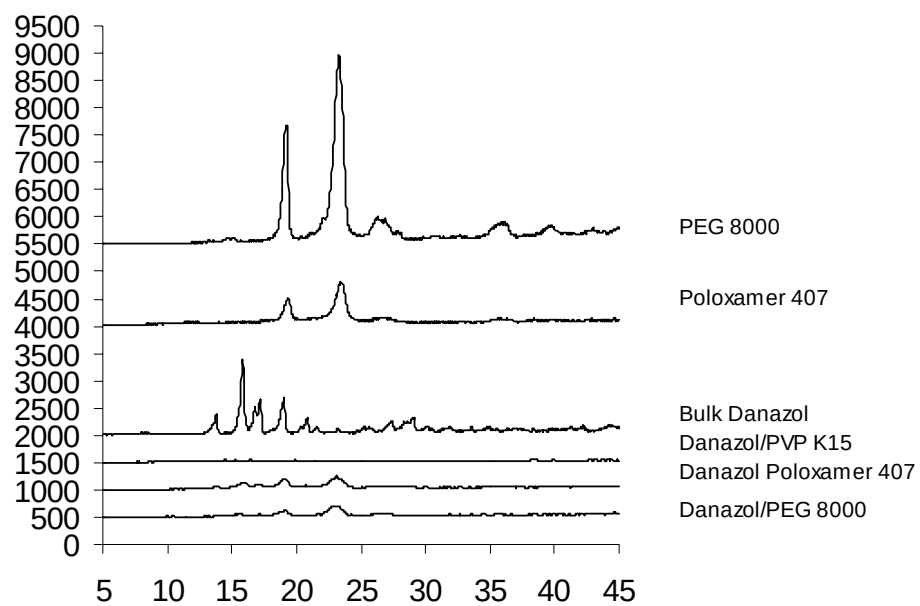


Fig. 5.1. Effect of excipients on the crystallinity of danazol in SFL powders by powder X-ray diffraction

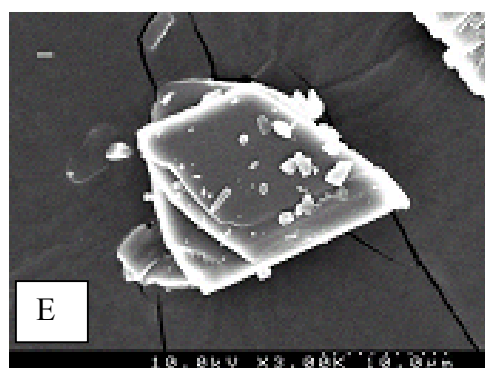
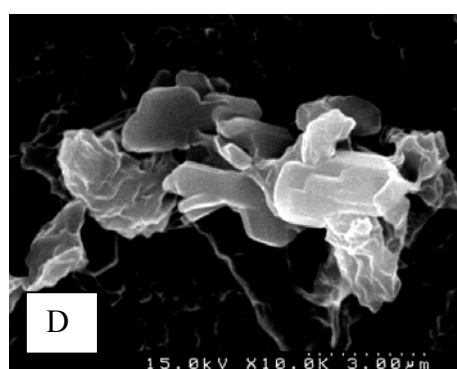
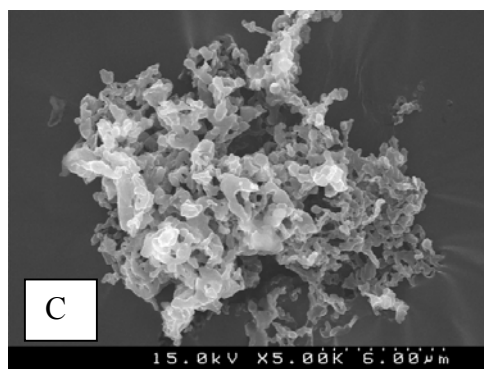
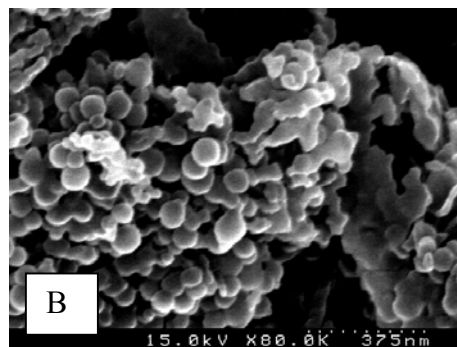
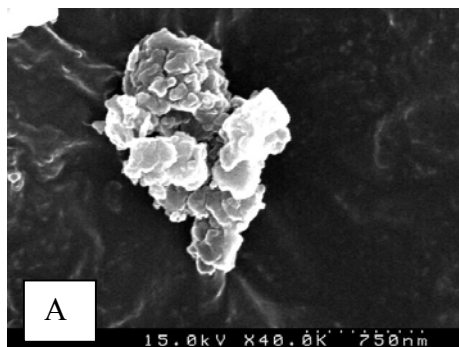


Fig. 5.2. SEM micrographs of SFL danazol/PVP k15 (Fig.5.2A and 5.2B), SFL danazol/PEG 8000 (Fig.5.2C), SFL danazol/poloxamer 407(Fig.5.2D), and bulk micronized danazol (Fig.5.2E).

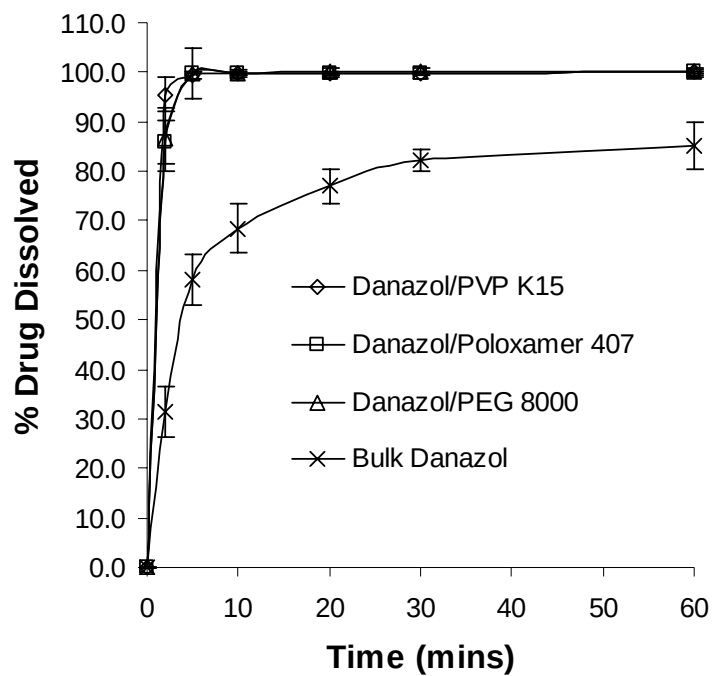


Fig. 5.3. Dissolution profiles (SLS 0.75%/Tris 1.21% buffer media, n=3) of SFL powders and micronized bulk danazol.

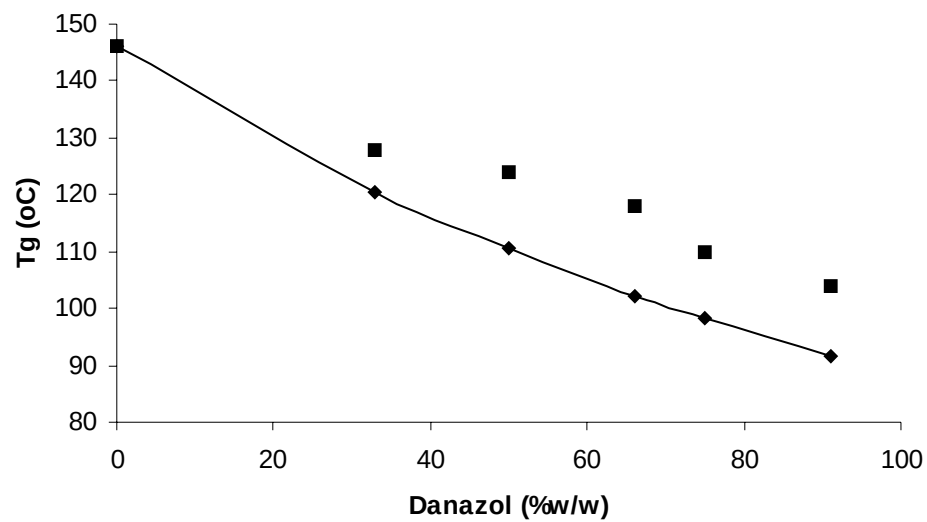


Fig. 5.4 Effect of danazol potency on the T_g of SFL danazol/PVP K-15 powders.

The solid line represents the predicted values from Gordon-Taylor equation. The symbols represent the measured T_g values.

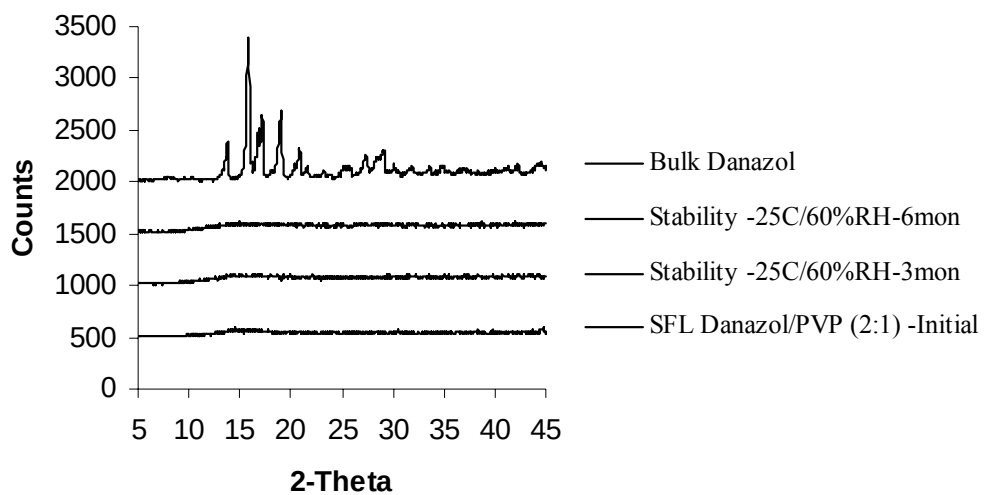


Fig. 5.5. Powder X-ray diffraction patterns of micronized bulk danazol, SFL danazol/PVP k15 (2:1) initial, and SFL danazol/PVP k15 six months at 25°C/60%RH

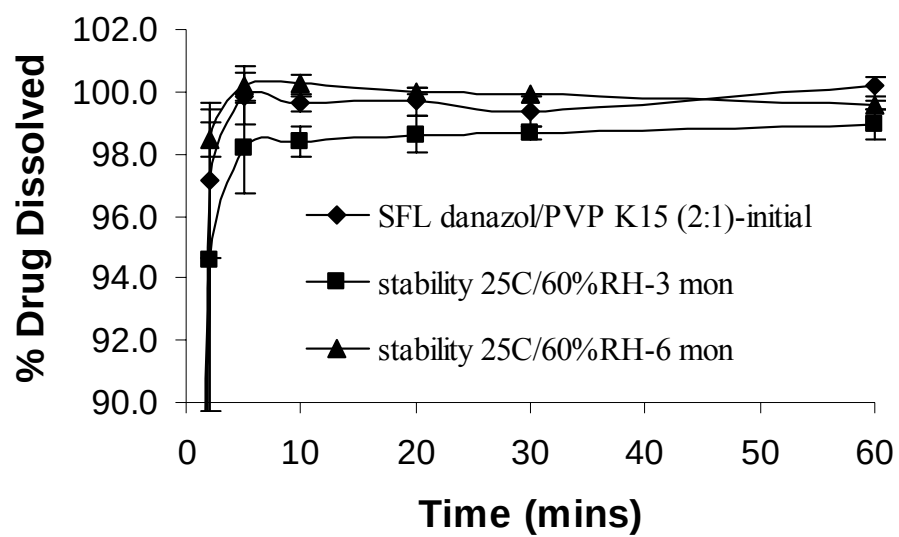


Fig. 5.6. Dissolution profiles (SLS 0.75%/Tris 1.21% buffer media, n=3) of SFL danazol/PVP k15 (2:1) initial and SFL danazol/PVP k15 six months at 25°C/60%RH

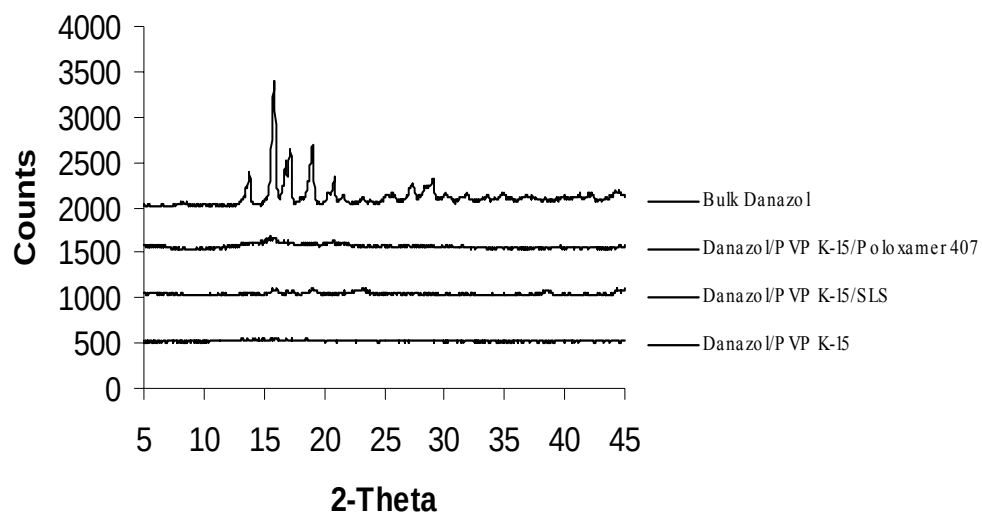


Fig. 6.1. Effect of excipients on the crystallinity of danazol in SFL micronized powder as determined by X-ray diffraction.

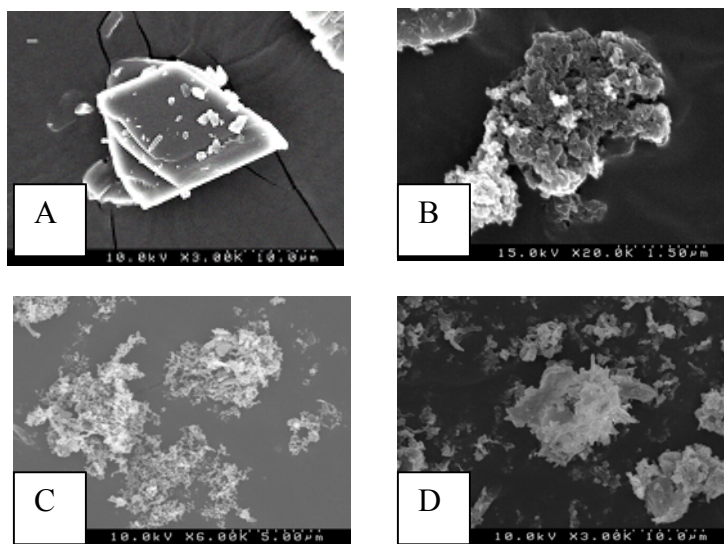


Fig. 6.2. SEM micrographs of bulk micronized danazol (Fig.6.2A), SFL danazol/PVP K-15 (2/1)(Fig.6.2B), SFL danazol/PVP K-15/SLS (4/1/1) (Fig.6.2C), SFL danazol/PVP K-15/Poloxamer 407 (4/1/1) (Fig.6.2D).

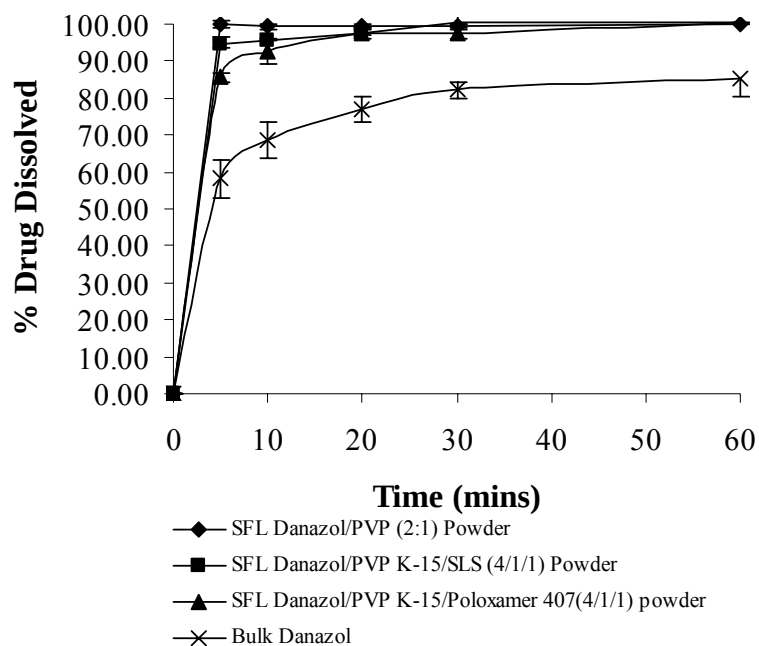


Fig. 6.3. Dissolution profiles (SLS 0.75%/Tris 1.21% buffer media, n=3) of SFL micronized powder with different excipients versus micronized bulk danazol

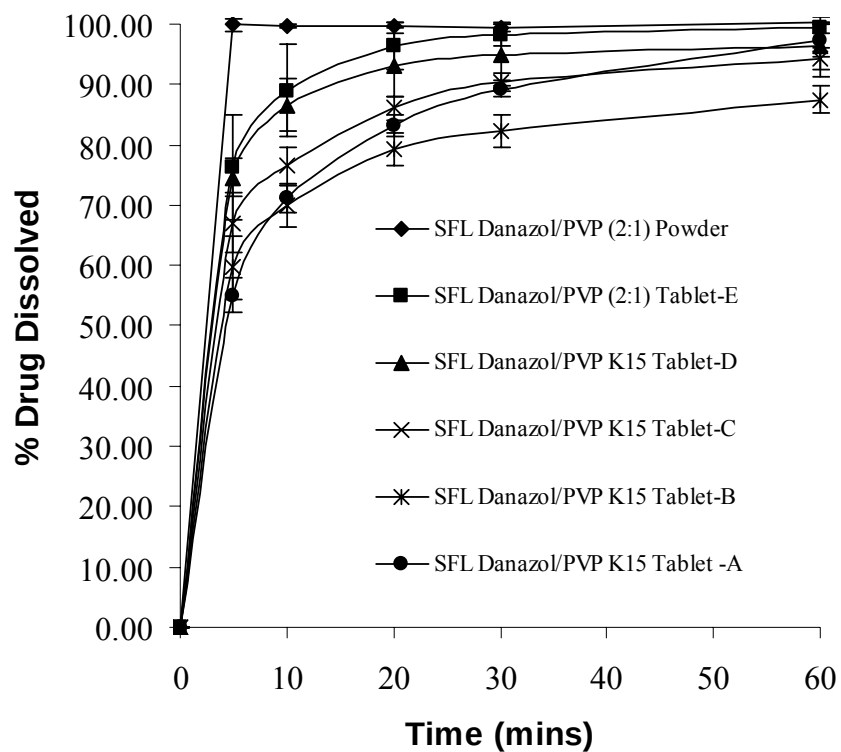


Fig. 6.4. Influence of tablet excipient compositions on the dissolution rates of danazol from tablets containing SFL danazol/PVP K-15 (2/1) powder (dissolution media: SLS 0.75%/Tris 1.21% buffer media, n=3).

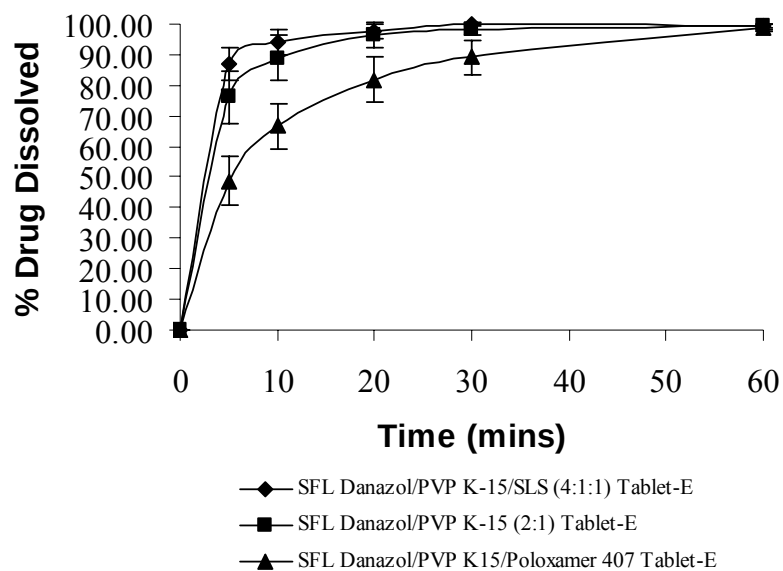


Fig. 6.5. Influence of SFL powder compositions on the dissolution rates of danazol from tablet formulation E containing SFL micronized powder (dissolution media: SLS 0.75%/Tris 1.21% buffer media, n=3).

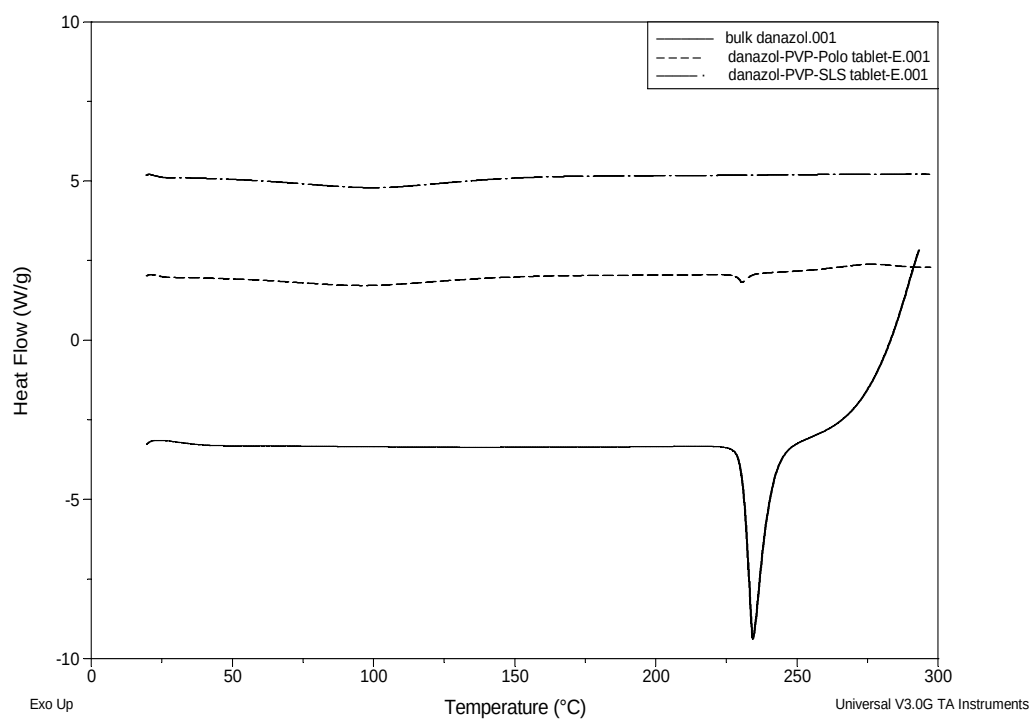


Fig. 6.6. Effect of direct compression process on the crystallinity of danazol in the tablets using DSC analysis.

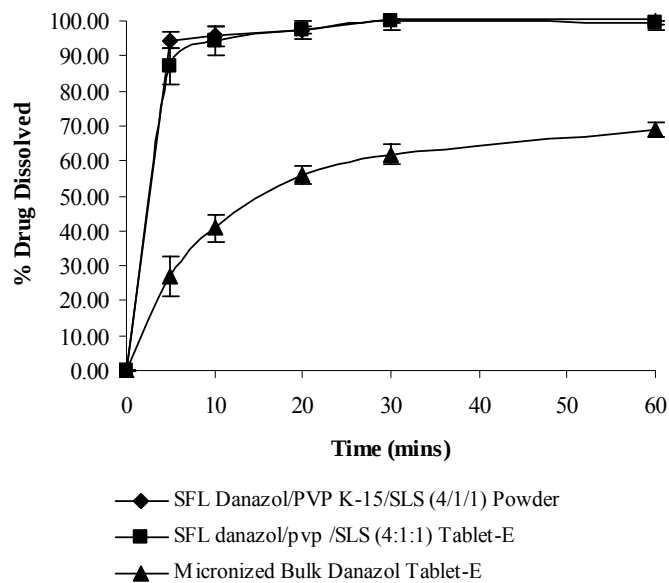


Fig. 6.7 Dissolution rates (SLS 0.75%/Tris 1.21% buffer media, n=3) of danazol from tablet formulation E containing SFL Danazol/PVP K-15/SLS (4:1:1) powder compared to micronized crystalline danazol tablet and SFL Danazol/PVP K-15/SLS (4:1:1) powder

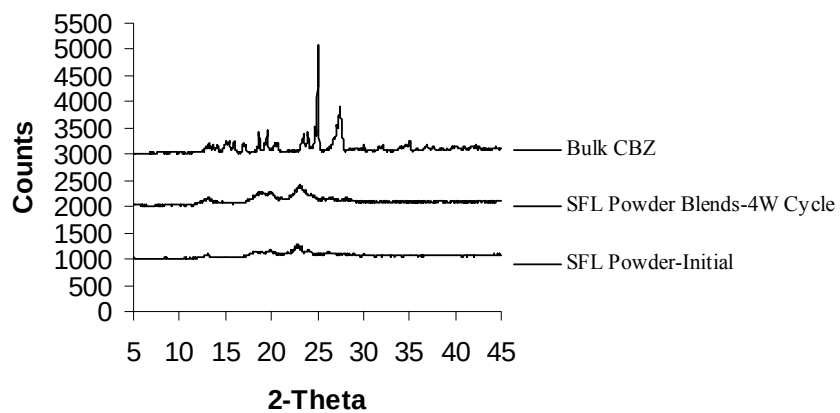


Fig. A1. Powder X-ray diffraction patterns of SFL CBZ/PVP K-15/Poloxamer 407 powder initial and SFL CBZ/PVP K-15/Poloxamer 407 after four weeks cycle stability.

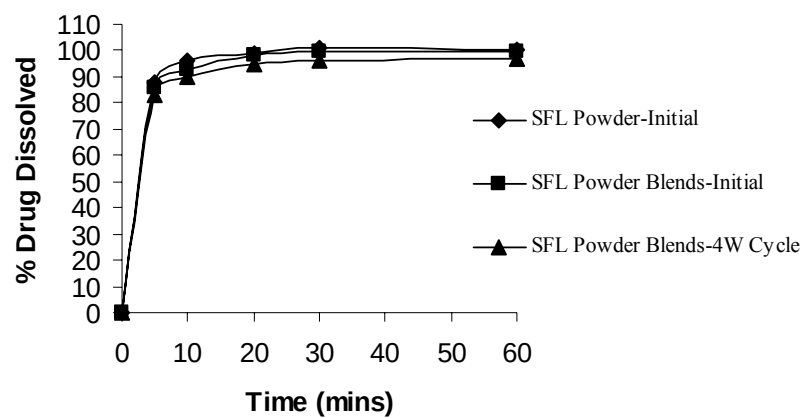


Fig. A2. Dissolution profiles (water, n=3) of SFL CBZ/PVP K-15/Poloxamer 407 powder initial and SFL CBZ/PVP K-15/Poloxamer 407 after four weeks cycle stability.

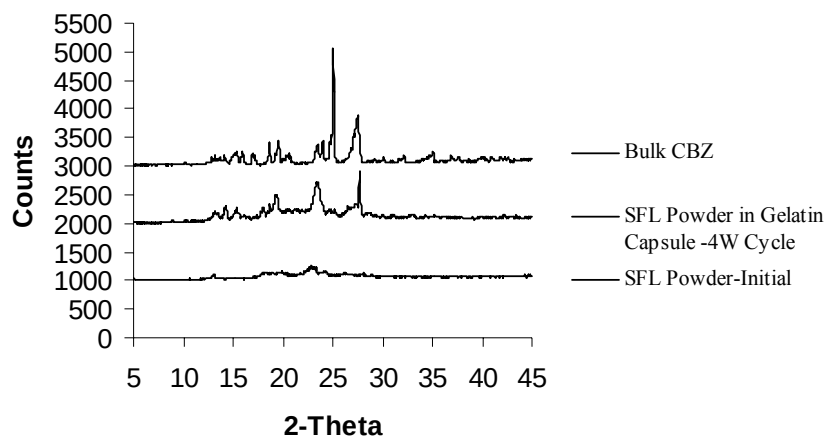


Fig. A3. Powder X-ray diffraction patterns of capsules containing SFL CBZ/PVP K-15/Poloxamer 407 powder initial and capsules after four weeks cycle stability.

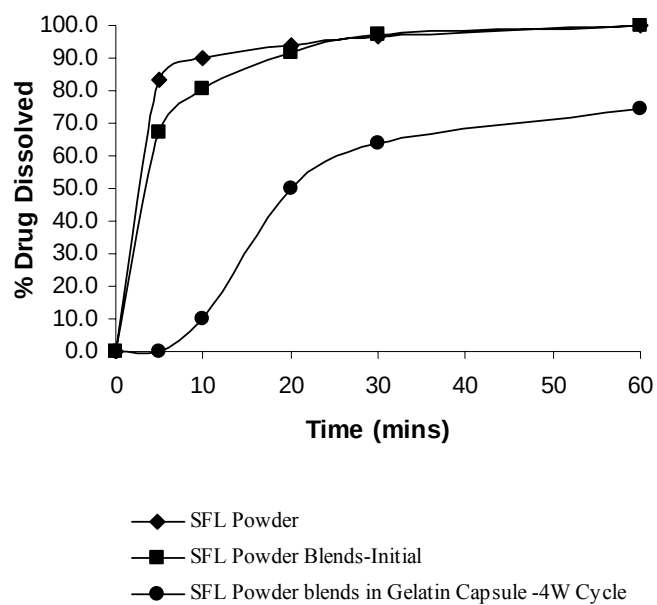


Fig. A4. Dissolution profiles (water, n=3) of capsules containing SFL CBZ/PVP K-15/Poloxamer 407 powder initial and capsules after four weeks cycle stability.

APPENDIX: Development of Rapid Release of Capsule

Formulation Containing SFL Micronized Powders

1. PURPOSE

The objective of the study in the Appendix was to prepare a rapid release capsule formulation containing SFL micronized powders and to investigate the stability of capsule formulation containing SFL micronized powder compositions.

2. MATERIAL AND METHODS

2.1 Materials

Marbamazepine (CBZ) was obtained as micronized powder from Spectrum Quality Products Inc. (Gardena, CA). Polyvinylpyrrolidone (PVP) K-15, and polyethylene-polypropylene glycol (Pluronic F127; poloxamer 407) were purchased from Spectrum Quality Products Inc. (Gardena, CA). Sodium starch glycolate (Explotab) were purchased from Penwest Pharmaceuticals Co. (Patterson, NY). Lactose Monohydrate, spray dried was from Foremost (Baraboo, WI). Colloidal silicon dioxide (Cab-o-sil M5P) was obtained from Cabot Corporation (Tuscola, IL). Hard gelatin size #1 capsule was obtained from Shionogi Qualicaps (Whitsett, NC). Acetonitrile was obtained from EM

Industries Inc. (Gibbstown, NJ). Purified water was obtained from an ultra-pure water system (Milli-QUV plus, Millipore, Molsheim Cedex, France).

2.2. Preparation of SFL micronized powders

CBZ, poloxamer 407 and PVP K15 (2:1:1) were dissolved in either acetonitrile or THF/water co-solvent (33% w/w) and processed by the SFL (Fig.1) technology (9). The SFL feed solution was placed into the solution cell (A). A constant pressure of 4000 PSI from the ISCO syringe pump (B) (Model 100 DX, ISCO Inc., Lincoln, NE) provided a flow rate of 11 mL/min (Table 1) for the SFL feed solution composed of THF/water co-solvent. A constant pressure of 2000 PSI provided a flow rate of 20 mL/min for the SFL feed solution composed of acetonitrile. The solution cell was connected to an insulated nozzle (C), which was positioned to atomize the SFL feed solution beneath the surface of the cryogenic liquid (D). In these experiments, liquid N₂ was used as the cryogenic liquid. The atomizing nozzle used was composed of polyetheretherketone (PEEK) tubing with an inner diameter of 63.5 µm. The SFL feed solutions were then sprayed through the nozzle and atomized directly into the liquid N₂ phase. Frozen particles formed instantaneously and were collected and dried by a VirTis Advantage Tray Lyophilizer (The VirTis Company, Inc. Gardiner, NY).

2.3. Preparation of Capsules

The capsule formulation containing SFL micronized powder is presented in Table 1. Firstly, all excipients were sieved through a 60 mesh screen. SFL micronized powder was then mixed with excipients (except for magnesium stearate) for 15 min. Magnesium stearate 0.5% (w/w) was added and blended for an additional 2 min. All mixtures were prepared by geometric dilution in a V-blender (Patterson Kelley, east Stroudsburg, PA). The blends were filled into capsules, which contained 50% SFL micronized powder.

2.4. Powder X-Ray Diffraction

Powder X-ray diffraction (XRD) was conducted using $\text{CuK}\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips 1720 X-ray diffractometer (Philip Analytical Inc., Natick, MA). The sample powders were placed in a glass sample holder. Samples were scanned from 5° to 45° (2 θ) at a rate of 0.05°/sec. For comparative purposes, the three highest values for relative peak intensity and their corresponding 2 θ positions were compared using the bulk CBZ as reference (15).

2.5. Dissolution Studies

The amount of API dissolved, as a function of time, was determined using USP Apparatus 2 method (Vankel 7000, Vankel Technology Group, Cary, NC).

All dissolution studies were conducted at sink conditions. SFL CBZ/poloxamer 407/PVP K15 powders or capsules containing approximately 12 mg CBZ were added to 900 mL of purified water (37°C). The paddle speed was 50 rpm. Five-milliliter samples in replicates of 3 (n=3) were taken at each time point, filtered through a 0.45µm filter, diluted with acetonitrile, filtered through a 0.45µm filter again, and then analyzed by HPLC (9, 18).

2.6. Stressed cycle stability study

SFL CBZ/PVP K-15/Poloxamer 407 powders and capsules containing SFL CBZ/PVP K-15/Poloxamer 407 powders were placed in glass vials (20 ml), sealed with desiccant containing in a temperature controlled oven. The temperature cycle was to increase temperature from –5°C to 40°C over 30 minutes, hold at 40°C for 2.5 hours and then decrease temperature from 40°C to –5°C over 30 minutes. The cycle was repeated 6 times per day. The duration of the stability study was four weeks.

3. RESULTS AND DISCUSSION

3.1 SFL Micronized Powder

3.1.1. Crystallinity

The crystallinity of CBZ in the SFL micronized powder was determined by powder X-ray diffraction (XRD). The high peak intensities of CBZ indicated a

high degree of crystallinity. However, the characteristic diffraction peaks of CBZ at 15.3, 25.0 and 27.6 (2θ) degrees were not evident in the SFL CBZ/PVP K-15/poloxamer 407 powders, indicating that CBZ contained in SFL powder was amorphous. (Fig. A1). This is because rapid freezing rates were achieved by atomizing the feed solution directly into liquid nitrogen during the SFL process. These freezing rates were fast enough to trap the CBZ in an amorphous state without allowing time for crystallization.

The stress imposed in stability testing is an important factor influencing the solid-state properties of amorphous powders. A stability study was conducted for the SFL CBZ/PVP K-15/poloxamer 407 powder at cycle stability conditions (-5°C to 40°C , 6cycles/day) for four weeks to examine any changes in crystallinity. Sample powders were stored in glass vials (20 mL) with aluminum-lined caps over a desiccant. The X-ray diffraction pattern (Fig.A1) exhibited no change in peak intensity for CBZ, suggesting that amorphous SFL powders were physically stable.

3.1.2. Dissolution Rate

The profiles presented in Figure A2 illustrate the dissolution of the SFL CBZ/PVP K-15/poloxamer 407 powders (water, $n=3$). SFL micronized powders displayed very rapid release profiles where greater than 90% CBZ was dissolved in only 10 minutes for the SFL CBZ/poloxamer 407/PVP K15 powders produced

from the initial sample or four weeks cycle for the stability sample. The increased dissolution rate of the SFL micronized powder was mainly attributed to amorphous CBZ, reduced particle size, enhanced surface area, and increased wettability.

3.2. Capsules Containing SFL Micronized Powder

3.2.1. Crystallinity

The stress imposed in stability testing is an important factor influencing the solid-state properties of amorphous powders. A stability study was conducted for the capsules containing SFL CBZ/PVP K-15/poloxamer 407 powder at cycle stability conditions (-5°C to 40°C , 6cycles/day) for four weeks to examine any changes in crystallinity. The X-ray diffraction pattern (Fig.A3) exhibited peak intensity for CBZ, suggesting that amorphous CBZ crystallized out during the cycle stability study. Capsule samples were stored in glass vials (20 mL) with aluminum-lined caps over a desiccant, which was the same package as SFL powder blends. The CBZ in the SFL powder blends was amorphous after four week cycle stability study, but the CBZ in capsule was crystallized out. This is because the water in the capsule shell was absorbed into SFL powder since the amorphous powder would absorb water spontaneously and this absorbed water led the crystallization of CBZ in capsules containing SFL micronized powder.

3.2.2. Dissolution Rate

The profiles presented in Figure A4 illustrate the dissolution of the SFL CBZ/PVP K-15/poloxamer 407 powders (water, n=3). About 95% CBZ was dissolved in 20 minutes from the capsules containing SFL CBZ/PVP K-15/poloxamer 407 powders, which is similar to the dissolution rate of SFL CBZ/PVP K-15/poloxamer 407 powders. However, after stored at cycle stability condition for four weeks, only 50% CBZ was dissolved from capsules. The decreased dissolution rate of CBZ from capsule formulations was mainly attributed to increased crystallinity of CBZ.

4.0 CONCLUSIONS

SFL powder blends demonstrated good stability in the cycle stability study. However, SFL capsule formulation exhibited increased crystallinity and decreased dissolution after four weeks exposure to the stressed cycle stability condition.

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