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Enhanced In Vivo Absorption of Itraconazole via Stabilization of Supersaturation Following Acidic-to-Neutral pH Transition

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Previous attempts to improve the dissolution and absorption properties of itraconazole (ITZ) through advanced formulation design have focused only on release in acidic media; however, recent reports indicate that absorption occurs primarily in the proximal small intestine. This suggests that enhancing supersaturation of ITZ in neutral aqueous environments is essential for improving absorption. The aim of this study was to evaluate different polymeric stabilizers with either immediate release (IR) (Methocel™ E5, Methocel™ E50, Kollidon® 12, and Kollidon® 90) or enteric release (EUDRAGIT® L 100-55, HP-55, and HP-55S) properties to determine the chemical and physical attributes of the polymeric stabilizers that promote supersaturation of ITZ in neutral media. Each amorphous composition was produced by hot-melt extrusion and characterized by differential scanning calorimetry. Dissolution testing by a supersaturated acidic-to-neutral pH change method was conducted on each composition. Testing of IR compositions revealed that Methocel™ was a superior stabilizer compared with Kollidon® owing to stronger intermolecular interaction with ITZ molecules in solution. Increasing the molecular weight of polymers was found to promote ITZ supersaturation and was most likely attributable to increased solution viscosity resulting in retention of ITZ molecules in an enthalpically favored association with the polymer for extended durations. Of the enteric polymeric stabilizers, EUDRAGIT® L 100-55 was found to be superior to both HP-55 grades because of its greater permeability to acid that allowed for improved hydration of ITZ in the acid phase as well as a greater number of free hydroxyl groups on the polymer backbone that presumably helped to stabilize ITZ in solution. The Methocel™ E50 and EUDRAGIT® L 100-55 formulations were evaluated for in vivo drug absorption in male Sprague–Dawley rats and were found to produce a threefold greater ITZ absorption over our previously reported IR formulations. The results of this study confirmed the hypothesis that supersaturation of ITZ following an acidic-to-neutral pH transition in vitro correlates directly to in vivo absorption.

Keywords itraconazole; supersaturation; in vivo; bioavailability; enteric; solid dispersion; solid solution; hot melt extrusion; amorphous

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INTRODUCTION

Numerous reports have indicated that mycotic infections have been occurring with increased frequency over the past four decades (Denning, 1998; Fridkin & Jarvis, 1996; Groll et al., 1996; Lin, Schranz, & Tentsch, 2001; Selik, Karon, & Ward, 1997; Yamazaki, Kume, Yamashita, Murase, & Arisawa, 1997). These reports also document a concurrent increasing trend in mycoses-related fatalities. The underlying cause of this trend has been attributed to the growing population of patients with compromised immune systems resulting from intensive chemotherapy treatments, bone marrow transplantation, organ transplantation, immunosuppressive drug treatments for autoimmune diseases, as well as the HIV/AIDS pandemic (Denning, 1998; Lin et al., 2001). Immunocompromised patients are especially susceptible to fungal infections as most mycotic infestations are opportunistic, relying on a weakened immune system for infection and proliferation. As the population of patients with immune system deficiencies continues to grow, so too will the number of fungal infestations and resulting fatalities. Hence, the demand for safe and effective antifungal drug treatments will continue to increase as this patient population expands.

Itraconazole (ITZ) is a commonly prescribed systemic antifungal drug treatment. ITZ is an orally active antifungal agent indicated for the treatment of a broad spectrum of fungal infections including: blastomycosis (pulmonary and extrapulmonary), histoplasmosis (including chronic cavitary pulmonary disease and disseminated, non-meningeal), aspergillosis (pulmonary and extrapulmonary), as well as onychomycosis of the finger and toenails (Janssen, 2006). Numerous reports have demonstrated ITZ to be an effective treatment against superficial and disseminated fungal infections (Denning et al., 1994; Grant & Clissold, 1998; Lewis, Wiederhold, & Klepser, 2005). Additionally, ITZ treatments have shown promise for the prophylaxis of opportunistic fungal infections in at-risk, immunocompromised patients (Bohme et al., 1996; Glasmacher et al., 2003; McKinsey et al., 1999; Mouy et al., 1994; Myoken,

Sugata, Kyo, Fujihara, & Mikami, 2002). Oral ITZ for the treatment of the aforementioned fungal infections is available commercially under the brand name Sporanox® as both a capsule and an oral solution formulation.

Although ITZ has been demonstrated to be effective in the treatment of fungal infections, the current oral delivery systems for ITZ are suboptimal. ITZ is a highly lipophilic compound that is completely insoluble in water, yet readily permeable to biological membranes (log $P_{o/w}$ = 5.66 at pH 8.1) (Janssen, 2006) indicating the absorption of ITZ is dissolution rate limited. The bioavailability of ITZ from oral solution is only 55%, hence current therapies must rely on high doses (200 mg once or twice daily) for therapeutic efficacy (Janssen, 2006). The absorption of ITZ from oral dosage forms has been characterized as erratic with high intra- and intersubject variability (Hardin et al., 1988; Janssen, 2006; McKinsey et al., 1999; Zhou et al., 1998). This combination of exaggerated doses and variable absorption is highly undesirable as toxic systemic concentrations could result in some patients. Additionally, the bioavailability of Sporanox® capsules has been demonstrated to be highly dependant on food intake (Janssen, 2006). This food effect represents another disadvantage of the current Sporanox® formulations as it decreases the dosing convenience that generally hinders patient compliance and reduces the success rate of drug therapy.

The need for an improved oral delivery system for ITZ is apparent and has prompted many researchers to investigate advanced formulation design to improve efficacy. Because thermodynamic stability of the ITZ crystal lattice and poor wettability are the primary barriers to dissolution, the utilization of solid dispersion technologies to produce composites of amorphous ITZ with hydrophilic excipients has been the strategy of choice for a vast majority of these researchers. Jung et al. utilized a spray-drying process to produce solid dispersions of ITZ in poloxamer 188, polyethylene glycol (PEG) 20 M, polyvinylpyrrolidone (PVP), hydroxypropyl methylcellulose (HPMC), as well as in the pH-dependent hydrophilic polymers polyvinylacetal diethylaminoacetate and Eudragit® E 100 (Jung et al., 1999). The use of hot-melt extrusion (HME) to produce amorphous ITZ in an EUDRAGIT® E 100 and a combination EUDRAGIT® E 100/PVP-co-vinyl acetate (PVPVA 64) carrier system was reported by Six et al. (2002), Six, Verreck, Peeters, Brewster, & Van den Mooter, (2004). In two separate reports, Verreck et al. produced amorphous ITZ solid dispersions in HPMC 2910 (5 cps) by HME as well as by an electrostatic spinning technique (Verreck et al., 2003; Verreck, Chun, Peeters, Rosenblatt, Brewster, 2003). Similarly, Rambali et al. produced solid dispersions of amorphous ITZ in a binary carrier system consisting of HPMC (5 cps) and hydroxypropyl-β-cyclodextrin (Rambali, Verreck, Baert, & Massart, 2003). The aerosol solvent extraction system (ASES) was used by Lee et al. to generate solid dispersions of amorphous ITZ in HPMC 2910 (Lee et al., 2005). Wang et al. produced an amorphous dispersion of ITZ in a binary carrier system consisting of PVPVA 64 and Myrj52 by solvent evaporation (Wang, Michoel, & Van den Mooter, 2005). Van den Mooter et al. reported the production of amorphous ITZ in a polymeric surfactant carrier known as Inutec SP1 by both spray drying and HME (Van den Mooter, Weuts, De Ridder, & Blaton, 2006). Amorphous dispersions of ITZ in Kollicoat™ IR were recently reported by Janssens, de Armas, Remon, & Van den Mooter (2007). Finally, Ye et al. produced amorphous ITZ/EUDRAGIT® E 100 composites by a high-shear mixing process (Ye, Wang, Heng, Chen, & Wang, 2007).

Each of these studies has in common the use of a carrier system that promotes immediate release (IR) of amorphous ITZ such that rapid dissolution rates and supersaturation of gastric fluids can be achieved following oral administration. In fact, all of the aforementioned studies demonstrate substantial improvements in the dissolution properties of ITZ in simulated gastric fluid. However, those studies that provide in vivo data report modest to no improvement over the Sporanox® system (Lee et al., 2005; Six et al., 2005; Yoo et al., 2000). In a clinical study conducted by Six et al., the rate of dissolution in simulated gastric fluid was found to be somewhat inversely proportional to in vivo ITZ absorption (Six et al., 2005). We reported a similar discrepancy between in vitro ITZ release in acid and in vivo performance in which formulations with substantially different extents of ITZ supersaturation in acid produced statistically equivalent in vivo absorption (Miller, McConville, Yang, Williams, & McGinity, 2007). By the use of a pH change dissolution methodology, it was revealed that although the two formulations had substantially different release profiles in the acid phase, catastrophic precipitation of ITZ following the transition from acidic to neutral pH was exhibited by both formulations. This effect results from the pH solubility profile of the weakly basic ITZ (pKa 3.7) as it is substantially more soluble in acidic media (4 μg/mL) than in neutral media (~1 ng/mL) (Janssens et al., 2007; Peeters, Neeskens, Tollenaere, Van Remoortere, & Brewster, 2002). These results from our previous study coupled with the results of Six et al. led to the conclusion that most ITZ absorption occurs in the proximal small intestine, and therefore, IR systems provide only a small window for absorption because supersaturated levels of ITZ in the gastric environment rapidly precipitate upon exit from the stomach. Hence, it was concluded that formulation design of oral ITZ formulations should focus on extent of supersaturation following acidic-to-neutral pH transition rather than a formulation's dissolution properties in acidic media because this in vitro dissolution metric is expected to correlate more closely to in vivo absorption.

The primary objective of this study was to investigate amorphous ITZ solid dispersion systems with different polymeric carriers with respect to the ability to produce or maintain supersaturation of ITZ following an acidic to neutral pH change. By evaluating polymeric carriers of differing chemical and physical properties, it was expected that the mechanism of stabilization of supersaturated ITZ in neutral media may be

elucidated. A secondary objective of this study was to investigate the in vivo absorption of ITZ from those formulations that generate the greatest extent of ITZ supersaturation in vitro following the acidic-to-neutral pH transition.

MATERIALS

ITZ, BP micronized was purchased from Hawkins, Inc. (Minneapolis, MN). Kollidon® 12 PF and 90 F (Povidone K 12 and K 90 USP) were kindly provided by BASF (Ludwigshafen, Germany). Methocel™ E5 and E50 Premium LV (Hypromellose 2910 5 and 50 cps) were kindly provided by The Dow Chemical Company (Midland, MI). HP-55 and HP-55S (Hypromellose phthalate, 40 cps viscosity grade and 170 cps viscosity grade, respectively) were kindly provided by Shin-Etsu through Biddle Sawyer Corporation (New York, NY). EUDRAGIT® L 100-55 was provided by Degussa GmbH (Linden, NJ). Triethyl citrate (TEC, NF) was provided by Vertellus™ Performance Materials, Inc. (Greensboro, NC). High-performance liquid chromatography (HPLC) grade Acetonitrile was purchased from EMD chemicals (Darmstadt, Germany). All other chemicals utilized in this study were of ACS grade.

METHODS

Hot-Melt Extrusion

All melt-extruded compositions presented in this study were produced with an HAAKE Minilab II Micro Compounder (Thermo Electron Corporation, Newington, NH) equipped with twin, co-rotating conical screws (5/14 mm diameter). All formulation components were premixed in a glass mortar and pestle before extrusion. For formulations requiring a plasticizer, the plasticizer and polymer(s) were blended in a glass mortar and pestle before the addition of ITZ. Powder blends were fed into the extruder barrel through the Minilab manual feeding device. No external die was applied at the outlet of the extruder barrel, and therefore, extruded materials were forced through the 1.0 × 4.0-mm rectangular outlet port. The operating parameters for each composition presented in this study are provided in Table 1. Following extrusion, extrudates were ground in a blade grinder (Capresso, Inc., Closter, NJ) for 2 min. The ground product was then passed through a 60-mesh sieve. The material that passed through the sieve was manually milled in a porcelain mortar and pestle for 1 min to yield a fine powder. All analyses were then conducted on this finely milled powder.

Differential Scanning Calorimetry

Modulated differential scanning calorimetry (DSC) analysis was conducted using a TA Instruments Model 2920 DSC (New Castle, DE) equipped with a refrigerated cooling system. Samples were weighed to 15 ± 5 mg in aluminum crimped pans (Kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT). Samples were heated at a ramp rate of 10°C/min from 5 to 215°C with a modulation temperature amplitude of 0.5°C and a modulation period of 40 s for all studies. Ultrahigh purity nitrogen was used as the purge gas at a flow rate of 40 mL/min. All data analyses were performed using TA Universal Analysis 2000 software. The thermogram for amorphous ITZ used in the DSC analysis of the solid dispersion formulations with EUDRAGIT® L 100-55, HP-55, and HP-55S was obtained on a second heating of crystalline ITZ following an initial heating to 215ºC followed by rapid cooling (20°C/min) to 5°C. The T_gs of the EUDRAGIT® L 100-55, HP-55, and HP-55S polymers were determined by first run DSC following preheating of the polymer powders to 90ºC for 15 min in an MF-50 model moisture analyzer (AND Company Ltd., Encino, CA) to remove residual moisture.

Dissolution Testing

Dissolution testing was performed according to USP 29 Apparatus 2 guidelines (paddle method) at 50 rpm in a Vankel 7000 Dissolution Tester (Vankel Technology Group, Cary, NC) equipped with a VK 8000 model autosampler. The dissolution method utilized was in accordance with the USP 29 dissolution-testing specifications for delayed-release dosage forms Method A. Specifically, formulations were first subjected to "acid stage" testing (2 h in 750 mL of 0.1 N HCl) followed by a pH adjustment to 6.8 ± 0.5 by the addition of

TABLE 1
Hot-Melt Extrusion Processing Parameters for Each Investigated Composition

Formulation	Extrusion Temperature (ºC)	RPM (min^{-1})	Torque (N·m)
ITZ : Methocel™ E50 (1:2)	180	100	150–200
ITZ : Methocel™ E5 (1:2)	180	300	100–150
ITZ : Kollidon® 90 (1:2)	180	300	100–150
ITZ : Kollidon® 12 (1:2)	180	300	150–200
ITZ : EUDRAGIT® L-100-55 [20% TEC] (1:2)	130	300	200–250
ITZ : HP-55 [20% TEC] (1:2)	135	300	100–150
ITZ : HP-55S [20% TEC] (1:2)	135	300	125–175

250 mL of 0.2 M tribasic sodium phosphate to start the "buffer stage" testing, which was conducted for 4 additional hours. The 250 mL of 0.2 M tribasic sodium phosphate was added to 750 mL of 0.1 N HCl rapidly from a graduated cylinder to avoid hot-spot formation during the pH adjustment phase (Miller, Gamba et al., 2007). The dissolution media was held at 37.0 ± 0.2ºC throughout the test procedure. No surfactant was included in either phase of dissolution testing. To each dissolution vessel, 180 mg of the milled extrudate powder was added (60 mg ITZ equivalent). This amount of solid addition corresponded to a theoretical 80 μg/mL ITZ concentration for the acid phase of testing, which represents a 20-fold level of supersaturation assuming an equilibrium solubility of 4 μg/mL in 0.1 N HCl (Peeters, Neeskens, Tollenaere, Van Remoortere, & Brewster, 2002). All aliquots of dissolution media were filtered using Acrodisc® CR 13-mm syringe filters with a 0.2-μm PTFE membrane (Pall Life Sciences, East Hills, NY). Filtered aliquots were then diluted in a 1:1 ratio with HPLC mobile phase.

Sampled aliquots of dissolution media were analyzed for drug content using a Waters (Milford, MA) HPLC system with a photodiode array detector (Model 996), and extracting at a wavelength of 263 nm (λ_{max}). An auto sampler (Model 717 Plus) was used to inject 200 μL samples, and the data were collected and integrated using Empower® Version 5.0 software. The column used was a Phenomenex® Luna 5 μm C18(2) 100A, 150 × 4.6 mm (Phenomenex®, Torrance, CA). The mobile phase consisted of 7:3 (vol/vol) acetonitrile : deionized water with 0.5 mL/L of diethanolamine. The retention time of ITZ was approximately 6 min. Linearity was demonstrated from 0.024 to 100 μg/mL ($r^2 \geq 0.999$) and the relative standard deviation of six injections was less than 0.5%.

In Vivo Studies

Institutionally approved in vivo studies were conducted using CD® IGS Sprague–Dawley rats (Charles River Laboratories, Inc., Wilmington, MA), which were pre-catheterized with a vascular catheter surgically inserted into the jugular vein. All rats received were between 275 and 325 g of total body weight. The catheter was flushed daily with 0.3 mL of 50 U/mL heparinized normal saline. After at least 3 days of acclimatization period, the rats were administered the aqueous dispersion of the formulations by oral gavage at a dose of 30 mg ITZ/kg body weight ($n = 4$). Each formulation was dispersed in deionized water just before dosing such that 400 μL of suspension contained a dose of 9 mg ITZ. Serial blood samples (approximately 0.3 mL each) were withdrawn through the jugular vein catheter at 0, 2, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 12, and 24 h after dosing, and placed into a pre-heparinized microcentrifuge tube. Equal volume of saline was replaced after each sampling. Plasma samples were harvested by centrifugation of the blood at 3000 × g for 15 min and were kept at –20°C until drug analysis.

Plasma Extraction and Chromatographic Analysis

Calibration standards and plasma samples were analyzed according to previously published methods (Gubbins, Gurley, & Bowman, 1998; Vaughn et al., 2006). Briefly, upon thawing, a volume of harvested plasma was transferred to a clean 1.5-mL microcentrifuge tube. Barium hydroxide 0.3 N (50 μL) and 0.4 N zinc sulfate heptahydrate solution (50 μL) were then added followed by vortex mixing for 30 s to precipitate water-soluble proteins. Acetonitrile (1 mL) containing 1200 ng/mL ketoconazole as an internal standard was added to each plasma sample followed by vortex mixing for 1.5 min. The samples were then centrifuged at 3000 × g for 15 min. The supernatants were then extracted and transferred to a clean 1.5-mL centrifuge tube and seated in an aluminum heating block (70°C) under a stream of nitrogen until dry. Samples were reconstituted with 250 μL mobile phase (62% acetonitrile: 38% 0.05 M potassium phosphate monobasic buffer adjusted to pH 6.7 with NaOH) and vortex mixed for 1 min. The samples were then centrifuged for an additional 15 min and subsequently a 150-μL aliquot of the supernatant was extracted and filled into low volume HPLC vial inserts. Each sample was analyzed using the previously described Waters HPLC system. A Phenomenex® Luna 5 μm C-18(2) 100A HPLC column (250 × 4.6 mm) was used in the analysis. The column was maintained at a temperature of 37°C for the duration of the injection set. The ITZ peak eluted at 14.6 min and the ketoconazole peak eluted at 5.3 min at a flow rate of 1.0 mL/min. The injection volume was 100 μL, and the wavelength of absorption was 263 nm. The limit of detection and quantitation for ITZ was 10 and 30 ng/mL, respectively.

Pharmacokinetic Analysis

Non-compartmental analysis for extravascular input was performed on the data using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA). By this method of analysis, T_{max} and C_{max} were determined directly from the empirical data, area under the plasma concentration–time curve (AUC) was calculated by the linear trapezoidal method, and $t_{½}$ was determined by calculation of the lambda z parameter. Statistical comparisons were performed by two-tailed Student's t-test assuming equal variances ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Rationale for Polymer Carrier Selection

A two-arm formulation strategy was employed in this study: (1) to identify IR formulations with polymeric carriers that would produce extensive supersaturation in acid and inhibit ITZ precipitation to maintain supersaturation following the acidic-to-neutral pH transition and (2) to develop delayed release formulations that would provide minimal ITZ release in acid and produce extensive supersaturation following the acidic-to-neutral pH transition. In addition to drug-release

properties, polymeric carriers were also selected according to their chemical and physical attributes in order to identify the properties of polymeric stabilizers that provide stabilization of ITZ supersaturation in neutral pH media. By identifying these key attributes, it was believed that the mechanism of stabilization of supersaturated ITZ solutions could be elucidated. To date, a complete explanation as to the mechanism(s) of stabilization of supersaturated drug solutions has not been made. Some researchers have speculated that improved supersaturation through polymeric additives to solutions is the result of intermolecular interactions in solution (hydrogen bonding), steric hindrance of recrystallization, or a combination thereof (Gao et al. 2004; Okimoto et al. 1997; Suzuki & Sunada, 1998; Yamashita et al. 2003; Yokoi et al. 2005).

To evaluate intermolecular interactions in solution as a mechanism of stabilization, povidone and hypromellose were selected as the two IR carrier polymers for evaluation as they have contrasting hydrogen-bonding characteristics. With carbonyl groups along the polymer backbone, povidone is a proton acceptor, whereas hypromellose contains free hydroxyl groups making it a proton donor. From the perspective of hydrogen bonding, these polymers should behave quite differently in solution, and thus are expected to exhibit very different stabilization characteristics of ITZ supersaturated solutions.

To evaluate the proposed steric hindrance mechanism of supersaturation stabilization, a low and a high molecular weight grade of both povidone and hypromellose were selected for evaluation. The reason for this choice being that solution viscosity increases with molecular weight corresponding to an increase in chain entanglements. It is expected that an increase in solution viscosity will result in greater steric hindrance of recrystallization, and thus by varying the molecular weight of each stabilizing polymer the viscosity effect on precipitation can be directly evaluated. For povidone, Kollidon® 12 PF (povidone K12, USP) and Kollidon® 90 F (povidone K90, USP) were selected as they have substantially different molecular weights of 2–3 kDa and 1,000–1,500 kDa corresponding to solution (5%) viscosities of 1–2 and 50–60 cps, respectively. For hypromellose, Methocel™ E5 and Methocel™ E50 were selected as the two grades to be evaluated because of their substantially different solution (2%) viscosities of 4–6 and 40–60 cps, respectively. By choosing these four polymers (Kollidon® 12, Kollidon® 90, Methocel™ E5, and Methocel™ E50) as IR carriers for amorphous ITZ solid dispersions and evaluating their ability to inhibit ITZ precipitation following the acidic-to-neutral pH transition, it was expected that the chemical and physical aspects of supersaturated ITZ solution stabilization could be elucidated.

The selection of modified release polymers for the second arm of ITZ solid dispersion formulation development focused firstly on polymers that would produce a drug-release profile that would target delivery to the proximal small intestine as this was the hypothesized optimal site of absorption. Therefore, enteric polymers with pH-dependant solubility, specifically those with onsets of dissolution at pH ≥ 5.5, were identified as primary candidates. Secondly, miscibility of ITZ with the selected polymers was also crucial as the intended final composition was an amorphous dispersion of ITZ. It is for these reasons that EUDRAGIT® L100-55 (Methacrylic Acid Copolymer, Type C USP/NF) and HP-55 (hypromellose phthalate, NF) were selected as the delayed release carriers for investigation as both polymers exhibit an onset of dissolution at pH ≥ 5.5, and miscibility of ITZ with these polymers has been previously demonstrated in the pharmaceutical literature (Overhoff, Moreno, Miller, Johnston, & Williams, 2007). Moreover, HP-55 is available in two different molecular weight grades, i.e., the standard HP-55 grade (M_w = 84 kDa) and the HP-55S grade (M_w = 132 kDa) (Petereit & Weisbrod, 1999). The molecular weights of HP-55 and HP-55S correspond to solution viscosities (10%) of 40 and 170 cps (Shin-Etsu, 2007). The selection of these two hypromellose phthalate grades with differing solution viscosities allows for the direct evaluation of steric hindrance as a mechanism for the stabilization of supersaturated concentrations of ITZ.

This choice of modified release polymers for investigation also presents an interesting comparison with regard to hydrogen bonding in solution. The degree of methacrylic acid substitution on EUDRAGIT® L 100-55 is 46.0–50.0% (dry basis), whereas the degree of phthalyl substitution on HP-55 is 27.0–35.0% according to USP requirements. Hence, EUDRAGIT® L 100-55 contains a greater number of free hydroxyl groups on the polymer backbone than HP-55 and may serve as a more potent hydrogen bond donor in solution.

DSC Analysis of Hot-Melt Extruded ITZ-Polymer Formulations

IR Formulations

All the IR formulations were processed by HME at 180ºC, which is above the melting point of ITZ. Miscibility of ITZ with povidone and hypromellose at drug concentrations of 50% (wt/wt) was demonstrated in our previous article (Miller, McConville et al., 2007). Therefore, melt processing of ITZ with Kollidon® and Methocel™ (33% drug loading) at 180ºC was expected to yield entirely amorphous compositions. The results of DSC analysis of these formulations and the associated physical mixtures are shown in Figure 1. As expected, DSC analysis demonstrated that ITZ was rendered entirely amorphous by HME processing for all formulations as indicated by the absence of the melting endotherm for crystalline ITZ at approximately 169ºC, which is seen with the physical mixtures of crystalline ITZ with Kollidon® and Methocel™.

Enteric Release Formulations

At temperatures above 150ºC, pendent groups begin to dissociate from the polymer backbone of EUDRAGIT® L100-55, and at temperatures above the melting point of ITZ, the polymer is rapidly and extensively degraded (Petereit & Weisbrod,

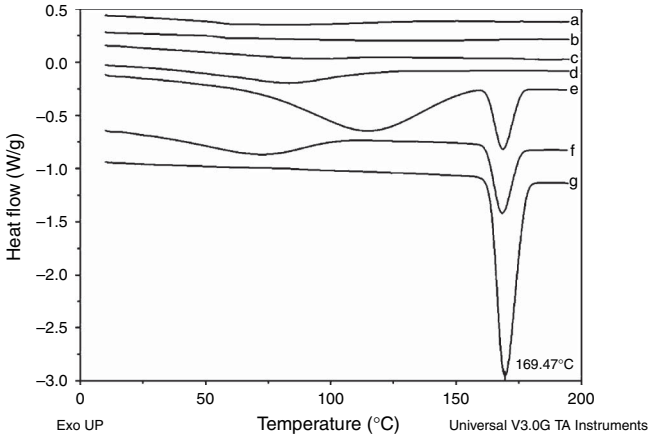

FIGURE 1. Differential scanning calorimetry (DSC) analysis of (a) hot-melt extrusion (HME)-processed itraconazole (ITZ) : Methocel™ E5 (1:2), (b) HME-processed ITZ : Methocel™ E50 (1:2), (c) HME-processed ITZ : Kollidon® 90 (1:2), (d) HME-processed ITZ : Kollidon® 12 (1:2), (e) ITZ : Kollidon® 90 (1:2) physical mixture, (f) ITZ : Methocel™ E50 (1:2) physical mixture, and (g) crystalline ITZ.

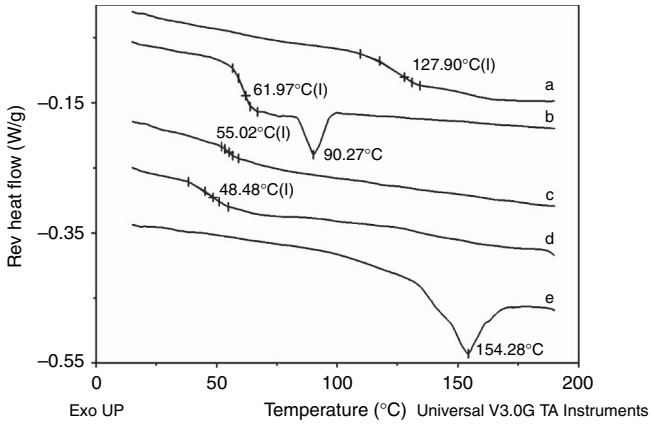

FIGURE 2. Differential scanning calorimetry (DSC) analysis of (a) EUDRAGIT® L 100-55 powder, (b) glassy itraconazole (ITZ), (c) placebo [hot-melt extrusion (HME)-processed EUDRAGIT® L 100-55 with 20% triethyl citrate (TEC) based on polymer mass], (d) HME-processed ITZ : [EUDRAGIT® L100-55 with 20% TEC] (1:2), and (e) physical mixture of ITZ : [EUDRAGIT® L100-55 with 20% TEC] (1:2).

1999). Similar thermal degradation of hypromellose phthalate was observed in HME trials conducted as part of the current study. Owing to the instability of the enteric polymers at elevated temperatures, HME processing could not be conducted above the melting point of ITZ. Moreover, the glass transition temperatures (T_g) of EUDRAGIT® L100-55 and HP-55 were determined to be 128 and 144ºC, respectively; thus, processing these polymers at temperatures even moderately above their T_gs would also cause thermal degradation.

Plasticization was evaluated as a means of lowering the processing temperatures to avoid polymer degradation as well as to reduce the molten viscosity of the polymers and facilitate extrusion at lower temperatures. To this end, trials were conducted to determine the level of plasticization required to enable melt extrusion of EUDRAGIT® L 100-55, HP-55, and HP-55S at temperatures below the onset of polymer degradation. It was determined that the incorporation of 20% (based on dry polymer mass) triethyl citrate (TEC) was sufficient to enable extrusion of EUDRAGIT® L100-55 at 130ºC and HP-55 and HP-55S at 135ºC without any apparent polymer degradation.

The results of the DSC analysis of HME-processed ITZ: [EUDRAGIT® L 100-55 with 20% TEC] (1:2) are shown in Figure 2. In this figure, it is seen that the T_g of EUDRAGIT® L 100-55 powder is reduced from approximately 128–55ºC by HME processing with 20% TEC demonstrating the plasticizing effect of TEC that enables HME processing at 130ºC. The thermogram of glassy ITZ shows a T_g at 62ºC followed by two endothermic transitions at 75 (not readily apparent on the displayed scale) and 90ºC. The occurrences of these thermal events associated with glassy ITZ are in close agreement with previous reports by Six, Verreck, Peeters, Augustijns et al. (2001), Six, Verreck, Peeters, Binnemans et al. (2001). In the latter of these two reports, the two endothermic events following the T_g were attributed to a monotropic mesophase (Six, Verreck, Peeters, Binnemans et al., 2001). This thermogram is included for comparison in order to differentiate a solid solution of ITZ in the carrier polymer versus a dispersion of domains of glassy ITZ.

The thermogram of the HME processed ITZ : [EUDRAGIT® L 100-55 with 20% TEC] (1:2) formulation shows a single T_g at 48.5ºC indicating the additional plasticizing effect of ITZ on TEC plasticized EUDRAGIT® L 100-55. This single T_g suggests the formation of a solid solution of ITZ in EUDRAGIT® L 100-55 as neither a secondary T_g nor a mesophase transition that would indicate the presence of glassy ITZ domains within the polymer matrix were observed. Additionally, a melting endotherm corresponding to crystalline ITZ as seen at 154ºC with the physical mixture that was not observed with the HME-processed formulation. The thermogram for the physical mixture provides an indication of the solubility of ITZ in molten EUDRAGIT® L 100-55 as the onset of the crystalline ITZ melting endotherm occurs immediately following the T_g of the polymer. Applying this result to the HME process, when ITZ and EUDRAGIT® L 100-55 with 20% TEC are processed at 130ºC/300rpm, the shear forces produced by the intermeshing co-rotating screws provide intimate mixing of the two components and facilitate the solubilization of ITZ by molten EUDRAGIT® L 100-55.

The results of DSC analysis for the HP-55 and HP-55S-based formulations are provided in Figure 3. The T_gs of HP-55 and HP-55S were found to be in the range of 143–145ºC. HME-processed HP-55 and HP-55S with 20% TEC (based on dry polymer mass) both exhibited T_gs of approximately 47ºC

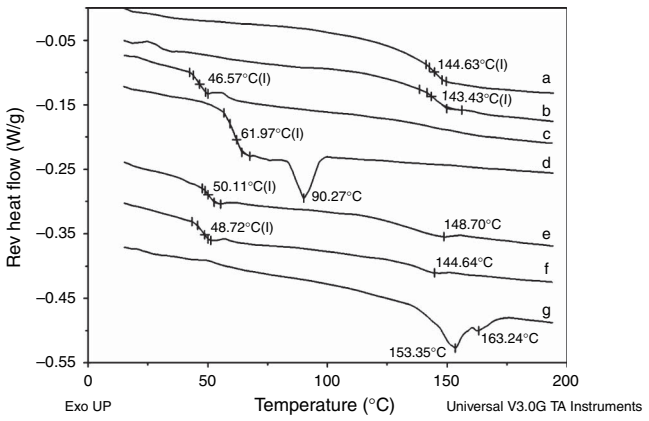

FIGURE 3. Differential scanning calorimetry (DSC) analysis of (a) HP-55 powder, (b) HP-55S powder, (c) placebo [hot-melt extrusion (HME)-processed HP-55S with 20% triethyl citrate (TEC) based on dry polymer mass], (d) glassy itraconazole (ITZ), (e) HME-processed ITZ : [HP-55 with 20% TEC] (1:2), (f) HME-processed ITZ : [HP-55S with 20% TEC] (1:2), and (g) physical mixture of ITZ : [HP-55S with 20% TEC] (1:2).

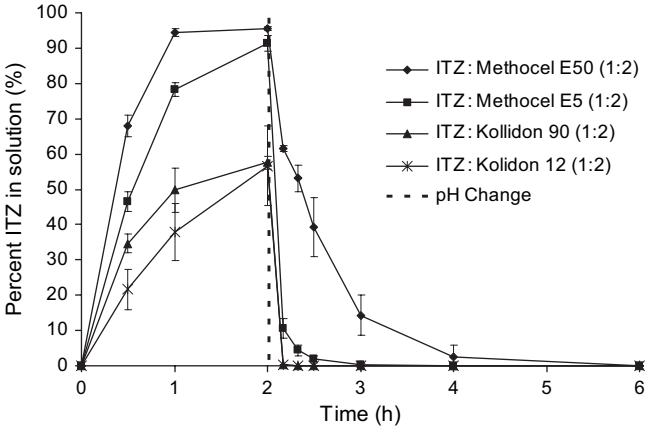

FIGURE 4. Supersaturation dissolution testing of the itraconazole (ITZ)-IR polymer hot-melt extrusion (HME)-processed compositions by pH change method. Each dissolution vessel (n = 3) contained 180 mg of the formulation (60 mg ITZ equivalent) corresponding to 20 times the saturation solubility of ITZ 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 of tribasic sodium phosphate solution.

demonstrating the plasticizing effect of TEC on hypromellose phthalate (HP-55 thermogram not shown). The HME-processed HP-55 and HP-55S formulations containing 33% ITZ both exhibited a T_g near 50ºC similar to the placebo extrudate. The similarity between the active and the placebo formulations with respect to this T_g indicate minimal plasticizing interaction of ITZ with hypromellose phthalate. The second transition observed with these formulations occurs in the temperature range of 140–150ºC with minima of 144 and 149ºC for HP-55S and HP-55, respectively. This event can be attributed to the melting endotherm of a small amount of crystalline ITZ. The two melting endotherms at 153 and 163ºC exhibited by the physical mixture indicate that 33% drug loading may exceed the miscibility of ITZ in molten hypromellose phthalate. This result supports the conclusion that the high-temperature transitions seen with the HME-processed ITZ/HP-55 and ITZ/HP-55S formulations are indicative of ITZ that was not rendered amorphous during processing. The presence of crystalline ITZ in the HME-processed formulations coupled with no apparent plasticizing interaction between the drug and the polymer suggests limited miscibility of ITZ in hypromellose phthalate.

Dissolution Testing with pH Change

IR Polymers

The results of dissolution testing with the IR carrier formulations are presented in Figure 4. In this figure, it is seen that the Methocel™ E50 formulation exhibited the most rapid dissolution rate with approximately 68% of ITZ in solution at 30 min. Whereas the Methocel™ E5, Kollidon® 90, and Kollidon® 12 formulations released only 47, 35, and 22% ITZ after 30 min in acid, respectively. The extent of ITZ supersaturation after 2 h in acid was similar between the Methocel™ E50 and E5 grades with respective percent release values of 96 and 92%. The Kollidon®-based formulations exhibited near-equivalent extents of supersaturation in acid with 57% ITZ released after 2 h. Following the acidic-to-neutral pH transition, the Methocel™ E50-based formulation provided the greatest stabilization effect against ITZ precipitation, with approximately 62% ITZ in solution 10 min after the pH transition. The Methocel™ E5-based formulation showed more extensive precipitation with only 11% ITZ remaining in solution 10 min after the pH change. Significant precipitation of ITZ was seen with both Kollidon® 90 and Kollidon® 12-based formulations as respective mean values of approximately 100 μg (0.17%) and 200 μg (0.33%) ITZ in solution were measured 10 min after the pH change. The Methocel™ E50-based formulation continued to show marked stabilization of supersaturated ITZ (1.4 mg in solution) up to 2 h after the pH transition. The Methocel™ E5-based formulation also produced substantial levels of ITZ in solution (32 μg) 2 h after the pH transition. Although these amounts of ITZ in solution may appear modest, when considering that the saturation solubility of ITZ in aqueous solution of neutral pH is estimated to be approximately 1 ng/mL (Janssens et al., 2007; Peeters et al., 2002), they represent considerable levels of ITZ supersaturation. Quantifiable levels of ITZ were not detected beyond 20 min after the pH change with either of the Kollidon®-based formulations.

To provide a more quantitative examination of these dissolution results, the area under the dissolution curve (AUDC) for the acid phase, buffer phase, and total dissolution test was calculated by the linear trapezoidal method. These values are presented in Table 2. The AUDC values further indicate the superiority of the Methocel® E50 formulation with respect to

TABLE 2
Area Under the Dissolution Curve (AUDC) Values for the Acid Phase, Neutral Phase, and Total Dissolution Test for Each Composition. Each Composition Contains 33% ITZ by Weight

Polymer Stabilizer	$AUDC_{acid}$ (mg·min)	$AUDC_{neutral}$ (mg·min)	$AUDC_{total}$ (mg·min)
Methocel™ E50	5,493 ± 66	1,960 ± 220	7,453 ± 194
Methocel™ E5	4,593 ± 131	399 ± 27	4,992 ± 153
Kollidon® 90	3,003 ± 180	175 ± 6	3,179 ± 182
Kollidon® 12	2,434 ± 522	171 ± 34	2,605 ± 556
EUDRAGIT® L100-55	628 ± 10	1,086 ± 36	1,714 ± 43
HP-55	51 ± 7	60 ± 8	111 ± 10
HP-55S	73 ± 7	90 ± 10	162 ± 17

the promotion of ITZ supersaturation in both acidic and neutral media. The Methocel™ E50 formulation exhibited 49, 134, and 186% greater mean $AUDC_{total}$ values than the Methocel™ E5, Kollidon® 90, and Kollidon® 12 formulations, respectively. In the acid phase of dissolution testing, the Methocel™ E50 formulation showed a 20, 83, and 126% increase in AUDC over the Methocel™ E5, Kollidon® 90, and Kollidon® K12, respectively. The most significant difference in dissolution performance between the IR formulations occurred following the acidic-to-neutral pH transition. The Methocel™ E50 formulation produced 391, 1,020, and 1,046% greater mean $AUDC_{neutral}$ values than the Methocel™ E5, Kollidon® 90, and Kollidon® 12 formulations, respectively.

The results of this dissolution study provide substantial insight as to how the attributes of polymeric stabilizers affect the dissolution properties of ITZ from amorphous solid dispersion systems. The differences in dissolution rate observed between these different stabilizing polymers indicate that both chemical and physical aspects of a polymeric stabilizer affect the rate of ITZ dissolution. Irrespective of molecular weight, Methocel™ was found to produce greater ITZ dissolution rates than Kollidon®. Because both polymers are readily water soluble, differences in wettability are an unlikely cause for this result. Rather, this result indicates stronger intermolecular interactions between ITZ and Methocel™ likely because of the numerous hydrogen bond donor sites on the polymer backbone. As a weakly basic molecule, ITZ is substantially more stable in aqueous solution in its protonated form. Because the three ionizable nitrogens on the ITZ molecule are not protonated above about pH 2 (Janssens et al., 2007), the molecule is only soluble in highly acidic aqueous media. When hypromellose is in solution in the immediate vicinity of dissolving ITZ, a proton-rich micro-environment is produced that is supplementary to the surrounding acidic aqueous medium. The presence of additional sites for proton interaction with ITZ in solution further promotes the ionization of ITZ, thereby reducing the enthalpy of hydration and accelerating the dissolution rate. The Kollidon® polymers do not contain free hydroxyl groups that would allow for hydrogen bonding in solution. In fact, the carbonyl groups present on the pyrrolidone ring are electronegative, hydrogen bond acceptors, and thus would compete with ITZ molecules for free protons in acidic solution. Therefore, povidone may act as a slight inhibitor of ITZ ionization in solution, which may explain the reduced dissolution rate with this stabilizer.

Contrary to expectations, higher molecular weight stabilizers were found to provide more rapid rates of supersaturation than lower molecular weight equivalents for both polymer types. This effect could be attributed to an increase in the local viscosity surrounding the dissolving drug provided by the higher molecular weight polymer. By increasing the local viscosity, the diffusion of solubilized ITZ molecules into bulk solution will be retarded, and hence, ITZ will remain in intimate association with the stabilizing polymer. When held in intimate contact with the stabilizing polymer in solution, the intermolecular interactions between ITZ and the polymer will provide stabilization of ITZ molecules in the thermodynamically unfavorable aqueous environment. As discussed above, the intermolecular attractions between ITZ and hypromellose in solution are stronger than those between ITZ and povidone, and hence, the effect of increasing molecular weight is more pronounced with the Methocel™ polymers than with the Kollidon® polymers.

The extent of supersaturation at 2 h in acid was seen to only depend on the chemical attributes of the polymer as the two grades within each polymer group reached approximately equivalent levels of ITZ supersaturation by the end of acid-phase testing. This result seems to indicate that intermolecular interactions between ITZ and the stabilizing polymer in solution solely govern the extent of supersaturation in acidic aqueous media. ITZ appears to interact more strongly with hypromellose in solution than with povidone, which is likely the result of hydrogen bonding with the free hydroxyl groups on the hypromellose polymer chain. As povidone does not contain free hydroxyl groups, hydrogen bonding is not possible and hence stabilization in solution is the result of weaker intermolecular interactions. With increasing stabilization by attractive intermolecular interactions between ITZ and the polymer in solution, the enthalpy of solution will decrease, thereby reducing the thermodynamic resistance to ITZ supersaturation. As a result, greater extents of ITZ supersaturation are possible with hypromellose than with povidone.

The behavior of these systems following the acidic-to-neutral pH transition further demonstrates the importance of both the chemical and the physical attributes of polymers for stabilizing supersaturated ITZ solutions. Following the pH transition, the Methocel™ E50 formulation provided substantial stabilization of supersaturated ITZ in neutral media whereas the Methocel™ E5 formulation showed only modest stabilization. The two Kollidon® formulations provided almost no inhibition of

precipitation. From comparison of these formulations, it is apparent that intermolecular interaction between ITZ and the polymer is essential to the prevention of precipitation of ITZ as seen by the different stabilizing properties between the hypromellose and the povidone polymers. Within the Methocel™-based formulation group, molecular weight was seen to have a substantial effect on the prevention of ITZ precipitation. This is most likely the result of increased solution viscosity with the Methocel™ E50 grade over the Methocel™ E5 grade that leads to a reduction in the diffusivity of ITZ in solution. By reducing the diffusivity of ITZ in neutral media, the molecules are able to remain in their intermolecular bonding positions on the polymer for longer durations rather than pass into the bulk, neutral pH medium where recrystallization will occur rapidly. Increased solution viscosity may also contribute to the prevention of ITZ precipitation by simple steric hindrance of nucleation and crystal growth. However, this effect seems to only contribute minimally as the Kollidon® 90 grade provided only slightly better stabilization of supersaturated ITZ than the Kollidon® 12 grade. Hence, reducing the molecular transport of ITZ in solution is not sufficient to stabilize supersaturation in neutral media; strong attractive intermolecular interactions must also exist for a polymer to provide adequate stabilization.

Enteric Release Polymers

The results of pH-modulated dissolution testing with the enteric release amorphous ITZ formulations are presented in Figure 5. The EUDRAGIT® L 100-55 formulation exhibited substantially greater ITZ release over the HP-55 and HP-55S formulations in both the acidic and the neutral pH phases of dissolution testing. In acid, the EUDRAGIT® L 100-55 formulation released approximately 17% ITZ after 2 h whereas the HP-55 and the HP-55S formulations released only 1.4 and 2%, respectively. All three enteric formulations produced maximum ITZ release 10 min after the pH change with the EUDRAGIT® L 100-55 formulation releasing 63%, the HP-55 formulation releasing 5%, and the HP-55S formulation releasing 8%. Thirty minutes following the pH transition, 32% ITZ remained in solution with the EUDRAGIT® L 100-55 formulation whereas the HP-55 and HP-55S formulations dropped to less than 1% ITZ in solution. Near-complete precipitation occurred for all formulations 1 h after the pH transition.

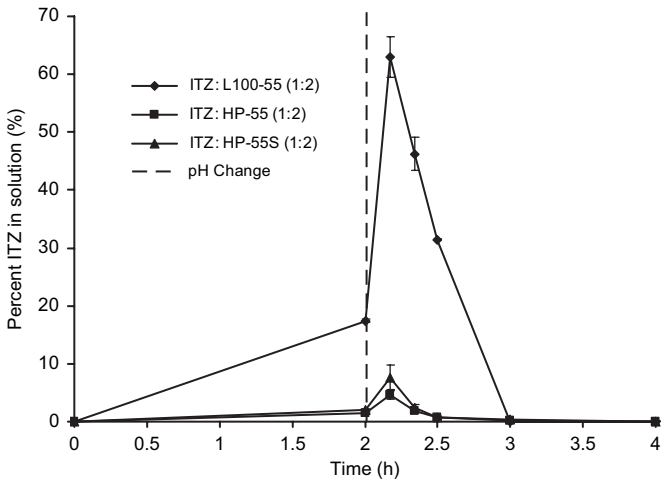

FIGURE 5. Supersaturation dissolution testing of the itraconazole (ITZ)-enteric polymer hot-melt extrusion (HME)-processed compositions by pH change method. Each dissolution vessel (n = 3) contained 180 mg of the formulation (60 mg ITZ equivalent) corresponding to 20 times the saturation solubility of ITZ in the acid phase. Testing was conducted for 2 h in 750 mL of 0.1 N HCl followed by pH adjustment 6.8 ± 0.5 with 250 mL of 0.2 M of tribasic sodium phosphate solution.

The AUDC values for the enteric formulations are provided in Table 2. The EUDRAGIT® L 100-55 formulation produced substantially greater ITZ release than the HP-55 and the HP-55S formulations in both phases of dissolution testing as indicated by a respective 15.4-fold and 10.6-fold greater $AUDC_{total}$ value. In the acid phase, the EUDRAGIT® L 100-55 formulation produced a 12.2-fold and an 8.6-fold $AUDC_{acid}$ increase over the HP-55 and the HP-55S formulations, respectively. The most significant difference between the three enteric formulations was seen in the neutral pH phase of dissolution testing where the $AUDC_{neutral}$ value for the EUDRAGIT® L 100-55 formulation was 18.2 times greater than that of the HP-55 formulation and 12.1 times greater than the HP-55S formulation. These results clearly indicate that the EUDRAGIT® L 100-55 formulation is superior to the HP-55 and HP-55S formulations with respect to providing supersaturation of ITZ following the acidic-to-neutral pH transition. With regard to the hypothesized in vivo correlation, this would indicate that the EUDRAGIT® L 100-55 formulation would provide substantially higher concentrations of ITZ in the lumen of the proximal small intestine than either of the HP-55 and of the HP-55S formulations.

The superior ITZ release in neutral pH of the EUDRAGIT® L 100-55 formulation compared with the HP-55 and HP-55S formulations may be due to its greater permeability to acid. When dissolution tests were conducted on these enteric formulations in pH 6.8 buffer without acid phase testing, no detectable amounts of ITZ were released suggesting that acid pretreatment of these enteric formulations is essential to achieving ITZ release in neutral pH media. During the acid phase of testing, acidic media permeates the EUDRAGIT® L 100-55 matrix and hydrates ITZ molecules contained within. Once hydrated, ITZ molecules are readily released into solution upon dissolution of the EUDRAGIT® L 100-55 matrix. Without acid pretreatment, this hydration is not achieved owing to the strong repulsive forces that exist between ITZ and aqueous media of neutral pH. In this case, once the enteric polymer dissolves, nucleation and precipitation of ITZ occur almost instantaneously. This suggests targeting ITZ to the proximal small intestine through an enteric-coated tablet or capsule would not be successful, as this mode of delivery eliminates

the essential acid-phase wetting of ITZ. This may also explain the poor dissolution performance of the HP-55 and HP-55S formulations in comparison with the EUDRAGIT® L 100-55 formulation. Greater release of ITZ in acid was observed with the EUDRAGIT® L 100-55 formulation than with the HP-55 and HP-55S formulations suggesting greater acid permeation of the enteric carrier matrix. In this case, ITZ was most likely hydrated to a greater degree before pH change in the EUDRAGIT® L 100-55 formulation than in the HP-55 and HP-55S formulations, resulting in more substantial ITZ release from the EUDRAGIT® L 100-55 formulation following the pH transition.

The presence of a greater number of acidic functional groups on the polymer chain of EUDRAGIT® L 100-55 than HP-55 and HP-55S could have also contributed to the greater extent of ITZ supersaturation in neutral pH media. As discussed previously, the stability of ITZ in solution is vastly improved by the presence of acidic functional groups, which provide a proton-rich microenvironment that stabilizes ITZ in aqueous solution through hydrogen bonding and/or protonization. With 46–50% methacrylic acid substitution for EUDRAGIT® L 100-55 versus 27–35% phthalyl substitution for HP-55, the EUDRAGIT® L 100-55 polymer contains substantially more free hydroxyl groups to stabilize ITZ in solution than HP-55.

Stabilizer molecular weight was also seen to promote ITZ supersaturation with the enteric formulations. The HP-55S (M_w = 132,000) formulation produced a 50% greater $AUDC_{neutral}$ value than the HP-55 (M_w = 84,000) formulation. The HP-55 and HP-55S polymers exhibit solution viscosities of 40 and 170 cps, respectively (Shin-Etsu, 2007; Petereit & Weisbrod, 1999). This result seems to support the previous conclusion that increased local viscosity extends the duration of intimate association between ITZ and the stabilizer thereby enhancing the stability of ITZ in solution and promoting increased levels of supersaturation.

Evaluation of In Vivo Absorption

Owing to considerably greater $AUDC_{neutral}$ values over the other investigated formulations, the Methocel™ E50 and EUDRAGIT® L 100-55 formulations were selected for in vivo evaluation in order to examine the hypothesis that the extent of ITZ supersaturation following acidic-to-neutral pH transition in vitro correlates directly to in vivo absorption. The ITZ plasma concentration versus time results are presented in Figure 6, and the results of the pharmacokinetic (PK) analysis are presented in Table 3. Most apparent when comparing the ITZ plasma concentration profile and the PK data between the two formulations is the considerably greater variability of ITZ

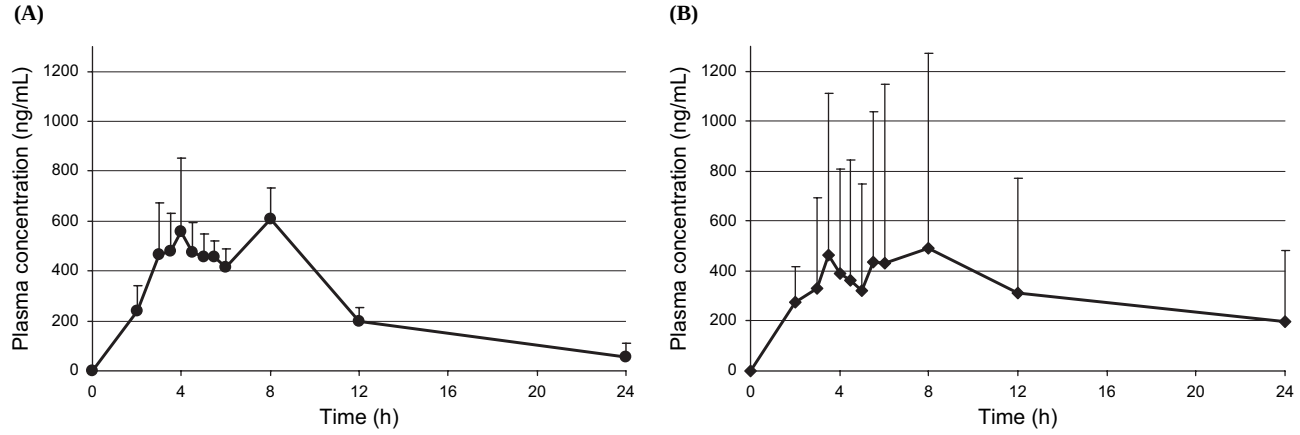

FIGURE 6. Plasma itraconazole (ITZ) concentration versus time from oral dosing of (A) hot-melt extrusion (HME)-processed ITZ : Methocel™ E50 (1:2) and (B) HME-processed ITZ : [EUDRAGIT® L 100-55 with 20% TEC] (1:2). The dose was administered by oral gavage in the amount of 30 mg ITZ/kg per subject (n = 4).

TABLE 3
Pharmacokinetic Data from the In Vivo Absorption Study with the ITZ : Methocel™ E50 and ITZ:EUDRAGIT® L100-55 Hot-Melt Extrusion-Processed Amorphous Solid Dispersion Formulations (n = 4 per group)

Formulation	C_{max} (ng/mL)	T_{max} (h)	AUC (ng·h/mL)	$T_{½}$ (h)
ITZ : Methocel™ E50 (1:2)	732 ± 187	4.9 ± 2.2	6,195 ± 1,134	6.7 ± 2.2
ITZ : EUDRAGIT® L100-55 (1:2)	630 ± 695	9.9 ± 9.7	7,335 ± 10,224	10.0 ± 2.3

absorption observed with the EUDRAGIT® L100-55 formulation over the Methocel™ E50 formulation. This is somewhat predicted by their in vitro release profiles as Methocel™ E50 was found to be a superior stabilizer of supersaturated levels of ITZ in neutral pH than EUDRAGIT® L100-55 as indicated by their respective $AUDC_{neutral}$ values of 1,960 and 1,086 mg. Additionally, the Methocel™ E50 formulation exhibited substantial levels of supersaturation (1.5 μg/mL) 2 h after the pH change whereas ITZ supersaturation with EUDRAGIT® L100-55 was negligible after only 1-h post-pH change. The difference between these two in vitro release profiles indicates a much larger window for ITZ absorption in the proximal small intestine with the Methocel™ E50 formulation than with the EUDRAGIT® L100-55 formulation providing more continuous absorption along the intestinal tract and hence less variability.

Although not statistically significant, it is interesting to note that the mean plasma concentrations at 12 and 24 h with the EUDRAGIT® L100-55 formulations are considerably greater than those with the Methocel™ E50 formulation. This may indicate late release of ITZ from the EUDRAGIT® L100-55 matrix and signal incomplete dissolution in the proximal small intestine. This effect could be the result of more acidic pH conditions in the upper small intestine of some of the animals and likely contributed to the variability associated with this formulation.

Another interesting aspect of the plasma concentration profiles presented in figure 6 is the peak–trough–peak shape seen with both formulations in which a second peak concentration is seen at about 8 h following the primary peak concentration at approximately 4 h. The maximum values of these peaks are within the standard error of the surrounding points and therefore statistically insignificant; however, the repetition of this profile shape for each subject substantiates the significance of this absorption profile. A similar dual peak effect was also reported by Hardin et al., which was attributed to enterohepatic recirculation of unmetabolized ITZ (Hardin et al., 1988). However, this result was only seen when patients were given a larger than normal dose of ITZ.

The mean C_{max} and AUC values for the Methocel™ E50 and EUDRAGIT® L100-55 formulations were determined to be 732 and 630 ng/mL and 6,195 and 7,335 ng h/mL, respectively. The similarity in these values indicates that comparable ITZ absorption is achieved on average with these two formulations. The greater mean T_{max} (9.9 versus 4.9 h) and $t_{½}$ (10 versus 6.7 h) of the EUDRAGIT® L100-55 formulation versus the Methocel™ E50 formulation may indicate that the enteric formulation produces a more delayed and sustained absorption profile; however, because of the variability in these parameters with the EUDRAGIT® L100-55 formulation, differences in these mean T_{max} and $t_{½}$ values are not statistically meaningful. Owing to substantially reduced variability, the Methocel™ E50 formulation is preferred over the EUDRAGIT® L100-55 formulation as more consistent blood levels would be expected.

The most interesting comparison of these in vivo results is with those from our previous study where two formulations that provided extensive supersaturation in acid, yet negligible stabilization of supersaturated ITZ following the acidic-to-neutral pH transition were evaluated for in vivo ITZ absorption under identical test conditions to the current study (Miller, McConville et al., 2007). As the variability associated with the EUDRAGIT® L100-55 formulation precludes meaningful comparison with these previous results, this comparison will only involve the Methocel™ E50 formulation. Both the mean C_{max} and AUC values achieved with the Methocel™ E50 formulation in this study were approximately three times greater than those achieved with the previously evaluated IR formulations signifying a substantial improvement in ITZ absorption. A similar improvement in ITZ absorption with the Methocel™ E50 carrier system is also seen in comparison with a study conducted by Lee et al. (2005). In this study, an amorphous dispersion produced by ASES of ITZ in a hypromellose carrier of equivalent grade to Methocel™ E5 was evaluated in vivo with male SD rats (~300 g) along with Sporanox® pellets for comparison. The C_{max} and AUC values obtained for the ASES formulation and the Sporanox® pellets were 173.5 and 179.2 ng/mL and 2301 and 2186 ng·h/mL, respectively. Hence, the Methocel™ E50 formulation from this study produced an approximate fourfold greater C_{max} value and an approximate threefold improvement in total AUC over both the ASES and the Sporanox® formulations. A 33% lower dose was administered in the study by Lee et al. than in this study; however, even on a dose-adjusted basis, the improvement in ITZ absorption generated by the Methocel™ E50 formulation remains substantial. The difference in ITZ absorption between the Methocel™ E50 formulation and the ASES and Sporanox® formulations is expected as the ASES and Sporanox® systems utilize low molecular weight grades of hypromellose as the stabilizing polymer. Based on the in vitro ITZ release comparison of the Methocel™ E50 and E5 formulations presented in this article, it is clear that the improvement in absorption produced by the Methocel™ E50 formulation over the ASES and Sporanox® formulations are the result of improved stabilization of supersaturated ITZ in neutral pH by the higher molecular weight stabilizer.

The results of this in vivo ITZ absorption analysis appear to confirm the hypothesis that the extent of supersaturation of ITZ following the acidic-to-neutral pH transition in vitro correlates directly to the extent of ITZ absorption in vivo. Relating the pH-modulated dissolution test method to its in vivo counterpart indicates that ITZ absorption occurs primarily in the upper small intestine. Therefore, oral ITZ formulation design should be directed by an $AUDC_{neutral}$-type metric and not by in vitro ITZ release in acid. Because the Methocel™ E50 and EUDRAGIT® L 100-55 formulations produced the greatest $AUDC_{neutral}$ values, these formulations provided substantial improvements in ITZ absorption over formulations providing supersaturation of ITZ only in acid. Although the Methocel™

E50 formulation was preferred over the EUDRAGIT® L 100-55 formulation because of reduced variability, the EUDRAGIT® L 100-55 formulation showed a very promising ITZ absorption profile, i.e., prolonged absorption. Future studies will therefore focus on identifying additives to the EUDRAGIT® L 100-55 formulation that will provide additional stabilization of supersaturated ITZ in neutral media so as to reduce variability and increase overall ITZ absorption.

CONCLUSIONS

The results of this study revealed that the strength of intermolecular interactions between ITZ and polymeric stabilizers largely determines the extent of supersaturation in aqueous media and the stability of supersaturated solutions. Increasing molecular weight of a polymeric stabilizer, as it relates to increasing solution viscosity, was also found to be critical to the stabilization of ITZ in solution owing to reduced diffusivity of ITZ and longer retention of the more enthalpicly favorable association between the drug and the polymer. This study also revealed that enteric release of ITZ for targeted delivery to the small intestine is contingent upon an acid pretreatment of the enteric composition to enable hydration of ITZ. Most importantly, this study confirmed the hypothesis that extent of ITZ supersaturation following an acidic-to-neutral pH transition in vitro directly correlates to in vivo drug absorption. Consequently, formulation design aimed at improving the oral absorption of ITZ must focus on this aspect of in vitro drug release for the results to be relevant to in vivo absorption.

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