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Amorphous Compositions Using Concentration Enhancing Polymers for Improved Bioavailability of Itraconazole

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Abstract: Amorphous engineered particle compositions of itraconazole (ITZ) and potential concentration enhancing polymers, cellulose acetate phthalate (CAP) and polyvinyl acetate phthalate (PVAP), were produced by ultra-rapid freezing to investigate the effect of these polymers on the bioavailability of ITZ solid dispersions. X-ray diffraction analyses of engineered particle compositions were shown to be amorphous. Modulated differential scanning calorimetry demonstrated that ITZ:CAP engineered particle compositions exhibited a strong correlation with the Gordon−Taylor relationship while ITZ:PVAP formulations exhibited positive deviations from predicted values attributed to hydrogen bonding interactions between the drug and polymer. Energy dispersive spectroscopy mapping demonstrated that the drug was homogenously distributed within all compositions, supporting the miscibility of the drug with the polymers. Scanning electron microscopy imaging of the particles demonstrated that the material existed in two general forms, discrete particles of approximately 5 μm and larger aggregates in excess of 30 μm, with engineered particle compositions having approximately 15 times higher measured specific surfaces areas compared to micronized ITZ. *In vitro* supersaturated dissolution results showed that all compositions provided significantly lower levels of supersaturation in acidic media and greater extents of supersaturation in neutral media compared to Sporanox pellets. ITZ:CAP formulations provided the greatest degree and extent of supersaturation in neutral media. Dissolution data were fitted to an exponential relationship based on a simplified model of particle growth, allowing for the determination of drug half-life in solution for evaluation of stabilization behavior. 1:2 ITZ:CAP showed superior *in vitro* performance compared to all other engineered particle compositions and was selected for *in vivo* testing. Although not fully elucidated, data indicated that the stabilization mechanism was due to interactions between the drug and polymer, primarily attributed to steric hindrance resulting from the molecular weight of the polymer chain and chemical composition of the polymer backbone relative to position of hydrogen bonding sites. *In vivo* testing conducted in Sprague−Dawley rats ($n = 6$) demonstrated a significant improvement in oral bioavailability from the 1:2 ITZ:CAP (AUC = 4,516 ± 1,949 ng·h/mL) compared to the Sporanox pellets (AUC = 2,132 ± 1,273 ng·h/mL) ($p \leq 0.05$). Additionally, the more rapid onset of action indicated superior targeting of the upper small intestines, and the prolonged half-life suggested the utility of CAP to maintain supersaturated concentrations, *in vivo*. These results demonstrated that amorphous compositions of ITZ and enteric concentration enhancing polymers provided improved bioavailability due to enhanced intestinal targeting and increased durations of supersaturation.

Keywords: Itraconazole; cellulose acetate phthalate; polyvinyl acetate phthalate; concentration enhancing polymers; supersaturation; bioavailability; in vivo; Sporanox; amorphous; ultra-rapid freezing; particle engineering

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Introduction

Itraconazole (ITZ) is a weakly basic triazole antifungal agent indicated in the treatment of both local and systemic fungal infections; however, successful treatment of infections is often complicated by its low aqueous solubility resulting in variable absorption and plasma concentration.[3,4] Classified as a BCS class II compound,[5] ITZ has a strongly pH dependent solubility ($pK_a \sim 3.7$) with reported solubilities in acidic and neutral media of approximately 4 μg/mL and 1 ng/mL respectively.[6] While limited by poor aqueous solubility, the highly lipophilic nature of the compound ($C \log P = 6.2$[6]) allows for high permeability of intestinal membranes. In order to maximize bioavailability and reduce *in vivo* variability, unique strategies for delivery of the marketed products have been utilized, including cyclodextrin complexation[7] and amorphous solid dispersion technology.[8]

Sporanox, the trade name of the currently marketed form of ITZ, is available in three formulations: a multiparticulate capsule, a 2-hydroxypropyl-β-cyclodextrin complexed oral solution and a solution for iv infusion. The capsule formulation is currently produced by drug layering of ITZ and hydroxypropyl methylcellulose (HPMC) onto sugar spheres using a dichloromethane:ethanol cosolvent system and is capable of providing rapid drug release in acidic media.[9,10] While this composition is able to provide adequate plasma levels for prophylaxis, it has shown both significant inter- and intrasubject variability.[8,11] ITZ pharmacokinetics have also been shown to be strongly affected by several factors including dose, diet and disease state, further complicating treatment. The dose dependent pharmacokinetics of ITZ have been well documented in the literature for both animal models[12] and human subjects,[8] with higher doses providing non-dose proportional increases in bioavailability. Furthermore, administration of Sporanox capsules in the fasted state[8] or with neutralizing agents such as antacids[13] has been shown to negatively affect bioavailability, while administration in conjunction with acidic beverages such as Coca-Cola has resulted in improved blood levels due to the pH dependent solubility of ITZ.[14] The cyclodextrin based formulation was developed, in part, to address these issues. In human trials with healthy volunteers the oral solution was shown to minimize the food effect and was also able to improve the bioavailability by 37% compared to the capsule based formulation.[4] Recent studies have shown that the ability of the cyclodextrin complexes to improve bioavailability may be a function of the ability of these materials to maintain solubility values in comparison to more conventional formulations which exhibit a decrease in drug concentration.[15] This formulation, however, does suffer from several side effects associated with the use of cyclodextrins which can reduce patient compliance, including poor taste, stomach pain and diarrhea.[11]

Although the oral solution has been able to address issues associated with limited and variable bioavailability, interest still exists in developing a solid dosage form with enhanced pharmacokinetic properties, including enhanced bioavailability and reduced variability. In an attempt to improve the bioavailability of ITZ solid dosage forms, numerous strategies have been investigated, including cyclodextrin complexed solid formulations,[16] capsule based self-emulsifying systems,[17] engineered particle compositions,[18−20] and amor-

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(1) DeSesso, J. M.; Jacobson, C. F. *Food Chem. Toxicol.* **2001**, *39* (3), 209–228.
(2) Six, K.; Daems, T.; de Hoon, J.; Van Hecken, A.; Depre, M.; Bouche, M.-P.; Prinsen, P.; Verreck, G.; Peeters, J.; Brewster, M. E.; Van den Mooter, G. *Eur. J. Pharm. Sci.* **2005**, *24* (2−3), 179–186.
(3) De Beule, K.; Van Gestel, J. *Drugs* **2001**, *61* (1), 27–37.
(4) Willems, L.; van der Geest, R.; de Beule, K. *J. Clin. Pharm. Ther.* **2001**, *26*, 159–169.
(5) Amidon, G. L.; Lennernäs, H.; Shah, V. P.; Crison, J. R. *Pharm. Res.* **1995**, *12* (3), 413–420.
(6) Peeters, J.; Neeskens, P.; Tollenaere, J. P.; Van Remoortere, P.; Brewster, M. E. *J. Pharm. Sci.* **2002**, *91* (6), 1414–1422.
(7) Barone, J. A.; Moskovitz, B. L.; Guarnieri, J.; Hassell, A. E.; Colaizzi, J. L.; Bierman, R. H.; Jessen, L. *Antimicrob. Agents Chemother.* **1998**, *42* (7), 1862–1865.
(8) Hardin, T. C.; Graybill, J. R.; Fetchick, R.; Woestenborghs, R.; Rinaldi, M. G.; Kuhn, J. G. *Antimicrob. Agents Chemother.* **1988**, *32* (9), 1310–1313.
(9) Gilis, P. M.; De Condé, V. F. V.; Vandecruys, R. P. G. Beads Having a Core Coated with an Antifungal and a Polymer. WO94/05263, 1997.
(10) Kapsi, S. G.; Ayres, J. W *Int. J. Pharm.* **2001**, (229), 193–203.
(11) Prentice, A. G.; Glasmacher, A. *J. Antimicrob. Chemother.* **2005**, *56* (Suppl. S1), i17–i22.
(12) Shin, J. H.; Choi, K. Y.; Kim, Y. C.; Lee, M. G. *Antimicrob. Agents Chemother.* **2004**, *48* (5), 1756–1762.
(13) Lohitnavy, M.; Lohitnavy, O.; Thangkeattiyanon, O.; Srichai, W. *J. Clin. Pharm. Ther.* **2005**, *30* (3), 201–206.
(14) Jaruratanasirikul, S.; Kleepkaew, A. *Eur. J. Clin. Pharmacol.* **1997**, *52* (3), 235–237.
(15) Brewster, M. E.; Vandecruys, R.; Peeters, J.; Neeskens, P.; Verreck, G.; Loftsson, T. *Eur. J. Pharm. Sci.* **2008**, doi:10.1016/j.ejps.2008.02.007.
(16) Shehatta, I.; Al-Marzouqi, A. H.; Baboucarr, Jobe; Dowaidar, A. *Can. J. Chem.* **2004**, (83), 1833–1838.
(17) Hong, J.-Y.; Kim, J.-K.; Song, Y.-K.; Park, J.-S.; Kim, C.-K. *J. Controlled Release* **2006**, *110* (2), 332–338.
(18) de Chasteigner, S.; Cavé, G.; Fessi, H.; Devissaguet, J.-P.; Puisieux, F. *Drug Dev. Res.* **1996**, *38* (2), 116–124.
(19) Hassan, A.; Tang, Y.-M.; Ayres, J. W. *Drug Dev. Ind. Pharm.* **2004**, *30* (10), 1029–1035.
(20) Vaughn, J. M.; Gao, X.; Yacaman, M.-J.; Johnston, K. P.; Williams, R. O., III *Eur. J. Pharm. Biopharm.* **2005**, (60), 81–89.

phous solid dispersions produced by melt extrusion[2,21−24] or spray drying.[25] While these systems have provided excellent dissolution properties in acidic conditions, the effect on bioavailability either was not assessed in an animal model or demonstrated limited improvement with no significant reduction in variability. In a study conducted by Miller and co-workers, limited bioavailability enhancement was observed from a system of engineered particles contained in a hydrophilic matrix designed to maximize supersaturation in acidic conditions.[26] *In vitro* supersaturated dissolution testing conducted under USP Method A enteric conditions demonstrated that upon pH change ITZ rapidly precipitated from the media, suggesting limited levels of drug available for absorption in the later stages of the GI tract. The authors hypothesized that the poor performance was due to the targeting of supersaturation to the low pH environment of the stomach.[26] In a subsequent study, solid dispersions of ITZ and enteric polymers were produced and compared to solid dispersions containing ITZ and hydrophilic polymers to study the bioavailability of compositions designed to provide supersaturation in neutral media. Although substantial variability was observed for the enteric solid dispersion, this work demonstrated that improved *in vitro* neutral media supersaturation correlated with improved *in vivo* plasma levels.[27] The variable plasma profiles observed for the enteric solid dispersion were hypothesized to be the result of the short duration of neutral media supersaturation. Eudragit L100-55 begins to dissolve at a pH of approximately 5.5 and has been reported to dissolve at a slower rate than enteric polymers containing phthalate groups,[28] which may result in variable efficiency in targeting the upper small intestine. Furthermore, the rapid *in vitro* precipitation of ITZ observed from these compositions suggests a lack of stabilizing function for the polymer which may also have contributed to variable performance.

The traditional development strategy for poorly water soluble compounds has long identified increased dissolution rates under sink conditions with improved bioavailability;[29]

Table 1. Lyophilization Conditions for Freeze Drying Engineering Particles

stage no.	duration (h)	shelf temp (°C)	vacuum (mTorr)	condenser temp (°C)
1	24	−20	<100	−80
2	36	−5	<100	−80
3	12	20	<100	−80

however, a number of researchers have begun investigating the use of concentration enhancing polymers to decrease precipitation rates and maintain supersaturation. By definition, concentration enhancing polymers provide increased levels of drug in solution in excess of the normal equilibrium solubility through either physical, chemical or a combination thereof of interactions with drug molecules that inhibits precipitation. Several examples for both solid and liquid phase formulations incorporating concentration enhancing polymers have been discussed recently in literature, providing significant improvements in bioavailability.[30−32] In the work of Gao and co-workers, HPMC was incorporated as an additive in self-emulsifying drug delivery system (SEDDS) formulations to increase the duration of supersaturation *in vitro* for PNU-91325 and paclitaxel. The improved duration of supersaturation was correlated with improved bioavailability in animal models for both molecules. Vandecruys et al. used a screening technique to determine which compositions provided improved solubilities and increased durations of supersaturation *in vitro* for a developmental compound.[32] Lead compositions selected based on solubility performance were tested in animal models and demonstrated enhanced bioavailability. In addition to published research papers, a number of companies have used this strategy to enhance the bioavailability of developmental compounds as described in the patent literature.[33−35] Similar to the research papers, the addition of concentration enhancing polymers provided increased *in vitro* AUC values which were correlated with enhanced bioavailability. Melt extruded compositions of ITZ, L100-55 and varying levels of Carbomer 974P were also investigated by Miller et al., for their application as a high viscosity stabilizing polymer and also potential mucoadhesive

(21) Six, K.; Berghmans, H.; Leuner, C.; Dressman, J.; Werde, K. V.; Mullens, J.; Benoist, L.; Thimon, M.; Meublat, L.; Verreck, G.; Peeters, J.; Brewster, M.; Van den Mooter, G. *Pharm. Res.* **2003**, *20* (7), 1047–1054.
(22) Six, K.; Leuner, C.; Dressman, J.; Verreck, G.; Peeters, J.; Blaton, N.; Augustijns, P.; Kinget, R.; Van den Mooter, G. *J. Therm. Anal. Calorim.* **2002**, *68* (2), 591–601.
(23) Six, K.; Verreck, G.; Peeters, J.; Brewster, M.; Van den Mooter, G. *J. Pharm. Sci.* **2004**, *93* (1), 124–131.
(24) Janssens, S.; Novoa de Armas, H.; Remon, J. P.; Van den Mooter, G. *Eur. J. Pharm. Sci.* **2007**, *30* (3−4), 288–294.
(25) Yoo, S. D.; Lee, S.-H.; Kang, E.; Jun, H.; Jung, J.-Y.; Park, J. W.; Lee, K.-H. *Drug Dev. Ind. Pharm.* **2000**, *26* (1), 27–34.
(26) Miller, D. A.; McConville, J. T.; Yang, W.; Williams, R. O., III; McGinity, J. W. *J. Pharm. Sci.* **2007**, *96* (2), 361–376.
(27) Miller, D. A.; DiNunzio, J. C.; Yang, W.; McGinity, J. W.; Robert O. Williams, I. *Drug Dev. Ind. Pharm.* **2008**, *34* (8), 890−902.
(28) Lamprecht, A.; Kawashima, Y. *J. Drug Delivery Sci. Technol.* **2006**, *16* (1), 25–34.
(29) Dressman, J. B.; Reppas, C. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl. 2), S73–S80.
(30) Gao, P.; Guyton, M. E.; Huang, T.; Bauer, J. M.; Stefanski, K. J.; Lu, Q. *Drug Dev. Ind. Pharm.* **2004**, *30* (2), 221–229.
(31) Gao, P.; Rush, B. D.; Pfund, W. P.; Huang, T.; Bauer, J. M.; Morozowich, W.; Kuo, M.-H.; Hageman, M. J. *J. Pharm. Sci.* **2003**, *92* (12), 2386–2398.
(32) Vandecruys, R.; Peeters, J.; Verreck, G.; Brewster, M. E. *Int. J. Pharm.* **2007**, *342* (1−2), 168–175.
(33) Crew, M. D.; Friesen, D. T.; Hancock, B. C.; Macri, C.; Nightingale, J. A. S.; Shankar, R. M. Pharmaceutical Compositions of a Sparingly Soluble Glycogen Phosphorylase Inhibitor. US 7,235,260 B2, 2007.
(34) Crew, M. D.; Shanker, R. M.; Smithey, D. T.; Miller, W. K.; Friesen, D. T. Stabilized Pharmaceutical Solid Compositions of Low-Solubility Drugs, Poloxamers and Stabilizing Polymers. W/O 2005/065656 A2, 2005.
(35) Gao, P.; Morozowich, W. Chemotherapeutic Microemulsion Compositions of Paclitaxel with Improved Oral Bioavailability. US 7,115,565 B2, 2006.

agent.[36] Results showed that the 20% Carbomer compositions provided the greatest duration of supersaturation and were also shown to provide the greatest improvement of *in vivo* AUC, suggesting that prolonged durations of supersaturation in neutral media provide enhanced bioavailability.

Based on the success of other researchers on enhanced ITZ bioavailability by maximizing neutral media supersaturation,[27,36] it was hypothesized that the formulation of ITZ with concentration enhancing polymers could further improve bioavailability by increasing the amount of drug available for absorption in the later stages of the GI tract. The purpose of this study was to investigate the effect of enteric polymers containing phthalate groups on the bioavailability of ITZ enteric solid dispersions. For this work CAP and PVAP were selected due to their lower pH of ionization (pH 5), more rapid polymer dissolution rate[28] and potential to function as a concentration enhancing polymer.[33] To study the effects of these polymers, amorphous compositions were manufactured using ultra-rapid freezing (URF) particle engineering technology[37,38] and characterized for their solid state properties by XRD, mDSC, Fourier transform infrared spectroscopy (FTIR), SEM, specific surface area analysis, transmission electron microscopy and EDS. *In vitro* dissolution testing was used to assess the performance of the compositions by evaluating the degree (C_{max}), duration ($t_{1/2}$) and extent ($AUC_{dissolution}$) of supersaturation. Based on the *in vitro* performance, the lead composition was dosed orally to Sprague−Dawley rats and compared to Sporanox pellets for evaluation of the relative oral bioavailability enhancement.

Materials and Methods

Materials. Itraconazole, BP was purchased from Hawkins, Inc. (Minneapolis, MN). Cellulose acetate phthalate (Cellacefate) was purchase from Spectrum Chemical Manufacturing Corporation (Gardena, CA). Polyvinyl acetate phthalate (pHthalavin 2138 clear) was donated by Colorcon, Inc. (West Point, PA). Sporanox capsules (Lot # 6MG457) were purchased from Janssen Pharmaceutica Products, L.P. (Titusville, NJ). Empty size 9 porcine gelatin capsules were purchased from Torpac, Inc. (Fairfield, NJ). 1,4-Dioxane was purchased from Fischer Chemical Co. HPLC grade acetonitrile was purchased from EMD chemicals (Darmstadt, Germany). All other chemicals utilized in this study were of ACS grade.

Methods. (a) Ultra-Rapid Freezing (URF). Samples of engineered particles were prepared using a thin film freezing technique.[37−40] Measured quantities of ITZ and enteric polymer (CAP or PVAP) were dissolved in 1,4-dioxane to produce a 1% w/v solution and slowly fed as discrete droplets onto a chilled rotating drum maintained at approximately −60 °C. The frozen material was removed from the drum by a scraper blade, collected and dried using a Virtis Advantage lyophilizer operated in a three stage drying program presented in Table 1.

(b) X-ray Diffraction (XRD). XRD testing was conducted using a Philips model 1710 X-ray diffractometer (Philips Electronic Instruments Inc., Mahwah, NJ). Samples of powder were placed into channeled stages, and the diffraction profile was measured from 5° to 50° using a 2θ step size of 0.05° and dwell time of 3s.

(c) Modulated Differential Scanning Calorimetry (mDSC). Modulated differential scanning calorimetry testing was performed using a TA Instruments model 2920 DSC (New Castle, DE) and analyzed using TA Universal Analysis 2000 Software. Prior to testing, polymer samples were preheated at 90 °C for 15 min using a model MF-50 moisture balance (AND Company Ltd. Encino, CA.) to remove excess residual moisture and weighed to 15 ± 2 mg in aluminum crimped pans (Kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT). Engineered particle samples were accurately weighed to 5 ± 1 mg in aluminum crimped pans. Testing was performed at a ramp rate of 10 °C/min from 5 to 215 °C using a modulation temperature amplitude of 0.5 °C and a modulation period of 40 s under nitrogen purge at a flow rate of 40 mL/min.

(d) Fourier Transform Infrared Spectroscopy (FTIR). Individual materials, physical mixtures and URF processed powders were analyzed by FTIR to evaluate potential interactions. Individual materials and physical mixtures were triturated in a mortar and pestle prior to pellet preparation. Physical mixtures of components were prepared by geometric dilution using a glass mortar and pestle. Samples for FTIR analysis were prepared by weighing approximately 4 mg of the material for analysis, mixing with 250 mg of dried KBr for approximately 2 min in a mortar and pestle, and compressing the mixture under vacuum with a compression force of 10 tons using a 13 mm diameter round flat face punch for three minutes to produce a pellet compacts. Samples were analyzed using a Thermo Mattson Infinity Gold FTIR with Spectra-Tech Thermal ARK module from 400 to 4000 cm^{-1} in transmission mode equipped with a KBr beamsplitter and DTGS detector having a resolution of 1 cm^{-1}. Each presented spectrum is the average of 64 scans.

(e) Brauner Emmitt Teller (BET) Specific Surface Area Measurements. Specific surface areas were measured using a Nova 2000 v.6.11 (Quantachrome Instruments,

(36) Miller, D. A.; DiNunzio, J. C.; Yang, W.; McGinity, J. W.; Robert O. Williams, I. *Pharm. Res.* **2008**, *25* (6), 1450−1459.
(37) Overhoff, K. A.; Engstrom, J. D.; Chen, B.; Scherzer, B. D.; Milner, T. E.; Johnston, K. P.; Williams, R. O., III *Eur. J. Pharm. Biopharm.* **2007**, *65* (1), 57–67.
(38) Overhoff, K. A.; Moreno, A.; Miller, D. A.; Johnston, K. P.; Williams, R. O., III *Int. J. Pharm.* **2007**, *336* (1), 122–132.
(39) Engstrom, J. D.; Lai, E. S.; Ludher, B. S.; Chen, B.; Milner, T. E.; Williams, R. O.; Kitto, G. B.; Johnston, K. P. *Pharm. Res.*, in press.
(40) Overhoff, K. A. *Improved Oral Bioavailability of Poorly Water Soluble Drugs Using Rapid Freezing Processes*; The University of Texas at Austin: Austin, 2006.

Boynton Beach, FL) with a nitrogen adsorbate gas. Powder samples of approximately 25 mg were placed into the sample bulb, degassed for a minimum of three hours at room temperature and measured using a six point pressure profile ranging from 0.05 to 0.30 psia. Specific and total sample surface areas were calculated from the BET model using NOVA Enhanced Data Reduction Software v. 2.13.

(f) Scanning Electron Microscopy (SEM). Prior to imaging, samples were mounted onto aluminum stages using double sided carbon tape and sputter coated using a Pelco model 3 equipped with an Au source for 20 s. Particle morphologies were evaluated by SEM using a Hitachi S-4500 scanning electron microscope (Hitachi, Ltd., Tokyo, Japan) operated at an accelerating voltage of 10 kV.

(g) Transmission Electron Microscopy (TEM) and Energy Dispersive Spectroscopy (EDS). Samples were prepared by dispersing powder samples onto a 200 mesh copper grid coated with a carbon support film (Electron Microscopy Sciences, Hatfield, PA). Imaging and EDS were conducted using a JEOL 2010F transmission electron microscope (JEOL USA, Inc., Peabody, MA) equipped with a HAADF STEM detector, Oxford spectrometer and GATAN digital imaging system operated at 200 kV.

(h) Supersaturated Dissolution Testing. Supersaturated dissolution testing was performed based on the USP XXIX method A enteric dissolution test using a VK 7010 dissolution apparatus (Varian, Inc., Palo Alto, CA) operating at 50 rpm paddle speed and VK 8000 autosampler (Varian, Inc., Palo Alto, CA). An equivalent amount of 37.5 mg $\pm$ 0.4 mg of ITZ ($\sim$10$\times$ 0.1 N HCl media equilibrium solubility) was weighed, prewetted with 15 mL of preheated (37 °C) 0.1 N HCl media and added to the dissolution vessel containing 735 mL of 0.1 N HCl media. After two hours, 250 mL of 0.2 M Na_3PO_4 solution was added to the dissolution vessel to achieve a pH of approximately 6.8. During testing 5 mL samples were removed from the dissolution vessels without replacement after 60, 120, 130, 150, 180, 240, 300, 360 and 1440 min. Samples were immediately filtered using 0.2 μm PTFE membrane, 13 mm Acrodisc filters (Pall Corporation, East Hills, NY) to minimize drug adsorption and precipitation, immediately diluted in a 1:1 ratio with mobile phase, vortexed mixed and transferred into 1 mL vials (VWR International, West Chester, PA) for analysis.

Dissolution samples were analyzed using a Waters (Waters Corporation, Milford, MA) high performance liquid chromatography (HPLC) system consisting of dual Waters 515 syringe pumps, a Waters 717 autosampler and a Waters 996 photo diode array extracting at a wavelength of 263 nm. The system was operated under isocratic flow at 1 mL/min using a mobil phase consisting of 70:30:0.05 acetonitrile:water:diethanolamine equipped with a Phenomenex Luna 5 μm C18(2) 100 Å, 150 mm $\times$ 4.6 mm (Phenomenex, Torrance, CA) HPLC column. Samples collected in the 0.1 N HCl media and neutralized media were injected in volumes of 50 and 200 μL respectively during testing. Data were collected and analyzed using Empower version 5.0 software. The retention time of ITZ was approximately 6 min. All analytical tests maintained system suitability limits for linearity from 0.024 to 100 μg/mL ($r^2 \geq 0.999$) and reproducibility of replicate injections (% RSD $\leq$ 2.0%).

(i) *In Vivo* Studies. Institutionally approved *in vivo* studies were conducted using jugular vein precatheterized CD IGS Sprague−Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing approximately 300 g. Throughout the study the animals were stored in individual cages, subjected to 12 h −12 h cycles of light and darkness, with access to food and water *ad libitum*. The catheters were flushed daily with 300 μL of 50 U/mL heparinized normal saline. A minimum of 72 h was allowed for acclimatization, after which time the rats were administered the formulations at a dose of 15 mg ITZ/kg body weight ($n = 6$). Prior to dosing, formulations of Sporanox pellets were filled into size 9 porcine gelatin capsules to achieve a target dose of 15 mg of ITZ/kg of body weight ($n = 6$). Briefly, the contents of ten Sporanox capsules were emptied and weighed (464 mg pellets/capsule), to determine a theoretical potency per pellet mass (216 mg of ITZ/g of pellets). An equivalent amount of 4.5 mg of ITZ was manually filled into the body of the capsule to achieve dosing of 15 mg of ITZ/kg for a 300 g animal. Closed capsules were dosed by oral capsule dosing syringe (Torpac, Inc., Fairfield, NJ) followed by administration of 200 μL of deionized water by oral gavage. Engineered particles were dispersed in deionized water prior to dosing at a concentration of 4.5 mg of ITZ/1 mL and dosed by oral gavage, providing volumetric doses below 4 mL/kg body weight to prevent spontaneous release through the pyloric sphincter.[41] Blood samples of approximately 300 μL were collected from the jugular vein catheter at 0, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12 and 24 h after dosing, placed into preheparinized 1.5 mL microcentrifuge tubes and replaced with equal volumes of heparinized saline. Blood samples were centrifuged at 3000*g* for 15 min and the plasma transferred to a clean 1.5 mL microcentrifuge tube. All samples were stored at −20 °C until HPLC analysis.

Prior to HPLC analysis, plasma samples were removed from frozen storage and allowed to equilibrate to room temperature, and a measured volume of plasma was transferred to a clean 1.5 mL microcentrifuge tube. To each microcentrifuge tube, 50 μL of 0.3 N barium hydroxide and 50 μL of 0.4 N zinc sulfate heptahydrate solution were added. Samples were then vortex mixed for 30 s, and 1 mL of acetonitrile containing 1200 ng/mL ketoconazole as an internal standard was added to each plasma sample. Samples were vortex mixed for an additional 90 s and centrifuged at 3000*g* for 15 min. From each vial the supernatant was extracted, transferred to a clean 1.5 mL centrifuge tube and dried in an aluminum heating block (70 °C) under a stream of nitrogen gas. Samples were reconstituted with 250 μL of mobile phase, vortex mixed for 60 s and centrifuged for an

(41) Alban, L.; Dahl, A. K.; Hansen, A. K.; Hejgaard, K. C.; Jensen, A. L.; Kragh, M.; Thomsen, P.; Steensgaard, P. *Anim. Welfare* **2001**, *10*, 303–314.

additional 15 min. An aliquot of the supernatant was extracted and filled into 150 μL HPLC vial inserts. Samples were analyzed at a wavelength of 263 nm using the previously described Waters HPLC system equipped with a Phenomenex Luna 5 μm C-18(2) 100 Å HPLC column (250 mm × 4.6 mm) maintained at a temperature of 37 °C using a 38:62 0.05 M phosphate buffer:acetonitrile mobile phase operated under isocratic flow of 1 mL/min. Sample injection volumes of 100 μL were utilized for testing, and the retention times of KTZ and ITZ were approximately 5.5 and 14.7 min, respectively. All analytical tests maintained system suitability limits for linearity ($r^2 \geq 0.999$) and reproducibility of replicate injections (% RSD ≤ 2.0%). The limit of detection and quantitation for ITZ was 10 ng/mL and 30 ng/mL, respectively.

(j) Pharmacokinetic Analysis. Plasma data was analyzed with WinNonlin v4.1 (Pharsight Corporation, Mountain View, CA) using noncompartmental analysis for extravascular input. Specifically, T_{max} and C_{max} were determined directly from empirical data, AUC was calculated by the linear trapezoidal method, and $t_{1/2}$ was determined by calculation of the lambda *z* parameter. For statistical analysis, encapsulated Sporanox pellets were used as the reference formulation.

(k) Statistical Analysis. Statistical analyses were conducted by ANOVA with Tukey comparison test or Student's *t* test, as identified in the respective section, using Minitab Release 14. For all tests, $p \leq 0.05$ was used as the criterion to assess statistical significance.

Results and Discussion

Solid State Characterization. In the development of amorphous compositions for enhanced bioavailability, several solid state properties are critical for product success including the amorphous nature, drug−polymer miscibility and product surface area.

Drug−polymer miscibility is essential for the stability of amorphous pharmaceutical compositions, as immiscibility can result in the formation of concentrated drug domains that may be prone to post production recrystallization. The miscibility of these pharmaceutical systems can be assessed by a variety of techniques including the use of the Gordon−Taylor equation,[42] presented in eq 1, wherein the physicochemical properties of the drug and the polymer are used to calculate the theoretical glass transition temperature of the mixture, with; T_{gi} indicating the glass transition temperature of component *i*, w_i indicating the weight fraction of component *i* in the mixture, ρ_i indicating the true density of component *i* and $\Delta\alpha_i$ indicating the change in thermal expansivity for component *i*.

$$T_{g12} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \qquad K = \frac{\rho_1 \Delta\alpha_1}{\rho_2 \Delta\alpha_2} \tag{1}$$

As values for $\Delta\alpha_i$ are not readily available for many materials, *K* is often approximated using the Simha−Boyer rule,[43] presented in eq 2.

$$K \cong \frac{\rho_1 T_{g1}}{\rho_2 T_{g2}} \tag{2}$$

By comparison of the calculated glass transition temperatures to the observed glass transition temperatures, the miscibility of the system can be assessed based on the correlation of the values. The observed glass transition values of engineered particle compositions were determined by mDSC as the midpoints of the observed transitions in reversing heat flow shown in Figure 1. In all cases, only a single transition was observed indicating that the systems existed as a single phase. All compositions below 80% drug loading did not exhibit an ITZ melting endotherm, further supporting the existence of a single drug−polymer phase. Compositions of 80% drug loading did exhibit a melting endotherm; however, prior to this event a recrystallization exotherm was observed in standard heat flow suggesting that the melting endotherm was due in part to recrystallization during the analysis. Standard heat flow also exhibited several broad endotherms during the initial phase of the testing not present in the reversing heat flow. These transitions were attributed to relaxation of the material, which is kinetically limited preventing these transitions from appearing in the reverse heat flow profiles. To further evaluate the miscibility, the calculated and observed glass transitions temperatures for ITZ:CAP and ITZ:PVAP compositions were plotted as a function of ITZ weight fraction, as shown in Figure 2. Compositions of ITZ:CAP showed an acceptable correlation ($r^2 = 0.984$) with the predicted glass transition values indicating drug and polymer miscibility. The observed glass transition temperatures of the ITZ:PVAP compositions demonstrated a lower correlation ($r^2 = 0.920$) and consistent positive deviation of the Gordon−Taylor relationship, indicating either hydrogen bonding interactions between the drug and polymer or immiscibility of the drug polymer system.[44]

The potential interactions between ITZ and PVAP were investigated further using FTIR, as shown in Figure 3. ITZ has numerous potential hydrogen bonding sites which may interact with enteric polymers such as PVAP, including the C═O group which functions as a hydrogen bond acceptor. Examination of the FTIR spectra for ITZ indicated an intense C═O stretch located at 1700 cm^{-1}, while a broader, less intense peak was observed for PVAP at 1735 cm^{-1}. The spectra for the 1:2 ITZ:PVAP physical mixture was similar to the additive profiles of the individual components with the intensities of the peaks in proportion to their concentration in the mixture, indicating that a simple admixture of the components did not induce a change in molecular interactions. Analysis of the 1:2 ITZ:PVAP engineered particle compositions showed a decrease in intensity and a broadening of the C═O stretch and a decrease in the C−H bond intensity compared to the physical mixture suggesting

(42) Gordon, M.; Taylor, J. S. *J. Appl. Chem.* **1952**, *2*, 493–500.
(43) Simha, R.; Boyer, R. F. *J. Chem. Phys.* **1962**, *37* (5), 1003–1007.
(44) Gupta, P.; Thilagavathi, R.; Chakraborti, A. K.; Bansal, A. K. *Mol. Pharmaceutics* **2005**, *2* (5), 384–391.

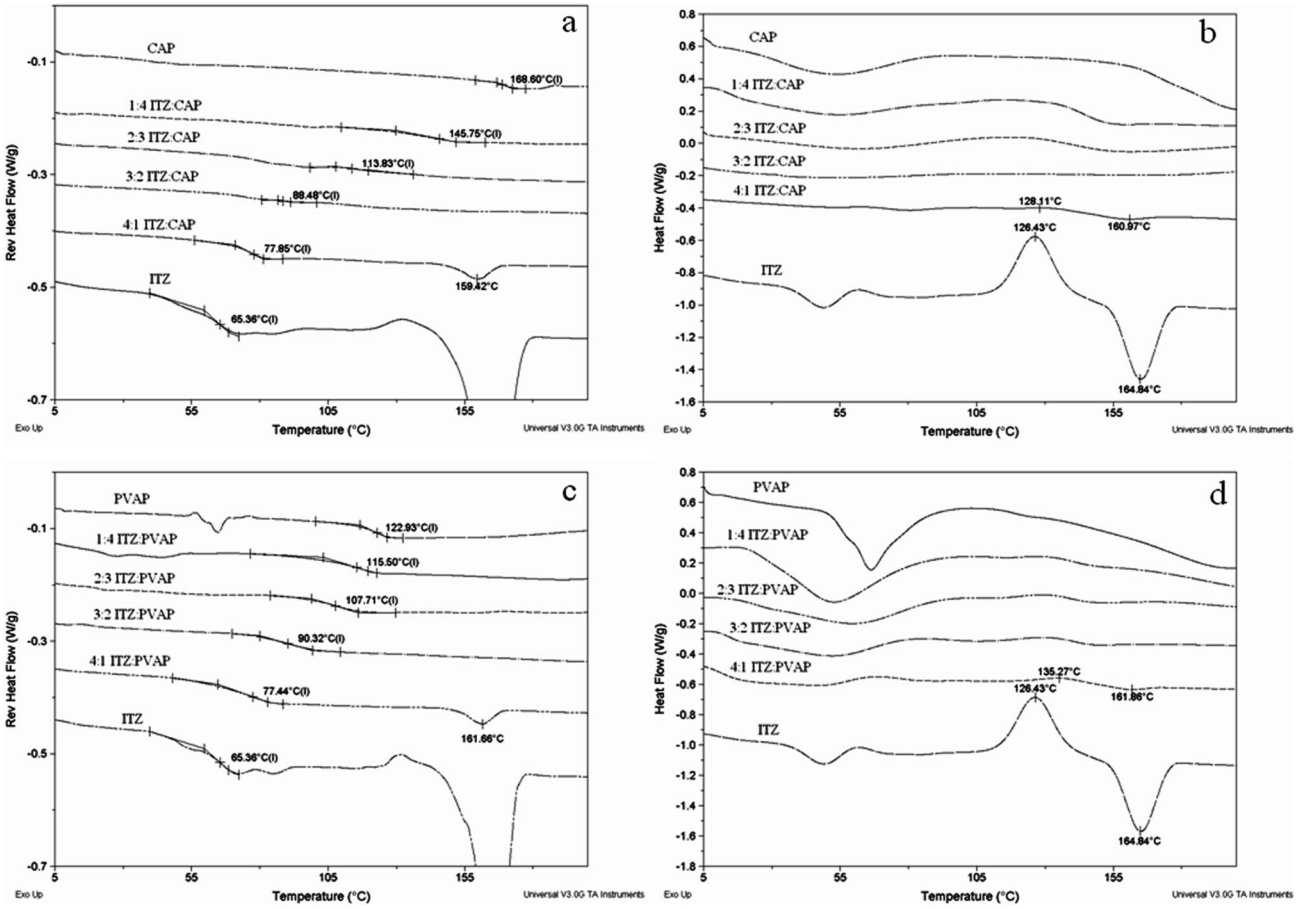

Figure 1. mDSC thermograms. (a) Reversing heat flow profile of ITZ:CAP compositions. (b) Heat flow profile of ITZ:CAP compositions. (c) Reversing heat flow profile of ITZ:PVAP compositions. (d) Heat flow profile of ITZ:PVAP compositions.

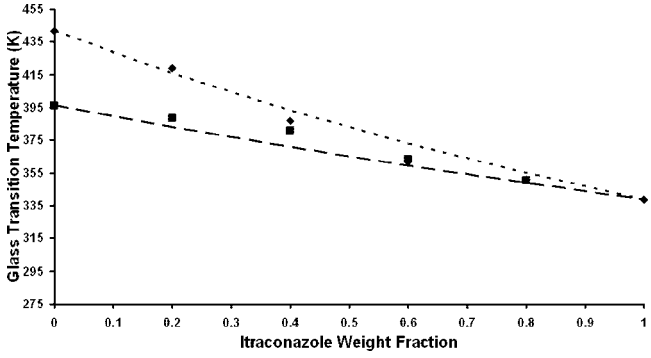

Figure 2. Gordon−Taylor relationship for engineered particle formulations. ITZ:CAP measured T_g (◆), ITZ:PVAP measured T_g (■), predicted ITZ:CAP Tg (•••), predicted ITZ:PVAP (− − −).

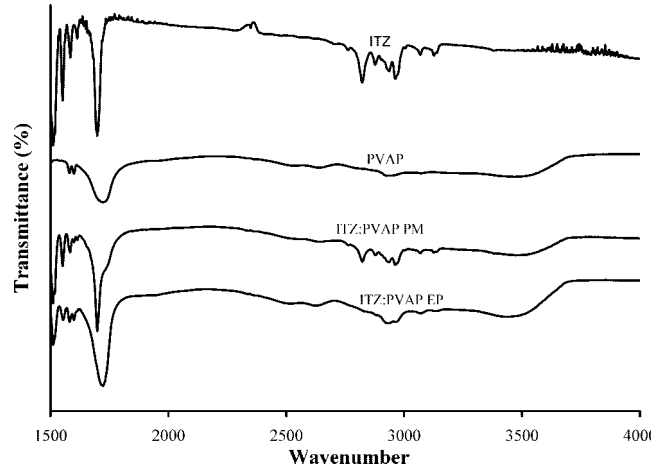

Figure 3. FTIR imaging of ITZ:PVAP compositions.

possible hydrogen bonding which may be responsible for the positive deviation observed in the Gordon−Taylor analysis.

ITZ has previously been reported to be immiscible with several polymers, including Eudragit EPO, resulting in observable phase separation.[22] Although only single glass transitions were observed by mDSC, EDS mapping was conducted to evaluate the qualitative distribution of ITZ in the composition by tracking the distribution of Cl, an atom unique to ITZ. EDS mapping of engineered particle compositions, presented in Figure 4, yielded a homogeneous distribution of ITZ with no observed regions of phase separation supporting the conclusion of acceptable drug-polymer miscibility. It can also be seen that as the ITZ concentration increased, the intensity of the Cl signal also increased.

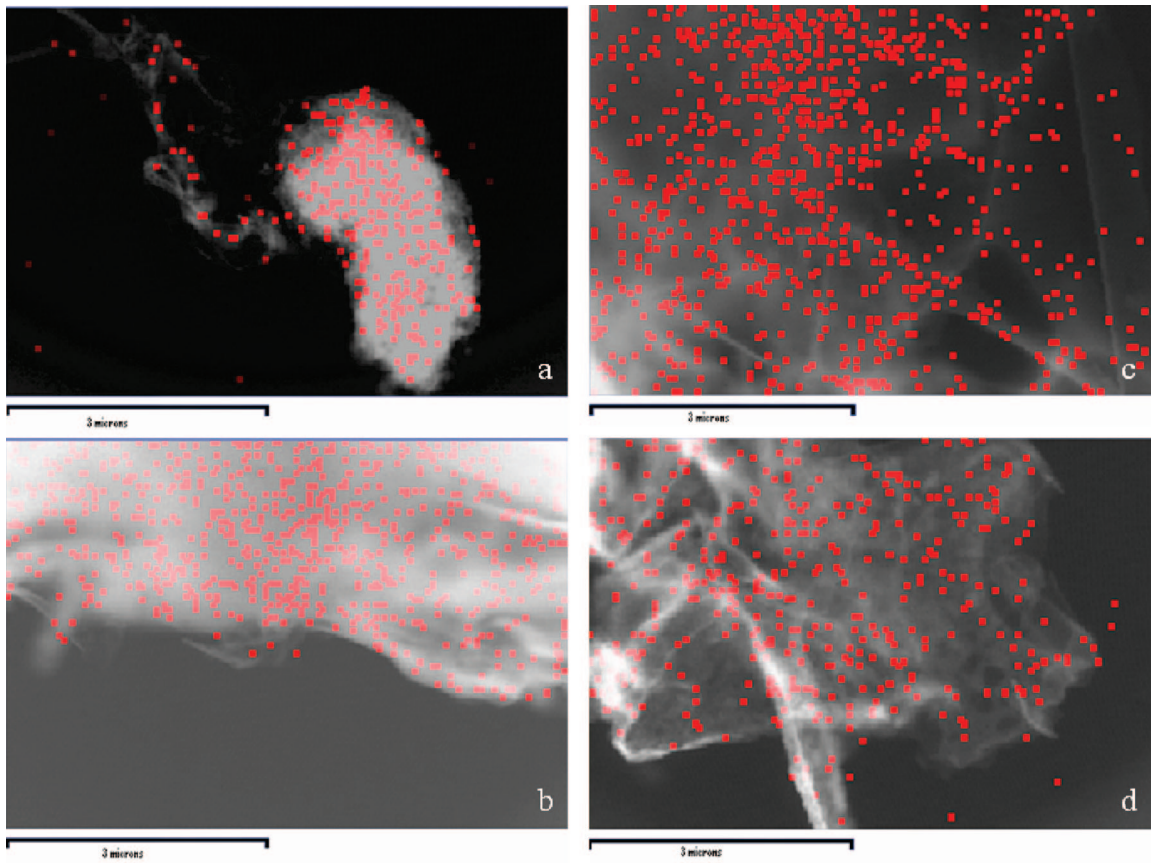

Figure 4. TEM imaging analysis of particle structure. Red squares indicate distribution of Cl atoms. (a) 1:2 ITZ:CAP. (b) 2:1 ITZ:CAP. (c) 1:2 ITZ:PVAP. (d) 2:1 ITZ:PVAP.

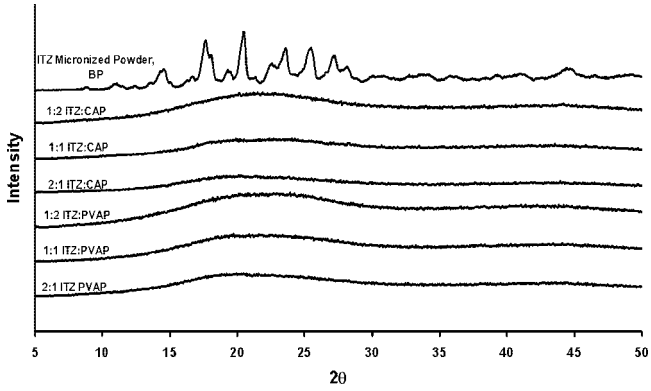

Figure 5. XRD patterns of formulations studied.

By producing amorphous compositions, the ability to effectively supersaturate aqueous media can be readily attained due to the thermodynamic reduction in the heat of fusion. To assess the amorphous nature of the compositions produced, pXRD testing was conducted, and the diffraction profiles are presented in Figure 5. ITZ exhibited several strong characteristic crystalline peaks at $2\theta = 14.4°$, 17.5°, 20.4°, 23.4°, 25.3° and 27.1°. Both polymers used for production of the engineered particles demonstrated amorphous halos, supported by an absence of melting endotherms during mDSC testing, which indicated their amorphous nature. Diffraction patterns of the engineered particle samples showed amorphous halos with an absence of the characteristic crystalline ITZ peaks, indicating that the ITZ was in an amorphous state. This was also supported by the mDSC analysis in which only a single glass transition temperature with no melting endotherm was observed for compositions below 80% ITZ loading.

The surface area and morphology of the engineered particles can significantly influence the drug release characteristics of the system through both kinetic and thermodynamic factors. It is generally recognized that increased surface area can provide enhanced mass transfer during the dissolution process. To examine the morphology and surface area of the particles, SEM and BET analyses were preformed. SEM imaging of the engineered particles, presented in Figure 6, revealed two distinct forms for all compositions studied: discrete particles of approximately 5 μm and larger particle aggregates in excess of 30 μm. In all cases, the particles exhibited a highly contoured surface providing significantly increased surface area compared to micronized powder, providing an order of magnitude increase in surface area, as shown in Table 2. These increased surface areas offered the potential for increased dissolution rates *in vitro* and *in vivo*.

***In Vitro* Dissolution.** To assess the performance of developmental compositions prior to animal testing, *in vitro* dissolution has routinely been used in the pharmaceutical industry. Dissolution studies reported in the literature and also testing recommended by the Food and Drug Adminis-

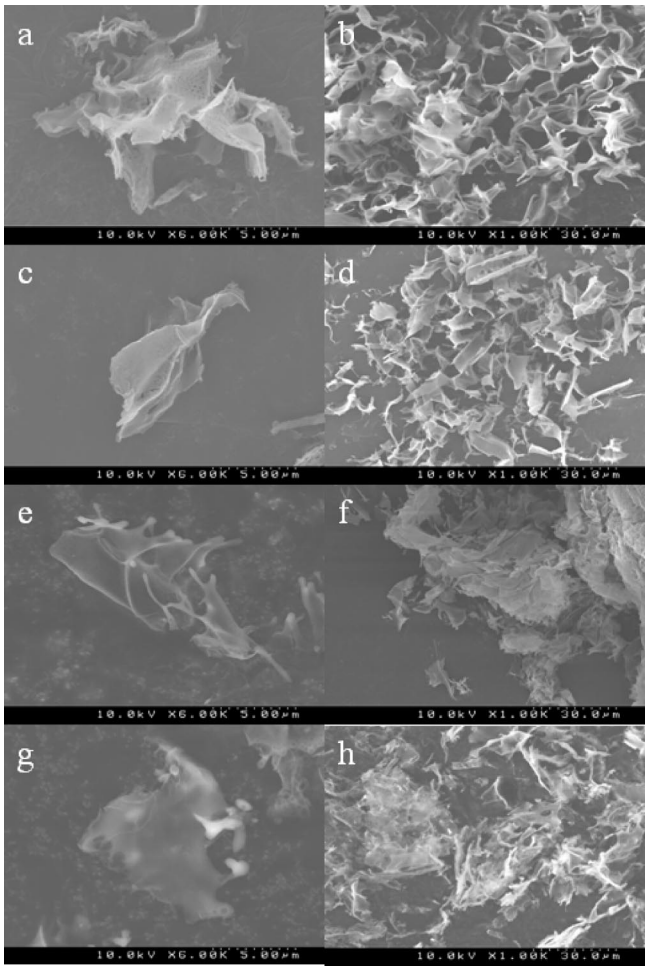

Figure 6. SEM images of engineered particle morphologies. (a and b) 1:2 ITZ:CAP. (c and d) 2:1 ITZ:CAP. (e and f) 1:2 ITZ:PVAP. (g and h) 2:1 ITZ:PVAP.

Table 2. Specific Surface Areas of Engineered Particles

composition	surface area (m^2/g)
ITZ (unprocessed)	2.90
1:4 ITZ: CAP	39.83
4:1 ITZ: CAP	55.91
1:4 ITZ: PVAP	47.53
4:1 ITZ: PVAP	61.26

tration (FDA)[45,46] have generally been conducted under sink conditions, wherein the concentrations are maintained at least three to five times below equilibrium solubility. Numerous articles have correlated the results of these tests to the *in vivo* performance of the formulations; however with amorphous compositions these tests neglect the ability of the formulation to supersaturate the dissolution media. Supersaturation can occur *in vivo* as well, necessitating the requirement for evaluation of the associated kinetics. For this study, the dissolution testing was conducted under supersaturated conditions in order to evaluate the supersaturation and precipitation dynamics of Sporanox pellets, ITZ:CAP engineered particles and ITZ:PVAP engineered particles (Table 3).

As discussed previously several recent papers have evaluated the dynamics of supersaturation,[26,27,30−32,35,36,47] however these analyses have focused primarily on the area under the curve (AUC), also referred to as extent of supersaturation. While this value can provide a general description of the *in vivo* bioavailability, it does not directly assess the stabilizing properties of the polymer. For the identification and characterization of concentration enhancing polymers, the rate of precipitation inhibition was identified by calculation of the half-life of drug in solution. During the growth phase of the precipitation process transport of drug to the precipitate surface is driven by the concentration of drug in solution, such that

$$G = \frac{\beta}{3\alpha} k_d \frac{\Delta C_s}{C_c} \tag{3}$$

where G is the growth rate of the particle, k_d is a constant, ΔC_s is the difference between measured concentration and equilibrium concentration, C_c is the precipitate density, α is the volume factor and β is the shape factor.[48] Assuming that the growth rate is proportional to the rate of concentration decrease and combining all constants into a lumped term, K', it can be shown that

$$\frac{\partial C}{\partial t} = K'(C - C_{eq}) \tag{4}$$

where C_{eq} is the equilibrium solubility. By identifying that $C \gg C_{eq}$ and integrating the equation it can be shown that the change in measured concentration followed an exponential relationship with time such that

$$C = C_o e^{-K't} \tag{5}$$

Assuming that the growth phase began at the maximum observed concentration and by plotting the dissolution data as an exponential function of the above form, the half-life of the formulation can be calculated by linear regression allowing for assessment of the polymer stabilizing properties.

Dissolution testing of the Sporanox pellets, presented in Figure 7, illustrated the ability of the formulation to rapidly and extensively supersaturate in acidic media, achieving near complete supersaturation. Following pH change, there was a rapid precipitation of drug from solution, with an observed $t_{1/2}$ of 6.7 ± 2.4 min, supporting the hypothesis of Miller et al., that the low and variable bioavailability of Sporanox was due to the inability of the formulation to maintain supersaturation in neutral media.[26] Although Sporanox pellets contain low viscosity HPMC E5 [9,10] and HPMC has been identified as a concentration enhancing polymer for several

(45) Guidance for Industry: Extended Release Oral Dosage Forms: Development, Evaluation, and Application of In Vitro/In Vivo Correlations, 1997, F.D.A, p 27.
(46) Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms, 1997, F.D.A, p 17.
(47) Gao, P.; Morozowich, W. *Expert Opin. Drug Delivery* **2006**, *3* (1), 97–110.
(48) Mersmann, A. *Chem. Eng. Process.* **1999**, *38* (4−6), 345–353.

Table 3. Summary of *In Vitro* Dissolution Testing Data with Reported Maximum Observed Concentrations (C_{max}), Observed Time To Achieve Maximum Concentration (t_{max}), Area under the Supersaturation Dissolution Profile for pH Change Testing ($AUC_{dissolution}$) and Observed Supersaturation Half-Life ($t_{1/2}$)

			$AUC_{dissolution}$ (mg·min)			
composition	C_{max} (mg)	t_{max} (min)	acid	neutral	total	$t_{1/2}$ (min)
Sporanox pellets	36.7 ± 1.6	120 ± 0	3023.6 ± 207.9	225.8 ± 23.1	3249.4 ± 222.6	6.7 ± 2.4
1:2 ITZ:CAP	10.8 ± 0.7	160 ± 17	469.6 ± 33.6	6290.9 ± 1183.0	6760.5 ± 1177.7	391.2 ± 30.7
1:1 ITZ:CAP	4.4 ± 0.6	120 ± 0	313.6 ± 44.6	2913.8 ± 463.7	3227.3 ± 502.4	441.7 ± 18.6
2:1 ITZ:CAP	5.7 ± 0.6	120 ± 0	375.3 ± 33.6	1538.5 ± 487.2	1913.8 ± 498.0	220.6 ± 20.4
1:2 ITZ:PVAP	3.9 ± 0.7	130 ± 0	219.4 ± 43.6	324.8 ± 243.5	543.9 ± 269.6	24.5 ± 6.7
1:1 ITZ:PVAP	1.7 ± 0.1	120 ± 0	130.9 ± 3.6	33.4 ± 17.2	164.3 ± 20.5	23.9 ± 16.3
2:1 ITZ:PVAP	1.3 ± 0.2	120 ± 0	84.9 ± 12.0	21.7 ± 6.8	106.6 ± 13.0	9.0 ± 0.7

compounds, [47] the viscosity of the material has been shown to significantly influence the supersaturation stabilization of ITZ in neutral media.[27] This suggests that a change in HPMC viscosity could improve the bioavailability of the commercial product.

Engineered particle compositions were also tested for *in vitro* dissolution performance. Dissolution profiles presented in Figure 8 and Figure 9 indicated that in all cases the engineered particle compositions provided significantly reduced release in acidic media, compared to Sporanox pellets due to minimal solubility of the enteric polymers in these conditions. Following pH change 1:2 ITZ:CAP and 1:2 ITZ:PVAP compositions showed the greatest increase of supersaturation in neutral media for the respective polymer classes due to ionization and subsequent dissolution of the enteric polymer, while compositions with higher ITZ:polymer ratios exhibited reduced degrees of supersaturation. Although reduced degrees of supersaturation can indicate immiscibility of the system caused by reduced wetting due to regions of hydrophobic drug, results from the solid state characterization indicated homogeneous distributions for both polymer systems, and this phenomenon was attributed to the higher drug loading which resulted in decreased particle wetting limiting the dissolution process. Differences in the degree of neutral media supersaturation were also observed for the CAP and PVAP compositions which were attributed to the substantially larger half-life of drug in solution and variations in the nucleation behavior of these systems. During testing both formulation types were observed to dissolve upon pH change, however the more rapid precipitation of PVAP compositions prevented identification of the true maximum amount of dissolved drug due to the short duration of the maximum. The differences in the measured values may also indicate a difference in the nucleation behavior of these systems, however insufficient data are available to assess this variation. Examination of $AUC_{dissolution}$ values showed that ITZ:CAP compositions provided statistically significant increases in extent of neutral media supersaturation compared to the other test formulations due to the prolonged half-life of drug in solution. Half-life values for CAP based compositions were significantly higher than those observed for the other tested compositions indicating that CAP was a superior concentration enhancing polymer, although the data does not conclusively indicate at which stage of the precipitation process (nucleation or growth) the polymer has its greatest effect.

Two mechanisms have been traditionally used to explain the stabilizing performance of excipients in supersaturated

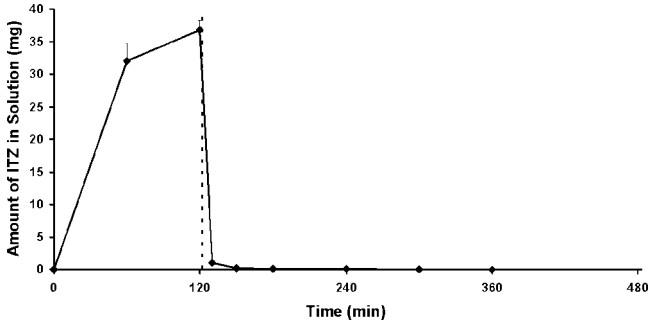

Figure 7. Supersaturated dissolution profile of Sporanox pellets. Each vessel (n = 3) contained 37.5 mg of ITZ equivalent corresponding to 10 times the equilibrium solubility of ITZ in the acid phase. Testing was conducted for 2 h in 750 mL of 0.1 N HCl followed by pH adjustment to 6.8 ± 0.5 with 250 mL of 0.2 M tribasic sodium phosphate solution. Dashed vertical line indicates the time of pH change.

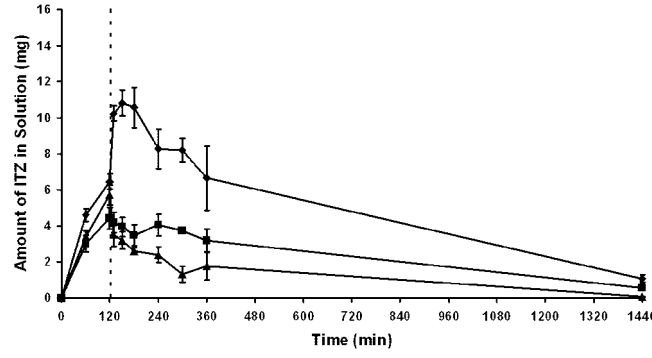

Figure 8. Supersaturated dissolution profile of ITZ:CAP formulations. Key: 1:2 ITZ:CAP (◆), 1:1 ITZ:CAP (■), 2:1 ITZ:CAP (▲). Each vessel (n = 3) contained 37.5 mg of ITZ equivalent corresponding to 10 times the equilibrium solubility of ITZ in the acid phase. Testing was conducted for 2 h in 750 mL of 0.1 N HCl followed by pH adjustment to 6.8 ± 0.5 with 250 mL of 0.2 M tribasic sodium phosphate solution. Dashed vertical line indicates the time of pH change.

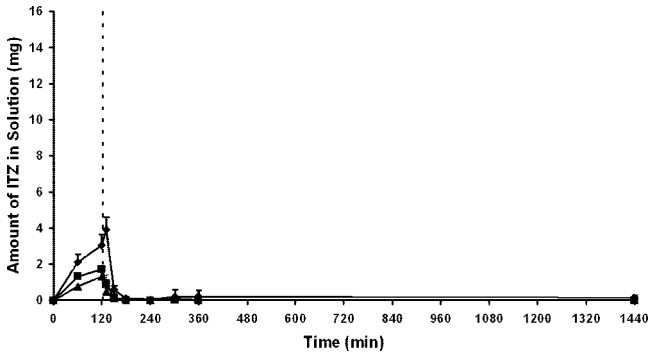

Figure 9. Supersaturated dissolution profile of ITZ:PVAP formulations. Key: 1:2 ITZ:PVAP (◆), 1:1 ITZ:PVAP (■), 2:1 ITZ:PVAP (▲). Each vessel ($n = 3$) contained 37.5 mg of ITZ equivalent corresponding to 10 times the equilibrium solubility of ITZ in the acid phase. Testing was conducted for 2 h in 750 mL of 0.1 N HCl followed by pH adjustment to 6.8 ± 0.5 with 250 mL of 0.2 M tribasic sodium phosphate solution. Dashed vertical line indicates the time of pH change.

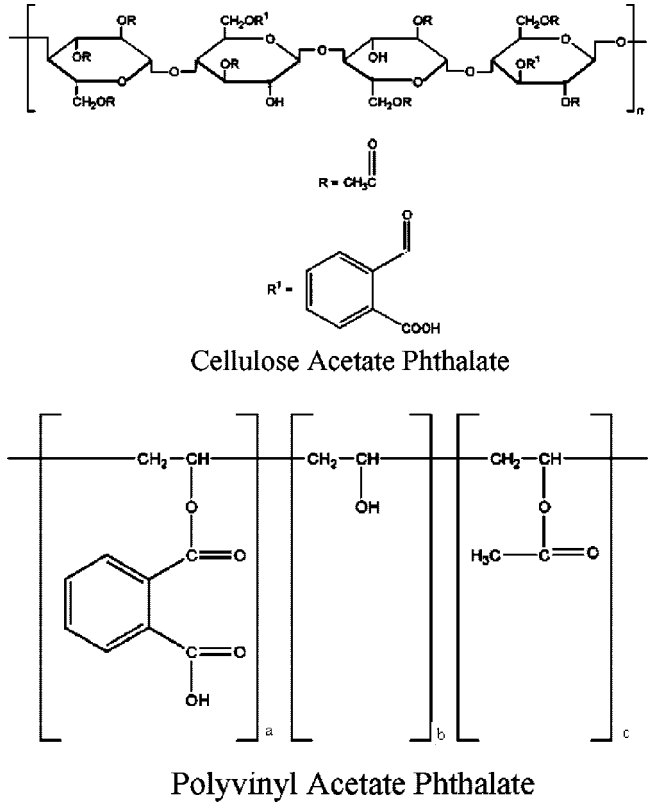

Figure 10. Molecular structure of concentration enhancing polymers.

solutions: hydrogen bonding and steric hindrance. Examination of the molecular structure of ITZ revealed numerous hydrogen bond acceptor sites that are capable of interacting with the hydrogen bond donor sites of the enteric polymers. The molecular structure of both polymers, presented in Figure 10, indicated that PVAP has more hydrogen bond donor sites per weight, suggesting that the magnitude of hydrogen bonding between the drug and polymer is not the mechanism

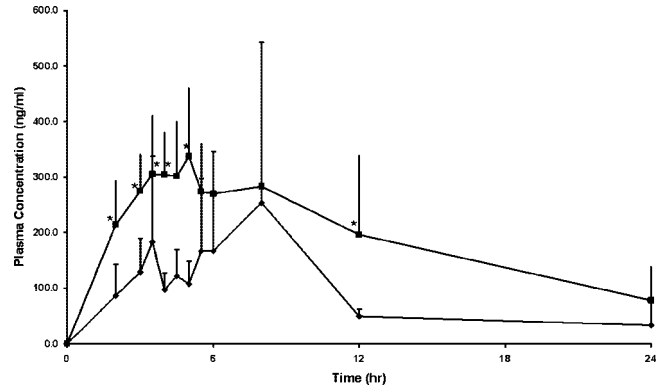

Figure 11. *In vivo* plasma profile. Key: Sporanox pellets (◆), 1:2 ITZ:CAP (■). Formulations were administered by oral gavage at a dose of 15 of mg ITZ/kg of body weight per rat ($n = 6$). * indicates statistically significant concentration difference between test and reference formulation as determined by one-way ANOVA with Tukey post hoc testing.

Table 4. Calculated Pharmacokinetic Parameters for Formulations Tested *in Vivo*

formulation	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-24h} (ng·h/mL)	$t_{1/2}$ (h)
Sporanox pellets	359.0 ± 261.0	5.5 ± 2.3	2132 ± 1273	4.9 ± 2.9
1:2 ITZ:CAP	381.4 ± 121.8	4.3 ± 0.6	4516 ± 1949	9.6 ± 1.9

of stabilization. A qualitative comparison of polymer solutions (2%) shows that CAP provides higher viscosities than PVAP,[49] suggesting that the stabilization may be due to steric hindrance. Furthermore, the molecular structure of CAP may also play a role in the stabilization. It has been observed that higher viscosities of HPMC, a cellulosic polymer, can provide improved stabilization of supersaturated ITZ solutions compared to comparable viscosities of PVP, a polymer with a polyalkylene backbone. The ability of these excipients, HPMC and CAP, to provide superior stabilization of supersaturation may also be due, in part, to the cellulosic polymer backbone which can provide additional shielding of ITZ from interactions that could result in recrystallization compared to polymers such as PVP and PVAP with a polyalkylene backbone due to differences in polymer rigidity.

In Vivo **Results.** Based on the superior *in vitro* dissolution performance of the 1:2 ITZ:CAP engineered particle composition, this formulation was selected for comparison to Sporanox pellets in a rat model. After dosing, plasma samples taken during testing were analyzed by HPLC and ITZ plasma concentrations were plotted as a function of time as shown in Figure 11. Both plasma profiles were analyzed by noncompartmental analysis for extravascular administration to determine the appropriate pharmacokinetic parameters of the two formulations, as presented in Table 4, and the data was analyzed statistically using the Student *t* test. Additional analysis of plasma profile time points was conducted using one way ANOVA with Tukey posthoc testing.

(49) United States Pharmacopeia. http://www.uspnf.com.

Statistically significant differences observed for both AUC_{0-24h} and $t_{1/2}$ values indicated that the engineered particle formulation, when compared to Sporanox pellets, provided greater oral bioavailability and suggested that larger concentrations of drug were available for absorption in the later stages of the GI tract due to the inhibition of drug precipitation, however one cannot rule out a potential contribution of metabolic conversion on the improvement of itraconazole bioavailability. Results also showed no statistically significant difference in measured C_{max} values, as well as a reduction in variability for the test product indicating a potential for increased oral bioavailability with a comparable or reduced side effect profile. Similarly, measured t_{max} values for the two formulations also showed no statistically significant difference, although the test formulation did exhibit lower variability. Statistical analysis of plasma profile time points collected from two to five hours during testing showed statistically significant differences in mean concentration, with engineered particle compositions providing greater concentrations within this time period which suggested superior targeting of the upper small intestine.

Comparing the *in vitro* dissolution results to the *in vivo* plasma profiles yields several interesting findings. First, the composition with the slower dissolution rate and lower overall degree of supersaturation provided the higher oral bioavailability. These results are similar in nature to those reported by Six et al.,[2] where *in vitro* dissolution rates did not linearly correlate to differences in oral bioavailability. Assessment of much of the currently available literature teaches that increased dissolution leads to increased bioavailability, however the results presented here contradict this hypothesis. When examining the physiological properties of the rat gastrointestinal tract, one sees that the approximate surface area of and residence time in the stomach are significantly lower than those of the intestinal tract,[1,50,51] allowing for increased absorption of drugs after transiting through the pyloric sphincter. *In situ* perfusion studies of ITZ in Sprague−Dawley rats demonstrated regional permeabilities and K_{obs} of approximately 5×10^4 cm/s and 1.3×10^{-3} respectively.[52] Although the permeability values decreased between the duodenum and the ileum, the relatively constant K_{obs} values suggested that absorption occurs throughout the intestine. Since the interaction of ITZ and CAP provided prolonged durations of supersaturation *in vitro*, it is reasonable to infer that a similar effect would occur in the neutral pH and highly permeable environment of the intestine,[52] resulting in a higher driving force for transport across the intestinal membrane thereby improving the absorption and bioavailability of ITZ. Products exhibiting longer durations of absorption, such as modified release tablets, exhibit longer *in vivo* half-lives when evaluated by noncompartmental analysis due to the combined mechanisms of absorption and elimination.[53] Examination of the $t_{1/2}$ values also showed that the ITZ:CAP composition provided an increased half-life, indicative of prolonged absorption in the later stages of the intestinal tract. Based on these results, for drugs such as ITZ that exhibit no regional absorption limitations, bioavailability can be enhanced by providing greater extents of supersaturation at lower maximal concentrations than by maximizing the dissolution rate when the drug substance is prone to precipitation under physiological conditions.

Another interesting observation was the statistically significant differences in the initial stages of the plasma profile for the two formulations, which suggested improved targeting of the upper small intestine by the ITZ:CAP composition. During testing gavage volumes were minimized to prevent spontaneous release through the pyloric sphincter, however variations in gastric emptying associated with the differences in dosage form[1] may have contributed to this disparity in performance between the formulations, as solid dosage forms have a longer intestinal residence time. Given the absence of a statistical difference observed in t_{max} values between formulations one can infer an underlying performance difference between compositions. Although the Sporanox pellets supersaturated the acidic media *in vitro*, rapid and complete precipitation to below the limit of detection was observed upon pH change above the pK_a. Analogously, *in vivo* the Sporanox pellets rapidly supersaturate the stomach, however the short residence time and minimal surface area limit absorption during this stage. Upon transiting through the pyloric sphincter the supersaturated solution encounters pH values above the pK_a causing the drug to un-ionize and rapidly precipitate from solution due to the absence of a strong stabilizing polymer. As a result of the precipitation in neutral media, limited and decreasing quantities of drug in solution are available to provide a driving force for diffusive transport in the highly permeable upper small intestine resulting in incomplete absorption. Conversely, the ITZ:CAP composition provided substantially lower levels of drug release in the acidic media representative of the stomach. When the stomach contents entered the upper small intestinal region, the pH increase ionized the enteric polymer allowing for maximal supersaturation in the region of highest permeability driving a more rapid increase in plasma concentration during the initial stages of the profile. Similar results have also been reported in the literature, for example where the use of enteric coprecipitates and intraduodenal administration of PVP coprecipitates resulted in improved bioavailability of the developmental drug HO-221 by targeting the upper small intestine,[54] further supporting the utility of site targeted supersaturation for enhancing bioavailability.

(50) Guignet, R.; Bergonzelli, G.; Schlageter, V.; Turini, M.; Kucera, P. *Neurogastroenterol. Motil.* **2006**, *18* (6), 472–478.
(51) Perry, R. L.; Carrig, C. B.; Williams, J. F.; Johnson, C. A.; Kaneene, J. B. *Lab. Anim. Sci.* **1993**, *43* (6), 586–593.
(52) Kim, Y. H.; Lee, Y. S.; Park, G. B.; Lee, K. P. *Yakche Hakhoechi* **1991**, *21* (4), 215–222.
(53) Rowland, M.; Tozer, T. N., *Clinical Pharmacokinetics: Concepts and Applications*; Lippincott, Williams & Wilkins: Philadelphia, PA, 1995.
(54) Kondo, N.; Iwao, T.; Hirai, K.; Fukuda, M.; Yamanouchi, K.; Yokoyama, K.; Miyaji, M.; Ishihara, Y.; Kon, K.; Ogawa, Y.; Mayumi, T. *J. Pharm. Sci.* **1994**, *83* (4), 566–570.

Conclusions

Solid state characterization demonstrated that the engineered particles were amorphous and provided increased specific surface areas which would provide thermodynamic and kinetic advantages during dissolution. It was also shown that the compositions were chemically homogeneous with only single glass transitions observed over a range of drug loadings, indicating miscibility of the drug−polymer systems studied and confirmed by EDS mapping. *In vitro* dissolution testing results indicated a reduced dissolution rate of the engineered particle systems compared to the Sporanox pellets, however ITZ:CAP compositions were able to more completely supersaturate the neutral pH environment and also provided greater extents of supersaturation due to the stabilizing effect of the polymer. As a result of the site targeting and improved extent of supersaturation, a statistically significant 2-fold improvement in bioavailability of itraconazole was observed over the currently marketed product in a rat model, suggesting a potential application of this formulation strategy in humans.

Clearly, these results indicate that the ability to provide enhanced bioavailability is dependent on more than the dissolution rate of the compositions and support the hypothesis that prolonged durations of neutral media supersaturation correlate with improved oral bioavailabilities. When formulating a poorly water soluble new chemical entity (NCE), it is essential to understand the dynamics of supersaturation and the regional absorption of the compound. Drug substances requiring supersaturation to improve absorption will ultimately be subjected to precipitation due to the metastability associated with the dissolution process. Identifying and incorporating materials capable of decreasing the rate of precipitation can provide increased bioavailability by prolonging the duration of supersaturation. Furthermore, site targeting of supersaturation using pH dependent polymers can be an effective way to provide the maximum driving force in the region of highest permeability. These results highlight the utility of this approach for the bioavailability enhancement of itraconazole, and similar strategies have been reported for other compounds in the literature, indicating that this technique can be an effective tool to improve the bioavailability of NCEs currently under development.

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