molecular pharmaceutics

Article

pubs.acs.org/molecularpharmaceutics

Formulation of a Danazol Cocrystal with Controlled Supersaturation Plays an Essential Role in Improving Bioavailability

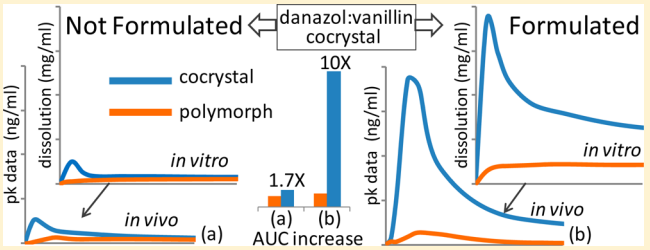
Scott L. Childs,*[,†,§] Praveen Kandi,[†] and Sreenivas Reddy Lingireddy[†,‡]

[†]Renovo Research, Atlanta, Georgia 30316, United States

[§]Triclinic Labs, West Lafayette, Indiana 47906, United States

Supporting Information

ABSTRACT: Cocrystals have become an established and adopted approach for creating crystalline solids with improved physical properties, but incorporating cocrystals into enabling pre-clinical formulations suitable for animal dosing has received limited attention. The dominant approach to *in vivo* evaluation of cocrystals has focused on deliberately excluding additional formulation in favor of "neat" aqueous suspensions of cocrystals or loading neat cocrystal material into capsules. However, this study demonstrates that, in order to take advantage of the improved solubility of a 1:1 danazol:vanillin cocrystal, a suitable formulation was required. The neat aqueous suspension of the danazol:vanillin cocrystal had a modest *in vivo* improvement of 1.7 times higher area under the curve compared to the poorly soluble crystal form of danazol dosed under identical conditions, but the formulated aqueous suspension containing 1% vitamin E-TPGS (TPGS) and 2% Klucel LF Pharm hydroxypropylcellulose improved the bioavailability of the cocrystal by over 10 times compared to the poorly soluble danazol polymorph. *In vitro* powder dissolution data obtained under non-sink biorelevant conditions correlate with *in vivo* data in rats following 20 mg/kg doses of danazol. In the case of the danazol:vanillin cocrystal, using a combination of cocrystal, solubilizer, and precipitation inhibitor in a designed supersaturating drug delivery system resulted in a dramatic improvement in the bioavailability. When suspensions of neat cocrystal material fail to return the anticipated bioavailability increase, a supersaturating formulation may be able to create the conditions required for the increased cocrystal solubility to be translated into improved *in vivo* absorption at levels competitive with existing formulation approaches used to overcome solubility limited bioavailability.



KEYWORDS: *cocrystal, co-crystal, pre-clinical formulation, supersaturation, danazol, solubility, dissolution, surfactant, precipitation inhibitor, bioavailability, spring and parachute*

INTRODUCTION

The acceptance of cocrystals as useful crystalline solid forms of active pharmaceutical ingredients (APIs) is well established in both industry and acadamia. Cocrystals have advantages that are inherent to crystalline materials, plus cocrystals can be designed with significantly higher solubility that can overcome solubility limited bioavailability problems.[1] In essence, the solubility improvement obtainable with a cocrystal occurs by directly altering the source of the solubility problem—the crystal structure of the API. Integrating an additional water-soluble component at the molecular level creates improved solubility at the macro level while still delivering the chemically unaltered API as a stable crystalline form in the final drug product.

There has been a commitment by the pharmaceutical industry to the use of cocrystals as an alternative solid form in pre-formulation studies; however, the selection of a cocrystal strategy at the pre-clinical formulation stage over other available formulation techniques for improving bioavailability has been relatively rare. The recognition and adoption of cocrystals as a robust alternative to competing formulation strategies for drugs' low bioavailability faces several obstacles. The pharmaceutical industry continues to have concerns about cocrystals with regard to industrial scale-up, long-term stability, coformer toxicity, and regulatory issues. However, these concerns will not be addressed in a systematic way until the industry is more confident that cocrystals can consistently perform at a level comparable to that of the established and proven formulation alternatives for poorly soluble drugs. In particular, cocrystals must demonstrate *in vivo* performance comparable to that of spray-dried dispersions (SDDs). Currently cocrystals are not considered to be a viable alternative that can routinely meet or exceed the performance of SDDs.

Current Approaches to Cocrystal Formulation. Current approaches to *in vivo* cocrystal evaluation have primarily relied on dosing the "neat" cocrystal material. Most published studies have deliberately excluded formulation in order to allow a direct comparison of the neat API and cocrystal materials. There are 10 published studies in the academic literature in which the

Received: March 21, 2013
Revised: June 26, 2013
Accepted: July 3, 2013
Published: July 3, 2013

bioavailability of cocrystals dosed to animals has been reported using either dogs or rats as the animal model.[2] Formulation methods reported are liquid suspensions dosed to rats or solid capsules or tablets dosed to beagle dogs. The majority of the suspensions utilize aqueous solutions designed to maintain a suspension of the neat crystalline solid using excipients such as methylcellulose (MC), polyvinyl chloride (PVP), or polyethylene glycol (PEG). Neat cocrystal material suspended in the presence of these excipients allows for a uniform dosing to rats by oral gavage. Solid dosage forms have contained neat cocrystal or lactose blends of neat cocrystal loaded in hydroxypropyl cellulose (HPMC) or gelatin capsules or in tablets. While the ability of cocrystals to improve bioavailability compared to that of the poorly soluble single-component crystal form of a drug has been clearly established by these studies, an examination of this literature and experience with cocrystal-based propriatary development projects led us to hypothesize that the plasma levels of compounds dosed as cocrystals in animal studies could be improved in many cases by integrating a suitable formulation strategy.

The current approach to pre-clinical cocrystal *in vivo* evaluation utilizing neat cocrystal material assumes that the inherent properties of the cocrystal solid form will translate directly into improved bioavailability. In this approach it is preferred to exclude formulation as a competing variable in order to focus on evaluating the effect of the cocrystal itself. If this approach fails, cocrystal development is typically halted without an examination of the effect of formulation on cocrystal bioavailability. However, in some cases a formulation strategy will be necessary in order to translate improved cocrystal solubility into a therapeutically relevant bioavailability increase. In these cases it will be necessary to consider form and formulation simultaneously.[3]

It is not being suggested that pharmacokinetic (pk) studies with neat cocrystal are not relevant. The use of neat cocrystal material incorporated into suspension or standard excipient mixtures used in direct compression tablets or loaded into capsules is still a preferred approach for performing pre-clinical and phase I studies as long as the cocrystal achieves the desired performance requirements. However, if the use of neat cocrystal fails to perform as expected, there are currently no published procedures or examples of how to systematically evaluate and optimize more complex formulation approaches specifically tailored to cocrystals. The study reported here is intended to begin filling this knowledge gap and provide a preliminary set of guidelines for developing more effective pre-clinical suspension formulations of highly soluble cocrystals of poorly soluble drugs.

Pre-clinical Formulation of Poorly Soluble Compounds. In order for cocrystals to become a more commercially relevant alternative for improving the bioavailability of poorly soluble compounds, the use of cocrystals as a robust and routine solution at the pre-clinical development phase must be established. For some cocrystal systems this will require a more advanced approach to cocrystal formulation in order to make cocrystals competitive with the alternative approaches for improving solubility that are traditionally used in pre-clinical formulation development. The most commercially relevant formulation strategies currently employed for poorly soluble molecules can be broadly categorized as particle size reduction, complexation (e.g., cyclodextrins), solubilizing formulations (simple oil in capsule to complex self-emulsifying formulations), and use of amorphous material (solid solutions and SDDs). Within each category there is varying degree of complexity.

The high attrition rate of APIs in development and resources required to investigate more complex dosage forms limit their use in pre-clinical formulation work when less complex solutions are available. However, when the less complex formulation methods fail, there is often no choice except to investigate more complicated approaches. Cocrystals that require additional formulation will likely be considered only after the less complex approaches have failed. The evaluation of amorphous SDDs also qualifies as a complex formulation approach for pre-clinical studies—one that is being applied at an accelerating rate in pharmaceutical research laboratories.

Cocrystals that fail to achieve adequate plasma levels when dosed as neat crystalline materials should also be reconsidered in parallel to other complex formulation strategies such as SDDs. Cocrystals offer an approach for overcoming solubility-limited bioavailability that has unique benefits compared to existing complex formulation technologies. This benefit is primarily due to the improved chemical and physical stability of a cocrystal compared to an amorphous or dissolved form of the drug. However, in order for those benefits to be realized, the ability of cocrystals to compete with these established formulation methods will have to be demonstrated.

A Supersaturating Drug Delivery System Design Strategy. There has been an increasing recognition of the advantages gained when a supersaturation design element is deliberately incorporated into formulation studies.[4] The concept of supersaturation is not itself a drug delivery strategy; rather it is a concept that can be applied to any formulation strategy that incorporates a high-energy form of the drug. Cocrystals should be considered in the context of a supersaturating drug delivery system when a more complex approach to cocrystal formulation is employed.[5] An optimized supersaturating formulation will be necessary to achieve high percent absorbed in situations where the neat cocrystal transforms rapidly to the low-solubility form of the drug and is unable to achieve the sustained solubility levels required to get improved absorption.

While it has been recognized that the concept of supersaturation applies to cocrystals, it has not been demonstrated with *in vitro* and *in vivo* data that a systematic approach to optimizing the cocrystal formulation can result in improved bioavailability. Perhaps more importantly, it has not yet been demonstrated that neglecting to account for or optimize supersaturation can lead to conversion of a cocrystal to a poorly soluble form and an *in vivo* result that underestimates the gain that can be obtained using a particular cocrystal. An important component of the experimental design in this study is to perform experiments illustrating that control over supersaturation levels achieved in cocrystal dissolution can be correlated to improved bioavailability compared to an "unformulated" or "neat" aqueous suspension. Additional *in vitro* and *in vivo* data will be obtained to probe the role of controlled supersaturation and inhibition of precipitation as enabling features of the formulated suspension. Powder dissolution will be performed under biorelevant non-sink conditions, and plasma concentrations of the model suspension drug formulations dosed to rats will be used to determine oral bioavailability.[6]

Danazol as a Model System. Danazol was selected as a model system to demonstrate the benefit of applying a designed supersaturating formulation with a pre-clinical focus to a highly

soluble cocrystal. Danazol is a well-studied BCS class II (low solubility/high permeability) compound with solubility-limited bioavailability. It is a non-ionizable compound that cannot form salts but is amenable to cocrystal formation. Danazol has an aqueous solubility of approximately 0.6 μg/mL.[7] Bioavailability studies with danazol indicate that blood levels do not increase proportionally with increases in the administered dose. A 2- to 4-fold difference in bioavailability between the fed and fasted states and a large inter-subject variation were identified in humans.[8] The solubility of danazol in biorelevant media has been reported.[9] Many established methods of improving bioavailability have been demonstrated using danazol, and our intention is to demonstrate that cocrystals can also create a therapeutically relevant bioavailability improvement with danazol.

The low solubility of 0.0067 mg/mL in fasted simulated intestinal fluid (FaSSIF) and proposed dose level of 20 mg/kg for pre-clinical studies make danazol a challenging system. The maximum absorbable dose (MAD) calculation[10] provides a way to estimate the maximum amount of drug absorbed after oral administration:

$$\mathrm{MAD} = C_s \times K_a \times V \times \mathrm{SITT} \quad (1)$$

where C_s is the saturated solubility, K_a is the absorption rate, V is the volume of intestinal fluid, and SITT is the intestinal transit time of the drug. The MAD equation can be rearranged to estimate the solubility that would be required to absorb the complete 20 mg/kg dose. In the context of a human dose, the MAD calculation the volume is fixed at 250 mL with a transit time of 270 min. The solubility requirement for complete absorption can be reduced to a dependency on dose and permeability.[6] Even using an optimistic (high) value for absorption of 0.05/min,[9b] the MAD calculation suggests that the complete absorption of a pre-clinically relevant dose level of 20 mg/kg for a 70 kg human would require a solubility level of approximately 0.4 mg/mL. This is around 60 times higher than the danazol solubility in FaSSIF of 0.0067 mg/mL.[11] The MAD calculations are not meant to be an absolute prediction of the required solubility to achieve complete absorption, but the values give a reasonable indication of the order of magnitude that is required in terms of solubility improvement that must be achieved experimentally. The estimate of a 60× increase in apparent solubility from the MAD calculation for a 20 mg/kg dose will be used to help guide the cocrystal formulation development.

EXPERIMENTAL SECTION

Reagents and Materials. Danazol was obtained from Yes Pharma Ltd. SIF powder for preparing FaSSIF was purchased from Biorelevant.com. Solvents and other reagents were purchased from commercial suppliers and used as received. Cremophor ELP, Solutol HS15, Cremophor RH 40, Lutrol E300, Lutrol F108NF, Lutrol F68NF, Soluplus, Kolliphor TPGS, Kollidon 25, Kollidon 90 F, and Kollidon 12 PF were obtained from BASF. Plasdone K29/32, Plasdone K12, and Plasdone S630 were obtained from ISP. Methocel E5 Prm, Methocel A15 Prm, and Methocel K3 Prm LV were obtained from Dow. Klucel LF Pharm hydroxypropylcellulose (HPC) and Klucel ELF Pharm HPC were obtained from Ashland Inc. Tween 80-NV-LQ-(AP) and Crodasol HS HP were obtained from Croda. Pharmatose 450M was obtained from DFE Pharma.

Cocrystal Synthesis and Characterization. Cocrystals of danazol were identified using screening methods that have been previously described.[12] The cocrystal with vanillin was scaled-up and characterized by X-ray powder diffraction (XRPD) and thermal analysis (see Supporting Information for DSC data). A two-week stability study at 40 °C and 75% relative humidity (RH) was conducted by exposing the cocrystal powder in a chamber containing saturated NaCl placed in a 40 °C oven. The XRPD patterns of the material before and after the stability study were compared, and no changes in the crystalline form of the material were observed. The 1:1 danazol:vanillin cocrystal was scaled-up with solution crystallization techniques utilizing non-stoichiometric amounts of the components. Solvent/anti-solvent combinations such as 2:1 ethyl acetate:heptane or 2:1 methyl ethyl ketone:isooctane were found to be suitable for crystallizing the pure-phase cocrystal. The anti-solvent was used to lower the solubility of both components so that excess coformer present in the solution at equilibrium would not be left in the wet filter cake during the vacuum filter isolation procedure. It was necessary to use a molar excess of coformer to produce the cocrystal as phase pure material. To a nearly saturated solution of coformer in the solvent mixture at room temperature was added a stoichiometric mixture of the API and coformer. The reactor was sealed and heated under pressure to dissolve the starting materials. The sealed reactor was allowed to cool slowly to room temperature. Sonication using a $^1/_8$-in. sonicating probe was used to induce crystallization after the supersaturated solution had cooled to room temperature. Smaller particles were made using longer sonication times at higher power (~1 min total sonication time), and larger cocrystals were formed using shorter sonication time (~15 s total sonication time at low power); the pure-phase cocrystal was the solid isolated at approximately 1.2 g batch sizes. The particle size of the cocrystal and danazol polymorph materials was determined using a Lasentec focused beam reflectance measurement (FBRM) probe in a heptane suspension of the crystalline material in combination with a calibrated microscope. Batches with 50, 30, and 15 μm average particle size were isolated as phase pure materials as determined by XRPD and HPLC. Materials with mean particle sizes of 50, 30, and 15 μm were sieved using sieve sizes of 75 (200 mesh), 45 (325 mesh), and 32 μm (450 mesh), respectively.

Single-Crystal X-ray Diffraction. Single crystals were grown by saturating vanillin in 4 mL of a 1:2 ethyl acetate:heptane mixture at room temperature. Twenty-five milligrams of the danazol:vanillin cocrystal was added, and the sealed vial was heated to dissolve the solid. The vial was allowed to cool slowly overnight in an incubated container. The resulting single crystals were isolated, and the single-crystal structure was determined. A crystal (colorless plate, 0.5 × 0.292 × 0.044 mm^3) was glued to a quartz fiber and placed in a nitrogen gas stream at 173(2) K on a Bruker D8 diffractometer with APEX2 detector, Cu Kα radiation, λ = 1.54178 Å. Data were measured using a series of combinations of φ and ω scans with 10 s frame exposures and 0.3° frame widths. A total of 14 181 reflections were collected, of which 4709 were unique (R_{int} = 0.0384). Final GooF = 1.045, $R1$ = 0.0384, $wR2$ = 0.0996, R indices based on 4427 reflections with $I > 2\sigma(I)$ (refinement on F^2), 450 parameters, 21 restraints. Lp and absorption corrections applied, μ = 0.673 mm^{-1}. More details can be found in the corresponding CIF file, which is included as Supporting Information. The XRPD pattern of the cocrystal

was calculated using Mercury V2.4 from the Cambridge Crystallographic Data Center.

X-ray Powder Diffraction (XRPD). XRPD data were obtained using a Scintag X1 powder diffractometer equipped with a peltier-cooled solid-state detector. Data were collected between 7° and 37° 2θ using a 0.05° step size and 15 min total run time for screening samples and between 3° and 40° 2θ using a 0.04° step size and 45 min total run time for material characterization. Data were collected using Cu Kα radiation, and the tube voltage and amperage were set to 45 kV and 40 mA, respectively. Instrument calibration was performed using a quartz reference standard.

HPLC Analysis. Samples for HPLC analysis were prepared by centrifuging for 3 min at 14 000 rpm in an Eppendorf 5415 centrifuge. The supernatant was filtered through a 0.2 μm nylon filter and diluted with 1 part HPLC grade methanol to prevent precipitation. The chromatographic system consisted of two Shimadzu LC-10 AT VP pumps (Shimadzu Corp., Kyoto, Japan) and a SPD-10 AV VP UV/visible detector. Separation was achieved using a Phenomenex Luna C18(2) column (75 × 4.6 mm, 3 μm) at room temperature. Methanol:water (85:15 v/v) was used as mobile phase with a flow rate of 1 mL/min. UV detection of danazol was performed at 285 nm. Analysis was performed using the Shimadzu LC Solutions software package.

Solubilizer Screening. The 10 solubilizing compounds (surfactants) selected for study were Lutrol F68NF, Lutrol F108NF, TPGS, Soluplus, Crodasol 68 (HS HP), Lutrol E300, Cremophor RH 40, Solutol HS15, Tween 80, and Cremophor ELP. Solutions of 0.25%, 1%, and 2.5% weight:volume (w/v) of each surfactant in water were prepared. The equilibrium solubility of danazol was determined by HPLC for each surfactant at the three different concentrations. The sealed samples were equilibrated for 48 h at 37 °C with stirring.

Inhibitor Screening. Ten polymeric compounds selected as potential precipitation inhibitors: Klucel LF-Pharma, Klucel ELF-Ph, Methocel K3 Prm LV, Kollidon 25, Plasdone K29/32, Plasdone K12, Kollidon 90 F, Kollidon 12 PF, Methocel E5 Prm LV, and Plasdone S630. A stock solution of danazol in 9:1 methanol:DMSO at 50 mg/mL was used to create supersaturated conditions (the "solvent shift" method) by adding small quantities of the stock solution to an aqueous solution using a calibrated pipet. Using additions of this stock API solution in the range of 1−30 μL in 1 mL of the solubilizer mixtures, supersaturation levels of up to 20× could be generated with minimal amounts of additional organic solvent being added to the test media. It was confirmed by HPLC that the addition of the 9:1 methanol:DMSO increased the equilibrium solubility by less than 3%. The resulting solutions were clear, but the danazol would precipitate over time. The rate of precipitation was determined visually by comparing the solution against calibrated standard samples under specific lighting conditions and documenting the time required for each experiment to reach a level of opacity that matched a standard sample. Four levels of opacity were recorded that corresponded to 25%, 50%, 75%, and 100% precipitation of the danazol sample. This approximate level of quantification was adequate for the purposes of identifying the onset of precipitation and measuring the rate of precipitation in order to select the most effective precipitation inhibitors. Experiments were conducted at 25 or 37 °C. Up to 16 individual experiments were prepared in a single run. Control experiments containing no inhibitor were run in duplicate with each run. The level of supersaturation was controlled on the basis of the amount of TPGS or Cremophor RH 40 present, the solution volume, and the amount of danazol stock solution added. The type and amount of the inhibitors were varied in order to compare the performance of the potential precipitation inhibitors. Representative solutions at the end of the experiment were equilibrated with stirring for 24−48 h, and the solubility of the final composition was determined by HPLC in order to calculate the supersaturation achieved.

Formulation Preparation. The two-part formulation was prepared as a dry powder solid component and a liquid component. The solid was added to the liquid component and stirred briefly to create a uniform suspension, and then the appropriate volume was dosed to rats by oral gavage. A solid powder of either cocrystal or danazol polymorph in micronized lactose constituted the dry solid powder, and the liquid contained the percentages of Vitamin E-TPGS and/or Klucel LF Pharma HPC in phosphate buffer at pH 6.5. The "unformulated" aqueous suspension was composed of the same solid mixture containing either the cocrystal or danazol polymorph in a solution containing 0.5% PVP K-25. The mixture of the cocrystal and danazol polymorph material in micronized lactose was prepared by physically mixing the components and passing the mixture through a 45 μm (325 mesh) sieve. The content uniformity and quantification of the amount of danazol present on a weight basis in the samples were determined by performing HPLC analysis of the material in triplicate. The amount of the physical mixture used in the *in vitro* and *in vivo* experiments was determined on the basis of the percent danazol present in each sample. The volume of the suspension dosed to each rat was determined on the basis of the weight of the animal. For example, a 20 mg/kg dose of the cocrystal at 15 mL/kg suspension volume for a 333 g rat would contain 9.67 mg of cocrystal (6.67 mg danazol and 3.00 mg vanillin) and 44.5 mg of micronized lactose in a solution of TPGS (1% wt:vol) and HPC (2% wt:vol) in 5 mL of phosphate buffer at pH 6.5. The corresponding dose with the danazol polymorph would be identical, with the exception of replacing the 9.67 mg of cocrystal with an equivalent amount (6.67 mg) of the danazol polymorph. This results in a 1.33 mg/mL of danazol in the suspension for a 20 mg/kg dose.

***In Vitro* Intrinsic Dissolution.** Intrinsic dissolution experiments were performed in a customized Argonaut 2410 personal screening synthesizer. The temperature of the FaSSIF medium was maintained at 37 °C ± 0.5 °C. The stirring rate was 100 rpm, and a custom magnetic stirring device was used. Discs of 9 mm diameter were compressed at 4 t/in.^{2}. The discs were carefully embedded in paraffin wax with only the top surface of the disc exposed. Aliquots of 0.5 mL were removed at each sample point (replaced by new FaSSIF at each time point) and filtered through a 0.2 μm nylon syringe filter. Experiments were performed in triplicate. The level of quantification (LOQ) for danazol using HPLC and UV detection was approximately 0.0001 mg/mL. The volume of medium used was established as 6 mL by considering the solubility of danazol in FaSSIF (~0.006 mg/mL) and the LOQ. The 6 mL volume maintained sink conditions for the danazol polymorph during the experiment and allowed quantification near the lower limit of detection. The concentrations of both danazol and vanillin were determined using separate HPLC methods. The concentration of danazol released from the cocrystal based on the vanillin coformer was calculated on the basis of the amount of vanillin detected converted to equivalent danazol concentrations by mass balance. *In situ* Raman data were obtained using a

Chromex Sentinel dispersive Raman instrument equipped with a 100 mW, 785 nm laser and a custom immersion probe. Spectra were obtained over 40 s at 5, 10, 20, 30, 45, 60, 90, and 120 min.

***In Vitro* Powder Dissolution.** Powder dissolution experiments were performed in a customized Argonaut 2410 personal screening synthesizer. The temperature was maintained at 37 °C ± 0.5 °C. The stirring rate was 100 rpm, and a custom magnetic stirring device was used. The volume used was 10 or 15 mL per experiment. Aliquots of 0.5 mL of solution were removed at each time point, and analysis was performed by HPLC. The dissolution medium was prepared using equal volumes of FaSSIF and phosphate buffer containing the specified amount of excipient (e.g., 1% TPGS and 2% HPC). The appropriate amount of powdered API or cocrystal material as a physical mixture with micronized lactose was added to the dissolution medium equilibrated at 37 °C. All figures with error bars represent experiments performed in triplicate. All figures without error bars are data from single experiments. All data were processed in Microsoft Excel.

***In Vivo* Pharmacokinetic Studies.** Pharmacokinetic studies in Sprague–Dawley rats were performed by Absorption Systems Inc. following Absorption System's Public Health Service (PHS) Approved Animal Welfare Assurance code A4282-01. The protocols used for the study were approved by Absorption System's Institutional Animal Care and Use Committee (IACUC). The studies were performed in compliance with all relevant laws and guidelines. Oral exposure of danazol was evaluated in male Sprague–Dawley rats following oral administration of six different formulations. Three rats (N = 3) were dosed for each formulation in a non-crossover study. The appropriate amount of the danazol polymorph or cocrystal lactose mixtures was added to aqueous solution containing pre-dissolved excipients. The suspension was stirred, and then the uniform suspension was dosed to the rats by oral gavage at 15 mg/kg volume and 20 mg/kg dose. Blood samples were collected up to 24 h post dose. Plasma samples were prepared for analysis by acetonitrile precipitation. Warfarin was added as an internal standard. Plasma concentrations of danazol were determined by LC-MS/MS. PKSolver,[13] a freely available menu-driven add-in program for Microsoft Excel, was used to perform the non-compartmental analysis of the pharmacokinetic data. Data for one rat in leg 6 (API in "not formulated" suspension) were excluded from the pk analysis because the data qualified as an outlier on the basis of the Q-test (p < 0.05).

RESULTS

A danazol:vanillin cocrystal was identified using a cocrystal screening process previously described.[12] The molecular structures of danazol and vanillin are shown in Figure 1. The danazol:vanillin cocrystal was determined to be a unique solid phase on the basis of XRPD data. The melting point of the danazol:vanillin cocrystal (124 °C) is between those of vanillin (81 °C) and danazol (225 °C). The single-crystal structure determination confirmed the stoichiometry of danazol:vanillin as 1:1 (Table 1). The hydrogen bonding between danazol and vanillin in the cocrystal is shown in Figure 2. The expected interactions of the best donor with the best acceptor and the weaker donor with the weaker acceptor are observed.[14] The calculated and experimental powder patterns are shown in Figure 3. On the basis of XRPD data, the cocrystal was determined to be stable after 2 weeks at 40 °C/75% RH. A stirred slurry of 10 mg of cocrystal in 3 mL of FaSSIF at 37 °C resulted in complete conversion to the poorly soluble danazol polymorph in less than 20 min, indicating that the danazol cocrystal was more soluble than the single-component danazol polymorph. This result can be predicted on the basis of the "rule of 10" that has been consistently effective in our experience for predicting the solubility of cocrystals compared to that of the corresponding API polymorph in water.[12c,15] This "rule of thumb" suggests that if the water solubility of the coformer is more than 10 times higher than the API polymorph solubility, then the cocrystal will also be more soluble than the API polymorph. The solubility of danazol is less than 0.001 mg/mL, and that of vanillin is 10 mg/mL, and the >10 000 times difference in solubility assures us that a cocrystal of danazol with vanillin will be more soluble than the danazol polymorph.

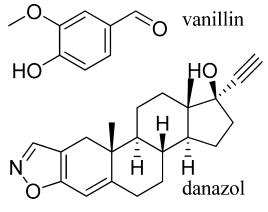


Figure 1. Molecular structure of danazol and vanillin.

Table 1. Crystal Structure Refinement Data for the 1:1 Danazol:Vanillin Cocrystal

wavelength	1.54178 Å	
crystal system	orthorhombic	
space group	$P212121$	
unit cell dimensions	a = 6.6170(3) Å	α = 90°
	b = 19.6403(10) Å	β = 90°
	c = 20.1853(9) Å	γ = 90°
Z, volume	4, 2623.3(2) Å^3	
reflections (tot., ind.)	14 181, 4709 [R(int) = 0.0384]	
data/restraints/param.	4709/21/450	
goodness-of-fit on F^2	1.045	
final R [$I > 2\sigma(I)$]	$R1$ = 0.0384, $wR2$ = 0.0996	
R indices (all data)	$R1$ = 0.0409, $wR2$ = 0.1016	

Vanillin is a non-ionizable coformer that has a favorable toxicology profile.[16] Vanillin is generally regarded as safe (GRAS) and has an acceptable daily intake (ADI) level of 0–10 mg/kg body weight based on an evaluation by the Joint FAO/WHO Expert Committee on Food Additives. Vanillin is non-ionizable at physiological pH, and in combination with the non-ionizable danazol molecule, the cocrystal will not have a significant pH-dependent dissolution profile.

Solubilizer Screening. The estimates of the MAD calculation illustrated in the Introduction indicate that, even with a high permeability value, the complete absorption of a pre-clinical immediate release dose of 20 mg/kg would require at least a 60-fold increase in solubility in FaSSIF. In practice, the solution concentration of a supersaturated API equal to 60 times the equilibrium solubility is unlikely, and a strategy that combines supersaturation with deliberate solubility adjustment using a surfactant was selected in order to maximize the percent of the dose absorbed. Our strategy to achieve the suggested MAD level is to combine a thermodynamic increase in

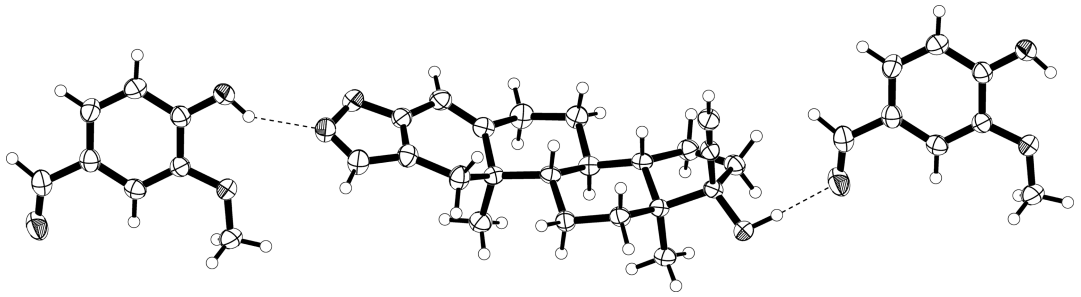


Figure 2. ORTEP (50% probability) drawing of the 1:1 danazol:vanillin cocrystal showing the hydrogen bonding between molecules.

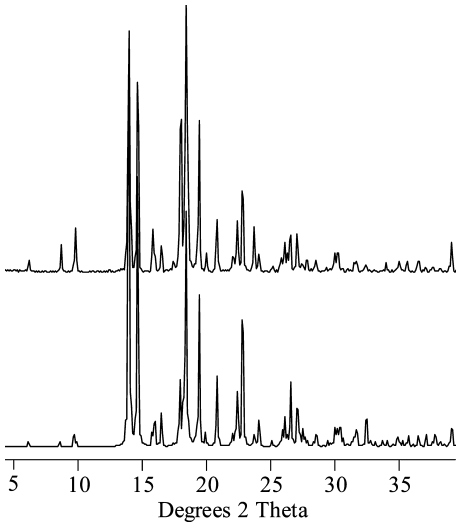


Figure 3. Calculated (bottom) and experimental (top) XRPD patterns for the 1:1 danazol:vanillin cocrystal.

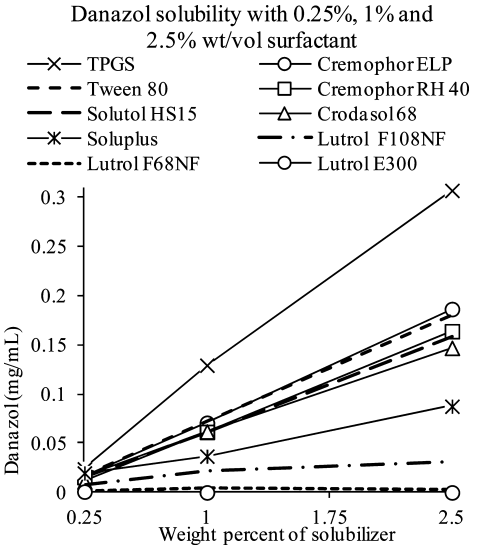


Figure 4. Solubility of danazol in the presence of selected surfactants.

solubility using micellar solubilization with controlled supersaturation achieved after the dissolution of a highly soluble danazol cocrystal. For purposes of formulation design, we targeted the increase in thermodynamic solubility multiplied by the apparent solubility at maximum supersaturation to be roughly equivalent to the MAD-calculated solubility of 0.4 mg/mL. The combination of solubilizers and precipitation inhibitors has received limited attention in the literature, but we believe this approach is generally applicable to cocrystal formulation.[4,17] This strategy of combining solubilizer, inhibitor, and cocrystal in a supersaturating drug delivery system establishes a design space that can be optimized in an iterative manner using appropriate *in vitro* powder dissolution experiments. The surfactant concentration, maximum achievable supersaturation (apparent solubility), and concentration of precipitation inhibitor needed to maintain the supersaturated state will be optimized to produce a suspension formulation that successfully translates the improved cocrystal solubility into improved bioavailability.

The process of developing this formulation first requires the identification of a suitable solubilizing excipient for danazol. The solubility of danazol in the presence of 10 pharmaceutically acceptable non-ionic solubilizing (surfactant) excipients[18] at 0.25%, 1%, and 2.5% w/v (weight of excipient to volume of water) was determined at 37 °C. All concentrations used were above the critical micelle concentration (cmc) for each excipient. The micellar solubilization of danazol in the presence of these solubilizing excipients increases linearly with concentration (Figure 4). Kolliphor TPGS is the BASF product Vitamin E TPGS or D-α-tocopherol polyethylene glycol 1000 succinate, which we will refer to simply as TPGS. Solubility of danazol with TPGS was the highest and TPGS was selected as the preferred solubilizing excipient. Cremophor RH40 was selected as the backup solubilizer on the basis of the relatively high solubility, favorable toxicity profile, and previous use in the published literature as a solubilizer for danazol.

Precipitation Inhibitor Screening. The supersaturated conditions that are anticipated when the danazol:vanillin cocrystal is dissolved must be maintained for a therapeutically relevant period of time in order for absorption to take place. This can be accomplished with the addition of a polymeric precipitation inhibitor. Identifying suitable inhibitors was performed using an empirical approach based on published methods.[19] A pharmaceutically acceptable set of polymeric excipients available from commercial suppliers and manufactured for use in pharmaceutical dosage forms was obtained from the excipient manufacturers. Thirteen water-soluble polymers were selected as a subset of these available polymers such that a broad range of polymer classes was represented.

The process of selecting a suitable crystallization inhibitor was initiated by generating supersaturation in an aqueous solution using the "solvent shift" method.[20] In this method a concentrated solution of danazol dissolved in a water miscible organic solvent is introduced to the aqueous solution. Based on the solubility of danazol in the aqueous solution (which was variable depending on the type and amount of surfactant used), the level of supersaturation generated can be controlled. Using this approach it was determined that a usable range of danazol

supersaturation was approximately 3−20×. "20×" in this context of supersaturation would mean that the total danazol concentration created by the solvent shift method is twenty times higher than the equilibrium solubility of danazol in the presence of the surfactant. A level of 1× would be equivalent to the equilibrium solubility. Screening for inhibitors at a generated supersaturation level greater than 20× resulted in very rapid precipitation regardless of inhibitor concentration because the higher supersaturation levels created a stronger driving force to precipitate.[5a,21] Based on these initial results in the context of the MAD calculation, it can already be determined that achieving complete absorption of a 20 mg/kg dose of danazol delivered as the cocrystal will be difficult because the supersaturation estimate of 60× that would be required to dissolve the entire dose in FaSSIF would lead to conditions that would strongly favor the rapid conversion of the danazol cocrystal into the poorly soluble danazol polymorph.

The solvent shift method was used to select a limited number of precipitation inhibitor candidates that were further evaluated using the dissolution of the cocrystal to create supersaturation. The results of a representative experiment with Cremophor RH 40 as the solubilizer at a supersaturation level of 4.5× (solvent shift method) using a set of potential inhibitors at 0.5% w/v is shown in Figure 5. Nucleation and growth were rapid for the majority of the polymers. In this case (Figure 5), nucleation was delayed with two Klucel HPC polymers (Klucel LF-Pharma and Klucel ELF-Pharma), Plasdone S630, and Methocel E5.

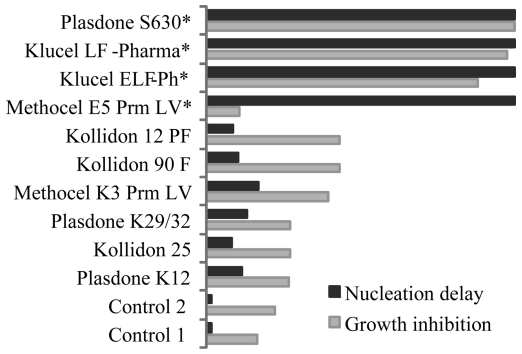


Figure 5. Representative precipitation inhibitor screening data with Cremophor RH 40 at 1.5% w/v, selected inhibitors at 0.5% w/v, and initial supersaturation fixed at 4.5×. Values are relative and normalized for comparison. * Nucleated manually after 15 min.

When these four experiments were seeded after 15 min, the precipitation in the presence of Methocel E5 was rapid, but growth in the presence of HPC or Plasdone S630 was slower.

The addition of the organic solvent in the solvent shift method of creating supersaturation did not affect danazol solubility by more than 2%. The presence of 2% w/v the HPC inhibitor also did not significantly affect the equilibrium solubility of danazol, although it did take up to 1 week for some slurry experiments with danazol and 2% inhibitor at 37 °C to reach equilibrium because a long-lived metastable state at approximately 2.5× supersaturation was consistently observed.

Experiments were conducted to evaluate the effect of using TPGS or Cremophor RH40 as the solubilizer at different concentrations and supersaturation levels, and with different inhibitor concentrations. Both the solvent shift and cocrystal were used to create supersaturation. The results using TPGS or Cremophor RH 40 were very similar and TPGS was selected as the preferred solubilizer due to the higher solubilization of danazol. Klucel LF Pharm hydroxypropylcellulose (HPC) manufactured by Ashland Inc. was the preferred inhibitor. The different molecular weights of Klucel were compared and Klucel LF-Pharma grade HPC was selected as the preferred polymeric precipitation inhibitor.

We found that although the solvent shift method of generating supersaturation is convenient and effective, it was essential to confirm the results using cocrystal as a starting material. A significant difference existed in the results obtained using the solvent shift method compared to generating supersaturation using the danazol:vanillin cocrystal. When using the solvent shift method, the supersaturated solution would remain clear (no precipitation) for a period of time before precipitation began. We did not observe this induction period using the cocrystal. Nucleation was immediate and not preventable using the cocrystal. This is interpreted as being due to nucleation and growth in the unstirred water layer (diffusion layer) on the surface of the cocrystal. It was noted in the literature that delay of nucleation was much more effective than slowed crystal growth in creating effective supersaturating felodipine formulations.[22] These results comparing the solvent shift method and cocrystal dissolution method of generating supersaturation suggest that there are still additional opportunities to improve this system by controlling the dissolution process of the cocrystal and achieving a seed free solution that persists for a longer period of time before nucleation occurs.

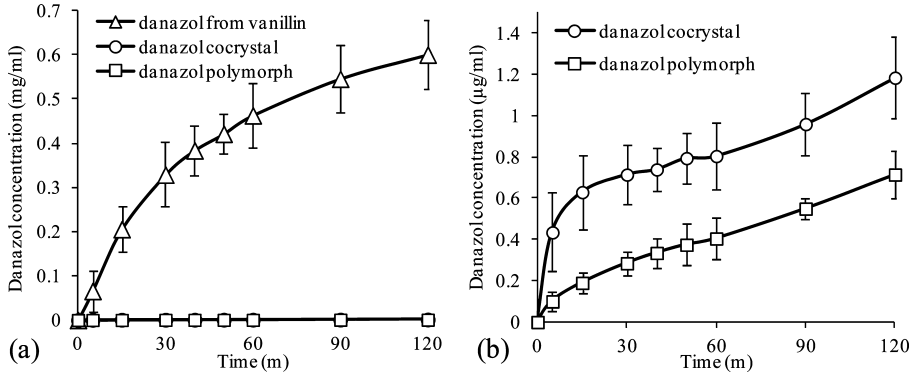


Figure 6. Intrinsic dissolution data for the danazol:vanillin cocrystal and the danazol polymorph. The theoretical concentration of danazol released from the cocrystal based on the measured concentration of the vanillin coformer in solution is shown in (a), while only the directly measured danazol concentrations are shown in (b). Note that the *Y*-axis scale is increased 500× in (b) compared to (a).

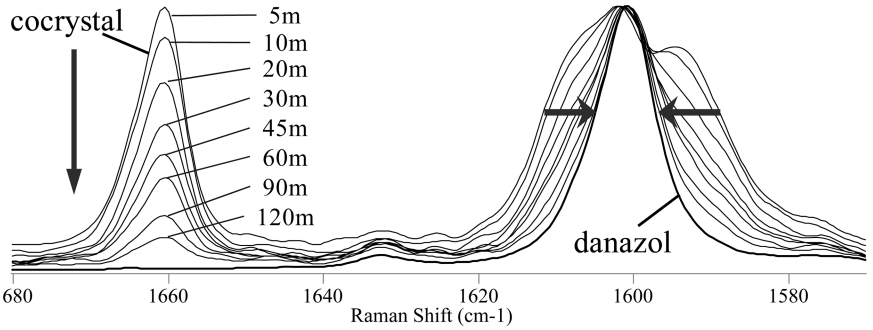


Figure 7. *In situ* Raman data for the danazol:vanillin cocrystal from the surface of an intrinsic dissolution disc obtained in parallel with solution concentration data. The arrows indicate the shifting of intensity over time as the cocrystal transforms into the danazol polymorph.

***In Vitro* Powder Dissolution.** Intrinsic dissolution experiments using the poorly soluble danazol polymorph and the danazol:vanillin cocrystal were performed under sink conditions. The results illustrate a common problem that can make interpretation of intrinsic dissolution data from cocrystals difficult. Figure 6a (triangle markers) shows the amount of danazol that has been theoretically released by cocrystal dissolution, but Figure 6b shows that the amount of danazol directly measured in solution is only a tiny fraction of the "danazol from vanillin" data shown in Figure 6a. This discrepancy is due to the crystallization of the danazol released from the dissolving cocrystal directly onto the surface of the intrinsic dissolution disc. The concentration of "danazol from vanillin" shown in Figure 6a plots the amount of danazol that has been released from the dissolving cocrystal at each time point as determined by mass balance and the measured concentration of vanillin in the dissolution media. It represents the amount of danazol that *should* be in solution if each molecule of vanillin released from the dissolving cocrystal also results in a molecule of dissolved danazol. Our interpretation of these data is that as the cocrystal dissolves, danazol becomes highly supersaturated in the microenvironment on the surface of the disc and immediately crystallizes as the single-component danazol polymorph. The danazol released from cocrystal dissolution is not able to reach the bulk solution because the mass transfer is diffusion rate limited within the unstirred water layer on the surface of the disc. Although the bulk solution maintains sink conditions (where danazol is undersaturated), the surface-mediated conversion of cocrystal to the danazol polymorph prevents the rapid cocrystal dissolution rate from resulting in significantly increased danazol solution concentrations.

The *in situ* Raman data shown in Figure 7 follow the conversion of the cocrystal into the danazol polymorph on the surface of the intrinsic dissolution disc. The conversion of the cocrystal to the poorly soluble API polymorph, presumably within the diffusion layer on the surface of the compressed disc, results in a dissolution rate that is characteristic of the poorly soluble polymorph and not the cocrystal. In Figure 6b the initial rate of cocrystal dissolution is faster than the danazol polymorph but the effective dissolution rate measured quickly becomes limited to that of the danazol polymorph after the exposed surface is essentially only danazol polymorph. The difference between the rapid limiting of the effective cocrystal dissolution rate in Figure 6b compared to the progressive transformation of the cocrystal based on the Raman data in Figure 7 is due to the penetration of the surface by the Raman instrument laser. This results in a Raman measurement through a finite thickness of the disc as opposed to only the surface. In contrast, the dissolution rate measurement is more sensitive to the composition of the exposed surface of the disc and becomes equivalent to the danazol polymorph as soon as the exposed surface is transformed.

While the intrinsic dissolution data based on the vanillin coformer concentration do suggest that the cocrystal dissolves very rapidly, the data do not support an accurate dissolution rate comparison. The effect of the transformation process occurring simultaneously with the cocrystal dissolution process and the unknown phenomena occurring in the surface microenvironment would make an estimate of the cocrystal dissolution rate speculative at best. We interpreted the intrinsic data as indicating that the cocrystal has the potential to release danazol at rates orders of magnitude faster than the danazol polymorph. Harnessing this potential cocrystal utility will require controlling and limiting the supersaturation in order to minimize the surface-mediated transformation that occurs during cocrystal dissolution.

These results suggest that, in general, cocrystal intrinsic dissolution data should be interpreted carefully when cocrystals of poorly soluble drugs are characterized. Additional data such as coformer concentrations or careful analysis of the sample surface should be obtained to confirm that the concentration of the drug measured in solution is truly representative of the cocrystal dissolution rate. Powder dissolution experiments are our preferred dissolution characterization method, as we do not find intrinsic dissolution to be consistently useful in the characterization of cocrystals that undergo rapid transformation to a less soluble form of the drug. As in the case of the danazol:vanillin cocrystal reported here, highly soluble cocrystals of poorly soluble drugs may (incorrectly) appear to have intrinsic dissolution rates that are essentially equivalent or only slightly higher than the poorly soluble API polymorph. This phenomenon has also been observed for other high-energy forms.[21]

***In Vitro* Powder Dissolution.** The solubilizer and inhibitor screening experiments resulted in the selection of an effective solubilizer (TPGS) and precipitation inhibitor (HPC) for danazol. Dissolution experiments can be used to evaluate the performance of solids in supersaturating systems; however, there is a risk that the rapid transformation to the low solubility form of the API will occur during the cocrystal dissolution experiment and this could lead to incorrect conclusions about the utility of the cocrystal. The evaluation of supersaturating systems benefits from the use of non-sink conditions that mimic the anticipated *in vivo* conditions as closely as possible. The optimization of a suspension formulation using these

excipients and the cocrystal was performed by evaluating the powder dissolution behavior of the system as key parameters were systematically varied. The selection of experimental conditions for dissolution experiments involving supersaturating systems must be done carefully.[21] The purpose of the dissolution experiments is to be predictive of the results of *in vivo* experiments. The extent of supersaturation generated in the system is determined by the selection and concentration of excipients, the dose level, media composition and volume, and the apparatus used. All of these parameters must be carefully evaluated and optimized in order to make the *in vitro* studies correlate with *in vivo* data.

An additional consideration in the design of appropriate experimental methods to evaluate supersaturating systems is the choice of analytical methods for determining drug concentration. We selected HPLC instead of *in situ* UV−vis measurements because the nanosized particles that can be generated in supersaturating experiments in the presence of inhibitors[23] has been shown to produce anomalous readings with *in situ* UV−vis dip probes.[24] HPLC results in fewer data points and is more labor intensive, but HPLC is less sensitive to the nature of the particulate material present in the experiment.

***In Vitro* Sink Condition Powder Dissolution.** Before the non-sink powder dissolution experiments were initiated, a set of powder dissolution experiments under sink conditions (in which all of the API added to the system is soluble at equilibrium) were performed in order to optimize the properties of the physical cocrystal sample that would be used in the dissolution and *in vivo* pk studies. The performance *in vitro* and *in vivo* will be affected by the particle properties of the cocrystal material that is used. We evaluated the powder dissolution profiles and dissolution behavior of three different batches of cocrystal material with the goal of selecting the most appropriate properties for the *in vitro* and *in vivo* studies. Cocrystal materials with average particle size of 50, 30, and 15 μm were isolated at gram quantities from solution. The 30 μm material was then ground by hand in a mortar and pestle to achieve a 10 μm particle size. All materials were sieved prior to use. Microscope observation of the dissolution of the 15 μm and ground 10 μm material showed poor wetting and agglomeration that prevented efficient dispersion. Samples of these materials mixed with 4 parts of milled lactose monohydrate (Pharmatose 450M) did not have agglomeration or wetting issues. The powder dissolution profile of four cocrystal samples is shown in Figure 8. The 15 μm material diluted into lactose was selected as the preferred material for delivering the cocrystal and was used in all subsequent experiments. Reference danazol material with 15 μm average particle was also diluted into an equivalent volume of lactose and this mixture was used as the danazol polymorph starting material. The use of API and cocrystal material with similar particle size is an important consideration in the performance of comparative *in vitro* and *in vivo* experiments.

The results with the 50 μm material in sink condition powder dissolution experiments highlight an important consideration when working with highly soluble cocrystals of poorly soluble drugs. XRPD data obtained from solids isolated at 45 min contain a mixture of cocrystal and the poorly soluble danazol polymorph. This is unexpected because under the experimental conditions the API polymorph is soluble. The observation is important because it implies that surface mediated transformation is occurring. This phenomenon is also likely responsible for the observation during the inhibitor screening

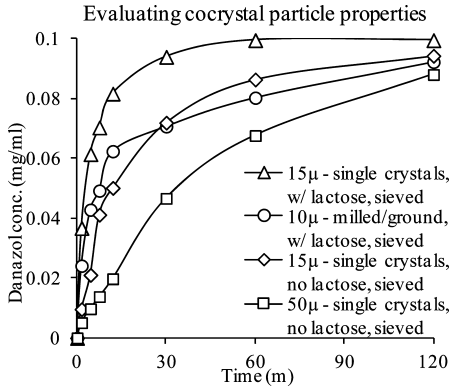


Figure 8. The cocrystal material used in the *in vitro* and *in vivo* experiments was selected by optimizing the powder dissolution profile under sink conditions.

process that no nucleation inhibition period was observed when cocrystal was used as the starting material instead of the solvent shift method.

The same surface- mediated precipitation phenomenon that was observed in the intrinsic dissolution experiments also affects the powder dissolution profile if the API precipitates before it can reach the bulk solution.[25] Shiraki et al.[26] investigated a similar situation where they found that surface-mediated transformation of a cocrystal enabled supersaturation to be achieved with small particle size but not with larger particle size. Microscope observations indicated that solvent-mediated transformation to the low-solubility API polymorphs was taking place on the surface of the cocrystal.

This phenomenon and the effect on the dissolution profile are not limited to cocrystals. Alonzo et al.[27] stated that amorphous material will be prone to surface-mediated precipitation, and this can cause the observed solubility of amorphous material to be essentially equivalent to that of the crystalline material if crystallization of the amorphous material is sufficiently fast upon contact with the dissolution medium. Control over the surface-mediated transformation of cocrystal into a lower solubility form of the API is a topic that requires further investigation, as it is the primary reason that cocrystals fail because researchers either do not see it or do not understand it.

***In Vitro* Non-sink Condition Powder Dissolution.** For the *in vitro* non-sink powder dissolution experiments, fasted state simulated intestinal fluid (FaSSIF) was selected as the dilution medium.[28] The dissolution medium composition was designed to simulate the administration of 15 mL/kg to a fasted rat. We selected a 1:1 combination of the formulation and FaSSIF at 37 °C as the medium for the powder dissolution experiments based on measured rat intestinal volumes and the 15 mL/kg dosage volume.[29] In all of the tables and figures referencing powder dissolution and *in vivo* data, the concentration of the excipients in the *formulation* is referenced, but the concentration of the excipients in the *dissolution experiments* are half of the concentration of the excipients in the formulation. All excipient concentrations in solution are expressed as a weight-to-volume percentage.

The purpose of the dissolution experiments was to use an iterative procedure to optimize the formulation and then to produce *in vitro* dissolution profiles for each pk study leg in triplicate. Our goal was to identify the "effective supersaturation limit" that could be achieved using the danazol:vanillin

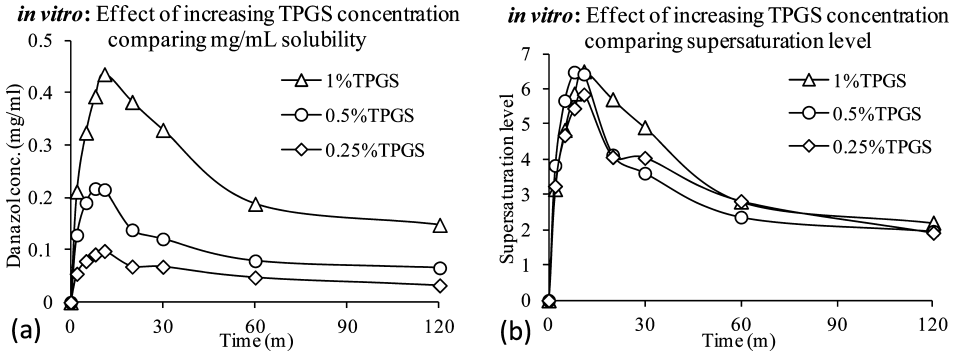


Figure 9. Variation in TPGS concentration and the resulting effect on danazol solution concentration (a) or supersaturation (b).

cocrystal as the starting material and then optimize the inhibition of precipitation to achieve a therapeutically relevant time period where the supersaturated state persisted and the apparent solubility of the cocrystal was consistent with the level of ~0.4 mg/mL for a 20 mg/kg dose suggested by the MAD calculations.

Variations in TPGS concentration with 2% HPC (Figure 9a) show that the danazol levels achieved in solution vary linearly with the TPGS concentration. When these data are plotted using supersaturation as the vertical axis (Figure 9b), it can be seen that supersaturation level achieved is the same, approximately 6×, regardless of TPGS concentration. A supersaturation of approximately 5−6× is the maximum level that could be achieved in our experiments with danazol. Figure 10 demonstrates that this is also the maximum supersaturation level achievable regardless of the amount of cocrystal put into the system. Addition of more cocrystal at a fixed TPGS concentration did not increase the apparent solubility. The iterative approach in *in vitro* testing resulted in selecting a formulation with a TPGS concentration of 1% and a HPC concentration of 2%. A 20 mg/kg dose and 15 mL/kg volume were selected as the fixed dosing parameters. *In vitro* dissolution data were collected in triplicate for each leg in the animal pk study.

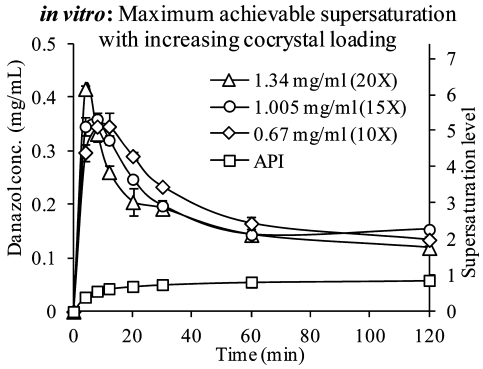


Figure 10. The maximum supersaturation achievable with increasing drug loading is limited to approximately 5.5× relative to the solubility of danazol. Dissolution medium contains a 1:1 mixture of FaSSIF and the formulated solution with 1% TPGS and 2% HPC at 37 °C.

The use of this formulation results in a 5.5× supersaturation at the maximum observed apparent solubility level in dissolution experiments (Figure 10). This 5.5× supersaturation level times the 10× solubility increase using TPGS results in an overall apparent solubility increase of 55×—very close to the 60× increase suggested by the MAD calculation. A 0.35 mg/mL maximum concentration of danazol was achieved, whereas the MAD calculation predicted a level of 0.4 mg/mL would be required for complete absorption.

There is often a concern in cocrystal studies that the presence of the coformer can be the cause of some observed physical property improvements or improved pk profiles. This concern can be addressed by evaluating the effect of a physical mixture of the API and coformer materials compared to the same materials in a cocrystalline form. The solubility of danazol in the presence of an equimolar concentration of vanillin in FaSSIF at 37 °C was effectively identical (within standard deviation) to the solubility of danazol without vanillin present. Likewise there was no change in the powder dissolution profile of the danazol polymorph in the formulated dissolution medium at 37 °C when evaluated as a physical mixture containing equimolar amounts of vanillin and danazol.

***In Vivo* Pharmacokinetic Studies.** The formulation dosed to rats was prepared by adding a solid phase to a liquid phase,

Table 2. Pharmacokinetic Data (Mean ± SD) for the 1:1 Danazol:Vanillin Cocrystal or the Danazol Crystalline Polymorph Dosed to Rats at 20 mg/kg[a]

parameter	unit						
crystal form		cocrystal	API	cocrystal	cocrystal	cocrystal	API
dose	mg/kg	20	20	20	20	20	20
TPGS amount	% w/v	1	1	0	1	0	0
HPC amount	% w/v	2	2	2	0	0	0
T_{max}	h	1.2 ± 0.3	1.3 ± 0.3	1.5 ± 0	0.42 ± 0.14	0.7 ± 0.3	1.5 ± 0
C_{max}	ng/mL	190 ± 11	14 ± 13	51 ± 16	85 ± 29	28 ± 5	9.5 ± 4
AUC 0−6 h	ng/mL·h	475 ± 42	46 ± 37	179 ± 59	115 ± 33	62 ± 24	36 ± 17
relative $F\%_{0-6h}$	%	100 ± 9	10 ± 8	38 ± 13	24 ± 6	13 ± 5	8 ± 5

[a] C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; AUC, area under the curve; AUC_{0-6}, AUC calculated from 0 to 6 h.

dx.doi.org/10.1021/mp400176y | *Mol. Pharmaceutics* 2013, 10, 3112−3127

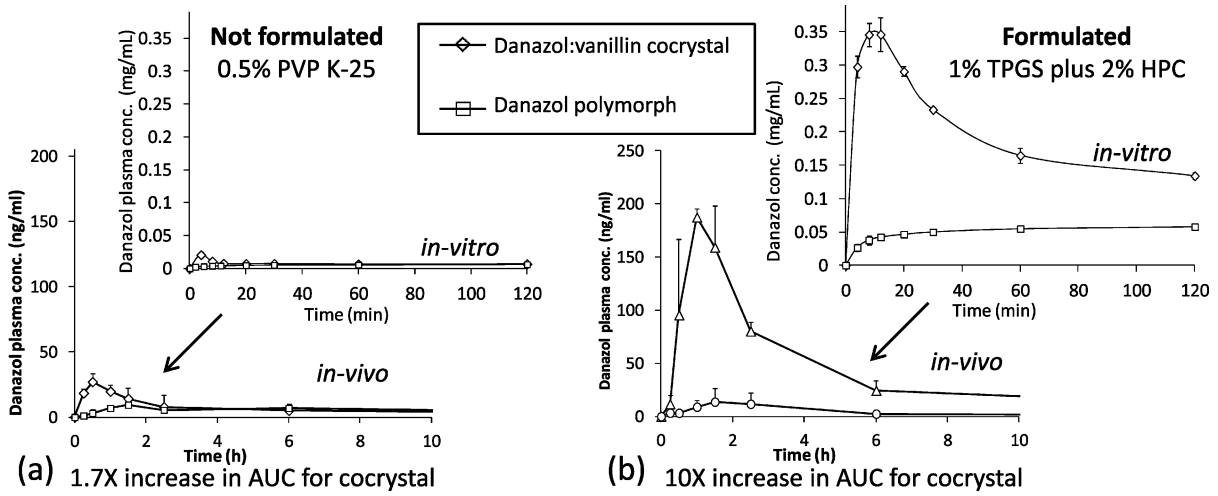


Figure 11. *In vitro* dissolution data and *in vivo* plasma concentration for the danazol cocrystal and polymorph, shown for the formulated suspension (a) containing 1% TPGS and 2% HPC, and the unformulated suspension (b) containing 0.5% PVP K-25 as a suspending agent.

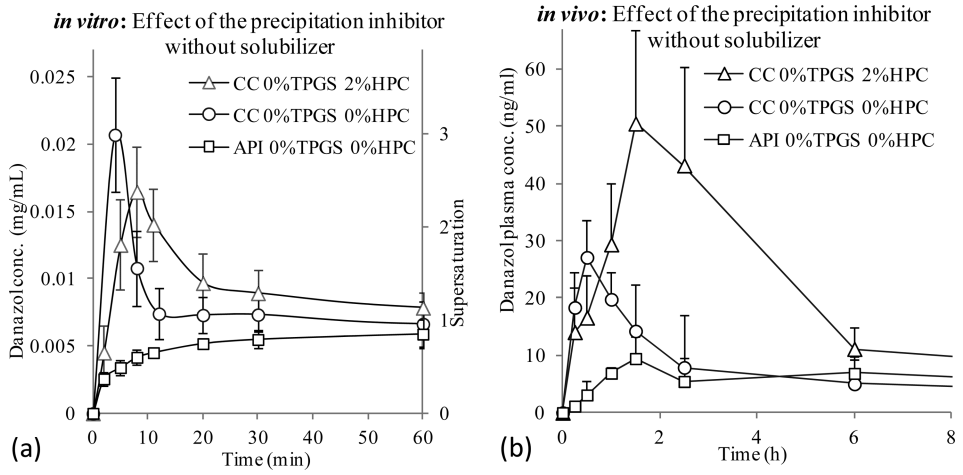


Figure 12. *In vitro* dissolution data (a) and *in vivo* plasma concentration (b) for the danazol cocrystal and polymorph with no TPGS present and either 2% HPC or 0% HPC included as a precipitation inhibitor.

stirring to create a uniform suspension, and then dosing the suspension to the rats by oral gavage at 15 mL/kg (equivalent to a 20 mg/kg dose). The solid phase was composed of either the cocrystal or danazol physically mixed into about 4 parts of micronized lactose monohydrate. The composition of the liquid phase varied while the solid phase remained constant. The weight percent of TPGS and/or HPC dissolved in phosphate buffer at pH 6.5 used in each pk study leg is indicated in Table 2. For the "unformulated" experiments (legs 5 and 6) the solution composition was 0.5% PVP K-25 dissolved in phosphate buffer at pH 6.5. The PVP functioned as a suspending agent to create a more uniform suspension.

Non-compartmental analysis was used to determine the pharmacokinetic data shown in Table 2. The results that most dramatically illustrate the degree of improvement that can be obtained by dosing a highly soluble cocrystal as a formulated suspension is shown in Figure 11. The "unformulated" aqueous suspension of the danazol:vanillin cocrystal (containing 0.5% PVP as a suspending agent) had a modest improvement of a 1.7× higher area under the curve (AUC) compared to the original poorly soluble API crystal form under identical conditions, but the formulated aqueous suspension containing 1% TPGS and 2% HPC improved the bioavailability of the cocrystal by over 10 times compared to the original API crystal form administered under identical conditions. These *in vivo* results are reflected in the *in vitro* dissolution profiles (Figure 11). These results suggest that when the danazol:vanillin cocrystal is dosed to rats as a simple aqueous suspension, the unformulated aqueous environment limits the solution concentration of danazol that can be achieved and promotes the rapid conversion of danazol to the low-solubility crystal form, which is subsequently not available for absorption.

Two independent control experiments using the danazol polymorph were performed. The polymorph was dosed in an aqueous suspension and in the presence of the formulation excipients. This demonstrates that the presence of the formulation excipients did not have a significant effect on the bioavailability of the poorly soluble danazol polymorph. The relative bioavailability of the danazol polymorph in the 1% TPGS:2% HPC formulation was 10%, and in the aqueous suspension of 0.5% PVP it was 8%.

Removing the TPGS surfactant and leaving the inhibitor (0% TPGS and 2% HPC) resulted in a greater than 10× reduction in *in vitro* danazol concentration. The *in vivo* reduction in AUC in the absence of TPGS was not as significant, dropping from 100% relative bioavailability to 38%. In the absence TPGS

dx.doi.org/10.1021/mp400176y | *Mol. Pharmaceutics* 2013, 10, 3112−3127

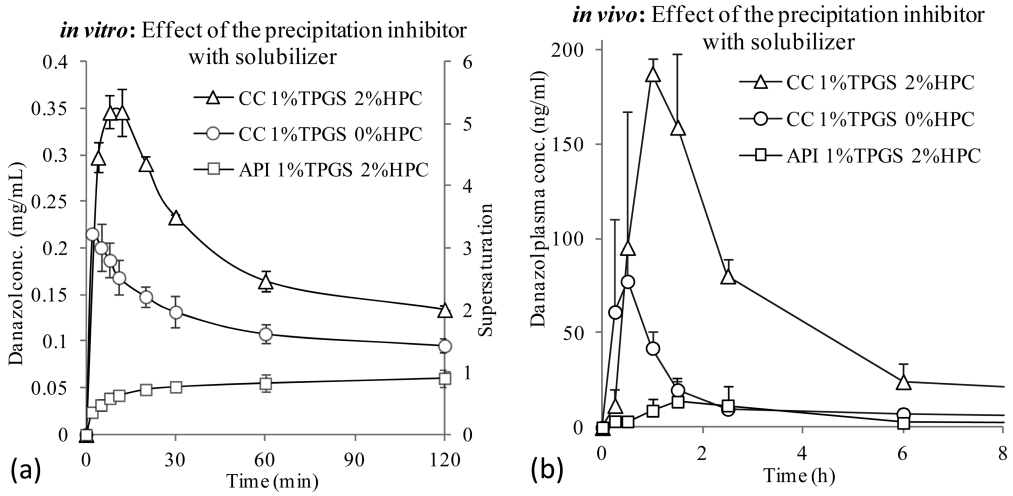


Figure 13. *In vitro* dissolution data (a) and *in vivo* plasma concentration (b) for the danazol cocrystal and polymorph are shown when 1% TPGS and either 2% HPC or 0% HPC is included as a precipitation inhibitor.

(Figure 12) or in the presence of TPGS (Figure 13), the T_{max} in both the *in vitro* and *in vivo* data correlates with the presence or absence of HPC. A longer time to T_{max} was observed in the presence of HPC compared the absence of HPC. The role of the viscosity of the formulation due to the presence of HPC was not investigated but may play a role.

The role of the inhibitor was probed by removing the HPC from the formulation and obtaining *in vitro* (Figure 13a) and *in vivo* (Figure 13b) data. Removing the HPC from the formulation (1% TPGS and 0% HPC) resulted in an approximately 50% reduction in the maximum solution concentration in powder dissolution experiments when compared to results with 1% TPGS and 2% HPC. Corresponding *in vivo* results when the inhibitor was removed were a reduction in the AUC from 100% to 24%.

A maximum of about 2.5−3× supersaturation was achieved *in vitro* when either TPGS or HPC was used alone, but a 6× supersaturation was achieved when the two excipients were used together. This is reflected in the *in vivo* data where the use of 1% TPGS resulted in a relative *F* of 24%, the use of 2% HPC resulted in a F of 38% but the combination of 1% TPGS and 2% HPC resulted in a F of 100%. If the effect of combining the excipients was strictly additive a relative bioavailability of 62% would be expected, but the resulting F of 100% suggests a synergy.

DISCUSSION

Danazol Is a Model System for Improving Bioavailability. Danazol is a BCS Class II compound with high permeability and solubility limited bioavailability. A broad array of formulation strategies have successfully demonstrated improved bioavailability in animal models. The successful techniques reflect the formulation methods that are typically considered at the pre-clinical development phase for poorly soluble compounds. Examples of successful *in vivo* formulation approaches include particle size reduction (micronization)[30] use of cyclodextrin inclusion complexes,[31] liquid filled capsules (oil and/or surfactant),[32] suspensions in surfactant,[33] SEDDS,[34] and an amorphous solid solution formed with Soluplus.[35] Supersaturating self-emulsifying drug delivery systems (S-SEDDS)[36] have also been demonstrated to be successful in animal pharmacokinetic studies.

Based on the results reported here, cocrystals can now be added to this list of successful approaches that have improved the bioavailability of danazol; however, without the use of the enabling formulation, the traditional neat cocrystal suspension would not be considered a successful result. In the case of the danazol:vanillin cocrystal, it was essential to consider both the solid form and the formulation simultaneously in order to achieve a successful bioavailability improvement with a cocrystal. A neat formulation of the danazol:vanillin cocrystal fails to significantly improve the bioavailability of danazol. We believe that continuing research will demonstrate that many neat dosage forms of cocrystals of poorly soluble drugs will systematically underperform and not represent the true bioavailability benefit that can be achieved with a cocrystal unless a suitable formulation is employed.

Cocrystals and Amorphous Forms Have Similarities. Cocrystals have the opportunity to be a viable alternative to spray-dried dispersions (SDDs) because there is considerable overlap of the molecules that could benefit from cocrystallization or SDDs. There are significant risks and potentially high costs associated with SDDs, primarily linked to stability concerns and the difficulty associated with the advanced manufacturing methods required to produce SDDs. If a cocrystal and a SDD were to demonstrate comparable bioavailability performance, the cocrystal could be the preferred solution because of the stability of the crystalline form compared to the amorphous material. Furthermore, if the cocrystal could be isolated using existing industrial crystallization techniques and be incorporated into a dosage form that can be manufactured using conventional equipment, the cost savings could be significant compared to a SDD.

The current perception of cocrystals in the pharmaceutical industry is that the field is still emerging and there is only limited awareness that cocrystals could be a feasible alternative to SDDs if they are properly formulated. Approaches to cocrystal drug delivery have not delivered convincing results that indicate cocrystals can be a rubust and reliable drug delivery strategy that can can deliver commercially relevant improvements over competing techniques used for poorly soluble drugs. The results presented here establish an awareness that achieving a therapeutically relevant bioavailability improvement with a cocrystal may require a more complex formulation

effort. It is anticipated that continued research along these lines will result in published accounts that demonstrate the ability of cocrystals to be competitive with amorphous forms.

The parallels between amorphous forms and cocrystals have been described by Babu and Nangia.[37] In particular they note that peak supersaturation levels of amorphous materials and cocrystals are comparable. This observation is reflected in the danazol system when the powder dissolution profiles of amorphous danazol and the danazol:vanillin cocrystal are compared. The theoretical solubility improvement of amorphous danazol compared to crystalline danazol has been reported in the literature.[38] The solubility improvement was calculated by Ozaki et al. to be 13.7 times higher, but experimentally they were only able to obtain a peak supersaturation after dissolving amorphous danazol in FaSSIF of 3.1×. Murdande et al. reported a similar result for amorphous danazol.[39] They calculated a 27× increase in solubility for amorphous danazol, but in a powder dissolution experiment in deionized water they also observed a ∼3× supersaturation maximum. These supersaturation levels are comparable to the results observed for the danazol:vanillin cocrystal when no excipients were used or only HPC was used (Figure 12a) or only TPGS was used (Figure 13a).

Supersaturation and Solubilization Are Complementary. In order for a supersaturating formulation to be robust, the generation and maintenance of supersaturation levels must be controllable.[40] Combining a rapidly dissolving cocrystal form of danazol with a surfactant and a precipitation inhibitor created a tunable system in which the contributions from the surfactant and precipitation inhibitor could be combined and optimized to yield an apparent solubility that was sufficient to achieve significantly improved absorption of danazol.

TPGS and HPC perform different functions in the formulation, and there was an observed benefit when the two excipients were combined both *in vitro* and *in vivo* compared to when they were used independently. The *in vitro* dissolution data for the danazol cocrystal and the two studies on amorphous danazol material suggest that a baseline supersaturation level achievable with danazol under a broad range of conditions is approximately 3×. The use of TPGS without HPC (Figure 13a) and the use of HPC without TPGS (Figure 12a) both give maximum supersaturation near 3×. However, when TPGS and HPC are combined, the observed supersaturation levels double to roughly 6× (Figures 9, 10, and 13a). The *in vivo* data parallel the *in vitro* observations, and the combination of TPGS and HPC resulted in a more significant benefit compared to the use of TPGS or HPC alone.

Surfactants and precipitation inhibitors are often considered independently; however, there are several published examples that demonstrate the utility of combining these two excipient types in the design of more effective formulations. The reported use of a surfactant and polymeric inhibitor combination in a formulation of an amorphous drug form resulted in an improved dissolution profile when combinations were used compared to the use of single excipients.[41] A felodipine formulation that combined surfactants and precipitation inhibitors was successful in creating a once-daily controlled-release tablet formulation.[42] Li et al. reported that a combination of surfactants to partially solubilize an API and precipitation inhibitors to minimize the precipitation resulted in improved oral bioavailability of a weakly basic compound that is soluble at gastric pH but then precipitates in more neutral environments.[43]

Use of Surfactants Raises Permeability Concerns. The effect of surfactants on cocrystal stability has recently been described by Huang and Rodriguez-Hornedo in a set of papers that has created a new addition to the crystal engineer's toolbox.[44] The ability to stabilize a pharmaceutical cocrystal in an aqueous environment that would otherwise transform to the poorly soluble polymorph of the drug is accomplished using surfactants. By preferentially solubilizing the API and not the coformer with a micelle forming surfactant, the phase diagram is shifted and, for cocrystals containing coformers that have a low affinity for partitioning into the micelle, the cocrystal will become stabilized if the surfactant concentration is high enough.

The benefit of using a surfactant in a formulation does not come without a price. A series of papers has highlighted the importance of considering the effect of surfactant use on permeability.[45] The presence of a surfactant will lead to reduced permeability, but this effect can be offset by the increased solubility of the drug. For highly permeable molecules like danazol the reduced permeability that occurs at typical surfactant concentrations utilized in formulations is offset by the increased solubility of the drug in the presence of the surfactant. The result is an overall increase in flux of danazol through the membrane despite the reduced permeability. The evaluation of the solubility/permeability interplay for danazol was performed in FaSSIF and compared to the standard transport medium used in Caco-2 monolayer assays.[46] The use of FaSSIF (which contains surfactants) instead of the standard transport medium (containing no surfactants) caused a reduction in the permeability of about 20 times. However, there was an approximately 100 times increase in the solubility and this improved the cumulative transport across the membrane by about 40% compared to the standard transport medium. In a related study the surfactants contained in aspirated human intestinal fluid showed similar results with danazol.[47,9b]

The reduced free fraction of drug in solution in a formulation that employs micellar solubilization has a significant influence on the observed permeability. The use of supersaturation to increase the apparent solubility of a compound increases the free fraction of the drug and thus there is less of an effect on permeability when a supersaturating formulation is used.[23b] The combination of supersaturation plus solubilization demonstrated in this study can be especially effective. In this study we attempted to minimize the use of the surfactant by maximizing the experimentally obtainable supersaturation level that could be achieved. It would be preferable to achieve the required MAD solubility level without the use of a surfactant at all, but in the case of danazol and a dose of 20 mg/kg that was not practical. In this case a combination of surfactant and controlled supersaturation levels was an effective approach to formulating the danazol cocrystal.

■ CONCLUSION

The screening, synthesis, and characterization of cocrystals has progressed significantly in the past decade; however, there is very limited published *in vivo* data suggesting that cocrystals can effectively compete with established technologies that are routinely used to provide improved formulations of poorly soluble drugs. Without the demonstrated ability to not only compete, but show advantages over existing formulation approaches for poorly soluble molecules, cocrystals will not be considered a preferred choice by pharmaceutical formulation

groups as they address the increasing number of poorly soluble compounds in industry development pipelines.

In some cases the requirements for a pre-clinical formulation of a poorly soluble drug can be met with a relatively simple formulation. However, it is not uncommon for drug delivery to be the limiting factor in the ability to determine the *in vivo* efficacy and toxicological profile of a drug candidate. Significant time and resources can be spent studying additional and increasingly complex drug delivery systems. When cocrystals are considered for pre-clinical animal studies, it may be possible to achieve the required bioavailability improvement with a simple suspension of the neat cocrystal material; however, if this approach fails then the results reported here suggest that the cocrystal should be further evaluated in the context of an enabling formulation.

In the case of the 1:1 danazol:vanillin cocrystal, an appropriate formulation was necessary in order to obtain a significant bioavailability improvement compared to the use of the poorly soluble crystalline danazol polymorph. When the danazol:vanillin cocrystal was dosed to rats as a simple aqueous suspension, the unformulated aqueous environment limited the solution concentration of danazol that could be achieved and promoted the rapid conversion of danazol to the low-solubility crystal form. The cocrystal as a neat suspension was subsequently not significantly absorbed based on the *in vivo* result of a 1.7 times increase in AUC for the cocrystal compared to the danazol polymorph in an unformulated aqueous suspension. However, when the danazol cocrystal was suspended in an aqueous phase that contained an excipient mixture designed to optimize the level of supersaturation achieved and maintain supersaturation for a therapeutically relevant period of time, the AUC for the cocrystal was 10 times higher than the AUC obtained for the danazol polymorph dosed in the same formulated solution. The formulation strategy relied on the combined use of a solubilizing surfactant (TPGS) and controlled supersaturation levels. The synergistic combination of TPGS as a solubilizer and hydroxyproplycellulose (HPC) as a precipitation inhibitor resulted in apparent solubility levels in *in vitro* powder dissolution profiles that correlated to danazol plasma levels in *in vivo* studies in Sprague–Dawley rats.

This is the first systematic investigation of a supersaturating cocrystal system where the goal was to simultaneously consider both the solid form and the formulation in order to generate commercially and therapeutically relevant blood levels in pharmacokinetic studies. It is believed that these results are generally applicable to cocrystals of poorly soluble APIs and cocrystals that are formulated as designed supersaturating systems will consistently show higher bioavailability and a higher percent of the dose absorbed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

X-ray crystallographic data for the crystal structure of 1:1 danazol:vanillin (CIF) and DSC thermogram for the danazol:vanillin cocrystal. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Renovo Research, 749 Moreland Ave. SE, Suite A201, Atlanta, GA 30316. E-mail: schilds@renovoresearch.com.

Present Address

‡S.R.L.: Eli Lilly and Co., Indianapolis, IN 99988.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation under Grant No. IIP-1143108.

■ REFERENCES

(1) Thakuria, R.; Delori, A.; Jones, W.; Lipert, M. P.; Roy, L.; Rodriguez-Hornedo, N. Pharmaceutical cocrystals and poorly soluble drugs. *Int. J. Pharm.* **2012**, DOI: 10.1016/j.ijpharm.2012.10.043.

(2) (a) McNamara, D. P.; Childs, S. L.; Giordano, J.; Iarriccio, A.; Cassidy, J.; Shet, M. S.; Mannion, R.; O'Donnell, E.; Park, A. Use of a glutaric acid cocrystal to improve oral bioavailability of a low solubility API. *Pharm. Res.* **2006**, *23* (8), 1888–1897. (b) Hickey, M. B.; Peterson, M. L.; Scoppettuolo, L. A.; Morrisette, S. L.; Vetter, A.; Guzman, H.; Remenar, J. F.; Zhang, Z.; Tawa, M. D.; Haley, S.; Zaworotko, M. J.; Almarsson, O. Performance comparison of a co-crystal of carbamazepine with marketed product. *Eur. J. Pharm. Biopharm.* **2007**, *67* (1), 112–119. (c) Bak, A.; Gore, A.; Yanez, E.; Stanton, M.; Tufekcic, S.; Syed, R.; Akrami, A.; Rose, M.; Surapaneni, S.; Bostick, T.; King, A.; Neervannan, S.; Ostovic, D.; Koparkar, A. The co-crystal approach to improve the exposure of a water-insoluble compound: AMG 517 sorbic acid co-crystal characterization and pharmacokinetics. *J. Pharm. Sci.* **2008**, *97* (9), 3942–3956. (d) Cheney, M. L.; Shan, N.; Healey, E. R.; Hanna, M.; Wojtas, L.; Zaworotko, M. J.; Sava, V.; Song, S.; Sanchez-Ramos, J. R. Effects of Crystal Form on Solubility and Pharmacokinetics: A Crystal Engineering Case Study of Lamotrigine. *Cryst. Growth Des.* **2010**, *10* (1), 394–405. (e) Jung, M.-S.; Kim, J.-S.; Kim, M.-S.; Alhalaweh, A.; Cho, W.; Hwang, S.-J.; Velaga, S. P. Bioavailability of indomethacin-saccharin cocrystals. *J. Pharm. Pharmacol.* **2010**, *62* (11), 1560–1568. (f) Smith, A. J.; Kavuru, P.; Wojtas, L.; Zaworotko, M. J.; Shytle, R. D. Cocrystals of Quercetin with Improved Solubility and Oral Bioavailability. *Mol. Pharm.* **2011**, *8* (5), 1867–1876. (g) Stanton, M. K.; Kelly, R. C.; Colletti, A.; Langley, M.; Munson, E. J.; Peterson, M. L.; Roberts, J.; Wells, M. Improved Pharmacokinetics of AMG 517 Through Co-crystallization Part 2: Analysis of 12 Carboxylic Acid Co-crystals. *J. Pharm. Sci.* **2011**, *100* (7), 2734–2743. (h) Venczel, M.; Szvoboda, I.; Podanyi, B.; Valente, D.; Menegotto, J.; Pintye-Hodi, K.; Ujhelyi, G. Formulation Possibilities of a Weak Base with a Narrow Solubility Range. *Cryst. Growth Des.* **2012**, *12* (3), 1101–1110. (i) Weyna, D. R.; Cheney, M. L.; Shan, N.; Hanna, M.; Zaworotko, M. J.; Sava, V.; Song, S.; Sanchez-Ramos, J. R. Improving Solubility and Pharmacokinetics of Meloxicam via Multiple-Component Crystal Formation. *Mol. Pharm.* **2012**, *9* (7), 2094–2102. (j) Zheng, W.; Jain, A.; Papoutsakis, D.; Dannenfelser, R.-M.; Panicucci, R.; Garad, S. Selection of oral bioavailability enhancing formulations during drug discovery. *Drug Dev. Ind. Pharm.* **2012**, *38* (2), 235–247.

(3) Palucki, M.; Higgins, J. D.; Kwong, E.; Templeton, A. C. Strategies at the Interface of Drug Discovery and Development: Early Optimization of the Solid State Phase and Preclinical Toxicology Formulation for Potential Drug Candidates. *J. Med. Chem.* **2010**, *53* (16), 5897–5905.

(4) Burton, L.; Ying, W.; Gandhi, R.; West, R.; Huang, C.; Zhou, S.; Shah, K.; Chen, J.; Shen, X. Development of a precipitation-resistant solution formulation to increase in vivo exposure of a poorly water-soluble compound. *Int. J. Pharm.* **2012**, *433* (1–2), 94–101.

(5) (a) Burton, L.; Ying, W.; Gandhi, R.; West, R.; Huang, C.; Zhou, S.; Shah, K.; Chen, J.; Shen, X. Development of a precipitation-resistant solution formulation to increase in vivo exposure of a poorly

water-soluble compound. *Int. J. Pharm.* **2012**, *433* (1−2), 94−101. (b) Brouwers, J.; Brewster, M. E.; Augustijns, P. Supersaturating Drug Delivery Systems: The Answer to Solubility-Limited Oral Bioavailability? *J. Pharm. Sci.* **2009**, *98* (8), 2549−2572.

(6) Thomas, V. H.; Bhattachar, S.; Hitchingham, L.; Zocharski, P.; Naath, M.; Surendran, N.; Stoner, C. L.; El-Kattan, A. The road map to oral bioavailability: an industrial perspective. *Exp. Opin. Drug Metab.* **2006**, *2* (4), 591−608.

(7) Kaukonen, A. M.; Boyd, B. J.; Charman, W. N.; Porter, C. J. H. Drug solubilization behavior during in vitro digestion of suspension formulations of poorly water-soluble drugs in triglyceride lipids. *Pharm. Res.* **2004**, *21* (2), 254−260.

(8) (a) Selinger, K.; Hill, H. M.; Anslow, J. A.; Gash, D. a Liquid-Chromatographic Method for the Determination of Danazol in Human Serum. *J. Pharm. Biomed. Anal.* **1990**, *8* (1), 79−84. (b) Charman, W. N.; Rogge, M. C.; Boddy, A. W.; Barr, W. H.; Berger, B. M. Absorption of danazol after administration to different sites of the gastrointestinal tract. *J. Clin. Pharmacol.* **1993**, *33* (12), 1207−1213. (c) Sunesen, V. H.; Vedelsdal, R.; Kristensen, H. G.; Christrup, L.; Mullertz, A. Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug. *Eur. J. Pharm. Sci.* **2005**, *24* (4), 297−303.

(9) (a) Vogt, M.; Kunath, K.; Dressman, J. B. Dissolution improvement of four poorly water soluble drugs by cogrinding with commonly used excipients. *Eur. J. Pharm. Biopharm.* **2008**, *68* (2), 330−337. (b) Vertzoni, M.; Markopoulos, C.; Symillides, M.; Goumas, C.; Imanidis, G.; Reppas, C. Luminal Lipid Phases after Administration of a Triglyceride Solution of Danazol in the Fed State and Their Contribution to the Flux of Danazol Across Caco-2 Cell Monolayers. *Mol. Pharmaceutics* **2012**, *9* (5), 1189−1198. (c) Annaert, P.; Brouwers, J.; Bijnens, A.; Lammert, F.; Tack, J.; Augustijns, P. Ex vivo permeability experiments in excised rat intestinal tissue and in vitro solubility measurements in aspirated human intestinal fluids support age-dependent oral drug absorption. *Eur. J. Pharm. Sci.* **2009**, *39* (1−3), 15−22.

(10) (a) Johnson, K. C.; Swindell, A. C. Guidance in the setting of drug particle size specifications to minimize variability in absorption. *Pharm. Res.* **1996**, *13* (12), 1795−1798. (b) Takano, R.; Kataoka, M.; Yamashita, S. Integrating drug permeability with dissolution profile to develop IVIVC. *Biopharm. Drug Disposition* **2012**, *33* (7), 354−365. (c) Curatolo, W. Physical chemical properties of oral drug candidates in the discovery and exploratory development settings. *Pharm. Sci. Technol. Today* **1998**, *1* (9), 387−393. (d) Ozaki, S.; Minamisono, T.; Yamashita, T.; Kato, T.; Kushida, I. Supersaturation-nucleation behavior of poorly soluble drugs and its impact on the oral absorption of drugs in thermodynamically high-energy forms. *J. Pharm. Sci.* **2012**, *101* (1), 214−222.

(11) Bevernage, J.; Brouwers, J.; Clarysse, S.; Vertzoni, M.; Tack, J.; Annaert, P.; Augustijns, P. Drug Supersaturation in Simulated and Human Intestinal Fluids Representing Different Nutritional States. *J. Pharm. Sci.* **2010**, *99* (11), 4525−4534.

(12) (a) Childs, S. L.; Hardcastle, K. I. Cocrystals of piroxicam with carboxylic acids. *Cryst. Growth Des.* **2007**, *7* (7), 1291−1304. (b) Childs, S. L.; Hardcastle, K. I. Cocrystals of chlorzoxazone with carboxylic acids. *CrystEngComm* **2007**, *9* (5), 364−367. (c) Childs, S. L.; Rodriguez-Hornedo, N.; Reddy, L. S.; Jayasankar, A.; Maheshwari, C.; McCausland, L.; Shipplett, R.; Stahly, B. C. Screening strategies based on solubility and solution composition generate pharmaceutically acceptable cocrystals of carbamazepine. *CrystEngComm* **2008**, *10* (7), 856−864. (d) Friscic, T.; Childs, S. L.; Rizvi, S. A. A.; Jones, W. The role of solvent in mechanochemical and sonochemical cocrystal formation: a solubility-based approach for predicting cocrystallisation outcome. *CrystEngComm* **2008**, *11* (3), 418−426.

(13) Zhang, Y.; Huo, M.; Zhou, J.; Xie, S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput. Methods Programs Biomed.* **2010**, *99* (3), 306−314.

(14) Etter, M. C. Encoding and decoding hydrogen-bond patterns of organic compounds. *Acc. Chem. Res.* **1990**, *23* (4), 120−126.

(15) Good, D. J.; Rodriguez-Hornedo, N. Solubility Advantage of Pharmaceutical Cocrystals. *Cryst. Growth Des.* **2009**, *9* (5), 2252−2264.

(16) (a) Ho, K.; Yazan, L. S.; Ismail, N.; Ismail, M. Toxicology study of vanillin on rats via oral and intra-peritoneal administration. *Food Chem. Toxicol.* **2011**, *49* (1), 25−30. (b) Mancebo, A.; Trapero, Y. M.; Gonzalez, Y.; Gonzalez, B. O.; Fuentes, D.; Hernandez, O.; Hernandez, J.; Gonzalez, C.; Hernandez, Y.; Arteaga, M. E.; Subiros, N.; Bada, A. M. Repeated dose oral toxicity (13 weeks) of 4-hydroxy-3-methoxy-benzaldehyde (vanillin) to Sprague Dawley rats. *Toxicol. Lett. (Shannon)* **2003**, *144* (Suppl. 1), s57−s57.

(17) Vandecruys, R.; Peeters, J.; Verreck, G.; Brewster, M. E. Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design. *Int. J. Pharm.* **2007**, *342* (1−2), 168−175.

(18) Strickley, R. G. Solubilizing excipients in oral and injectable formulations. *Pharm. Res.* **2004**, *21* (2), 201−230.

(19) (a) Yamashita, T.; Kokubo, T.; Zhao, C.; Ohki, Y. Antiprecipitant Screening System for Basic Model Compounds Using Bio-Relevant Media. *Jala* **2010**, *15* (4), 306−312. (b) Yamashita, T.; Ozaki, S.; Kushida, I. Solvent shift method for anti-precipitant screening of poorly soluble drugs using biorelevant medium and dimethyl sulfoxide. *Int. J. Pharm.* **2011**, *419* (1−2), 170−174. (c) Dai, W.-G. In vitro methods to assess drug precipitation. *Int. J. Pharm.* **2010**, *393* (1−2), 1−16.

(20) Warren, D. B.; Benameur, H.; Porter, C. J. H.; Pouton, C. W. Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: A mechanistic basis for utility. *J. Drug Targeting* **2010**, *18* (10), 704−731.

(21) Hawley, M.; Morozowich, W. Modifying the Diffusion Layer of Soluble Salts of Poorly Soluble Basic Drugs To Improve Dissolution Performance. *Mol. Pharmaceutics* **2010**, *7* (5), 1441−1449.

(22) Alonzo, D. E.; Raina, S.; Zhou, D.; Gao, Y.; Zhang, G. G. Z.; Taylor, L. S. Characterizing the Impact of Hydroxypropylmethyl Cellulose on the Growth and Nucleation Kinetics of Felodipine from Supersaturated Solutions. *Cryst. Growth Des.* **2012**, *12* (3), 1538−1547.

(23) (a) Alonzo, D. E.; Gao, Y.; Zhou, D.; Mo, H.; Zhang, G. G. Z.; Taylor, L. S. Dissolution and Precipitation Behavior of Amorphous Solid Dispersions. *J. Pharm. Sci.* **2011**, *100* (8), 3316−3331. (b) Miller, J. M.; Beig, A.; Carr, R. A.; Spence, J. K.; Dahan, A.; Win-Win, A. Solution in Oral Delivery of Lipophilic Drugs: Supersaturation via Amorphous Solid Dispersions Increases Apparent Solubility without Sacrifice of Intestinal Membrane Permeability. *Mol. Pharm.* **2012**, *9* (7), 2009−2016.

(24) Van Eerdenbrugh, B.; Alonzo, D. E.; Taylor, L. S. Influence of Particle Size on the Ultraviolet Spectrum of Particulate-Containing Solutions: Implications for In-Situ Concentration Monitoring Using UV/Vis Fiber-Optic Probes. *Pharm. Res.* **2011**, *28* (7), 1643−1652.

(25) Greco, K.; Bogner, R. Solution-mediated phase transformation: Significance during dissolution and implications for bioavailability. *J. Pharm. Sci.* **2012**, *101* (9), 2996−3018.

(26) Shiraki, K.; Takata, N.; Takano, R.; Hayashi, Y.; Terada, K. Dissolution improvement and the mechanism of the improvement from cocrystallization of poorly water-soluble compounds. *Pharm. Res.* **2008**, *25* (11), 2581−2592.

(27) Alonzo, D. E.; Zhang, G. G. Z.; Zhou, D.; Gao, Y.; Taylor, L. S. Understanding the Behavior of Amorphous Pharmaceutical Systems during Dissolution. *Pharm. Res.* **2010**, *27* (4), 608−618.

(28) (a) Boni, J. E.; Brickl, R. S.; Dressman, J.; Pfefferle, M. L. Instant FaSSIF and FeSSIF-Biorelevance Meets Practicality. *Dissolut. Technol.* **2009**, *16* (3), 41−45. (b) Clarysse, S.; Brouwers, J.; Tack, J.; Annaert, P.; Augustijns, P. Intestinal drug solubility estimation based on simulated intestinal fluids: Comparison with solubility in human intestinal fluids. *Eur. J. Pharm. Sci.* **2011**, *43* (4), 260−269.

(29) McConnell, E. L.; Basit, A. W.; Murdan, S. Measurements of rat and mouse gastrointestinal pH fluid and lymphoid tissue, and

implications for in-vivo experiments. *J. Pharm. Pharmacol.* **2008**, *60* (1), 63−70.
(30) (a) Liversidge, G. G.; Cundy, K. C. Particle size reduction for improvement of oral bioavailability of hydrophobic drugs. *Int. J. Pharm.* **1995**, *125* (1), 91−97. (b) Takano, R.; Furumoto, K.; Shiraki, K.; Takata, N.; Hayashi, Y.; Aso, Y.; Yamashita, S. Rate-limiting steps of oral absorption for poorly water-soluble drugs in dogs; Prediction from a miniscale dissolution test and a physiologically-based computer simulation. *Pharm. Res.* **2008**, *25* (10), 2334−2344.
(31) (a) Badawy, S. I. F.; Ghorab, M. M.; Adeyeye, C. M. Bioavailability of danazol-hydroxypropyl-beta-cylodextrin complex by different routes of administration. *Int. J. Pharm.* **1996**, *145* (1−2), 137−143. (b) Badawy, S. I. F.; Ghorab, M. M.; Adeyeye, C. M. Characterization and bioavailability of danazol-hydroxypropyl beta-cyclodextrin coprecipitates. *Int. J. Pharm.* **1996**, *128* (1−2), 45−54.
(32) Erlich, L.; Yu, D.; Pallister, D. A.; Levinson, R. S.; Gole, D. G.; Wilkinson, P. A.; Erlich, R. E.; Reeve, L. E.; Viegas, T. X. Relative bioavailability of danazol in dogs from liquid-filled hard gelatin capsules. *Int. J. Pharm.* **1999**, *179* (1), 49−53.
(33) Larsen, A.; Holm, R.; Pedersen, M. L.; Muellertz, A. Lipid-based Formulations for Danazol Containing a Digestible Surfactant, Labrafil M2125CS: In Vivo Bioavailability and Dynamic In Vitro Lipolysis. *Pharm. Res.* **2008**, *25* (12), 2769−2777.
(34) Van Speybroeck, M.; Williams, H. D.; Tri-Hung, N.; Anby, M. U.; Porter, C. J. H.; Augustijns, P. Incomplete Desorption of Liquid Excipients Reduces the in Vitro and in Vivo Performance of Self-Emulsifying Drug Delivery Systems Solidified by Adsorption onto an Inorganic Mesoporous Carrier. *Mol. Pharmaceutics* **2012**, *9* (9), 2750−2760.
(35) Linn, M.; Collnot, E.-M.; Djuric, D.; Hempel, K.; Fabian, E.; Kolter, K.; Lehr, C.-M. Soluplus (R) as an effective absorption enhancer of poorly soluble drugs in vitro and in vivo. *Eur. J. Pharm. Sci.* **2011**, *45* (3), 336−343.
(36) (a) Kataoka, M.; Sugano, K.; Mathews, C. d. C.; Wong, J. W.; Jones, K. L.; Masaoka, Y.; Sakuma, S.; Yamashita, S. Application of Dissolution/Permeation System for Evaluation of Formulation Effect on Oral Absorption of Poorly Water-Soluble Drugs in Drug Development. *Pharm. Res.* **2012**, *29* (6), 1485−1494. (b) Anby, M. U.; Williams, H. D.; McIntosh, M.; Benameur, H.; Edwards, G. A.; Pouton, C. W.; Porter, C. J. H. Lipid Digestion as a Trigger for Supersaturation: Evaluation of the Impact of Supersaturation Stabilization on the in Vitro and in Vivo Performance of Self-Emulsifying Drug Delivery Systems. *Mol. Pharmaceutics* **2012**, *9* (7), 2063−2079.
(37) Babu, N. J.; Nangia, A. Solubility Advantage of Amorphous Drugs and Pharmaceutical Cocrystals. *Cryst. Growth Des.* **2011**, *11* (7), 2662−2679.
(38) Ozaki, S.; Kushida, I.; Yamashita, T.; Hasebe, T.; Shirai, O.; Kano, K. Evaluation of drug supersaturation by thermodynamic and kinetic approaches for the prediction of oral absorbability in amorphous pharmaceuticals. *J. Pharm. Sci.* **2012**, *101* (11), 4220−4230.
(39) Murdande, S. B.; Pikal, M. J.; Shanker, R. M.; Bogner, R. H. Solubility Advantage of Amorphous Pharmaceuticals: II. Application of Quantitative Thermodynamic Relationships for Prediction of Solubility Enhancement in Structurally Diverse Insoluble Pharmaceuticals. *Pharm. Res.* **2010**, *27* (12), 2704−2714.
(40) Gao, P.; Guyton, M. E.; Huang, T. H.; Bauer, J. M.; Stefanski, K. J.; Lu, Q. Enhanced oral bioavailability of a poorly water soluble drug PNU-91325 by supersaturatable formulations. *Drug Dev. Ind. Pharm.* **2004**, *30* (2), 221−229.
(41) Shanbhag, A.; Rabel, S.; Nauka, E.; Casadevall, G.; Shivanand, P.; Eichenbaum, G.; Mansky, P. Method for screening of solid dispersion formulations of low-solubility compounds—Miniaturization and automation of solvent casting and dissolution testing. *Int. J. Pharm.* **2008**, *351* (1−2), 209−218.
(42) Basalious, E. B.; El-Sebaie, W.; El-Gazayerly, O. Application of Pharmaceutical QbD for Enhancement of the Solubility and Dissolution of a Class II BCS Drug using Polymeric Surfactants and Crystallization Inhibitors: Development of Controlled-Release Tablets. *AAPS Pharmscitech* **2011**, *12* (3), 799−810.
(43) Li, S.; Pollock-Dove, C.; Dong, L. C.; Chen, J.; Creasey, A. A.; Dai, W.-G. Enhanced Bioavailability of a Poorly Water-Soluble Weakly Basic Compound Using a Combination Approach of Solubilization Agents and Precipitation Inhibitors: A Case Study. *Mol. Pharmaceutics* **2012**, *9* (5), 1100−1108.
(44) (a) Huang, N.; Rodriguez-Hornedo, N. Effect of Micelliar Solubilization on Cocrystal Solubility and Stability. *Cryst. Growth Des.* **2010**, *10* (5), 2050−2053. (b) Huang, N.; Rodriguez-Hornedo, N. Engineering Cocrystal Solubility, Stability, and pH(max) by Micellar Solubilization. *J. Pharm. Sci.* **2011**, *100* (12), 5219−5234. (c) Huang, N.; Rodriguez-Hornedo, N. Engineering cocrystal thermodynamic stability and eutectic points by micellar solubilization and ionization. *Crystengcomm* **2011**, *13* (17), 5409−5422.
(45) (a) Dahan, A.; Miller, J. M. The Solubility-Permeability Interplay and Its Implications in Formulation Design and Development for Poorly Soluble Drugs. *AAPS J.* **2012**, *14* (2), 244−251. (b) Dahan, A.; Miller, J. M.; Hoffman, A.; Amidon, G. E.; Amidon, G. L. The Solubility-Permeability Interplay in Using Cyclodextrins as Pharmaceutical Solubilizers: Mechanistic Modeling and Application to Progesterone. *J. Pharm. Sci.* **2010**, *99* (6), 2739−2749. (c) Miller, J. M.; Beig, A.; Carr, R. A.; Webster, G. K.; Dahan, A. The Solubility-Permeability Interplay When Using Cosolvents for Solubilization: Revising the Way We Use Solubility-Enabling Formulations. *Mol. Pharmaceutics* **2012**, *9* (3), 581−590. (d) Miller, J. M.; Beig, A.; Krieg, B. J.; Carr, R. A.; Borchardt, T. B.; Amidon, G. E.; Amidon, G. L.; Dahan, A. The Solubility-Permeability Interplay: Mechanistic Modeling and Predictive Application of the Impact of Micellar Solubilization on Intestinal Permeation. *Mol. Pharmaceutics* **2011**, *8* (5), 1848−1856.
(46) Ingels, F.; Beck, B.; Oth, M.; Augustijns, P. Effect of simulated intestinal fluid on drug permeability estimation across Caco-2 monolayers. *Int. J. Pharm.* **2004**, *274* (1−2), 221−232.
(47) Brouwers, J.; Tack, J.; Lammert, F.; Augustijns, P. Intraluminal drug and formulation behavior and integration in in vitro permeability estimation: A case study with amprenavir. *J. Pharm. Sci.* **2006**, *95* (2), 372−383.