A Solid-State Approach to Enable Early Development Compounds: Selection and Animal Bioavailability Studies of an Itraconazole Amorphous Solid Dispersion

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Received 22 February 2010; revised 14 April 2010; accepted 23 April 2010

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22233

ABSTRACT: A solid-state approach to enable compounds in preclinical development is used by identifying an amorphous solid dispersion in a simple formulation to increase bioavailability. Itraconazole (ITZ) was chosen as a model crystalline compound displaying poor aqueous solubility and low bioavailability. Solid dispersions were prepared with different polymers (PVP K-12, K29/32, K90; PVP VA S-630; HPMC-P 55; and HPMC-AS HG) at varied concentrations (1:5, 1:2, 2:1, 5:1 by weight) using two preparation methods (evaporation and freeze drying). Physical characterization and stability data were collected to examine recommended storage, handling, and manufacturing conditions. Based on generated data, a 1:2 (w/w) ITZ/HPMC-P dispersion was selected for further characterization, testing, and scale-up. Thermal data and computational analysis suggest that it is a possible solid nanosuspension. The dispersion was successfully scaled using spray drying, with the materials exhibiting similar physical properties as the screening samples. A simple formulation of 1:2 (w/w) ITZ/HPMC-P dispersion in a capsule was compared to crystalline ITZ in a capsule in a dog bioavailability study, with the dispersion being significantly more bioavailable. This study demonstrated the utility of using an amorphous solid form with desirable physical properties to significantly improve bioavailability and provides a viable strategy for evaluating early drug candidates.
 J Pharm Sci 99:3901–3922, 2010

Keywords: itraconazole; X-ray amorphous; solid dispersion; phase separation; glass transition; spray drying; X-ray powder diffraction; bioavailability

INTRODUCTION

The high cost of development has motivated the pharmaceutical industry to explore new strategies to accelerate the drug development process in order to bring promising drug candidates on the market sooner and decrease development costs.[1] The FDA has defined the critical path of drug development as the time between discovery and launch, where the principle issues were identified as ensuring product safety, demonstrating medical utility, and industrialization.[1] Currently, a new candidate entering Phase I, after 10 years of preclinical screening and testing, is estimated to have only an 8% chance of making it to the market, down from 14%.[1] A 10% improvement in anticipating failures early in development could save $100 million in development costs per molecule.[1]

A number of approaches have been reported in order to decrease development time, including reengineering large pharmaceutical development organizations,[2] differentiating the focus of early stage development from late stage development,[3] using decision trees for first-in-human (FIH) formulations,[4] and improving properties using solid-state chemistry.[5] Many times promising candidates are not advanced from preclinical into FIH formulations due to intrinsic solid-state properties, such as solubility, and changes in the molecular structure or a switch to another compound is necessary.[6] It is

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Journal of Pharmaceutical Sciences, Vol. 99, 3901–3922 (2010)

estimated that 25–30% of the candidates nominated from discovery are poorly soluble,[7] with estimates as high as 60% reported within companies,[8] which challenges conventional development approaches and makes fast development more difficult.

The most well-known classification system for pharmaceutical compounds considers solubility and permeability in the Biopharmaceutical Classification System (BCS).[9] This classification divides compounds into four quadrants: high solubility and high permeability (Class 1), low solubility and high permeability (Class 2), high solubility and low permeability (Class 3), and low solubility and low permeability (Class 4). Another classification system which considers solubility and metabolism has also been developed in the Biopharmaceutics Drug Disposition Classification System (BDDCS).[10] This classification divides compounds into the following four quadrants: high solubility and extensive metabolism (Class 1), low solubility and extensive metabolism (Class 2), high solubility and poor metabolism (Class 3), and low solubility and poor metabolism (Class 4). The use of different solid forms (e.g., salts, co-crystals, amorphous solid dispersions) can be used to overcome the poor solubility of Class 2 and possibly Class 4 compounds.

The present study evokes the use of solid-state chemistry as a means to improve bioavailability and was completed as an essential part of an internal proof-of-concept program intended to demonstrate a pathway for accelerating early drug candidates, even those that are poorly bioavailable, into FIH clinical studies by reducing time from bulk active pharmaceutical ingredient (API) to regulatory submission (e.g., IND and CTA). While it is acknowledged that additional process and product development may be needed in advance of commercialization, performing necessary solid-state screening early will result in a solid form with desirable properties and a formulation suitable for early clinical evaluation. The solid form could include the most physically stable crystalline form, a salt, a co-crystal, amorphous, or an amorphous solid dispersion. A variety of simple formulation approaches would be available to further compensate for properties not inherent to the selected solid form. Utilization of quality by design (QbD)[11] and process analytical technologies (PAT)[12] concepts provide better process understanding to aid the accelerated development program. Using this solid-state approach provides the opportunity to move a larger number of new chemical entities (NCE) into early clinical evaluation and to determine if further development of the candidate should be pursued.

While a variety of solution formulation approaches are available,[4] amorphous solid dispersions are being pursued for many poorly soluble compounds as an alternative for early clinical studies. In some cases, amorphous solid dispersions have been shown to achieve better bioavailability than solution formulations in animal studies.[13] An amorphous solid dispersion consists of an amorphous active API stabilized by a polymer, with the amorphous form of the drug providing increased *apparent* solubility. The benefit of these materials is the increased concentration in solution and supersaturation relative to the crystalline material. It has also been suggested that the presence of the polymer may help prevent crystallization and maintain the supersaturation.[14] This can be an important factor in animal and human studies for poorly soluble compounds; it is important to keep the material in solution and prevent the crystalline API from precipitating in the stomach or intestines. While *in vitro* tests are frequently not predictive of performance *in vivo*, keeping the material in solution provides the best opportunity to achieve maximum exposure in the biological studies, giving more information on performance parameters such as bioavailability and efficacy.

The model compound chosen for the study was itraconazole (ITZ) (Fig. 1), which is commercially available and has many of the physical properties that present challenges to early clinical development. It is a very poorly soluble weak base with an aqueous solubility estimated at approximately 1 ng/mL at neutral pH and approximately 4 μg/mL at pH 1.[15] The calculated log*P* is 6.2 and is classified as a BCS Class 2 drug. Extensive formulation development was needed to overcome the poor aqueous solubility of crystalline ITZ during development. The marketed product is Sporanox® and the oral formulation consists of a three layer bead consisting of a round core, a coating film, and a seal-coating polymer layer.[16] The coating film contains hydroxypropyl methylcellulose and ITZ as a molecularly dispersed solid solution. The seal-coating polymer layer, polyethylene glycol (PEG), is applied to the drug coated cores to prevent sticking of the beads, which would have the undesirable effect of a decrease in the dissolution rate and of bioavailability. The oral solution consists of hydroxypropyl-β-cyclodextrin as a solubilizer, an aqueous hydrochloric acid medium as a bulk liquid carrier to achieve an optimum pH

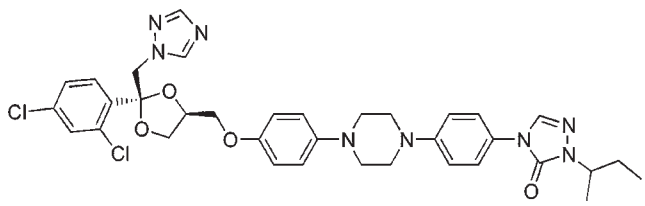

Figure 1. Chemical structure of itraconazole.

 DOI 10.1002/jps

around 2, and an alcoholic cosolvent propylene glycol to dissolve the compounds.[17]

A number of other, possibly more complicated formulation strategies have been reported to improve the *apparent* solubility (or increase the dissolution rate) of ITZ including nanoparticles,[18] ordered mesoporous silica,[19] cyclodextrin based nanosponges,[20] self-emulsifying formulations,[21] laponite-based nanohybrid,[22] and electrospun nanofibers.[23,24] In-situ micronization[25] and evaporation precipitation for rapidly dissolving particles[26] have also been proposed. The poor solubility of crystalline ITZ makes it an ideal model compound for developing and testing these novel formulations or manufacturing processes.

Amorphous solid dispersions of ITZ have also been studied using a number of polymers as stabilizing agents including polyethylene glycol[27] (PEG 6000,[28] PEG 20000[29]), polyvinylpyrrolidone (PVP),[30] hydroxy propyl methyl cellulose (HPMC),[31] hydroxyl propyl cellulose (HPC),[30] ethylcellulose (EC 20 cps),[32] Eudragit® E100,[33,34] polyvinylacetal diethylaminoacetate (AEA®),[29] polyoxyethylene–polyoxypropylene copolymers (Poloxamer® 188),[29] Inutec SP1,[35] polyvinylpyrrolidone vinyl acetate (PVPVA64),[36] Kollicoat IR®,[37] and hydroxypropylmethylcellulose phthalate (HP55).[38] Mixtures of polymers have also been evaluated such as Eudragit® E-100/polyvinyl PVPVA64,[39,40] hydroxypropyl-β-cyclodextrin (HP-β-CD)/HPMC,[41] d-alpha-tocopheryl polyethylene glycol (TPGS 1000)/PVPVA64,[42] PEG 6000/HPMC 2910 E5,[43,44] and PVPVA64/Myrj 52.[45] Techniques to form the dispersions have included hot melt extrusion,[46] spray drying,[29] ultra-rapid freezing,[38] high shear pelletization,[47] and supercritical carbondioxide.[36] This is not meant as an exhaustive list, only a sampling of the work that has been performed on ITZ.

As stated, the purpose of the present study was to apply a solid-state approach to find a solid amorphous dispersion that would overcome the poor solubility of a BCS Class 2 drug and to use it in a simple formulation suitable for FIH clinical trials. In the present study, ITZ was treated as a new chemical entity (NCE) with only an HPLC method being taken from the literature. The intent was to test this approach for rapidly identifying a prototype solid form, specifically an amorphous solid dispersion that is suitable for preclinical evaluation of bioavailability, not to develop a better formulation specifically for ITZ. Techniques readily available in our laboratories were chosen and more complicated formulation approaches were not included. It should also be emphasized that while the solid form screen described is aimed at finding a suitable prototype, if successful in FIH clinical trials, additional formulation and process development would be needed for later phase studies.

This report describes the characterization of a model compound, ITZ, including the preparation of an amorphous form. Selection of suitable amorphous solid dispersion compositions and processes was conducted based on attempts to prepare X-ray amorphous materials and the inherent physical stability on exposure to temperature, humidity, and nonideal drying conditions to narrow the number. One dispersion composition with acceptable stability was scaled-up, and a dog bioavailability study performed to assess the *in vivo* performance relative to the crystalline solid form. The findings reported herein were conducted as part of a proof of concept study to demonstrate that an amorphous solid dispersion of a poorly water soluble API could be identified through screening, the selected dispersion scaled-up and used to manufacture clinical supply material, and an investigational new drug (IND) application prepared in a time frame of approximately 6 months. While it was found to be possible, it is important to note that dispersion selection is only one part of bringing an NCE into early clinical evaluation, and many factors, including manufacture of the clinical supply material, need to be considered.

EXPERIMENTAL

Materials

ITZ was purchased from Spectrum (Gardena, CA). A variety of polymers were selected as stability agents to generate ITZ solid dispersions: three grades of PVP, K-12, 29/32, and 90; and a vinyl acetate (PVP VA) derivative, grade S-630 were obtained from International Specialty Products (ISP; Wayne, NJ); hydroxypropyl methylcellulose phthalate (HPMC-P), grade 55; and hydroxypropyl methylcellulose acetate succinate (HPMC-AS), grade HG were obtained from Shin-Etsu (distributed by Biddle Sawyer; New York, NY). All materials were used as received unless stated otherwise.

Methods

Preparation of Amorphous Itraconazole

Cryogrinding was used to obtain an X-ray amorphous sample of ITZ for use in computational analysis (described below). ITZ was charged into a small polycarbonate cylinder with removable stainless steel ends. A stainless steel rod was added and the cylinder capped. The assembled grinding container was mounted on a Model 6750 Freezer Mill (SPEX CertiPrep, Metuchen, NJ) and processed at liquid nitrogen temperatures for a total 30 or 60 min consisting of 15 or 30 cycles respectively; for each cycle, grinding for 2 min and hold for 1 min was used. Liquid nitrogen was refilled every five cycles during

the process. The ground solid was isolated and stored at −20°C over desiccant until analyzed.

Amorphous solids of ITZ were also prepared by quench-cooling of the melt. Crystalline ITZ in a clean vial was immersed in a silicon oil bath maintained at approximately 10°C above the melting temperature. The molten liquid was poured into a mortar containing liquid nitrogen and was ground into powder using a pestle. The quench-cooled solid was collected and stored at −20°C over desiccant until analyzed.

Composition and Process Selection

Solubility Assessment

Approximate solubilities of crystalline ITZ as received were estimated in various solvents and compared with known solubilities of polymers. Selection of the solvents used in the screen was based on the need to dissolve both ITZ and polymer. Approximate solubilities were determined by adding measured aliquots of solvent to weighed amounts of ITZ and sonicating the mixture between aliquot additions. Dissolution was judged by visual inspection.

Flash Evaporation

Concentrated ITZ solutions were prepared in acetone or 3:1 (v/v) acetone/ethanol mixtures in clean glass vials. Polymers of different types were added into the solution to make dispersions with various ITZ/polymer ratios (5:1 (83.3% ITZ), 2:1 (66.7% ITZ), 1:2 (33.3% ITZ), 1:5 (16.7% ITZ), by weight). The vial or flask was attached to a rotary evaporator and the solvent was evaporated to dryness. The water bath was heated to a temperature near the boiling point of solvent so that the solvent can rapidly evaporate under vacuum; this technique was used during initial composition screening and is termed "rotovap-screen" elsewhere in this report. After rotary evaporation, the vial or flask was secondarily dried in a vacuum oven at 40°C for 24 h as a precaution to remove residual solvent. The solids were isolated and stored at −20°C over desiccant.

Freeze Drying

Concentrated ITZ/polymer solutions with different polymer types and drug/polymer ratios were prepared in *p*-dioxane in clean glass vials. The solution was frozen in a thin layer on the walls of the vial by rotating in a bath of liquid nitrogen or dry ice/isopropanol. The vial containing the frozen sample was placed into a lyophilizing container, which was then attached to a Flexi-Dry manifold lyophilizer (SP Industries, Stone Ridge, NY) for 1–3 days. The temperature of the cold finger was maintained at −50°C for the duration of the experiment. The solids were isolated and stored at −20°C over desiccant.

Physical Stability Assessment

Thermal Stress

Samples of amorphous solids were stressed at 40°C over desiccant up to 16 days to examine excursions from ambient storage temperature possible on storage of packaged materials. Solids were packed between 3-μm thick polymer films (to facilitate for XRPD analysis), which were placed in a vial inside a larger jar containing desiccant and stored at controlled temperature. At designated time intervals, the prepared sample was analyzed by XRPD for the evidence of crystalline material.

Relative Humidity Stress

Samples of amorphous solids were exposed to 40°C/75% relative humidity (RH) for 4 h in uncapped vials. As with thermal stress, short duration excursions from more protective storage conditions (e.g., desiccant) or open handling during downstream manufacture (e.g., encapsulation) were investigated. At designated time intervals, samples were analyzed by XRPD and polarized light microscopy to inspect for evidence of crystalline material.

Kinetic Stress

ITZ/polymer solutions were prepared with different polymer types at a 1:2 (w/w) ITZ/polymer ratio (by weight) in acetone or 3:1 (v/v) acetone/ethanol solution in clean 1000 mL flasks. A 250 mL flask was attached to a rotary evaporator and immersed in water bath heated to a temperature around the boiling point of the solvent. The solution was transferred from 1000 mL flask to 250 mL (receiving) flask through clean, narrow-bore polymer tubing under vacuum. The flow rate of solution to the receiving flask was controlled by adjusting the length and diameter of the tubing. After evaporation termed "rotovap-spray" elsewhere in this report, the deposited material was dried under vacuum at 40°C for 24 h as a precaution to remove residual solvent. The solids were isolated and stored at −20°C over desiccant.

Solid Dispersion Scale-Up

Spray Drying

Spray drying was conducted at ISP Pharma Technologies Division (Columbia, MD). The spray solution was prepared from ITZ and polymer at 0.8% solids in acetone; higher solids concentrations were possible by heating feed solution. Using a cocurrent, two-fluid nozzle, several development experiments were performed using a NIRO Mobile Minor spray dryer (Columbia, MD) operated in an open cycle configuration to establish suitable spray drying conditions. The

dried solid particles were separated from the process gas flow by a cyclone, with the exhaust gas directed through a small baghouse assembly and vented to carbon bed absorbers. Tests were also made to verify the proposed process parameters during extended run time (200 g theoretical batch size) for production of cGMP material; inlet temperature (120°C), outlet temperature (60°C), and atomizing gas pressure (0.8 bar). Production of cGMP material (1 kg theoretical batch size) was completed using a NIRO PSD-1 spray dryer and the parameters determined from the scale-up experiments. The spray solution was prepared with continuous stirring at approximately 30°C and maintained at approximately 25–30°C during processing. Secondary drying of the cGMP material was carried out by fluid bed drying at 40°C (1–4 h).

Instrumental Techniques

X-Ray Powder Diffractometry (XRPD)

X-ray powder diffractometry (XRPD) analysis was conducted using either a Model XRD-6000 diffractometer (Shimadzu, Kyoto, Japan) or Model D-8 Discover diffractometer (Bruker AXS, Madison, WI) with Cu Kα radiation. Selection of the instrument used was determined by the amount of sample available. For both diffractometers, a silicon standard was analyzed using the known silicon 111 peak position at 28.441° 2θ to within ± 0.01° to verify the accuracy of the diffractometer optics.

The Shimadzu XRD-6000 diffractometer is equipped with a long fine focus X-ray tube. The tube voltage and amperage were set to 40 kV and 40 mA, respectively. The divergence and scattering slits were set at 1° and the receiving slit was set at 0.15 mm. Diffracted radiation was detected by a NaI scintillation detector. A θ–2θ continuous scan at 1°/min (2.4 s/0.04° step) from 2.5 to 60° 2θ was used. A silicon standard was analyzed to check the instrument alignment. Data were collected and analyzed using XRD-6100/7000 v. 5.0. Samples were prepared for analysis by placing them in an aluminum holder with silicon insert.

The Bruker D-8 Discover diffractometer is equipped with Bruker's General Area Diffraction Detection System (GADDS, v. 4.1.20). An incident beam of Cu Kα radiation was produced using a fine-focus tube (40 kV, 40 mA), a Göbel mirror, and a 0.5 mm double-pinhole collimator. The sample was packed between 3-μm thick films to form a portable disc-shaped specimen. The prepared specimen was loaded in a holder secured to a translation stage and analyzed in transmission geometry. The incident beam was scanned and rastered to optimize orientation statistics. A beam-stop was used to minimize air scatter from the incident beam at low angles. Diffraction patterns were collected using a Hi-Star area detector located 15 cm from the sample and processed using GADDS. The intensity in the GADDS image of the diffraction pattern was integrated using a step size of 0.04° 2θ. The integrated patterns display diffraction intensity as a function of 2θ.

Optical Microscopy

Optical microscopy was performed using a Leica DM LP microscope equipped with Spot Insight color camera (model 3.2.0). A 20 × 0.4 N.A. objective, or 10 × 0.25 N.A. objective in some cases, was used with cross polarizers to view samples.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was performed using a Model 2920 differential scanning calorimeter (TA Instruments, New Castle, DE). The sample was placed into an aluminum DSC pan, and the weight accurately recorded. The pan was covered with a lid then crimped or perforated with a laser pinhole to allow for pressure release, and then hermetically sealed. The sample cell was equilibrated at −50°C and heated under a nitrogen purge at a rate of 20 or 10°C/min, up to a final temperature of 350°C (except for crystalline ITZ, where the final temperature was 220°C). Indium metal was used as the calibration standard. Reported temperatures are at the transition onset point and maxima.

For studies of the glass transition temperature (T_g) of the amorphous ITZ, a cyclic temperature program was followed. The sample cell was equilibrated at 25°C, and then heated under nitrogen at a rate of 20°C/min, up to 175°C. The sample cell was held at this temperature for 2 min and then allowed to cool and equilibrate at −50°C for 5 min. The sample cell was then heated at 20°C/min up to a final temperature of 325°C. The T_g is reported from the half-height of the transition.

Modulated Differential Scanning Calorimetry (MDSC)

Modulated differential scanning calorimetry data were obtained on a Model 2920 differential scanning calorimeter (TA Instruments) equipped with a refrigerated cooling system (RCS). The sample was placed into an aluminum DSC pan, and the weight accurately recorded. The pan was covered with a lid and then crimped. MDSC data were obtained using a modulation amplitude of ±0.8°C and a 60 s period with an underlying heating rate of 1°C/min. Various temperature ranges were used. The temperature and the heat capacity were calibrated using indium metal and sapphire as the calibration standards, respectively. The reported glass transition temperatures

(T_g) are obtained from the half-height of the step change in the reversible heat flow signal.

Thermogravimetry (TGA)

Thermogravimetric analyses were performed using a Model 2950 thermogravimetric analyzer (TA Instruments). Each sample was placed in an aluminum sample pan and inserted into the TGA furnace. The furnace was first equilibrated at 25°C, and then heated under nitrogen at a rate of 10°C/min, up to a final temperature of 350°C. Nickel and Alumel™ were used as the calibration standards.

Water Vapor Sorption/Desorption Analysis (DVS)

Moisture sorption/desorption data were collected on a Model SGA-100 vapor sorption analyzer (VTI Corporation, Hialeah, FL). Sorption and desorption data were collected over a range of 5–95% RH at 10% RH intervals under an air or nitrogen purge. Samples were not dried prior to analysis. Equilibrium criteria used for analysis were <0.01% weight change in 5 min, with a maximum equilibration time of 3 h if the weight criterion was not met. Data were not corrected for the initial moisture content of the samples. NaCl and PVP were used as calibration standards.

Computational Analysis

Semi-Quantitative Analysis by PDF Method

Analysis of amorphous solid dispersion based on the linear combination of pair distribution function (PDF) derived from the XRPD data was carried out using a previously published procedure.[48] PDFs for each component, ITZ and polymer, and the amorphous solid dispersion sample were calculate from high-quality XRPD patterns using custom-developed software, PatternMatch v2.4.0 (SSCI, West Lafayette, IN). A minimization procedure was used to fit the sum of PDFs for the components to the PDF for the amorphous solid dispersion by varying the contribution of the PDF for each component. The quality of the fit was evaluated by calculating the difference between the sum-squared intensities of the calculated PDF and the PDF from measured XRPD data for the dispersion sample.

Dog Bioavailability Studies

The research protocol of the animal experimentation was approved by the Department of Drug Metabolism Animal Ethics Committee at Aptuit, Ltd (Riccarton, Scotland). All studies were conducted in accordance with the Animals (Scientific Procedures) Act 1986, with the UK Home Office Guidance on the implementation of the Act,[49] and with all applicable Codes of Practice for the care and housing of laboratory animals. Aptuit is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Dosing

Dose Preparation

All capsules were prepared on the day prior to dose administration and were stored at refrigerated temperatures, over desiccant, and protected from light until use. For Stage 1, an appropriate weight of the selected amorphous solid dispersion was dispensed directly into a gelatin capsule (torpac lock ring capsule, size 11). Each capsule contained approximately 152 mg of amorphous solid dispersion (equivalent to 50 mg ITZ). For Stage 2, an appropriate weight of crystalline ITZ was dispensed directly into a gelatin size 11 capsule. Each capsule contained approximately 50 mg ITZ.

Dose Administration

Three nonnaive male beagle dogs weighing 14.4–16.3 kg and ranging in age from 23 to 24 months were used for the study. The animals had been used on previous studies conducted at the test facility, although a minimum of 4 weeks was allowed between previous use and use on this study. Animals were identified uniquely by ear tattoo and animal numbers (001M–003M) were allocated randomly. Actual body weights were recorded within a 24 h period prior to dose administration. Each animal received a daily allowance of 400 g of pelleted dog diet, Harlan Teklad 2021 dog maintenance diet for Stages 1 and 2, with the exception of a predose overnight fast and a 2 h postdose fast for each stage. Dose weights were not altered to take into account the weight of the animal at the time of dose administration and all animals received a dose of approximately 50 mg ITZ, regardless of body weight. The actual dose received by each animal was calculated from, the animal weight, the weight of dose administered and the number of capsules administered.

The oral dose was administered via hard gelatin capsule. Capsules were placed at the top of the throat, the mouth held closed and the throat gently massaged until the animal was seen to swallow. A small quantity of tap water (approximately 5 mL) was given to each animal immediately postdose to ensure that each capsule had been swallowed. For Stage 1, three nonnaive male beagle dogs each received a single oral administration of ITZ amorphous solid dispersion (equivalent to 50 mg crystalline ITZ). Blood samples were collected at various time points up to 72 h postdose. Following a minimum wash out period of approximately 1 week following Stage 1, the same three beagle dogs each received a single oral administration of crystalline ITZ at a target dose level

of 50 mg. Samples were collected as detailed for Stage 1.

Sample Collection

Following dose administration, serial whole blood samples (approximately 1 mL), were removed from the jugular vein of each animal predose and at each of the following time points postdose (actual times were recorded postdose): 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 48, and 72 h. All blood samples were thoroughly mixed following collection and stored on wet ice prior to centrifuging, at approximately 4°C, within 1 h of collection, and the plasma transferred to appropriately labeled plain tubes. Residual blood cells were discarded.

Bioanalysis

HPLC–MS/MS

Parent concentrations of ITZ, in each plasma sample were determined by high performance liquid chromatography followed by tandem mass spectrometric detection (HPLC–MS/MS). While it is acknowledged that ITZ is extensively metabolized to hydroxyitraconazole in humans and beagle dogs, information on metabolized products may not always be available at an early stage of development. Since ITZ was being treated as a NCE with limited information available, only the ITZ levels were measured in this study.

Preparation of Calibration Samples

The bioanalytical method with a range of 5–2500 ng/mL was used and accuracy and precision were determined. The quantitative measurement of ITZ in dog plasma samples was carried out. Chromatograms and integration of the peaks were reviewed by visual inspection to ensure the integration was performed correctly.

Pharmacokinetic Parameters

PK parameters were calculated by noncompartmental analysis using WinNonlin Pro (v4.0.1 or higher). The parameters were derived, where appropriate, from the individual ITZ plasma concentration time profiles following single oral administration of two different forms of ITZ (crystalline and amorphous solid dispersion). Actual sampling times were used for calculation of the PK parameters and calculations were performed on nonrounded data. Values for t_{max} were displayed as actual times.

RESULTS

Crystalline and Amorphous Itraconazole

ITZ is an anhydrous, crystalline solid form with the XRPD pattern (Fig. 2) displaying sharp peaks indicative of the form reported in the Cambridge Structural Database (TEHZIP).[50] By DSC (data

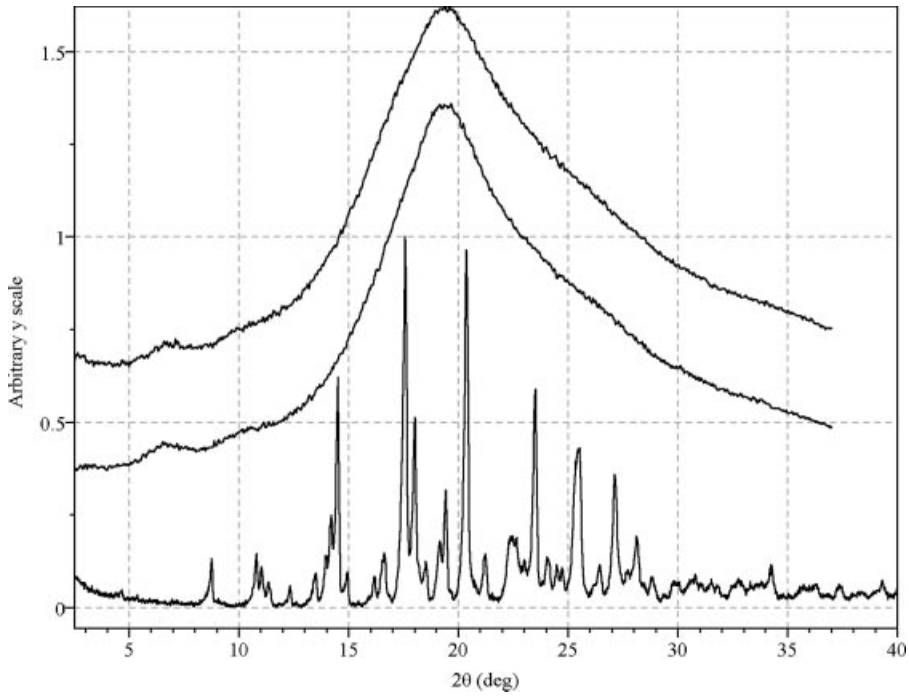

Figure 2. Overlay of XRPD patterns (top to bottom); melt-quench cooled ITZ, cryo-ground ITZ at 30 min, and crystalline ITZ as received.

not shown), a single endotherm at 166°C (onset; ΔH_f: 84 J/g) corresponds to a solid to liquid transition confirmed by hot stage microscopy. Loss of volatiles by TGA from ambient through the melt was <0.1 wt%, consistent with an anhydrous material.

Kinetic solubility was determined to select solvents suitable for preparing amorphous solid dispersion samples. ITZ is sparingly soluble (≤1 mg/mL) in many pure organic solvents, including acetone, methanol, and ethanol with increased solubility in solvent mixtures, such as (3:1; v/v) acetone/ethanol (4.5 mg/mL), at ambient temperature. Slurrying of ITZ in acetone and 9:1 (v/v) acetone/methanol at 20, 30, and 40°C was also conducted for 24 h to inspect for possible crystal form changes. Under the conditions examined, no change in the solids recovered was found by comparison to XRPD and DSC data of ITZ as received.

X-ray amorphous ITZ was obtained by cryo-grinding of crystalline ITZ and by quench-cooling of molten ITZ. Attempts to prepare amorphous material were conducted to assess if amorphous material could be generated (in the absence of a stabilizing agent). If successful, characterization data are used for evaluation of amorphous solid dispersion samples by thermal analysis, where the presence of the T_g for amorphous ITZ suggests phase separation in the amorphous solid dispersion, and in computational analysis as the XRPD pattern for amorphous ITZ. As shown in Figure 2, XRPD patterns for both cryo-ground ITZ at 30 min and quench-melt are nearly identical, owing to the shape and packing arrangement of the molecule in the amorphous solid. For the cryo-ground specimen, a series of endothermic events at 47 (onset), 71, and 86°C, were found by DSC on heating with an asymmetric exotherm, at approximately 95°C (onset), presumably due to crystallization. Using temperature cycling, the *apparent* T_g of a quench-cooled melt by DSC was approximately 60°C (midpoint), with other events at 71 and 90°C similar to those found for the cryo-ground specimen on heating. These data were also noted in the heat flow signal on cooling and are in good agreement with previous literature reports where amorphous ITZ prepared from the melt displayed an *apparent* T_g of 58°C and other thermal events at 74 and 90°C,[51] characteristic of a possible mesophase. The absence of possible crystallization for the quenched melt specimen on heating lends support for the well-established observation that different preparation methods can yield materials with different properties. Evidence of crystallization serves as motivation for selection of a suitable stabilizing agent, given that insufficient stability is displayed by the amorphous material alone.

Amorphous Solid Dispersion Selection

Selection of an amorphous solid dispersion composition and method for preparation was conducted with emphasis on solid-state properties and physical stability. While *in vitro* performance testing is acknowledged as potentially useful criteria to compare prototype compositions, there is no guarantee that a model can be developed to correlate these results to anticipate *in vivo* exposure upon administration. Given that amorphous materials are metastable relative to the crystalline state, with a thermodynamic tendency to crystallize, thus offsetting the potential beneficial effects of greater *apparent* solubility, solid-state attributes important for storage, handling, and processing were investigated initially.

In this study, parameters examined included polymer type, API/polymer ratio, solvent type, and method of preparation. A number of small-scale dispersion samples were produced to cover a wide experimental space with minimal material to get as much information as possible early in development.

For ITZ, various polymers, PVP K-12, K-29/32, and K-90, HPMC-P, and HPMC-AS, were used to produce samples during the screen at four API/polymer concentrations and two preparation methods (evaporation and freeze drying); samples containing PVP VA were only prepared by freeze drying. Polymers were chosen based on commercial availability and effectiveness as stabilizing agents in literature reports for other APIs and in other amorphous solid dispersion screens conducted in our laboratory. Although a large number of other polymers are available, the screen was limited to commonly used polymers that were also amenable to scale-up techniques available. Different grades of PVP were used for comparison. Measured T_g and volatile content values for each polymer prepared by the rotovap-screen technique during screening are shown in Table 1. All polymers used display a measured T_g greater than the T_g of amorphous ITZ; therefore,

Table 1. Physical Characterization of 1:2 (w/w) ITZ/Polymer Solid Dispersions Prepared during Screening

	Polymer		Dispersion			
Type	T_g	(wt%)[a]	T_g	T_g'–T_g''[b]	ΔC_p[c]	(wt%)[a]
HPMC-AS	120	1.2	84	16	0.42	0.6
HPMC-P	138	2.8	107	14	0.34	1.4
PVP K-12	125	4.2	97	18	0.40	3.6
PVP K-29/32	164	4.4	57/155	3/3	0.03/0.05	8.0
PVP K-90	173	5.5	127	34	0.41	5.2

[a]Percent weight loss to 100°C by TGA.
[b]Width of the glass transition region.
[c]Heat capacity change at T_g by mDSC.

these polymers would be expected to increase the T_g of an intimate mixture of the two components.

API/polymer ranges covering a range of API loadings (5:1 (w/w) ITZ/polymer or 83.3 wt% ITZ) to low values (1:5 (w/w) ITZ/polymer or 16.7 wt% ITZ) were included in the screen. Low polymer loading is often sufficient to achieve a physically stable, X-ray amorphous solid; however, higher polymer concentration is often needed to maintain supersaturation and prevent precipitation of the API on exposure to biorelevant media.[14] While it is acknowledged that a wide range of API/polymer concentrations is advantageous, efforts were focused on compositions of 1:2 and 2:1 (w/w) API/polymer. Small-scale preparation methods, such as evaporation amenable to scale-up (e.g., spray drying) were used.[52] Other methods, such as melt techniques, which can be scaled using hot melt extrusion, were not included in this screen due to availability of suitable equipment, but this and other preparation methods have since been added to the screening process in our laboratory.

Initial assessment of the samples was conducted by visual inspection of XRPD patterns and by polarized light microscopy. Only X-ray amorphous materials with no evidence of birefringence (with extinction) were given further consideration. The T_g values of these amorphous samples were then obtained using modulated DSC. By the classical interpretation, a single T_g intermediate between the T_g values of the individual components indicates an intimate mixture or "miscible" dispersion. Alternatively, multiple T_g values suggest the system is at least partially phase separated. Based on this convention, samples with a single T_g are desirable and a T_g of 50°C above ambient temperature has been suggested to achieve long-term stability.[53] Whenever possible, samples with low-volatile content as prepared or low propensity to sorb ambient moisture were chosen to help minimize plasticization effects and to decrease molecular mobility that can contribute to physical instability.

While all freeze dried samples exhibited sharp peaks by XRPD corresponding to crystalline ITZ by XRPD (data not shown) and were not pursued further, X-ray amorphous dispersions were generated by flash evaporation using PVP (all grades), HPMC-AS, and HPMC-P. Overlays of XRPD patterns for 1:2 and 2:1 (w/w) ITZ/polymer samples are shown in Figure 3 along with the XRPD patterns for polymer and amorphous ITZ. Each polymer displays two broad halos. For each mixture, the relative intensity of the broad halo near 10° 2θ shows an apparent composition dependent change (e.g., decreased relative intensity) as ITZ loading increases. This trend provides an initial indication that the components are not intimately mixed and packing of the individual components is likely similar to that of a physical mixture.

The measured T_g for dispersions at 1:2 (w/w) ITZ/polymer prepared by the rotovap-screen technique is shown in Table 1. With the exception of PVP K-29/32, a single measured T_g intermediate to that of amorphous ITZ and the polymer was found for all dispersion samples, with a similar heat capacity change at T_g (0.34–0.42 J/(g°C)). For PVP K-29/32, two T_g values near the measured values of the components were measured, a clear indication of phase separation. For PVP K-90, the width of the glass transition region, T_g'–T_g'', was found to be significantly greater than other samples displaying a single T_g. Although other explanations are possible, this result has been described for polymers as a possible early indicator of phase separation.[54] Volatiles loss on heating (by TGA) for ITZ/PVP samples ranged from approximately 4–8 wt% and was greater than the loss for dispersions containing HPMC derivatives (0.6–1.4 wt%); these values correspond with the relative magnitude of the volatiles content for the polymers used (Tab. 1). Lower volatile content is desirable to reduce the propensity for physical change due to increased molecular mobility.

Based on the available characterization data for samples prepared by a rotary evaporation technique, the effect of temperature, RH, and nonideal drying stress was evaluated for three different polymers (PVP K-12, HPMC-AS, and HPMC-P) at two ITZ/polymer loadings. PVP K-29/32 was omitted from further study given evidence of two T_gs. PVP K-90 was omitted given the high-volatiles content and width of the glass transition region (e.g., possible indicator of phase separation) relative to the other polymer options displaying a single T_g.

Physical Stability

Each ITZ/polymer dispersion sample was stressed at two conventional conditions, 40°C/dry and 40°C/75% RH, to assess physical stability due to possible excursions from more protective storage conditions. These data are summarized in Table 2 for selected ITZ/polymer compositions. Volatiles lost on heating (presumed to be water sorption) at elevated RH conditions was also obtained. All samples stored dry at elevated temperature showed no change by XRPD after 16 days. Under elevated humidity, XRPD data for all samples with the exception of 1:2 (w/w) ITZ/HPMC-AS and ITZ/HPMC-P exhibit sharp peaks corresponding to crystalline ITZ. By TGA (data not shown), the volatiles loss for samples generated from PVP, HPMC-AS, and HPMC-P is near 1 wt% or less.

A rotary evaporation spray technique (denoted rotovap-spray) was also devised as part of the selection process to prepare dispersions at 3 g scale. While this was not intended to mimic the kinetics of spray drying, the continuous spray of ITZ/polymer solution applied to deposited solids created a nonideal

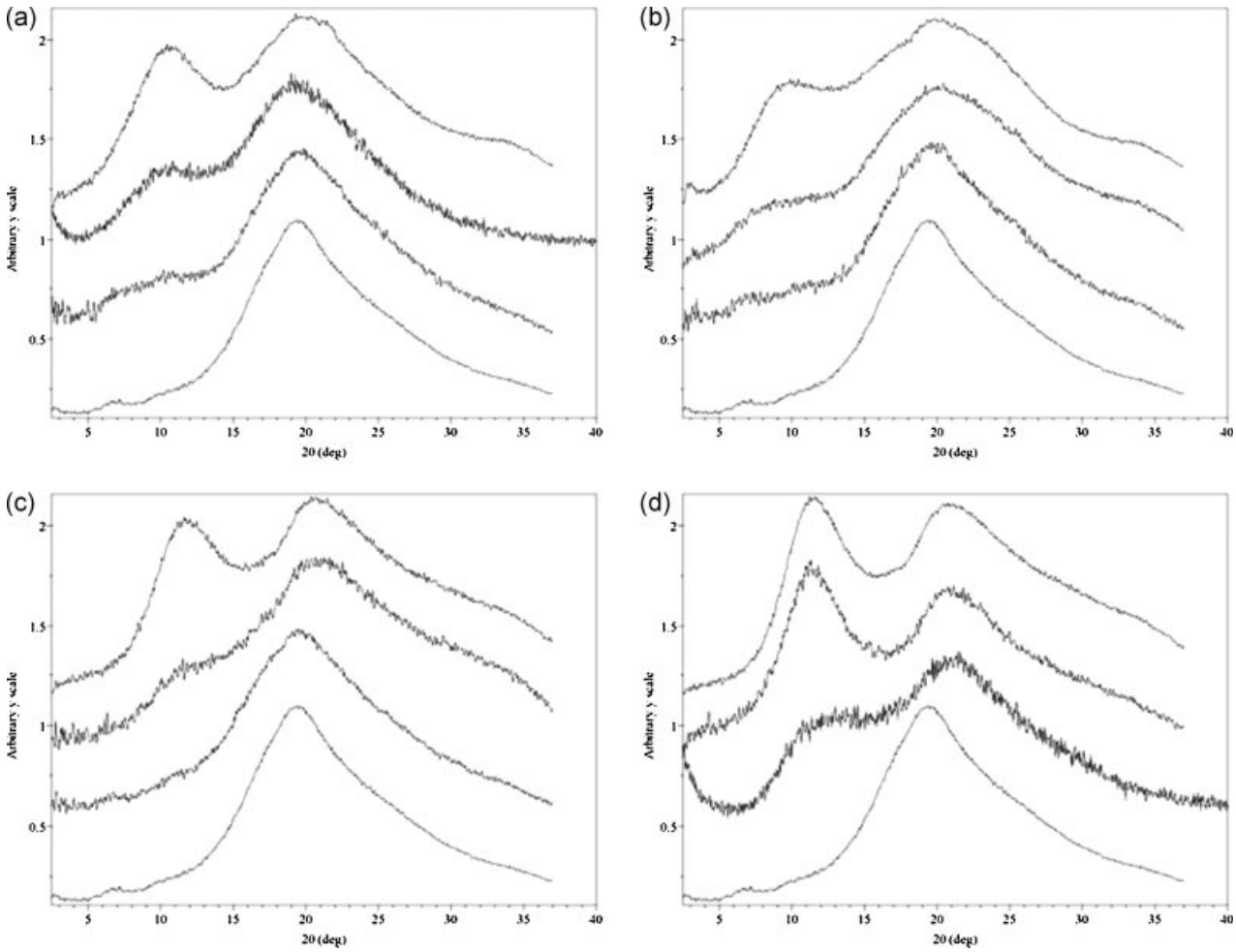

Figure 3. Overlay of XRPD patterns for ITZ/polymer dispersions; (a): HPMC-AS, (b): HPMC-P, (c): PVP K-12, and (d): PVP K-90. For each panel (top to bottom); polymer only, 1:2 and 2:1 (w/w) ITZ/polymer, and X-ray amorphous ITZ prepared by melt-quench.

drying condition (e.g., a kinetic stress), potentially useful for evaluating other methods of manufacture (e.g., fluid bed coating). Under these conditions, only the 1:2 (w/w) ITZ/HPMC-P dispersion was X-ray amorphous. All other samples exhibited evidence of sharp peaks by XRPD corresponding to crystalline ITZ. This suggests that the HPMC-P dispersion is readily produced even under nonideal drying kinetics.

Using the criteria of an X-ray amorphous solid with a single T_g >70°C that is physically stable on exposure to elevated temperature and humidity, and a nonideal drying condition, 1:2 (w/w) ITZ/HPMC-P dispersion was chosen for scale-up and *in vivo* performance testing.

Table 2. Summary of Characterization Data for Selected ITZ/Polymer Solid Dispersions after Physical Stress

		X-Ray Amorphous (Yes or No)			
Polymer	Ratio[a]	40°C/16 day|	40°C/75% RH/4 h	(wt%) [b]	Rotovap-Spray
PVP K-12	1:2	Yes	No	1.3	No
	2:1	Yes	No	—	—
HPMC-AS	1:2	Yes	Yes	0.6	No
	2:1	Yes	No	<0.1	—
HPMC-P	1:2	Yes	Yes	1.2	Yes
	2:1	Yes	Yes	0.1	—

[a]API: polymer ratio, by weight.
[b]Weight loss to 100°C by TGA, after storage at 40°C/75% RH.

Characterization of 1:2 (w/w) Itraconazole/HPMC-P

Process parameters used for manufacture of 1:2 (w/w) ITZ/HPMC-P dispersion were defined during a series of development experiments. Given the low solids solution concentration in acetone, the resultant product was a low bulk (0.10 g/mL) and tapped (0.15 g/mL) density, fine particle powder. Secondary drying was performed at 40°C under vacuum, where the difference in acetone level before

and after vacuum drying was 0.1 wt%. Spray drying was conducted at 200 g scale to confirm the process parameters during an endurance run of approximately 5 h; a yield of 156 g (78%) was collected at the cyclone (yield at the cyclone is attributed to the small particles produced due to low solids content). Chamber deposits and material accumulated on the nozzle during both spray-drying campaigns were typical of this type of product.

The 1:2 (w/w) ITZ/HPMC-P dispersion spray dried at 200 g scale was characterized by a variety of methods and compared to the initial material produced during the screen. Data are compared in Table 3. The XRPD patterns for samples prepared by the rotovap-screen technique, rotovap-spray technique, and spray drying are shown in Figure 4 and were X-ray amorphous. For the rotovap-spray sample, it is interesting to note that the primary halo near 20° 2θ appears shifted to higher scattering angles. By XRPD, the position of the primary halo and overall shape of material prepared by the rotovap-screen and spray-drying techniques is consistent with similar materials being produced by different methods.

Volatile contents measured by TGA ranged from 0.8 to 1.2 wt% and the values decreased for the sample prepared using larger scale processes. Specifically, the morphology of the solids (e.g., flakes or films by rotovap-screen and fine, discrete particles by spray drying) likely contributed to the efficiency by which volatiles were removed during drying. Following spray drying, the 200 g scale material was vacuum dried whereas the 1 kg scale material was fluid bed dried. This suggests that the secondary drying (after spray drying) can be a critical step during manufacture. No major differences were found by water sorption/desorption isotherms for the material produced by rotovap-spray technique and spray drying and physical stability was similar to screening samples.

By modulated DSC, one T_g was measured for all ITZ/HPMC-P samples prepared by different methods, as shown in Figure 4. The width of the glass transition region (T_g'–T_g'') and the specific heat change (ΔC_p) at T_g for samples prepared by the rotovap-screen technique (107°C) and spray dried at two different scales (108°C) were similar; however, the rotovap-spray technique showed a lower T_g at 89°C. While the lower value does not correlate with a higher volatiles content (e.g., plasticization leads to decreased T_g), the greater value of T_g'–T_g'' offers a potential early indication of phase separation as noted previously. The nonideal drying conditions for the rotovap-spray technique as compared to the other techniques provide a clear example that the preparation method, or sample history, can influence the properties of amorphous materials.

Computational studies were also performed for the 1:2 (w/w) ITZ/HPMC-P dispersion. These studies are based on the linear combination of PDF traces derived from the XRPD data. Details of the computational analysis of dispersions have been reported.[48] If the experimental data for the dispersion sample can be described by amorphous API and the polymer data, the material is considered to be phase separated. Alternatively, if the experimental data for the amorphous solid dispersion cannot be described by the individual components, the dispersion is considered miscible or the data for the individual components does not reflect the local structure of the mixture. Amorphous ITZ was produced by cryogrinding and the as-received polymers were dissolved and evaporated under similar conditions used for the dispersion samples in order to reproduce any possible changes due to processing. These processed polymer samples were used as reference materials for the

Table 3. Characterization Data for 1:2 (w/w) ITZ/HPMC-P Solid Dispersion Prepared by Different Techniques

	Method of Preparation (Scale)			
Test	Rotovap-Screen (50 mg)	Rotovap-Spray (3 g)	Spray Drying (200 g)	Spray Drying (1 kg)
XRPD	X-ray amorphous	X-ray amorphous	X-ray amorphous	X-ray amorphous
T_g (°C)	107	89	108	108
T_g'–T_g'' (°C)[a]	14	42	17	15
ΔC_p (J/g°C)[b]	0.34	0.48	0.38	0.37
TGA (wt %)[c]	1.2	1.0	0.8	0.4
DVS analysis				
Gain (5–95% RH) (wt%)	—	5.2	5.5	5.6
Loss (95–5% RH) (wt%)	—	5.5	5.5	5.5
X-ray amorphous (yes or no)				
After DVS	—	Yes	Yes	—
40°C/ 14–16 days	Yes	—	Yes	—
40°C/75% RH/4 h	Yes	—	Yes	—

[a]Width of glass transition region.
[b]Heat capacity change at T_g by mDSC.
[c]Weight loss to 100°C by TGA.

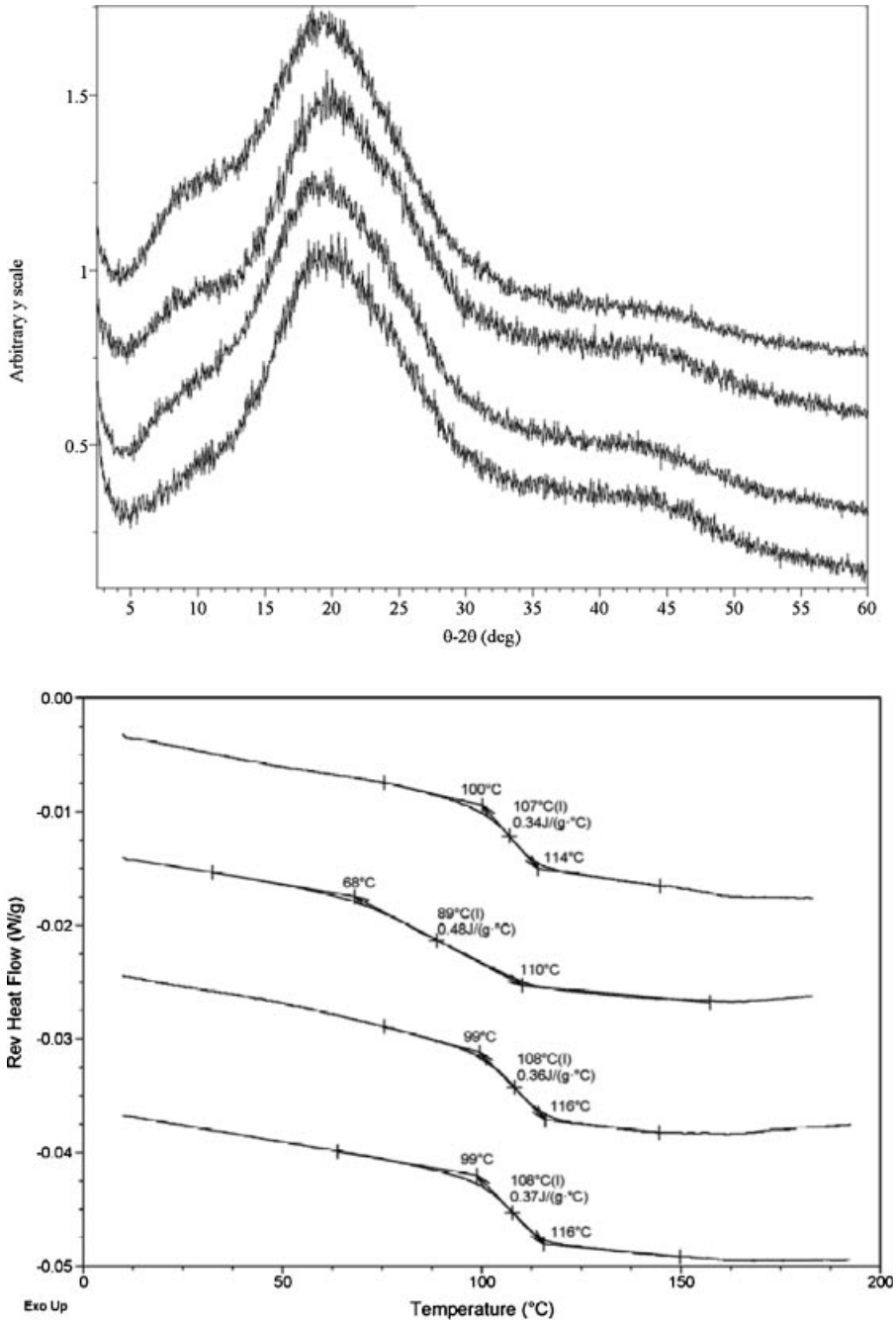

Figure 4. Overlay of XRPD patterns (top panel) and reversing heat flow signal by modulated DSC (bottom panel) for 1:2 (w/w) ITZ/HPMC-P samples, from top to bottom: screening (50 mg scale), rotovap-spray (3 g scale), spray dried (200 g scale), and spray dried (1 kg scale).

analysis method. The computational analysis results using PDF are shown in Figure 5. For the 1:2 (w/w) ITZ/HPMC-P dispersion, the PDF can be readily described by the individual components, which suggest that the sample is phase separated.

The conclusion derived from PDF analysis clearly indicates the 1:2 (w/w) ITZ/HPMC-P dispersion is phase separated and is in *apparent* contrast to the single T_g classically interpreted as an indication of a "miscible" system. Since DSC cannot detect two T_g values when phase separation produces amorphous domains with sizes less than approximately 30 nm,[55] it is concluded that the local structure of the 1:2 (w/w) ITZ/HPMC-P specimen is likely a phase separated mixture having nanosize domains of amorphous ITZ and HPMC-P. This has been reported for the trehalose/dextran system as a solid nanosuspension[48] and other examples have been

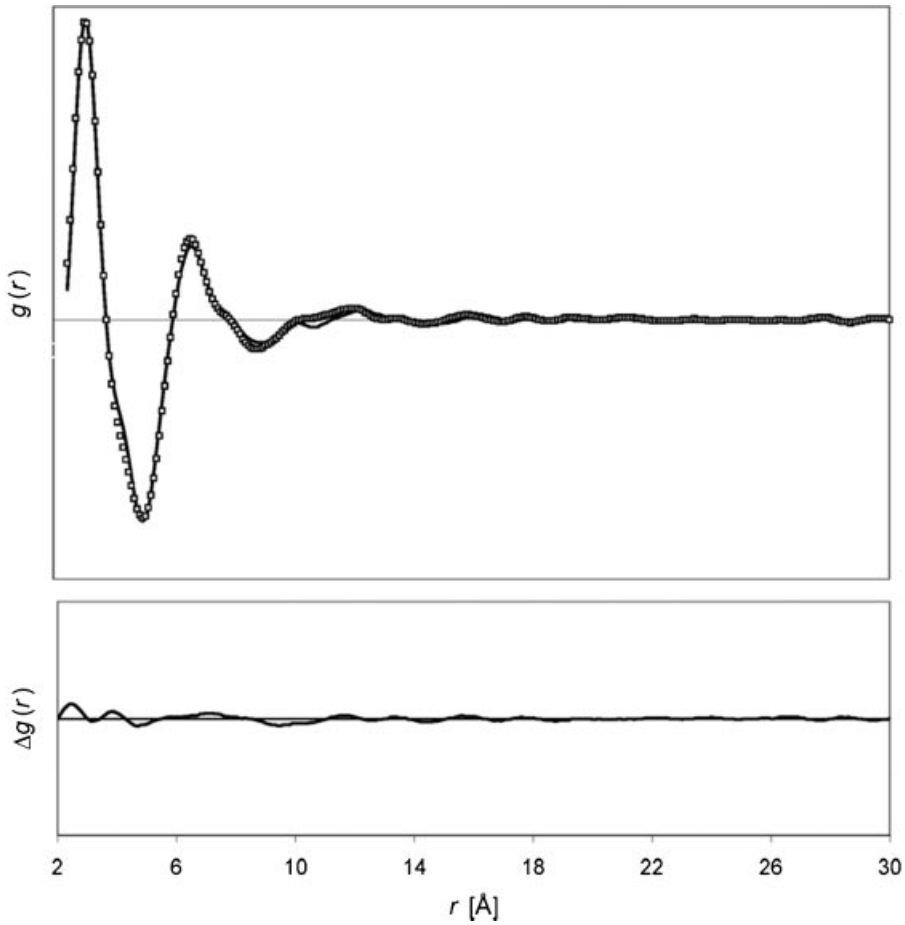

Figure 5. Computational analysis of 1:2 (w/w) ITZ/HPMC-P amorphous solid dispersion by PDF method. The PDF acquired from measured data (bold line) is described by the PDF calculated from the individual components (squares), suggesting the sample is a phase-separated mixture.

found in our laboratory. Therefore, by hypothesis, such systems would be expected to have properties intermediate to those observed for miscible and macroscopically phase separated solid dispersions and could provide adequate stability for development.

Performance: Dog Bioavailability Study

A bioavailability study in dogs was performed to compare the 1:2 (w/w) ITZ/HPMC-P dispersion to crystalline ITZ. The focus in this study was to improve the solid-state properties to increase *apparent* solubility and ultimately the bioavailability. A very simple formulation (drug in capsule) was used to directly evaluate the improvement found by changing the solid form from a crystalline solid to the dispersion.

The results of the dog study are shown in Figure 6 and summarized in Table 4, where C_{max} is the maximum observed concentration, T_{max} is the time of occurrence of C_{max}, and F_{rel} is the relative oral bioavailability of ITZ as an amorphous solid dispersion compared to crystalline ITZ. A suffix "C" for crystalline ITZ and "D" for amorphous solid dispersion is added to the coding assigned to each animal to facilitate understanding. Following single oral administration of ITZ as an amorphous solid dispersion, ITZ plasma concentrations were measurable up to 72 h postdose in all animals, indicating continuous systemic exposure to ITZ over the entire blood-sampling period. By contrast, following single oral administration of ITZ as a crystalline form, ITZ plasma concentrations were measurable up to 24 or 72 h postdose in animals 001MC and 003MC, respectively. It should be noted that animal 002M ITZ plasma concentrations were all below the lower limit of quantification of the assay. Maximum ITZ plasma concentrations (C_{max}), were observed at 1–2 h postdose for the dispersion. For the crystalline material, C_{max} was observed later at approximately 4 and 8 h postdose in animals 001MC and 003MC, respectively. It is noteworthy that C_{max} was 34.9- and 16.7-fold greater for the dispersion compared to the crystalline material in animals 001MD and 003MD, respectively. Following single oral administration of

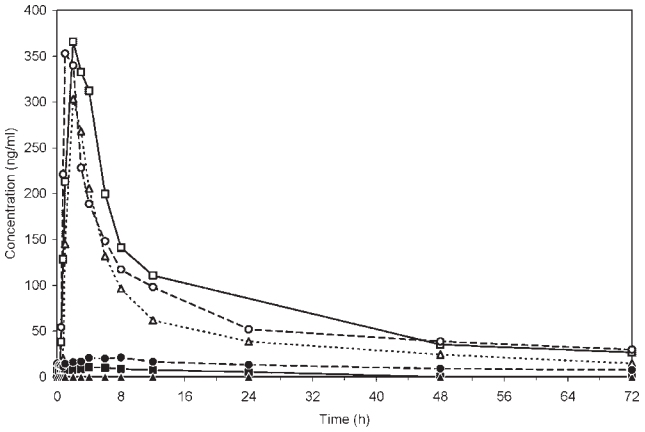

Figure 6. Dog bioavailability data for crystalline ITZ in a capsule (001MC: ■, 002MC: ▲, 003MC: ●) and 1:2 (w/w) ITZ/HPMC-P (001MD: □, 002MD: △, 003MD: ○) in a capsule.

50 mg ITZ as an amorphous solid dispersion, systemic exposure to ITZ was 551–3115% of that observed following administration of 50 mg crystalline ITZ (F_{rel}; i.e., 5.5- to 31.2-fold greater).

Results of this study show that significant gains in bioavailability are possible by simply changing the solid form and using a very simple formulation of drug in a capsule. It should be noted that this increase could be achievable for BCS Class 2 and 4 compounds where the bioavailability will be affected by solubility.

DISCUSSION

The purpose of this study was to apply a solid-state approach to identify an amorphous solid dispersion with potentially useful properties, relative to the crystalline form, using ITZ as a model compound. It was conducted as part of an internal proof of concept program intended to accelerate early drug candidates. An amorphous solid dispersion was selected to provide ITZ in a more bioavailable solid form to maximize systemic exposure. The use of alternate solid forms to overcome solubility deficiencies has been demonstrated with polymorphs,[56,57] salts,[58] and co-crystals.[59] Polymorphs do not always provide the large solubility increase needed for very poorly soluble compounds, and therefore can be of limited use for challenging compounds.[60] Salts can be a viable alternative for compounds containing acidic and basic groups, but cannot be used for neutral pharmaceutical materials. Co-crystals can be used for both ionizable and neutral compounds; however, they will not always provide the desired increase in solubility.[61,62] Amorphous solid dispersions are a viable fourth option. An amorphous solid dispersion consists of an amorphous API stabilized by a polymer or polymers, with the high energy, amorphous form of the API providing increased *apparent* solubility.[63] Since the API is in an amorphous state, dramatic increases in *apparent* solubility and dissolution rate can be obtained when compared to the crystalline compound. The increase in *apparent* solubility can lead to an increase in bioavailability as well; a number of examples of increased bioavailability of amorphous solid dispersions have been reported.[64–66] The polymers added to the dispersion can improve stability of the amorphous API when favorable combinations of components are found. The marketed product Kaletra®, a combination product containing ritonavir and liponavir, is an amorphous solid dispersion produced by hot melt extrusion.[67,68] It exhibits improved bioavailability over crystalline ritonavir as well as acceptable shelf life for a marketed drug product. While its *in vivo* performance can be attributed in part to the dispersion, Kaletra® achieves significant improvement in bioavailability by coformulating with a second active to reduce metabolic activity – an approach that is potentially useful for BCS Class 4 compounds.[69] Other examples of commercial products developed using an amorphous solid dispersion include: Cesamet® (nabilone); Gris-PEG® (griseofulvin); Isoptin® SR-E 240 (verapamil); and Nimotop® (nimodipine).[70]

For the solid form selection and performance evaluation part of the internal program, key milestones included finding an acceptable amorphous solid dispersion with properties (e.g., *apparent* solubility and physical stability), needed for early development, defining suitable processing technology for scale-up and manufacture of a solid dispersion, and demonstrating improved performance by improved bioavailability in dogs. The time required to achieve each milestone is illustrate in Figure 7. It should be noted that the spray-dried material was ultimately formulated into a simple powder in capsule (PIC) dosage form and packaged; however, these details are beyond the scope of this paper. An

Table 4. Pharmacokinetic Parameters for Bioavailability Study

Animal No.	C_{max} (ng/mL)[a]	t_{max} (h)[b]	F_{rel} (%)[c]
Dispersion			
001MD	366	2.0	3115
002MD	303	2.0	NC
003MD	353	1.0	551
Crystalline			
001MC	10.5	4.0	—
002MC	0	NC[d]	—
003MC	21.1	8.0	—

NC, not calculated.
[a]Maximum observed concentration.
[b]Time of occurrence of C_{max}.
[c]Relative oral bioavailability of ITZ as an amorphous solid dispersion compared to crystalline ITZ.

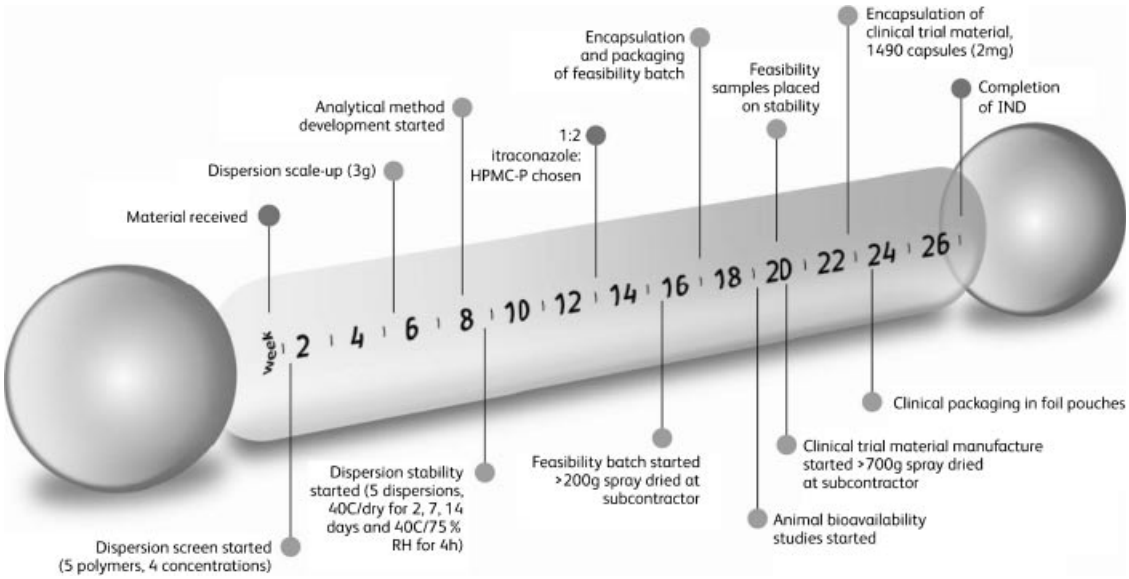

Figure 7. Summary of proof of concept program.

amorphous solid dispersion was chosen to overcome the deficiency in aqueous solubility (and bioavailability) of a poorly soluble model compound, ITZ. The aim was to select an amorphous solid dispersion that could be prepared by available solvent-based approaches, display acceptable physical stability, and would achieve maximum exposure in preclinical assessment of a BCS Class 2 drug. Ultimately, this composition could be used in a simple formulation for FIH clinical trials. ITZ was chosen as the model compound because it represented a poorly soluble API with many of the properties that challenge rapid early development studies. Even though complex formulation strategies have been reported for improving the *apparent* solubility of ITZ and other poorly soluble compounds, the purpose here was to leverage an understanding of solid-state properties, specifically for amorphous materials, to achieve reduced timelines and accelerated time to submission. In this study, a useful solid dispersion was selected within 12 weeks of receipt of the API. Process development to enable scale-up and delivery of spray-dried amorphous solid dispersion for capsule manufacture was achieved in <3 weeks. Finally, *in vivo* performance assessment in dogs was conducted within 20 weeks of the start of the screening study. This also included the development of a bioanalytical method to detect the compound in the plasma.

Solid form selection involving (crystalline) polymorphs, salts, or co-crystals requires comparison of numerous properties, such as stability, solubility, melting point, and a variety of other characteristics.[71–73] Similar circumstances arise when choosing an amorphous solid dispersion. The requisite list can be dependent on the dosage form targeted, the properties desired, issues with the original compound, as well as many others. Decision trees have been developed to capture relevant questions, and are useful to narrow the possibilities during form selection[71–73] and the same concept can be used for amorphous solid dispersions. For these systems, physical properties, stability, processing, and performance are usually key considerations. An example decision tree is given in Figure 8 covering these properties. This decision tree can be modified and properties can be added or changed depending on the developmental challenges of the compound. The general concept is to use an initial assessment of physical properties to identify potentially useful dispersion compositions, investigate stability and processing to narrow the choices, and determine performance by an applicable method, such as animal bioavailability testing, to determine the lead dispersion. This approach can cover a wide range of polymers and concentrations to meet specific requirements, as well as focus effort on the main issues that need to be addressed in developing a robust amorphous solid dispersion; that is, processing, stability, and performance.

Physical Properties

Finding an amorphous solid dispersion with the desirable physical properties usually starts with a screening activity. Solid dispersion screening is conducted similarly to polymorph or salt/co-crystal screens conducted in our laboratory; a wide experimental space is explored using a variety of variables and material sparing techniques. For polymorphs, screening is intended to identify the thermodynamically most stable form and to find metastable and *pseudo*-forms (e.g., hydrates and solvates) under conditions for anticipated for production and use.

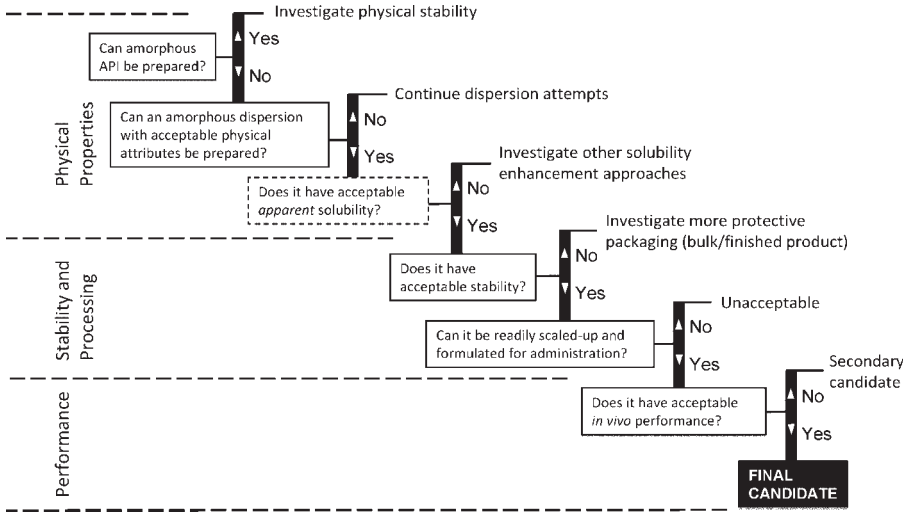

Figure 8. Decision tree for dispersion selection.

Salt and co-crystal screens involve the same parameters (e.g., a wide range of solvents, temperatures, and crystallization procedures) along with counterions or guests selected based on inspection of the molecular structure. While polymorphism is not predictable, screens for crystalline solid forms are directed toward identifying the thermodynamically most stable form and developing an awareness of other possible crystalline forms (including solvates and hydrates) from solvents used in the final isolation step, formed under different storage (stress) conditions, or by interactions with excipients selected for drug product formulation. For amorphous solid dispersion screens, the variables include polymer types, API/polymer ratios, solvents, temperature, and preparation methods. Screens can be strictly empirical containing a large number of common polymers or polymer choices can be narrowed based on common interactions, or the lack of interactions, between the known functional groups in the API and polymers. For instance, interactions between carboxylic acids to form dimers, as seen with indomethacin and PVP,[74] represent a common combination to try. Other common hydrogen bonding motifs,[75,76] along with ion–dipole interactions,[77] have been proposed between polymers and APIs to explain the stability of the amorphous solid dispersions. Additional approaches for choosing polymers include miscibility and solubility using Flory–Huggins theory,[78] molecular mobility,[79] melting point (T_m), and glass transition (T_g) ratio (T_m/T_g),[80] Hansen solubility parameters,[81] and viscoelastic properties.[82]

Dispersion screens have the same advantages as other solid form screens: cover a wide space (in this case by using multiple polymers and concentrations), use small amounts of material for initial studies, narrow down choices based on acceptable properties, and patent new compositions early. Screening methods for dispersions usually employ manual methods on the milligram scale, such as solvent evaporation, melting, or comilling. Properties then need to be assessed, such as crystallinity, *apparent* solubility, etc. in order to narrow down the possible choices. Scale-up is then an important consideration to produce additional material for further characterization, formulation, and performance studies. Common laboratory scale approaches include melt methods, rotary evaporation, and spray drying. The most popular large-scale manufacturing methods include melt extrusion and spray drying. Automated screens have also been reported for finding amorphous solid dispersions.[70,83] One screen used an approach that is amenable for melt extrusion using a solvent casting screen in plates for the initial experiments, followed by a small-scale melt-press method for dispersions with increased *apparent* solubility.[70] Scale-up using melt extrusion was then used to produce material for animal bioavailability studies. A second study also employed solvent casting in plates for initial experiments, but then made larger quantities by spray drying.[83] Both studies showed that automated screening was an efficient tool to rapidly survey a large number of combinations with minimal sample.

Physical property assessment is an important aspect of the dispersion selection process. The ideal dispersion would have the following characteristics: X-ray amorphous or noncrystalline solid, increased *apparent* solubility, a T_g greater than ambient temperature, low-volatile content, and nonhygroscopic. Depending on the scale of the experiments, DSC data can be collected to determine T_g and use the classical interpretation to inspect for evidence of miscibility (e.g., one T_g); however, exceptions have

been reported for this criteria.[84] Volatile content data can be used to assess the solvent present in the material that can contribute to increased mobility and to help better define drying and storage conditions. In most cases, higher solvent levels will result in increased mobility, lower T_g values, and a greater probability for crystallization. In many cases, an ideal dispersion may not be found. However, a dispersion composition suitable for evaluation of bioavailability improvement (relative to the crystalline form) and, if successful, FIH clinical evaluation is possible, if suitable controls are put in place, such as low-temperature storage for low T_g dispersions or special handling/packaging to mitigate exposure to RH.

The ITZ dispersion screen performed here was a targeted study using manual solvent methods leading to scale-up of up to 1 kg using spray drying. Dispersions were tested initially to inspect for evidence of crystalline content. Amorphous samples were then tested by DSC and TGA for T_g, and volatile content, respectively. Samples with acceptable T_g ($>70^\circ$C) and low-volatile contents were then taken to the next level of stability and processing.

While miscible dispersions are desired, this criterion may not be achievable in all cases. Previous work performed on dispersions has shown that even if one T_g is observed for an amorphous solid dispersion, modeling studies suggest that it may exist as phase separated, nanosize amorphous domains of the individual components, amorphous API in the polymer.[84] A solid nanosuspension is a physical mixture of the amorphous API and the polymer, but due to the very small domain size (estimated as <30 nm), only one T_g is evident by DSC. The amorphous nature of the API results in higher *apparent* solubility and even though it is a physical mixture, there can be a significant stability improvement with these types of systems. A solid nanosuspension may have suitable properties to enable preclinical and early clinical evaluation and would need to be studied further to determine if it was suitable for later clinical studies. In this study, the 1:2 (w/w) ITZ/HPMC-P dispersion was found to be a solid nanosuspension based on thermal analysis and inspection of the local structure through limited modeling studies. The physical properties (XRPD, T_g, volatile content) were all found to be acceptable for an early development candidate, even though it was not considered a miscible dispersion.

Stability and Processing

Physical stability of the amorphous dispersion is a major consideration during development since crystallization of the amorphous API is not acceptable, but is a thermodynamic expectation. In some cases, an increased amount of polymer will result in improved physical stability.[85] Other studies show dispersion samples can remain amorphous for 3 years at elevated temperature and RH conditions.[86] Other materials have been reported to remain amorphous during storage, but exhibit two rather than the original one T_g, indicating a possible phase separation between the amorphous drug and polymer.[87] These and other examples indicate that improved physical stability can be achieved with amorphous solid dispersions, but samples will need to undergo testing to determine initially if there is improvement and then the extent of the improvement.

Both temperature and RH can play a role in the stability of an amorphous solid dispersion.[88] It has been suggested that storage of an amorphous solid at approximately 50°C below T_g will contribute to the stability of the amorphous material and will help prevent crystallization.[53] In amorphous solid dispersions, the addition of the polymer helps increase T_g of the system which can significantly add to the physical stability. The effect of RH also needs to be considered. Water is a known plasticizer; therefore, when water is absorbed by the amorphous solid dispersion, the T_g will decrease. This decrease in T_g can be related to increased mobility and possible crystallization in some systems. To ensure the physical stability of amorphous solid dispersions, the effect of temperature and RH need to be assessed for each system.

Handling of the amorphous solid dispersion during the formulation process is another important consideration for these materials. In many cases, low-RH conditions would be ideal when filling the dispersion into capsules or for other processing steps. This may not be possible in all cases, but should be considered when developing these materials. For the ITZ/HPMC-P dispersion, the environmental conditions during encapsulation were to be between 30% and 60% RH. These conditions were not expected to be detrimental for this dispersion based on the early stability work presented and were found not to be an issue during the actual processing (data not shown). This may not be the case for all dispersions and early stability work should help delineate conditions that will be needed.

Packaging is also of concern for the drug product. Most amorphous solid dispersions should be protected from extreme RH conditions. There are a number of ways to do this (foil pouches, blister packs, etc.) that can be explored for the compound being developed. Additional drug product stability studies were performed in our laboratory using spray dried ITZ/HPMC-P material produced at the 200 g scale. The amorphous solid dispersion was filled into size 1 gelatin capsules (6 mg dispersion per capsule), the capsules were sealed in foil pouches (10 capsules per pouch), and the packaged samples were placed on stability at 25°C/60% RH and 40°C/75% RH. The samples remained amorphous at the 1-month time point even with 1–2% uptake measured for the

powder. The water in the gelatin capsules did not appear to have a deleterious effect on the solid and the packaging provided suitable protection at the high RH conditions.

Scale-up of amorphous solid dispersions, to amounts necessary for clinical trials, needs to be considered during selection and can affect how screening experiments are performed. The common methods to produce kilogram quantities of dispersion are melt extrusion and spray drying.[89] Melt extrusion involves melting of the polymer and API to form the solid amorphous dispersion. While appropriate melting temperatures and the lack of degradation at elevated temperatures of the formulation components are considerations for melt extrusion technology, it is also possible to generate dispersions by processing below the melting temperature of the pure API.[90] Spray drying is a solvent based technique where the solvent is rapidly removed to produce the amorphous solid dispersion. Solubility of both the API and polymer(s) in the solvent, as well as the ability of the solvent to volatize are examples of the properties that need to be evaluated for the spray drying technology. Studies have shown that the method used to produce the amorphous solid dispersion can have an effect on the properties of the material. An amorphous solid dispersion of a development compound with HPMC-AS produced by melt extrusion and coprecipitation resulted in materials that had similar physical properties (based on T_g, spectroscopic data, water uptake, and physical stability in the solid-state), but different surface areas, surface porosity, dissolution rates, and stability in an aqueous suspension formulation.[91] Another study involved melt extrusion, spray drying, and ball milling to produce dispersions of three compounds using PVP. The study showed again that physical stability was not affected by the production method, but differences in dissolution were observed, with the spray-dried material exhibiting the slowest dissolution.[92]

Performance

Performance testing for amorphous solid dispersions includes the normal evaluation for pharmaceutical formulations, such as disintegration, dissolution, and ultimately bioavailability. However, some additional considerations may be needed when conducting these tests on amorphous solid dispersions.

Disintegration is an important parameter that can be overlooked for simple formulations, such as powder in a capsule. Visually observing a filled capsule immersed in relevant media can give insight into possible performance. Some amorphous solid dispersions may readily disperse in the media and remain dispersed during the course of the experiment. Others may form a gel-like appearance and remain at the bottom of the vessel, which could slow the dissolution of the API. Adding a disintegrant to a simple capsule formulation may help performance by forcing the amorphous solid dispersion to disperse in the medium being used. Other amorphous solid dispersions may readily disperse, but may not wet sufficiently, resulting in solid floating to the surface and "sticking" to equipment, such as mixing shafts, resulting in inaccurate readings during testing.

Determining how much of the dispersion dissolves in relevant media (such as simulated gastric or intestinal fluid) can help rank the dispersions in terms of improvement over the crystalline material. Extent of dissolution is commonly used as an initial indicator of exposure potential, although good *in vitro–in vivo* correlation (IVIVC) is still lacking.[63] This may be due to the as yet unclear role that the polymer can play in the gastrointestinal tract, either as a crystallization inhibitor, solubilization agent, adsorption aid, or other functional moiety. Rate of crystallization can also be investigated and used to rank the amorphous solid dispersions. Fast crystallization could result in lower bioavailability due to a large presence of crystalline rather than amorphous API. Solution calorimetry is a method that has been used to analyze amorphous materials[93] and can be applied to amorphous dispersions. It can be used to investigate dissolution as well as crystallization in relevant media over a time period of hours to simulate the transit time in the digestive tract. Although all these tests provide conventional information on the material, none of them provide the maximum exposure information needed for moving the compound into clinical trials.

The ultimate performance test is a bioavailability study to determine systemic absorption of the API. In order to reduce time during development, it can be advantageous to move quickly into an animal model to test systemic exposure and bioavailability of the dispersion. Common performance tests, such as dissolution, commonly provide minimal information on bioavailability, however, significant time is usually spent developing an assay that may not correlate to bioavailability. As a way to save development time, an alternate approach is to develop a full dissolution assay after performance is demonstrated. This test can then be used to show process control and consistency of the preparation method.

Animal model selection in screening dispersions prior to FIH studies can be an important consideration due to differences in oral absorption in various species and the correlation with human absorption.[94] In this study, bioavailability was measured in dogs to compare the 1:2 (w/w) ITZ/HPMC-P dispersion to crystalline ITZ. The bioavailability of crystalline ITZ is extremely low[15] and, as discussed earlier, a variety

of formulation approaches have been used to try to increase the bioavailability. The focus in this study was to change the solid form to ultimately increase bioavailability. A very simple formulation (drug in capsule without excipients) was used to directly evaluate the improvement found by changing the solid form from a crystalline solid to the amorphous solid dispersion. It should be noted that the ITZ/HPMC-P dispersion in this study was a solid nanosuspension of amorphous drug and polymer rather than a miscible dispersion, and the bioavailability advantage was still significant. Other amorphous dispersions have also shown an increase in bioavailability for a variety of compounds.[64–66] The major increase in bioavailability found in this study for a simple PIC formulation containing ITZ/HPMC-P illustrates that a solid-state approach using simple formulations may be a realistic path in getting poorly soluble drugs into human clinical trials faster.

CONCLUSIONS

It was shown in this study that modifying the solid-state properties of a poorly soluble, crystalline, BCS Class 2 compound using an amorphous solid dispersion in a simple formulation (drug in capsule) can result in a significant improvement in bioavailability. The intent of this study was to test a solid-state approach for rapidly identifying a prototype solid form for a model compound, not to develop a better formulation specifically for ITZ. Physical characterization, stability, and processing were investigated to help identify a prototype formulation suitable for preclinical and clinical use. The studies described here were part of an accelerated approach to decrease early development time. For each compound, formulation design attributes are established based on a solid-state approach (decision trees) developed to meet the requirements of preclinical, clinical, and commercial drug product. What has been reported here is a process of systematically choosing a solid dispersion of a poorly soluble API for use in early animal and FIH studies, as reflected in the program, with the potential to expand to a marketed product if deemed necessary. While this approach is suitable for enabling early drug candidates, it is recognized that additional process and product development effort is required to move this prototype formulation into later stage clinical development and product commercialization. Based on this study and other reports, amorphous solid dispersions are a viable option to help maximize exposure in preclinical and FIH studies without overly complicated formulation development.

ACKNOWLEDGMENTS

The authors would like to acknowledge Analytical Resources Group at SSCI, West Lafayette for the solid-state characterization data presented here ISP Pharma Technologies Division Columbia, MD for the manufacture of spray dried material used in this study, and Pharmaceutics Group at Aptuit, Inc, Kansas City, MO for conducting capsule stability work referenced. The authors would also like to thank Prof G. Zografi and Dr. S. Bethune for their helpful suggestions in the compilation of this work.

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