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ORIGINAL ARTICLE

Production of advanced solid dispersions for enhanced bioavailability of itraconazole using KinetiSol® Dispersing

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Abstract

Objectives: To investigate the ability of KinetiSol® Dispersing to prepare amorphous solid dispersions of itraconazole using concentration-enhancing polymers. *Methods*: Concentration-enhancing nature of several cellulosic polymers (HPMC, hypromellose acetate succinate) was studied using a modified in vitro dissolution test. Solid dispersions were prepared by KinetiSol® Dispersing and characterized for solid-state properties using X-ray diffraction and differential scanning calorimetry. Potency and release characteristics were also assessed by high-performance liquid chromatography. Oral bioavailability of lead formulations was also assessed in animal models. *Results*: Screening studies demonstrated superior concentration-enhancing performance from the hypromellose acetate succinate polymer class. Data showed that stabilization was related to molecular weight and the degree of hydrophobic substitution on the polymer such that HF > MF ≈ LF, indicating that stabilization was achieved through a combination of steric hindrance and hydrophobic interaction, supplemented by the amphiphilic nature and ionization state of the polymer. Solid dispersions exhibited amorphous solid-state behavior and provided neutral media supersaturation using a surfactant-free pH change method. Rank-order behavior was such that LF > MF > HF. Addition of Carbopol 974P increased acidic media dissolution, while providing a lower magnitude of supersaturation in neutral media because of swelling of the high viscosity gel. In vivo results for both lead compositions displayed erratic absorption was attributed to the variability of gastrointestinal pH in the animals. *Conclusions*: These results showed that production of amorphous solid dispersions containing concentration-enhancing polymers through KinetiSol® Dispersing can provide improved oral bioavailability; however, additional formulation techniques must be developed to minimize variability associated with natural variations in subject gastrointestinal physiology.

Key words: *Bioavailability; concentration-enhancing polymers; hydroxypropyl methylcellulose acetate succinate; itraconazole; solid dispersion; supersaturation; thermal processing*

Introduction

Oral bioavailability limitations for developmental drugs are becoming more frequent and it has been reported that above 90% of the developmental compounds today exhibit properties that can limit absorption[1]. To this end, solubility enhancement techniques have emerged as a primary method for improving pharmacokinetic behavior. The term solubility enhancement refers to the capability of a composition to improve the apparent solubility of an active ingredient and can be achieved by two specific mechanisms: alteration of equilibrium solubility and temporary establishment of elevated metastable solubility. Alteration of equilibrium solubility is achieved by the addition of solubilizing excipients that modify the thermodynamic properties of the system to increase the

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equilibrium solubility of the drug substance. Such techniques include formulation of emulsions[2,3] or addition of complexing agents such as cyclodextrins[4–7]. In such systems, the active component becomes dispersed within the oil phase or complex and results in an equilibrium between the concentration of the two phases (oil/complex and aqueous). For such systems, oral absorption may still be limited as a result of partitioning of the drug between the oil and aqueous phase or the analogous complexed and noncomplex form. The development of such systems has been successfully applied to a variety of molecules including itraconazole (ITZ)[8], paclitaxel[9], and cyclosporine[10]. Alternatively, drug products may be developed to achieve a greater metastable solubility through modification of the component morphology or change in the material properties. Such techniques include the development of nanoparticles[11–16] to provide increased surface area for dissolution and the generation of amorphous solid dispersions[17–21] to alter the free energy of the system. Numerous studies of such compositions have described their use in dissolution rate enhancement and oral bioavailability improvement; however, it is important to note that solubility gains for such processes are transient and equilibrium solubility will eventually be reached.

The application of amorphous pharmaceutical production techniques has been gaining increased industrial acceptance, with several commercially marketed formulations currently available and numerous others in development. Effectively designing such formulations requires optimization of both magnitude and duration of supersaturation. Special consideration must also be given to account for the physicochemical properties of the moiety administered and the primary site of absorption for the compound. Although many studies have described techniques for maximizing the magnitude of supersaturation, several other studies have detailed techniques for providing site-targeted delivery[22,23] and stabilized supersaturation[13,21]. Molecular properties, such as acid/base nature of the compound, play a significant role in the design of such compositions. Many pharmaceutically active molecules can be classified as weak acids or weak bases although the majority of ionizable compounds are classified as weak bases. For weakly acidic compounds, greater solubility will generally be achieved in the later stages of the gastrointestinal tract where biological design facilitates absorption. This may limit the need for solubility enhancement although this will also be a function of the pK_a and the magnitude of solubility in the given environments. For weakly acidic and weakly basic compounds having a pK_a above the physiological range, solubility behavior within the gastrointestinal tract will be similar to the behavior of neutral compounds. In such cases, formulations may be designed to supersaturate in acidic or neutral pH environments of the intestinal tract without concern for changing solubility as a function of regional pH. Stabilization and site-targeting components for such systems may also be included based on the precipitation kinetics and regional absorption behavior of the moiety. Weak bases exhibiting low pK_a values (≤ 5) present a unique situation because such compounds will exhibit a substantial decrease in equilibrium solubility during intestinal transit. The anatomical construction of the body is designed to facilitate absorption of most nutrients at the upper small intestine, which presents a significant surface area relative to other regions as a result of villi and microvilli, which compose the intestinal surface[24–28]. Designing such systems to rapidly supersaturate in the acidic environment of the stomach may provide the greatest dissolution rate and magnitude of supersaturation as a result of the greater solubility in these conditions; however, this will also result in oversaturation, resulting in higher crystallization driving force during transit. As a result, when reaching the primary site of absorption, the rate of nucleation and growth will be amplified, resulting in rapid precipitation of the drug from solution. For such systems, site targeting of supersaturation can be an effective method of limiting the oversaturation of the system. Kondo et al.[22] demonstrated that this approach could successfully be applied to improve the oral bioavailability of HO-221, an experimental anticancer compound, by preparing solid dispersions of the drug in enteric hydroxypropyl methylcellulose phthalate[22]. In a similar study Miller et al. prepared enteric solid dispersions of itraconazole and Eudragit® L100-55 to improve the oral bioavailability of the antifungal agent[23]. Although mean bioavailability improvements were observed in the Miller et al. study, significant variability was also noted and attributed to the rapid precipitation of the drug from solution. Designing formulations by incorporation of concentration-enhancing polymers for extended durations of supersaturation can also be an effective method of improving oral bioavailability of all classes of pharmaceutical actives. The presence of these materials reduces precipitation rates by physical, chemical, or a combination thereof of interactions that can affect component solubility, solid–liquid boundary layer viscosity, molecular mobility, and interfacial solvation to thereby alter the nucleation and growth rates. Expanding on previous studies, Miller et al.[29] incorporated Carbomer 974P, a cross-linked high-viscosity acrylic acid polymer, into solid dispersion formulations of ITZ and Eudragit® L100-55. In vitro dissolution testing showed lower magnitudes and longer durations of supersaturation from compositions containing Carbomer 974P following pH change, suggesting a concentration-enhancing potential of the formulations. Oral bioavailability assessed in a Sprague–Dawley rat model showed that bioavailability was improved and variability reduced in comparison to the previous formulation. In another study, DiNunzio et al.[21] demonstrated

that engineered particle formulations of amorphous ITZ dispersed in cellulose acetate phthalate, a concentration-enhancing polymer, could provide improved oral bioavailability compared to the commercially marketed solid dispersion. Similar studies have shown incorporation of concentration-enhancing excipients strategy to be an effective technique to improve oral bioavailability for marketed and developmental compounds including tacrolimus[13,20], paclitaxel[9], celecoxib[30], PNU-3125[31], and RS-8359[32].

For this study ITZ, a weakly basic triazole antifungal agent, was selected to serve as the model's poorly water-soluble active ingredient and has been extensively studied in a variety of bioavailability-enhancing platforms. Owing to the extremely low and pH-dependent solubility, which has been reported to be as low as 4 μg/mL in acidic media and 5 ng/mL in neutral media, ITZ exhibits low and variable absorption[6,33,34]. Absorption of ITZ is also affected by extensive metabolism of the molecule because of cytochrome P-450 enzymes in the intestinal wall and liver[35]. In this study, it was hypothesized that the production of ITZ solid dispersions containing hypromellose acetate succinate (HPMCAS) by KinetiSol® Dispersing could provide enhanced oral bioavailability. Additionally, incorporation of Carbomer 974P (C974) was included to confirm whether previously reported concentration-enhancing properties or secondary properties of the material, such as mucoadhesion or paracellular transport increases, were responsible for bioavailability improvement[36–38].

Materials and methods

Materials

ITZ, BP micronized, was purchased from Hawkins, Inc. (Minneapolis, MN, USA). Hydroxyitraconazole was purchased from BDG Synthesis (Wellington, New Zealand). HPMCAS was generously donated in three grades (LF, MF, and HF) by Shin Etsu Chemical Co. (Tokyo, Japan). Carbomer® 974P (C974) was generously provided by Lubrizol Advanced Materials Inc. (Cleveland, OH, USA). Hypromellose (HPMC) was generously donated in three grades (E3, E50, and F50) by the Dow Chemical Company (Midland, MI, USA). HPLC grade acetonitrile was purchased from EMD Chemicals (Darmstadt, Germany). All other chemicals utilized in this study were of ACS grade.

Methods

KinetiSol® Dispersing

KinetiSol® Dispersing was performed using a custom built compounder designed for pharmaceutical processing applications by DisperSol Technologies, L.L.C. (Austin, TX, USA) as described previously[39,40]. Before processing, drug and polymer compositions were accurately dispensed into an impact mill and premixed for 1 minute before being charged into the compounder. During processing, temperature and rotational speeds were monitored with material discharged immediately upon achieving the target-processing temperature. During production, all compositions were prepared using a maximum rotational speed of 2200 rpm. Following discharge, the material was quench pressed between two chilled plates, ground using an impact mill (Capresso Inc., Closter, NJ, USA), and passed through a 60-mesh (250 μm) screen before further testing. In-process temperature profiles were smoothed using a five-point mean value algorithm and plotted using Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA).

Potency testing

Processed powder samples were accurately weighed to 10.0 ± 0.1 mg theoretical equivalent of ITZ and transferred to a 100-mL volumetric flask. Powder samples were dissolved in a 70:30:0.05 acetonitrile : water : diethanolamine solution and diluted to volume. Samples were analyzed by HPLC for potency by comparing to a known standard and adjusting for recorded sample weight. HPLC analysis was conducted as described in the 'HPLC Analytical Method' section.

X-ray diffraction

X-ray diffraction (XRD) testing was conducted using a Philips Model 1710 X-ray diffractometer (Philips Electronic Instruments Inc., Mahwah, NJ, USA) operating at an accelerating voltage of 40 kV and 30 mA. Samples of powder were placed into channeled stages and the diffraction profile was measured from 2.5° to 60° using a 2θ step size of 0.04° and dwell time of seconds.

Modulated differential scanning calorimetry

Modulated differential scanning calorimetry (mDSC) testing was performed using a TA Instruments Model 2920 DSC (New Castle, DE, USA) and analyzed using TA Universal Analysis 2000 Software. Samples were weighed to 15 ± 2 mg in aluminum-crimped pans (Kit 0219-0041, Perkin-Elmer Instruments, Norwalk, CT, USA) and tested under a ramp rate of 10 °C/min from 5°C to 215°C using a modulation temperature amplitude of 0.5 °C and a modulation period of 40 seconds under nitrogen purge at a flow rate of 40 mL/min. For unprocessed polymer samples, the first run was processed to remove the thermal history of the material and the second run analyzed to determine thermal properties.

Supersaturation stabilization studies

Polymer supersaturation stabilization studies were conducted by accurately weighing 75 ± 1 mg of selected polymers (HPMC E3, HPMC E50, HPMC F50, HPMCAS-LF, HPMCAS-MF, HPMCAS-HF) into 1000 mL of pH 6.8 phosphate media prepared by the addition of 250 mL of 0.2 M Na_3PO_4 buffer into 750 mL of 0.1 N HCl followed by neutralization with 1 N sodium hydroxide. ITZ was dissolved into 1,4-dioxane at a concentration of 18.75 mg/mL. Testing was conducted by preheating the dissolution apparatus to 37.0 ± 0.3 °C using a VK 7010 dissolution apparatus (Varian, Inc., Palo Alto, CA, USA). For testing, 2 mL aliquots of the ITZ solution were injected by pipette into the 1000 mL dissolution vessels containing predissolved polymer. Samples of approximately 5 mL were taken after 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 1440 minutes without replacement using a VK 8000 autosampler (Varian, Inc.), immediately filtered using 0.2-µm 13-mm PVDF membrane filters (Whatman Corporation, Florham Park, NJ, USA) and diluted 1:1 with 70:30:0.05 acetonitrile:water: diethanolamine mobile phase. Filtered and diluted samples were then transferred to 1-mL HPLC vials (VWR International, West Chester, PA, USA) for analysis, using the procedure described in the 'HPLC Analytical Method' section.

Supersaturated dissolution testing

Supersaturated dissolution testing was performed based on the USP XXIX method A enteric dissolution test using a VK 7010 dissolution apparatus and VK 8000 autosampler. An equivalent amount of solid dispersion having 37.5 ± 0.4 mg ITZ (~ 10 × 0.1 N HCl media equilibrium solubility) was weighed and added to the dissolution vessel containing 750 mL of 1 N HCl media. After 2 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, 125, 130, 135, 150, 180, 240, 300, 360, and 1440 minutes. Samples were immediately filtered using 13-mm 0.2-µm PVDF membrane filters, diluted in a 1:1 ratio with mobile phase, vortex mixed, and transferred into 1-mL vials for HPLC analysis.

Biosimilar media dissolution testing

Biosimilar media, fasted state simulated intestinal fluid (FaSSIF), was prepared using a method adapted from the method presented by Mellaerts et al.[41] Briefly, 6.96 g of NaOH and 123.72 g of $NaH_2PO_4H_2O$ were dissolved in 20 L of purified water. Additional 1 N NaOH was added as necessary to achieve a pH of 6.5. To a 1.2 L aliquot of pH 6.5 phosphate buffer, 9.66 g of sodium taurocholate and 3.54 g of lecithin were added and allowed to mix overnight. Before testing, 200 mL of the sodium taurocholate was brought to 1000 mL volume by the addition of pH 6:5 phosphate buffer. The diluted solutions were added to the dissolution apparatus and this procedure was repeated as necessary to prepare dissolution media. Dissolution testing was performed based on the USP XXIX apparatus II test using a VK 7010 dissolution apparatus and VK 8000 autosampler. An equivalent amount of solid dispersion having 37.5 ± 0.4 mg ITZ was weighed and added to the dissolution vessel containing 1000 mL of biosimilar media maintained at approximately 37.0°C. During testing 5-mL samples were removed from the dissolution vessels without replacement after 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 1440 minutes. Samples were immediately filtered using 13-mm 0.2-µm PVDF membrane filters, diluted in a 1:1 ratio with methanol, vortex mixed, and transferred into 1-mL vials for HPLC analysis. Preliminary studies indicated that the method of media preparation (i.e., dichloromethane emulsion or mixing overnight) did not provide a significant difference in release profile.

HPLC analytical analysis

Dissolution samples were analyzed using a Waters (Waters Corporation, Milford, MA, USA) 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 mobile phase consisting of 70:30:0.05 acetonitrile: water: diethanolamine equipped with a Phenomenex Luna 5 µm $C_{18}(2)$ 100Å, 150 mm × 4.6 mm (Phenomenex®, Torrance, CA, USA) 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 minutes. 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 ≤ 2.0%).

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, USA) weighing approximately 300 g. Throughout the study the animals were kept in individual cages, subjected to 12–12 hour 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 hours was allowed for acclimatization, after which time the rats were administered the formulations at a dose of 15 mg ITZ/kg bodyweight (n = 6). Solid dispersion formulations were dispersed in deionized water before dosing at a concentration of

4.5 mg ITZ/400 μL and dosed by oral gavage. 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 hours 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 minutes and the plasma transferred to a clean 1.5-mL microcentrifuge tube. All samples were stored at −20°C until HPLC analysis.

Before HPLC analysis, plasma samples were removed from frozen storage, 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 0.4 N zinc sulfate heptahydrate solutions were added. Samples were then vortex mixed for 30 seconds 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 seconds and centrifuged at 3000 g for 15 minutes. 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 mobile phase, vortex mixed for 60 seconds, and centrifuged for an additional 15 minutes. 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 C18(2) 100Å 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, OH-ITZ, and ITZ were approximately 5.5, 7.5, and 14.7 minutes, respectively. All analytical tests maintained system suitability limits for linearity ($r^2 \geq 0.999$) and reproducibility of replicate injections (% RSD ≤ 2.0%). The limits of detection and quantitation for both ITZ and OH-ITZ were 10 and 30 ng/mL, respectively.

Pharmacokinetic analysis

Plasma data were analyzed with WinNonlin v4.1 (Pharsight Corporation, Mountain View, CA, USA) 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 $t_{½}$ was determined by calculation of the lambda *z* parameter.

Statistical analysis

Statistical analyses of precipitation inhibition, nonsink dissolution, and pharmacokinetic data were conducted by one-way analysis of variance with Tukey comparison test using Minitab® Release 14. For all tests, $P \leq 0.05$ was used as the criteria to assess statistical significance.

Results and discussions

Precipitation inhibition studies

Successful development of formulations utilizing concentration-enhancing polymers requires identification of the carrier excipient that will provide the greatest degree of stabilization; however, only limited works have discussed preformulation techniques for assessing maintenance of supersaturation. Screening was conducted using two major classes of polymers, HPMC and HPMCAS, each having three distinct types of material. The HPMC group consisted HPMC E3, HPMC E50, and HPMC F50 to study the effect of molecular weight and functional group contributions to the stabilization of supersaturation. For the HPMCAS group, three grades consisting of LF, MF, and HF having varying ratios of succinoyl:acetyl groups were used. During testing, rapid precipitation of all formulations was observed because of the significant magnitude of supersaturation which was estimated to be approximately 10,000 times equilibrium solubility. Following rapid nucleation and initial growth, measured concentrations of the formulations reached a period of gradual decrease and continued to decrease over the 24-hour testing period. Concentration data measured during the study were used to calculate area under the dissolution curve (AUDC), as presented in Table 1.

Data showed that the HPMCAS polymers as a group provided greater stabilization of supersaturation than the HPMC polymers. Further examination showed that the stabilization within each group was directly related to the number of hydrophobic functional groups on the polymer. Within the HPMCAS category, the HF grade provided significantly greater stabilization than either the LF or MF grade materials. Differences in molecular weight could not explain this behavior because all grades have been reported to have similar molecular weights[42]. Examination of the molecular structure for these materials based on manufacturer specifications revealed that the level of methoxyl and hydropropoxyl substitutions are nearly the same; however, acetyl substitutions (ACS) increase and succinoyl substitutions (SUS) decrease when comparing the LF grade (ACS = 7.0, SUS = 6.0) to MF grade (ACS = 9.0, SUS = 12.0) to HF grade (ACS = 12.0, SUS = 6.0). This indicated a correlation between hydrophobic functional groups on the polymer and stabilization of supersaturation. A comparison of HPMC E50 to HPMC F50 revealed similar behavior. For E chemistry, HPMC functional substitutions are

Table 1. Polymer screening results and chemical properties for selected polymers and their concentration-enhancing effect.

Polymer	Methyl	Hydroxypropyl	Succinoyl	Acetyl	AUDC (ng · min/mL)
None	—	—	—	—	152,060 ± 98,350
HPMC E3	29.0	8.5	—	—	169,168 ± 26,277
HPMC E50	29.0	8.5	—	—	517,434 ± 195,710
HPMC F50	28.0	5.0	—	—	201,332 ± 43,338
HPMCAS-LF	22.0	7.0	16.0	7.0	481,859 ± 28,322
HPMCAS-MF	23.0	7.0	12.0	9.0	419,661 ± 115,182
HPMCAS-HF	24.0	8.0	6.0	12.0	2,921,246 ± 371,143

$n = 3$. Values reported from manufacturer technical packages.

29% methoxy1 and 10% hydropropoxy1 while F chemistry is composed of 28% methoxy1 and 5% hydropropoxy1. The common behavior across polymer classes of increasing stabilization with greater hydrophobicity suggested a hydrophobic interaction between the drug and polymer, which contributed to the observed concentration-enhancing effect. Further comparison of the different E chemistry molecular weight grades showed that higher molecular weight polymers provided superior stabilization compared to the lower molecular weight analogues. This indicated that a component of the stabilization was due to steric hinderance. In a previous study investigating the use of polyviny1 acetate phthalate (PVAP) and cellulose acetate phthalate (CAP) as concentration-enhancing polymers for ITZ, it was shown that CAP provided superior stabilization to PVAP, which was partially attributed to differences in polymer backbone[21]. Combining this information, one can hypothesize that the mechanism of stabilization of ITZ in solution is because of the combination of steric hinderance and hydrophobic interaction, supported by the capability of the polymer to also interact with water because of the presence of hydrophilic groups. In such a scenario, polymers that orient at the solid–liquid boundary layer provide an increased diffusional barrier because of molecular weight and entropic restrictions limiting molecular motion resulting from backbone rigidity. Additional intermolecular interactions that could result in precipitation are further hindered by partial shielding because of polymer backbone. Increasing the hydrophobic component of the polymer results in greater drug–polymer interactions as the ITZ molecules diffuse to the solid surface for recrystallization. Further stabilization of the ionic polymer class may also be explained by the ionization state of the polymeric stabilizers, which prevents the rapid collapse of the colloidal precipitate. Using such a model, it is possible to explain stabilization behavior observed in this study, as well as previous studies of ITZ supersaturation stabilization.

Results from this study allowed for selection of the primary carrier for prototype formulation development based on stabilization; however, they did not provide information on the dissolution behavior of the amorphous solid dispersions. In a recent paper by Vandecruys et al.[43], they examined the stabilization potential of multiple carriers on a set of 25 active ingredients of varying physicochemical properties in a high-throughput setting by applying a similar technique in which drug was dissolved in an organic solvent and added dropwise to the aqueous polymer solution until precipitation was observed. Samples were subsequently measured periodically to establish the magnitude of supersaturation and the ability to stabilize supersaturation. While this technique provided additional information on the magnitude of supersaturation that could be achieved from such compositions, it did not account for kinetic factors associated with the solid dispersion. Similarly, the method applied within this study did not account for such factors and therefore the development of prototype formulations was necessary to establish supersaturation behavior. For prototype formulation development, HPMCAS was selected based on the greater stabilization of supersaturation in neutral media.

Solid-state characterization

Prototype solid dispersions were prepared using KinetiSol® Dispersing. This technology is classified as a fusion production process, which has recently been reported for a range of applications including hydrophilic solid dispersion production, plasticizer-free solid dispersion manufacture, and solid dispersion processing of temperature-sensitive active ingredients[39,40,44]. During processing, material temperatures were rapidly raised through the generation of frictional energy within the equipment while being thoroughly mixed as a result of the shear developed. The process resulted in rapid cycle times, as shown in Figure 1, with all batches produced in under 20 seconds. Exposure to elevated temperature conditions was also minimized because of the rapid temperature increase, measured to be approximately 35°C/s during the primary working phase, followed by cooling application of a quench press technique after

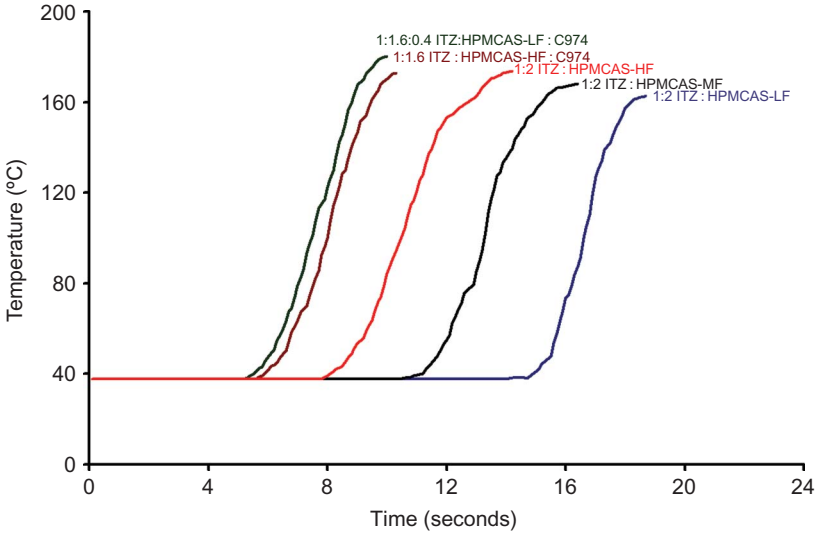

Figure 1. KinetiSol® processing temperature profiles for manufactured formulations.

material discharge upon reaching approximately 160°C. Interestingly, during production of HPMCAS solid dispersions, a rank-order behavior of frictional processing duration was observed. Frictional processing occurs during the initial stage of production before entering the working phase where heat is rapidly generated as viscoelastic material properties change. The time required for frictional energy generation to enter the working phase was observed such that LF > MF > HF. This indicated a potential variation in underlying material feed properties that may impact processing profiles. Compositions containing C974 showed extremely short frictional and working stages similar to the behavior observed in previously reported studies[40], further suggesting that material properties play a role in the duration of these stages during KinetiSol® Dispersing. Product potency testing also revealed that compositions provided prepared by KinetiSol® Dispersing exhibited values between 95.0% and 105.0%, as shown in Table 2, conforming to the acceptance criteria listed in the USP.

Processed powders were examined by mDSC and XRD to study the solid-state characteristics of these systems. Thermal analysis was performed to identify the presence of residual crystalline ITZ in the dispersion and also to identify the formation of an amorphous glass solution, indicated by single-phase behavior of the system. In Figure 3a, crystalline ITZ exhibited a strong melting endotherm at 170°C which was not present in the processed solid dispersions. This indicated that the compositions were amorphous, which was further confirmed using XRD. As presented in Figure 2, XRD profiles showed that all compositions yielded an amorphous halo with no crystalline peaks of ITZ visible, verifying the amorphous nature of the compositions. Total and reversing heat flow profiles for the compositions tested are presented in Figure 3a and b, respectively. Reversing heat flow profiles were utilized to deconvolute thermal events associated with specific heat that are characteristic of glass transition events. Amorphous ITZ, prepared as a second heating sequence of crystalline ITZ as described in the methods section, revealed a glass transition at approximately 58.5°C and subsequent rearrangement of the liquid crystalline phase at 75°C and 90°C. Although the temperature magnitude for these events was similar to those reported by Six et al.[45], the exothermic behavior of the chiral nematic mesophase to isotropic liquid conversion observed in this study was because of the mDSC heating rate. At reduced heating rates, this event is endothermic and similar to the results presented by Six and coworkers. HPMCAS polymer samples also exhibited amorphous behavior, providing glass transition temperatures of approximately 120°C. Previously reported studies on C974 have shown a glass transition temperature of approximately 130°C[29]. For all processed compositions, a single primary T_g value was observed at approximately 85°C. This indicated the formation of a homogeneous amorphous solid dispersion

Table 2. Potency values for compositions produced by KinetiSol® Dispersing.

Composition	Potency (%)
1:2ITZ:HPMCAS-LF	104.7
1:2ITZ:HPMCAS-MF	101.9
1:2 ITZ:HPMCAS-HF	102.5
1:1.6:0.4 ITZ:HPMCAS-LF:C974	102.2
1:1.6:0.4 ITZ:HPMCAS-HF:C974	98.0

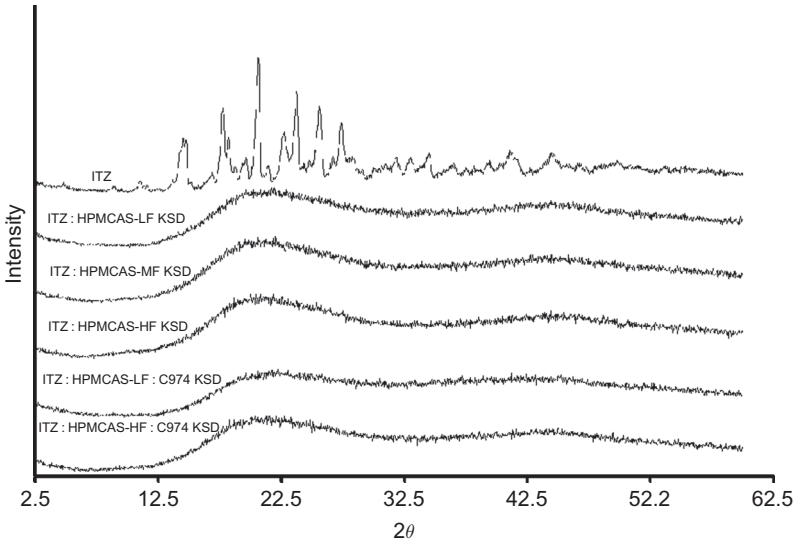

Figure 2. XRD profiles of KSD processed batches.

and was similar to control compositions prepared by hot melt extrusion using a Haake minilab which also exhibited single-phase behavior (data not reported).

In vitro dissolution studies

Application of screening studies provided insight into the stabilization properties of the polymers selected; however, issues regarding dissolution rate behavior accounting for kinetic factors could not be assessed using the screening technique employed. To assess the behavior of the solid dispersions prepared, samples were subjected to pH change supersaturated dissolution testing using surfactant-free media. Dissolution profiles comparing the behavior of compositions containing LF, MF, and HF grade of HPMCAS are presented in Figure 4, with critical formulation metrics presented in Table 3. During the acidic period, all compositions exhibited partial release, achieving approximately 50% of the equilibrium solubility value during the 2-hour period, which was most likely because of the presence of accessible amorphous ITZ located in the surface region of the particles. Following pH change, particle dissolution was observed, with the rate of dissolution (LF > MF > HF) and the extent of supersaturation (LF ≈ MF > HF) being correlated to the grade of HPMCAS used in solid dispersion production. This release behavior was most likely because of the ratio of succinoyl: acetyl functional groups on the polymer which increased with greater dissolution rates and also contributed to the pH onset of dissolution, which has been reported by the manufacturer as pH 5.5, 6.0, and 6.8, respectively. The extent of supersaturation in neutral media also showed similar performance of the LF and MF formulations, as indicated by the $AUC_{dissolution}$ in neutral media. This behavior was most likely because of a combination of factors, including the similar stabilization properties reported during screening, as well as the lower maximum concentration in solution observed for the MF grade which provided a reduced driving force for nucleation and growth. For compositions prepared using LF and MF grade material, maximum amounts of drug in solution were observed following pH change within 60 minutes; however, the HF grade material that provided the greatest stabilization of supersaturation failed to provide adequate release to substantially supersaturate neutral media. As a result of the superior dissolution rate of the LF grade and the similar stabilization properties of the LF and MF grade material, A_{max} and $AUC_{dissolution}$ values showed that the LF composition provided the greatest magnitude of supersaturation with similar extents of supersaturation observed for LF and MF formulations.

The effect of incorporation of C974 into the solid dispersions on in vitro dissolution performance was also assessed. In a previous study by Miller et al.[29], C974 was observed to provide concentration-enhancing properties to ITZ in solution when used in solid dispersions and this in vitro behavior was attributed to the improved oral bioavailability of the formulation. Carbomer has been reported to provide other functions in vivo, specifically yielding mucoadhesive properties and facilitating transport through GI membranes[37,38]. Incorporation of this material as an additive into a solid dispersion composed of a concentration-enhancing polymer carrier would allow study of the contribution of these secondary properties. In vitro dissolution profiles of formulations prepared containing 20% C974 on a polymer weight basis are presented in Figure 5, along with the profile for the HPMCAS-LF composition as

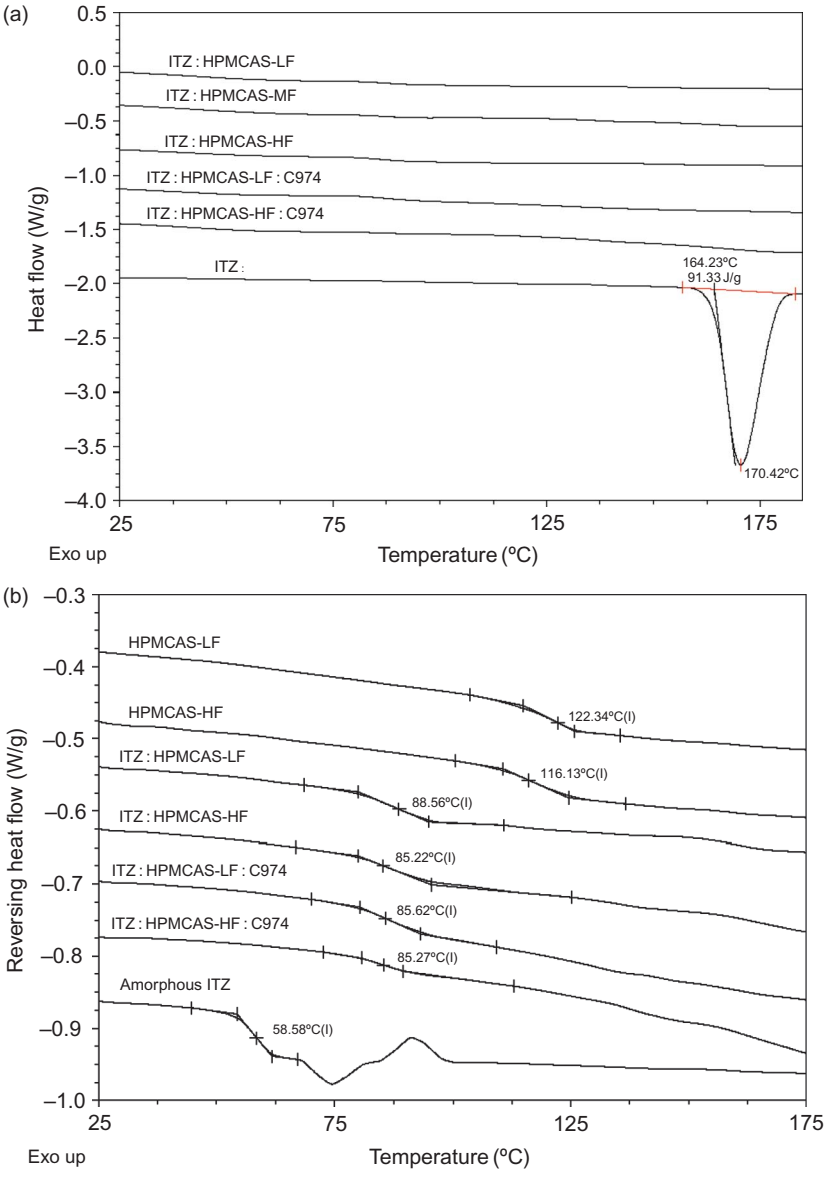

Figure 3. mDSC profiles for KSD processed solid dispersions, unprocessed polymers, and API: total heat flow profile (a) and Reversing heat flow profile (b).

reference. Critical dissolution metrics for these formulations are also provided in Table 3. The presence of C974 in the dispersion increased the release in acidic media, resulting in slightly supersaturated media (~ 2 × equilibrium solubility). Previous reports incorporating C974 into solid dispersions have shown greater drug release in acidic media because of increased permeability resulting from swelling and erosion[46]. Swelling and interfacial erosion of the particles would provide a larger quantity of accessible amorphous ITZ, contributing to the release in acidic media. The presence of C974 also affected supersaturation behavior in neutral media. Solid dispersions containing HPMCAS-LF and C974 showed significantly reduced dissolution rates when compared to C974-free material. As C974 is a high-molecular-weight cross-linked polymer, an increase in solid–liquid boundary layer viscosity most likely contributed to the muted release by reducing diffusion of elevated pH bulk media to the particle surface while simultaneously limiting drug and concentration-enhancing polymer diffusion from the solid dispersion surface. As a result of the lower drug release rate, concentrations of drug in solution never reached sufficient levels to maximize $AUC_{dissolution}$ values. In the case of HPMCAS-HF and C974 compositions, contribution of swelling and erosion imparted by the acrylic acid polymer increased acidic and neutral media drug release. As HPMCAS-HF has a dissolution onset of

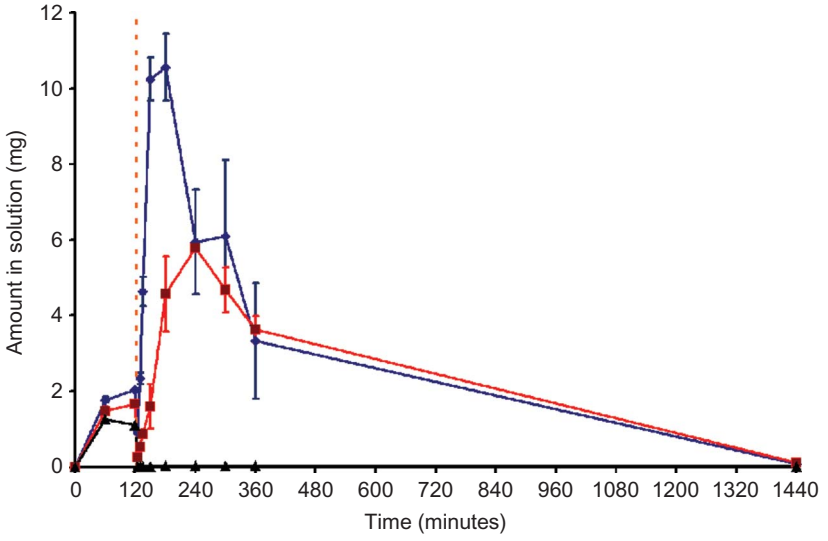

Figure 4. In vitro dissolution testing compositions prepared with HPMCAS-LF (◆), HPMCAS-MF (■) and HPMCAS-HF (▲). Each vessel ($n = 3$) contained 37.5 mg ITZ equivalent corresponding to 10 times the equilibrium solubility of ITZ in the acid phase. Testing was conducted for 2 hours in 750 mL of 0.1 N HCl followed by pH adjustment to approximately 6.8 with 250 mL of 0.2 M tribasic sodium phosphate solution. Dashed vertical line indicates the time of pH change.

Table 3. Summary of in vitro dissolution testing data with reported maximum observed amounts (A_{max}), observed time to achieve maximum amount (t_{max}), and area under the supersaturation dissolution profile for pH change testing (AUC) in respective media.

Composition	A_{max} (mg)	t_{max} (minutes)	$AUC_{dissolution}$ (mg·min) ACID	Neutral	Total
1:2 ITZ:HPMCAS-LF	10.6 ± 0.9	170 ± 17	168 ± 4	3440 ± 1067	3608 ± 1070
1:2 ITZ:HPMCAS-MF	5.8 ± 0.1	240 ± 0	139 ± 10	3021 ± 272	3160 ± 267
1:2 ITZ:HPMCAS-HF	1.3 ± 0.1	60 ± 0	110 ± 6	41 ± 15	151 ± 16
1:1.6:0.4 ITZ:HPMCAS-LF:C974	5.4 ± 0.3	130 ± 17	354 ± 32	1129 ± 194	1483 ± 186
1:1.6:0.4 ITZ:HPMCAS-HF:C974	7.4 ± 0.6	120 ± 0	503 ± 37	665 ± 44	1167 ± 81

$n = 3$, Values represent mean ± SD.

approximately pH 6.8, testing in media having a pH of 6.8 provides only partial ionization of the polymeric chains and results in slower dissolution. By incorporating the C974, the solid dispersion particles become partially swollen and more porous as the polymer swells and diffuses into the bulk media. This allows for greater specific surface to facilitate drug dissolution following pH change. Even with this behavior, however, this formulation still fails to significantly supersaturate the dissolution media.

Application of the surfactant-free pH change dissolution method has been applied extensively to characterize the dissolution behavior of enteric ITZ solid dispersions; however, the media used does not accurately reflect the contents of the upper small intestine that contain bile salts and micellular complexes[47,48]. To better understand the effect of intestinal contents on the dissolution and supersaturation behavior of concentration-enhancing polymer solid dispersions, lead compositions consisting of ITZ:HPMCAS-LF and ITZ:HPMCAS-LF:C974 were tested in FaSSIF media having a pH of 6.5. Dissolution profiles of both compositions and critical statistical metrics are presented in Figure 6 and Table 4, respectively. Behavior of both formulations in pH 6.5 biosimilar media showed rapid and extensive supersaturation, with a high degree of similarity between the two profiles. In previous studies of other enteric polymer compositions [23], addition of ITZ : L100-55 solid dispersions directly into neutral media have shown minimal drug release. For ITZ : L100-55 solid dispersions, this behavior was ascribed to the permeability of the matrix under acidic conditions, which allowed the drug to solvate in the more favorable low pH conditions. HPMCAS solid dispersions, however, showed the ability to supersaturate biosimilar media having a pH of 6.5, as well as pH 6.5 media without biosimilar components (data not shown). This suggested that the presence of HPMCAS may also affect the solvation of ITZ because of the amphiphilic nature of the polymer, which allowed hydrophobic domains of the

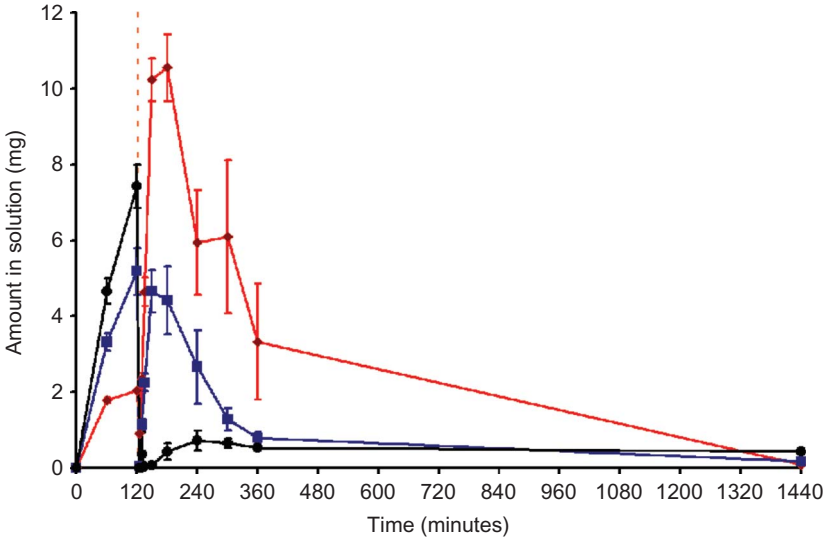

Figure 5. In vitro dissolution profiles for compositions containing Carbomer 974P. 1:2 ITZ:HPMCAS-LF (◆), 1:1.6:0.4 ITZ:HPMCAS-LF:C974P (■), 1:1.6:04 ITZ:HPMCAS-HF:C974P (●) Each vessel ($n = 3$) contained 37.5 mg ITZ equivalent corresponding to 10 times the equilibrium solubility of ITZ in the acid phase. Testing was conducted for 2 hours in 750 mL of 0.1 N HCl followed by pH adjustment to approximately 6.8 with 250 mL of 0.2 M tribasic sodium phosphate solution. Dashed vertical line indicates the time of pH change.

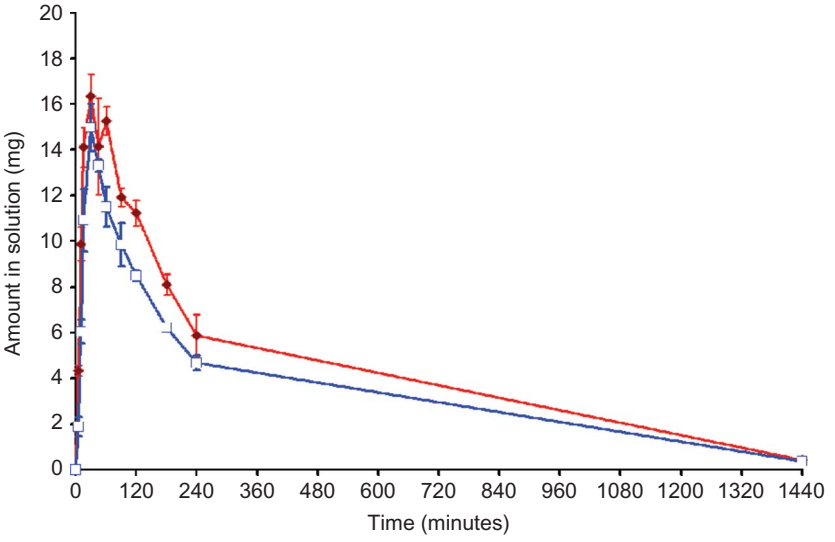

Figure 6. In vitro dissolution profile of lead compositions tested in FaSSIF. Key: 1:2 ITZ:HPMCAS-LF (◆), 1:1.6:0.4 ITZ:HPMCAS-LF:C974 (□). Each vessel (n = 3) contained 37.5 mg ITZ equivalent. Testing was conducted using biosimilar media under USP Apparatus II at 50 rpm and 37°C.

polymer to interact with ITZ, whereas hydrophilic groups on the polymer provided favorable interactions with water that prevented rapid aggregation and precipitation of the dissolved drug. Stabilization properties of the tested formulations exhibited greater similarity than observed during the pH change method although $AUC_{dissolution}$ differences were still statistically significant. The much more rapid dissolution of compositions containing C974 in biorelevant media was one of the most interesting characteristic differences in material behavior between the two tests. Under biosimilar testing conditions, materials were added directly to pH 6.5 media, which prevented gelling of C974 under acidic conditions. This suggested that the muted release after neutralization observed in pH change conditions was primarily because of a residual low pH microenvironment as a result of limited diffusion, while a contribution of microenvironmental viscosity increase to reduced release of ITZ also could not be ruled out. In both cases compositions tested using biosimilar media showed

Table 4. Summary of in vitro dissolution testing data measured in pH 6.5 biosimilar media with reported maximum observed amounts (A_{max}), observed time to achieve maximum amount (t_{max}), and area under the supersaturation dissolution profile for pH change testing (AUC) in respective media.

Composition	A_{max} (mg)	t_{max} (minutes)	$AUC_{dissolution}$ (mg·min)
1:2ITZ:HPMCAS-LF	16.5 ± 0.9	35 ± 9	6482 ± 603
1:1.6:0.4ITZ:HPMCAS-LF:C974	15.0 ± 1.0	30 ± 0	5051 ± 107

n = 3, Values represent mean ± SD.

greater $AUC_{dissolution}$ and A_{max} values. Intestinal fluids are known to contain bile salt micelles that are capable of solubilizing hydrophobic substances. Improvements in these metrics suggested that physiological media would facilitate the solubilization of ITZ, improving the oral bioavailability over what would be predicted from surfactant-free tests.

Formulation comparison within test methods was also strongly influenced by the media and procedure employed in the study. Application of the pH change method without surfactant predicted a significant difference in oral bioavailability of ITZ based on formulation, indicating substantial improvements for compositions prepared without C974. Using biosimilar media, differences were again predicted; however, the magnitude was significantly reduced. Although both methods predicted improvements from compositions without C974, the true predictive power of the methods needed to be verified using an animal model.

In vivo bioavailability

Lead formulations of ITZ:HPMCAS-LF and ITZ:HPMCAS-LF C974 were selected based on in vitro behavior and dosed to Sprague–Dawley rats by oral gavage at a dose of 15 mg/kg. During the study, blood samples were taken periodically and analyzed for plasma concentration of ITZ and the primary metabolite OH-ITZ. Plasma levels measured during the study are presented in Figure 7, with calculated pharmacokinetic data shown in Table 5. Upon oral administration, ITZ was rapidly absorbed from both formulations with greater oral bioavailablities based on AUC values for both compositions compared to hydrophilic solid dispersions of ITZ and Sporanox® pellets [21,39]. As predicted by both models, the mean AUC for ITZ:HPMCAS compositions was greater than that observed for the C974 formulation; however, the extensive variability observed in both compositions made it impossible to establish statistical significance.

Similar high variability for pH-sensitive solid dispersions was also noted in a series of studies by Miller et al.,[23,29] in which formulations containing L100-55 had significant AUC variability, measuring over 100%, which was attributed to the absence of concentration-enhancing polymers. The variability of the composition was significantly reduced to approximately 30% by the addition of C974 and attributed to the supersaturation stabilization effect of the material. In this study, C974 failed to improve variability because concentration-enhancing polymers were already present in both formulations. This indicated that the behavior of C974 as a concentration-enhancing polymer, described by Miller et al., was correct and not associated with secondary properties of the material, such as mucoadhesion or increased membrane transport. It also suggested that the variability observed in this study was not because of the stabilization of supersaturation. Examination of the in vitro dissolution profiles for C974-free formulations showed that the time to achieve maximum concentration was approximately 45 minutes once entering neutral media. In the case of the C974 composition measured under pH change, maximum concentrations were actually observed in acidic media. This suggested that a kinetic component may be partially responsible for the observed variation. Additionally, examination of rat intestinal physiology shows that pH is variable in the duodenum and jejunum, which can be further impacted by the food intake of the animal. In a recent study by McConnell et al.[49] the pH of intestinal fluids were measured in fed and fasted rats along various segments of the intestinal tract. Results showed duodenal pH values ranging from 5.0 ± 0.3 in the fed state to 5.9 ± 0.3 in the fasted state, whereas jejunal values were measured to be 5.1 ± 0.3 and 6.1 ± 0.3 in the fed and fasted states, respectively. Interestingly, these measured values fall within the dissolution onset value for the HPMCAS polymer used. As rats were administered food adlibitum during the study, it was postulated that the intestinal pH values in the primary regions of absorption would cover the range reported in the McConnell study. If so, dissolution of some compositions would be hindered as a result of the variable pH observed. An additional contribution may also stem from the extent of metabolic conversion of ITZ to OH-ITZ. Pharmacokinetic profiles of OH-ITZ for both formulations exhibited similar behavior and also similar mean values, although high variability was again observed. As ITZ is extensively metabolized in the gut wall as well as the liver[35], the amount of drug in solution will be related to the extent of metabolic conversion. For cases where low concentrations are observed, metabolism is expected to be extensive; however, if the drug is highly supersaturated the extent of metabolic conversion may be limited because of saturation. Interestingly, these OH-ITZ values were also similar in magnitude to enteric ITZ solid dispersions prepared by particle engineering technologies (data not presented), whereas ITZ levels

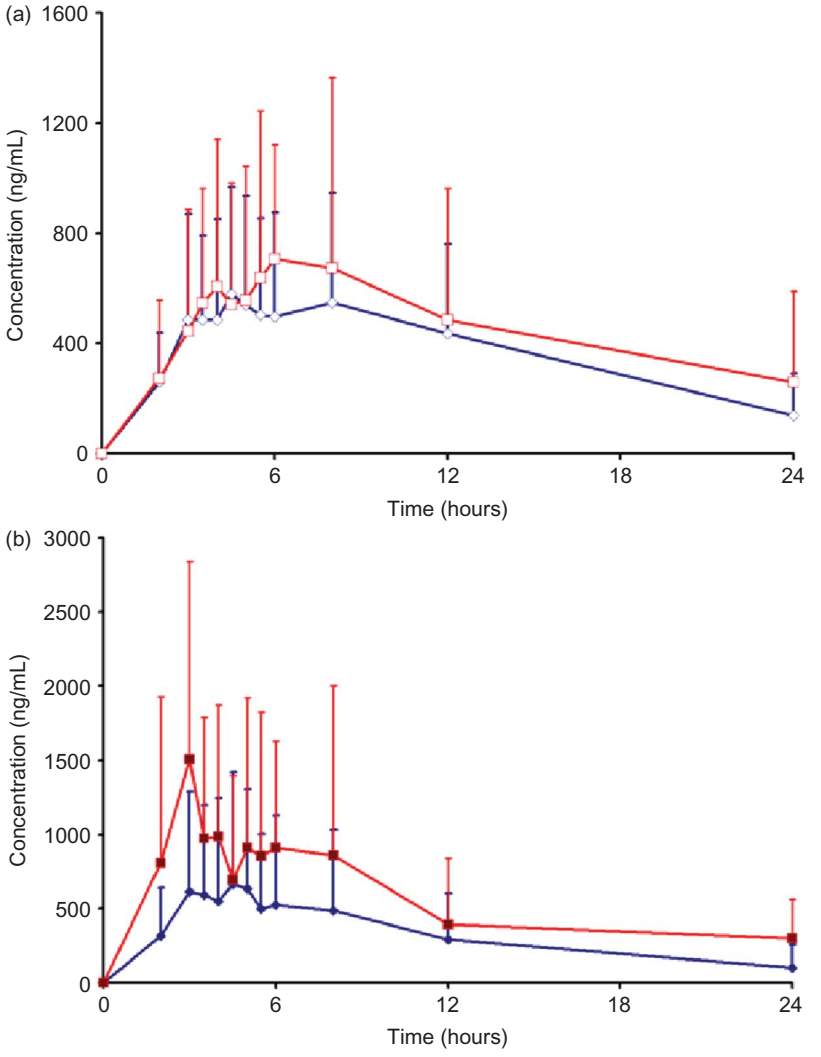

Figure 7. In vivo plasma profile of hydroxy-itraconazole (a) and itraconazole (b). Key:1:2 ITZ:HPMCAS-LF (■/□) and ITZ:HPMCAS-LF:C974 (◆/◇). Formulations were administered by oral gavage at a dose of 15 mg ITZ/kg bodyweight per rat ($n = 6$).

Table 5. Calculated pharmacokinetic parameters for formulations tested *in vivo*.

Compound	Formulation	C_{max} (ng/mL)	t_{max} (hours)	$AUC_{0-24\ hours}$ (ng·hr/mL)	$t_{½}$ (hours)
	1:2ITZ:HPMCAS-LF	1003.4 ± 504.5	5.9 ± 1.9	10,546 ± 9,847	7.6 ± 6.3
OH-ITZ	1:1.6:0.4 ITZ:HPMCAS-LF:C974P	675.2 ± 391.5	4.1 ± 1.2	8586 ± 6,520	8.0 ± 3.1
	1:2 ITZ:HPMCAS-LF	2288.9 ± 1,018.6	4.4 ± 2.2	13,225 ± 11,368	5.8 ± 3.1
ITZ	1:1.6:0.4 ITZ:HPMCAS-LF:C974P	798.4 ± 731.8	3.8 ± 1.4	7345 ± 8,126	6.8 ± 5.4

$n = 6$, Values represent mean ± SD.

were significantly higher in this study. This suggested that the gut wall metabolism may be saturated at higher levels of ITZ supersaturation, and it would also stand to reason that if supersaturation was highly variable as a result of pH so would be the extent of metabolic conversion, thereby contributing to the highly variable AUC values.

Although in vitro dissolution tests did correctly predict the magnitude of AUC improvement, they failed to provide an indication of variability. Because solid dispersions prepared with ionic polymers are highly sensitive to environmental pH, patient-to-patient differences of gastrointestinal physiology may strongly affect pharmacokinetic behavior and clinical outcome. When

developing such systems, examination of drug release at multiple pH conditions is recommended to establish pharmacokinetic variability that can be anticipated within a study.

Conclusions

Oral bioavailability of many developmental compounds can be limited because of reduced solubility; however, formulation techniques designed to increase and maintain metastable solubility can provide improved bioavailability of many poorly water-soluble drugs. Based on the results presented, it can be concluded that KinetiSol® Dispersing is a viable manufacturing technique for producing concentration-enhancing solid dispersions for improved oral bioavailability. Additionally, this study also validated previous work, attributing enhanced oral bioavailability to the concentration-enhancing nature of the polymeric carriers. However, as a result of the pH-dependent nature of drug release from these system testing methods must be modified to assess the impact of patient-to-patient variability on formulation performance when developing such systems. Furthermore, continued work is necessary to develop formulations capable of providing improved oral bioavailability with reduced variability.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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