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Research paper

Performance comparison of a co-crystal of carbamazepine with marketed product

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Abstract

The carbamazepine: saccharin co-crystal (**1**) was studied in terms of a series of attributes, including suitability for multi-gram scale-up, propensity for crystal polymorphism, physical stability, *in vitro* dissolution and oral bioavailability, with the goal of comparing **1** with the marketed form of carbamazepine (Tegretol®). Preparation of **1** was achieved on a 30 g scale with a conventional cooling crystallization process from alcohol solution without seeding. The compound is not overtly polymorphic. This finding is in contrast to the form diversity of pure carbamazepine, which has four known polymorphs and a host of solvates, including a dihydrate, which is the stable form in the presence of water. Physical and chemical stability of the co-crystal is also shown to be quantitatively similar to the pure drug in the marketed product (Tegretol®). Finally, comparison of oral bioavailability of **1** with Tegretol® tablets in dogs shows the co-crystal to be a viable alternative to the anhydrous polymorph in formulated solid oral products. The balance of properties and performance of **1** as a model co-crystal is discussed.

Keywords: Polymorphs; Pharmaceutical; Co-crystal; Saccharin; Dihydrate; Dissolution; Stability; Bioavailability

1. Introduction

Crystal form can be crucial to the performance of a dosage form [1]. This is especially true for compounds that have intrinsic barriers to drug delivery, such as low aqueous solubility, slow dissolution in gastrointestinal media, low permeability and first-pass metabolism. According to the Biopharmaceutics Classification System (BCS) [2], the nature of the physical form and formulation tends to exhibit the greatest effect on bioavailability parameters of water-insoluble compounds that need to be given orally in high doses.

Carbamazepine [2] (Chart 1), an important anti-epileptic agent that has been in use for over 30 years, is an example of a water-insoluble drug that has a high dose requirement (>100 mg/day) for therapeutic effect. Carbamazepine poses multiple challenges for oral drug delivery, including a narrow therapeutic window, autoinduction of metabolism and dissolution-limited bioavailability [3,4]. Form diversity of the pure compound is also well documented [5]. Carbamazepine crystallizes in at least four anhydrous polymorphic modifications and has been shown to form several solvates, including a stable dihydrate from aqueous solutions [4,6]. Pharmacokinetic (PK) studies in dogs using polymorphs, the dihydrate and oral solutions of carbamazepine have revealed the influence of physical form and formulation on oral bioavailability. Solutions made with cyclodextrin [7] or PEG [8] show the highest bioavailability (in terms of AUC exposure), while suspensions give high peak

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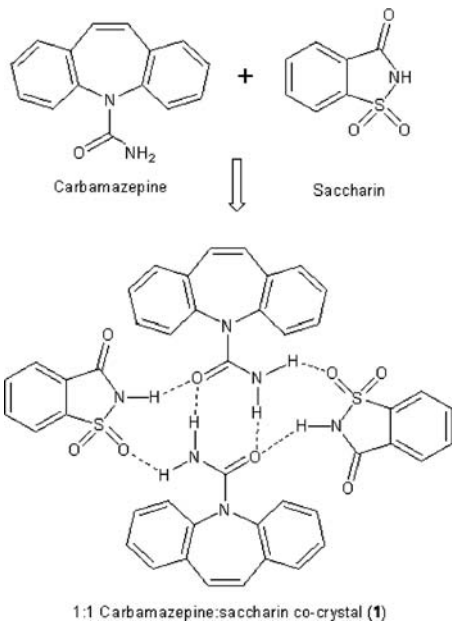

Chart 1. Components and schematic packing motif present in co-crystal **1**.

plasma concentrations. Polymorphs and hydrates differ in their PK characteristics in a manner that is correlated with dissolution profiles [8]. The strong correlation between oral bioavailability and dissolution parameters of carbamazepine tablets has been linked to moisture impact on the dosage form (slowing dissolution due to formation of the dihydrate) and particle size reduction of the drug substance (enhancing dissolution) [9]. Control over form and formulation is therefore critical to achieving the desired biopharmaceutical performance of carbamazepine oral products.

Pharmaceutical co-crystals have recently been suggested as promising materials in drug discovery and development [10]. Co-crystals are self-assembled at the molecular scale and can significantly expand the number of crystal forms of a given API over polymorphs, solvates and salts [10]. Co-crystals may also play a role in modulating the physical properties of an API, including stability, solubility and dissolution [11]. A number of co-crystals of carbamazepine have been reported [6]. The carbamazepine: saccharin co-crystal (**1** in Chart 1) has been prepared from methanol solutions and will be the focus of this paper. As yet, the area of co-crystals is relatively unexplored, especially with regards to issues of development and application in dosage forms [11].[1] For this reason, the questions of scale-up, polymorphism propensity, biopharmaceutics, and other performance parameters of 1 will be discussed. The paper will also report a performance comparison of the co-crystal to the marketed form of carbamazepine (Tegretol®, containing carbamazepine form 3).

[1] While under review, an article on the use of a co-crystal to improve the oral bioavailability of an API was published and is cited in Ref. [11]c.

2. Materials and methods

2.1. Physical characterization

Powder X-ray diffraction (PXRD) patterns were measured on a Rigaku D/Max Rapid image plate diffractometer (Rigaku/MSC, Woodlands, TX) employing Cu/Kα radiation with a 0.3 mm collimator and a 2.0 kW source, operating at 46 kV/40 mA. Data were collected in transmission mode, while oscillating about the ϕ-axis from 0 to 5° and spinning 360° about the ϕ-axis at 2 deg/s. Some PXRD patterns were also measured on a Bruker AXS D8 Discover X-ray Diffractometer. The instrument was equipped with GADDS™ (General Area Diffraction Detection System) software, a Bruker AXS HI-STAR Area Detector at a distance of 15.05 cm as per system calibration, a copper source (Cu/K_α 1.54056 Å), automated *x-y-z* stage, and 0.5 mm collimator. Thermal analyses were performed on a Q1000 mDSC and Q500 TGA (TA Instruments, Wilmington, DE). Heating rates of 10 °C/min were employed. The DSC was calibrated with a single-point method using the extrapolated onset of the melting point of a 0.275 mg sample of indium. Microscopy was performed using a Zeiss Axioplan microscope fitted with a Zeiss Axiocam type 1 SNO641 (Zeiss, Thornwood, NY). Raman acquisition was made using an Almega™ Dispersive Raman (Almega™ Dispersive Raman, Thermo-Nicolet, 5225 Verona Road, Madison, WI 53711-4495) system fitted with a 785 nm laser source. Full characterization of the co-crystal using the techniques described above is available in the supplementary material.

2.2. Crystallization of *1*

Small-scale preparation of **1** has been described [6]. Scale-up crystallization was performed in a 500 mL water-jacketed glass crystallization vessel (Part No. 607280-1024, Kontes, Vineland, NJ). Temperature was maintained by a circulating water bath. A reflux column, digital thermometer, and overhead stirrer (Arrow Engineering, Model FR4, Serial No. KG03062021) with a glass shaft and Teflon blade were attached to vessel ports. Anhydrous carbamazepine (21 g, 0.089 mol, Sigma–Aldrich, Lot 013K1381) and saccharin (16.3 g, 0.089 mol, Spectrum, Lot PTO842) were added to a reaction vessel. The solids were dissolved in 280 mL of a 62.5/37.5% v/v ethanol/methanol (both from EM Science) mixture and heated to 70 °C for 1 h under reflux. Temperature was decreased in 10 °C increments to induce precipitation in a stirred, unseeded system. Appearance of the co-crystal solid phase was first observed in the range of 60–50 °C. The temperature was further lowered to 30 °C to drive additional precipitation. Following equilibration at 30 °C, solids were isolated using a Büchner funnel and rinsed with cold ethanol. Solid recovery was 28 g (76%). Further cooling of the filtrate to enhance solids recovery was not performed. The collected colorless solid was dried in air and characterized as the

co-crystal using powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC, m.p. 174–176 °C) to confirm the identity of the co-crystal product.

An additional experiment was performed on a smaller scale to make larger crystals. To carbamazepine (85.7 mg, 0.363 mmol) and saccharin (66.2 mg, 0.362 mmol) was added an ethanol–methanol solution (62.5/37.5%v/v, 1 mL). The solution was heated to 85 °C to dissolve the solids and was then equilibrated at 70 °C for 1 h. Temperature was decreased to 30 °C in 10 °C increments at 1 h intervals. Final equilibration was allowed to occur at room temperature. Clear colorless single crystals of **1** were obtained and characterized using PXRD and DSC.

2.3. Stability

Chemical stability was determined using a gradient HPLC technique and an Atlantis dC18 5 μm 150 × 4.6 mm column, kept at 30 °C and eluted with binary mixtures of 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B) at a flow rate of 1.0 mL/min. The gradient used was from 5 to 50% B over a period of 13 min. An injection volume of 10 μL was used. Detection wavelength in the UV–visible was set at 280 nm. Linear ranges were determined by standard injections to be 2.5–250 μg/mL for carbamazepine (retention time = 2.68 min) and 2.5–250 μg/mL for saccharin (retention time = 4.78 min). Crystalline samples of the co-crystal were weighed into glass vials, capped and incubated at 5 °C, 25 °C/60% RH, 40 °C/ambient RH, 40 °C/75% RH, and 60 °C for 8 weeks and were checked for chemical stability by HPLC at 2, 4, and 8 weeks. Samples of **1** and carbamazepine form 3 were incubated at 25 °C/60% RH, 40 °C/ambient RH, and 40 °C/75% RH for 2 weeks before physical stability assessment by PXRD using a Bruker GADDS system. A sample of the co-crystal, prepared by dry-grinding with microcrystalline cellulose (Avicel, PH-102), was incubated at 5 °C, 25 °C/60% RH, 40 °C/ambient RH, 40 °C/75% RH, and 60 °C for 1 week.

2.4. In vitro dissolution of dosage forms

Dissolution of carbamazepine into SGF was measured using USP dissolution apparatus (Vankel, paddle method) connected to a Cary 50 spectrophotometer (Varian, Palo Alto, CA) at a wavelength of 320 nm. Absorbance was monitored at 320 nm in order to eliminate the effect of saccharin, which does not absorb significantly at that wavelength. Bath temperature was maintained at 37 °C and absorbance was measured at 5, 10, 15, 20, 25, 30, 40, 50, 60, 90, and 120 min. SGF was prepared with 2 g/L NaCl and 1 g/L Triton X-100, and acidified to pH 2 with HCl. Samples of **1** were prepared from crystallization of ethanol solutions at different temperatures and cooling ramps. Crystallization batches were combined and separated via metal mesh sieves (VWR, mesh sizes: 53, 150, 300, 500, and 1000 μm) and were measured into gelatin capsules. Eighteen capsules were prepared with particle sizes ranging from less than 53 μm to greater than 1000 μm (three capsules for each particle size). Capsules containing 355 mg (±3.5 mg) of **1** (corresponding to 200 mg of carbamazepine) were suspended in 900 mL of paddle-stirred SGF at a stir speed of 100 rpm.

2.5. Polymorphism study

High-throughput crystallization trials were performed using CRYSTALMAX®technology [12]. A total of 480 crystallization trials of carbamazepine with saccharin in various solvents and solvent mixtures were carried out in five 96-well blocks. The individual mixtures were dispensed into the tubes from an organic solvent using Tecan miniprep (TECAN, Durham, NC) and dried under a stream of nitrogen (TurboVap 96, Zymark Corp., Hopkinton, MA). Water and/or organic solvents were dispensed combinatorially by a Cartesian SynQuad 32-channel dispenser (Cartesian Technologies, Irvine, CA). The mixtures were heated to 70 °C for 2 h followed by a 1 °C/min cooling ramp to 5 °C. Triplicates of each composition were prepared; identical samples were located in different arrays in each case. Each tube in a 96-tube array was sealed within 15 s of combinatorial dispensing with a Teflon-coated crimp top to avoid evaporation of organic solvents. A selection of 12 solvents was used as single solvents or as various binary solvent combinations. The samples were checked daily to identify solid materials that had crystallized over time. A total of 156 solid materials were recovered over a period of 2 weeks and were collected, quenched and characterized using Raman spectroscopy and PXRD.

Mechanical grinding experiments were conducted by adding a small molar excess of saccharin to solid carbamazepine. Typical experiments contained 10 mg of carbamazepine and 5–10 mg of saccharin. A total of 24 solvents (10 μL) were used as additives in experiments designed to identify alternative polymorphs of the carbamazepine: saccharin co-crystal. The samples were ground for 20 min using a mechanical shaker and powders characterized using PXRD.

Slurry conversion experiments in seven solvents were conducted. Solvent (100 or 200 μL) was added to the co-crystal (20 mg) and the resulting suspension was stirred at room temperature for four days. After four days, the solvent was decanted and the solid material was dried under a flow of nitrogen for 5 min. The remaining solids were then characterized using PXRD. A second slurry experiment was carried out in water: carbamazepine dihydrate (110 mg), carbamazepine: saccharin (170 mg) and saccharin (70 mg) were slurried in water (10 mL) for one day. After one day a sample was removed from the slurry and was centrifuged in an eppendorf tube equipped with a filter (0.2 μm). The solid sample was then characterized by PXRD as the carbamazepine: saccharin co-crystal.

2.6. Dog bioavailability study

The pharmacokinetics of **1** was evaluated in four fasted beagle dogs at MDS Pharma Services (Montreal, Canada). Facility meets the requirements for the care and use of experimental animals established by the Canadian Council on Animal Care (CCAC), protocol #AA18272. Tegretol® 200 mg tablets (immediate release formulation) were obtained from a pharmacy and used as the reference dose. A prototype oral dosage form in an HPMC capsule (Shionogi Qualicaps) containing **1** was prepared by mixing α-lactose monohydrate (2.52 g, 0.0069 mol, Sigma, Lot 43254338) with **1** (3.654 g, 0.087 mol, particle size <53 μm) and blending the two solids in a mortar and pestle. Capsules containing 600 mg of formulated co-crystal (corresponding to 200 mg of carbamazepine) were prepared. Both dosage forms were administered orally to four animals in a 2-way cross-over design. Plasma samples were collected pre-dose and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, and 12 h post-dose. Plasma samples were analyzed by a validated liquid chromatography-tandem mass spectrometry (LC/MS/MS) (Applied Biosystems/MDS Sciex API 365, equipped with a Turbo Ionspray source operating in positive ion mode). Sample preparation was performed using protein precipitation coupled with on-line column switching. The analytical reference standard of carbamazepine (Lot No. 29H0459) was used for all the analytical support work carried out in this study. The precursor-to-product ion transitions were used as follows: carbamazepine at m/z 237.0 → 194.1 and Internal Standard (Lorazepam) at m/z 321.0 → 275.1. Pharmacokinetic parameters were calculated (non-compartmental analysis) using Excel software.

3. Results and discussion

In an evaluation of a new crystal form for development of a solid oral dosage form, a series of questions require consideration: (1) Can the material be scaled up with a process that can ultimately be transferred to a large scale? (2) Does the compound display polymorphism, and if so, how does a change in physical form affect performance criteria? (3) Can the material be formulated and processed without change in physical form? (4) Does the compound have the appropriate oral PK performance? These questions prompted us to assess the 'developability' [13] of the 1:1 carbamazepine: saccharin co-crystal (**1**) [6]. Specifically, we sought to address the suitability of a model co-crystal as an alternative to the form in currently marketed versions of carbamazepine. What follows is our comparative analysis of **1** with marketed tablets of carbamazepine (Tegretol®, carbamazepine form 3).

3.1. Scale-up of *1* for pre-clinical studies

The primary objective of crystallization scale-up was to identify a conventional process for making **1** from solution. Based on solubility measurements of carbamazepine in ethanol and methanol as a function of temperature, it was found that **1** could be crystallized by cooling alcohol solutions containing the dissolved components. Scale-up of **1** was successfully achieved on a 30 g batch size using a 62.5% ethanol/37.5% (V/V) methanol mixture. The procedure requires no seeding, as the 1:1 co-crystal is the phase that nucleates in the solution in the supersaturated state. Fig. 1 shows photomicrographs of products from two different crystallization trials. As expected, crystal growth was found to be a function of cooling rate and incubation time. Near-saturation crystallization resulted in very large (millimeter size) crystals of the desired product, as shown in Fig. 1a. Rapid cooling of alcohol solutions lead to smaller crystals, as shown in Fig. 1b. The yield of co-crystals was not optimized, since it was not the goal of the study to generate a commercial process. Nonetheless, a 76% mass recovery of pure **1** was readily demonstrated. In general, plate-like morphology was observed from all crystallization trials. The conclusions from exploratory crystallization scale-up are: (a) significant quantities of **1** can be readily obtained by a conventional cooling crystallization from alcohol solution in a controlled process, and (b) further scale-up and optimization appears feasible to provide even larger scale batches of the material.

3.2. Polymorphism tests with the co-crystal

In our crystallization experiments only a single crystalline form of **1** was obtained, based on PXRD[2]. A question remains whether a co-crystal of carbamazepine has reduced polymorphism tendency relative to pure carbamazepine, which has four known polymorphs [5]. In order to further assess the polymorphism potential of the co-crystal, several experiments including a high-throughput solvent-mediated crystallization screen, grinding experiments in the presence of diverse solvents and slurry conversion in various solvents were carried out. Several forms of carbamazepine were obtained from these experiments, including two of the anhydrous polymorphs (2 and 3) [5], the dihydrate, two known solvates [6] and **1**. The co-crystal material consistent with the published structure [6] was obtained from several different solvents and was the only form of 1 observed based on PXRD analysis, as shown in Fig. 2. Known forms, such as the dihydrate, solvates and anhydrous polymorphs of the parent carbamazepine and saccharin, could account for all other patterns (Table 1).[3]

Formation of **1** was also probed using mechanical grinding [14]. Fig. 3 shows an overlay of PXRD patterns of the co-crystal obtained from evaporation of methanol

[2] See supplementary material for full characterization data of the 1:1

[3] The powder patterns for all forms of carbamazepine, carbamazepine dihydrate, co-crystal **1** and solvates can be obtained from the single crystal data available in the Cambridge Structural Database: ConQuest v. 1.8; CSD v. 5.27; May 2006 update.

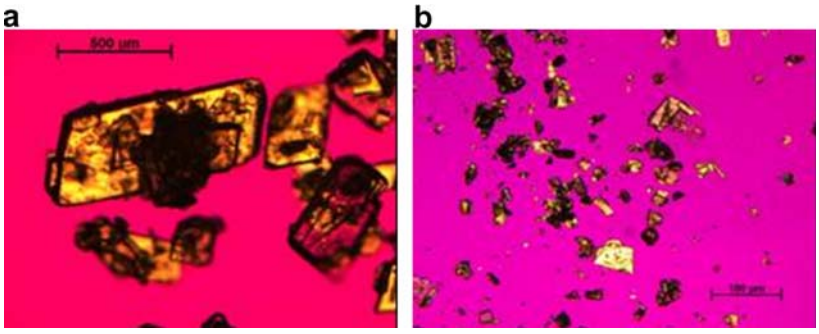

Fig. 1. Photomicrographs (5× objective) of samples of **1** obtained from cooling crystallization experiments from ethanol/methanol solutions. (a) 500–1000 µm particle size. (b) 53–150 µm particle size.

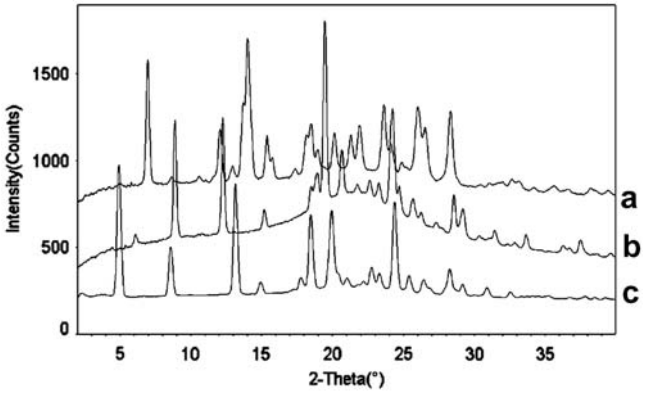

Fig. 2. Overlay of PXRD patterns for several forms of carbamazepine. (a) Carbamazepine: saccharin co-crystal; (b) carbamazepine dihydrate; (c) stable form of carbamazepine (form 3). The different forms are easily distinguished from the PXRD patterns, with a peak at $2\theta = 7.0°$ used to identify presence of co-crystal **1** and a peak at $2\theta = 8.9°$ used to identify presence of the dihydrate of carbamazepine.

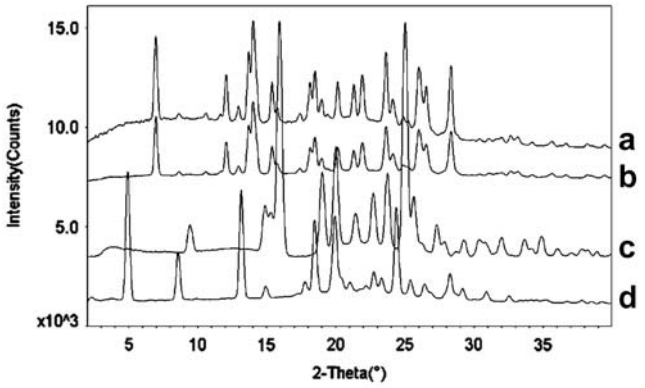

Fig. 3. Representative PXRD patterns of carbamazepine saccharin co-crystal and starting materials. (a) **1** obtained from solution; (b) **1** obtained from grinding; (c) pure saccharin; (d) pure carbamazepine form 3.

Table 1
Summary of forms obtained from the HT polymorph screen

Solvent conditions	Formation of co-crystal **1**	Form obtained
Ethyl acetate, isopropyl acetate, dichloromethane, methanol, and others	Yes	Carbamazepine: saccharin co-crystal [6] (**1**)
Toluene	No	Carbamazepine form 2
THF	No	Carbamazepine form 3
Water[a]	No	Carbamazepine dihydrate
DMSO[a]	No	Carbamazepine: DMSO solvate [6]
Acetic acid[a]	No	Carbamazepine: acetic acid solvate [6]

Form assignment was made based on the previously published characterization data for the polymorphs, solvates and co-crystal of carbamazepine.

[a] Some vials contained other solvents in addition to those listed in the table.

solutions, co-crystal from grinding, pure saccharin and carbamazepine form 3. The grinding experiments were carried out in the presence of 23 solvents plus the dry condition. In all grinding experiments, only **1** was obtained, as identified by PXRD.

Solvent-mediated polymorph transformations are an effective method to screen for both metastable and thermodynamically stable polymorphs [15]. Slurry conversion was attempted in an effort to find a polymorph of **1**. This experiment also served to measure the physical stability of **1** in seven different solvents. No discernible form conversion or dissociation was observed in water, acetone, ethanol, methanol, isopropanol, cyclohexane, and *n*-heptane at room temperature for four days (based on PXRD). The observation that no dissociation occurs in the solvents indicates that the co-crystal is resistant to conversion to carbamazepine or a solvate of carbamazepine under those conditions. Strikingly, no detectable conversion to the carbamazepine dihydrate was observed when stirring 20 mg of **1** in approximately 200 µL of water for four days at room temperature. Clearly, in concentrated aqueous suspension (less than 5% of pure drug dissolved), **1** is resistant to formation of the dihydrate. This finding is in contrast to published results on the rapid conversion of polymorphs of pure carbamazepine to the dihydrate [8,16]. This is likely due to the fact that the slurry experiments were carried out at room temperature in aqueous solutions which did not contain any surfactants, which have been shown to

accelerate the rate of conversion of anhydrous carbamazepine to the dihydrate [8,16]. In order to determine whether the co-crystal or the dihydrate is the stable form in aqueous suspensions, a slurry experiment was carried out with one part each of co-crystal **1**, carbamazepine dihydrate and saccharin. PXRD analysis of a sample removed after 24 h indicated that the co-crystal had formed in the slurry and the dihydrate was not detectable by PXRD. In summary, the polymorph screening experiments indicate that from a total of about 550 experiments, only one form of **1** was obtained and **1** possesses physical stability relative to the dihydrate in concentrated aqueous suspension at room temperature.

3.3. Stability and in vitro dissolution

Co-crystal **1** was found to have comparable chemical stability to carbamazepine form 3 (the polymorph found in Tegretol® tablets). Neither material chemically degrades to a measurable extent in the solid-state, based on HPLC analysis of stability samples after two months in capped, unsealed vials at 5, 40, and 60 °C at ambient humidity. Both carbamazepine form 3 and the co-crystal degrade slowly in open vials at the higher humidity conditions of 25 °C/60% RH and 40 °C/75% RH. Both the co-crystal and carbamazepine form 3 exhibit the same degradation pattern. The ratio of degradate peaks is essentially the same for both materials and the total area percentage of degradates after two months at 40 °C/75% RH is 0.12 and 0.15% for carbamazepine form 3 and **1**, respectively, representing a slight growth from initial samples.

The physical stability of **1** toward dihydrate formation was compared to anhydrous carbamazepine form 3. The co-crystal and form 3 were stored in open containers at 25 °C/60% RH, 40 °C/ambient RH, and 40 °C/75% RH for 2 weeks. The samples were characterized using PXRD after 1, 2, 4, 7, and 14 days. No form change could be detected by PXRD (within an estimated 5% detection limit) in samples of **1** or of the marketed form 3, after 2 weeks at 40 °C/75% RH. Both forms appear to be physically stable at accelerated stability conditions. From the experiment described above, it is concluded that **1** is resistant to hydrate formation over a period of 2 weeks under direct (unpackaged) exposure to ICH storage conditions. This finding is consistent with observations made on a shorter time scale in slurry conversion experiments (vide supra).

Dissolution experiments in SGF were performed in order to understand the relationship between particle size and the dissolution profile of **1**. These studies focused on the initial rate of dissolution, which has been shown to correlate to increased overall absorption and improved bioavailability [17]. As expected, faster initial dissolution was observed with smaller particle size of **1**, presumably as a result of increased surface area. Sieved co-crystal fractions of particles less than 150 μm showed the fastest initial dissolution, as shown in Fig. 4a. All suspensions were supersaturated with respect to the dihydrate, yet dissolution

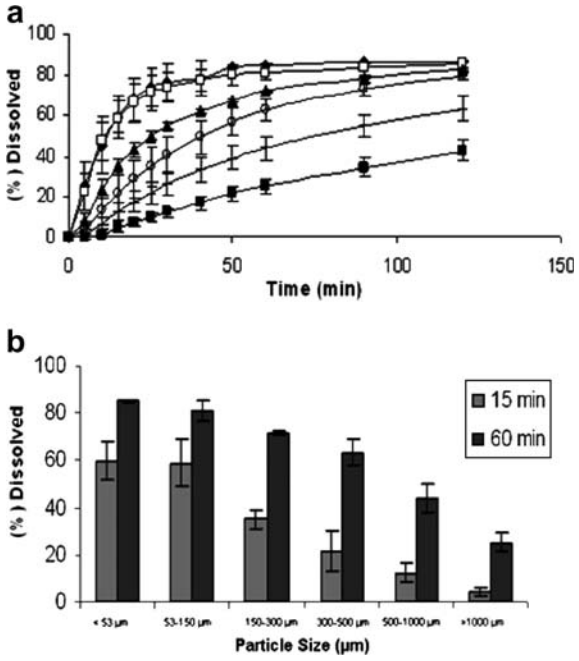

Fig. 4. (a) Mean dissolution profiles ($n = 3$, ~355 mg of co-crystal corresponding to 200 mg of pure drug used for all sieved fractions) of various sizes of **1** in SGF at 37 °C (■, >1000 μm; ×, 500–1000 μm; ○, 300–500 μm; ▲, 150–300 μm; □, 53–150 μm; ♦, <53 μm). (b) Extent of dissolution at 15 and 60 min for various particle sizes of **1** in SGF at 37 °C. Grey bars: mean % ($n = 3$) carbamazepine from **1** dissolved at 15 min; black bars: mean % ($n = 3$) of carbamazepine from **1** dissolved at 60 min.

was essentially complete in the cases where particle size is smaller than 500 μm. The dissolution rates for sieved fractions greater than 500 μm are slowed to the point that carbamazepine concentrations are significantly lower than those from the smaller particles after 2 h at 37 °C. Fig. 4b represents a bar graph of percentage dissolved vs. particle size for all sieved fractions at two time points, 15 and 60 min. Fig. 4b indicates that sieved fractions greater than 500 μm were less than 50% dissolved after 60 min, whereas sieved fractions of less than 150 μm particles were greater than 80% dissolved at the same time point. Solids from the larger particle size crystals (500 μm to greater than 1 mm) were recovered after being suspended in SGF overnight and were found to contain a mixture of co-crystal 1 and carbamazepine dihydrate by PXRD. Presence of the dihydrate was confirmed from the diffraction peak at $2\theta = 8.9°$ (see Fig. 2). It is likely that conversion to the dihydrate on the surface of the larger crystals is responsible for the lower concentrations of carbamazepine observed. Further detailed evaluation of conversion to dihydrate as a function of time and particle size was not conducted as part of this study.

The overall conclusions regarding the stability of **1** are that (i) chemical stability in the solid-state appears to be similar to the marketed form of carbamazepine, (ii) physical stability of **1** is comparable to the anhydrous polymorph form 3, and (iii) **1** shows a dependence of dissolution rate on particle size above 150 μm and a resistance of the

smaller particle sizes to conversion to dihydrate in aqueous suspension on the time scale of drug absorption. Regarding the last point, it is apparent that aqueous solubility of carbamazepine has not been affected by co-crystal formation to such a degree to completely relieve the known dissolution rate limitation of carbamazepine. The test of this prediction is an oral bioavailability comparison of **1** with carbamazepine.

3.4. Comparison of bioavailability of *1* and Tegretol® in dogs

A probe into the oral pharmacokinetics (PK) of **1** was conducted in dogs comparing the co-crystal with a commercial formulation (Tegretol® immediate release tablets, 200 mg strength). For the purpose of this preliminary PK study, ground **1** (less than 53 μm particle size by microscopy, consistent with good dissolution in SGF; see Fig. 4a) was mixed with lactose in a dry blending step and placed in HPMC capsules for dosing. In vitro dissolution experiments carried out on formulated co-crystal containing lactose showed no effect on the rate of dissolution or the concentration of carbamazepine as a result of adding lactose. Release from the capsule was also found to be consistent with that of the micron-sized co-crystal (e.g., Fig. 4a). The study was conducted at a dose equivalent to 200 mg of anhydrous carbamazepine. Comparison of plasma concentration of carbamazepine as a function of time for Tegretol® and **1** in fasted beagle dogs is shown in Fig. 5.

Much like previous studies using polymorphs of carbamazepine [7,8], absorption of carbamazepine from **1** occurs relatively quickly in dogs, with the peak levels observed within about 1 h on average (individuals ranged in Tmax from 0.25 to 2 h). Comparison of PK of **1** vs. Tegretol® tablets shows AUC[4] and Cmax values were higher for **1** with similar Tmax values (1.0 h for the co-crystal vs. 1.75 h for Tegretol®). As an average trend, the co-crystal shows higher plasma levels than Tegretol®, however, no statistically significant differences were observed between pharmacokinetic parameters of Tegretol® and **1**, as suggested by calculated p values (Student's t test). We conclude from this preliminary PK study with four dogs that the PK parameters of carbamazepine are comparable when the drug is administered as **1** or as Tegretol® tablet.

Current marketed carbamazepine formulations include several generic versions of carbamazepine and two controlled release formulations (Tegretol XR® and Carbatrol®) and a recently introduced formulation for bipolar disorder (Equetro™). All of these formulations are ostensibly based on the use of anhydrous polymorphs (presumably form 3), and none contain saccharin. The amount of saccharin delivered by **1**, assuming the highest approved dose of the drug, would be close to current limits based on acceptable use levels [18]. At this point, other biopharmaceutical and pharmacological considerations, such as a narrow therapeutic index [19], dose-dependent autoinduction of metabolism [20] and efflux [21], appear to overshadow issues of dissolution, physical stability and polymorphic tendency of the pure drug. Hence, the co-crystal in this case serves as a model for addressing the physicochemical problems of a pharmaceutical material, but cannot overcome issues of metabolism and pharmacology.

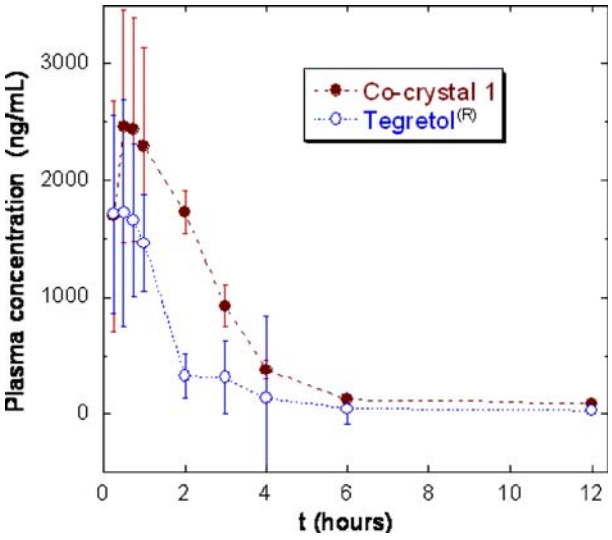

Fig. 5. Average plasma time curves of carbamazepine concentrations (±SEM) from a cross-over experiment in fasted beagle dogs ($n = 4$) given oral doses of 200 mg of the active drug as Tegretol® tablets and co-crystal **1**.

4. Conclusions

The present study illustrates the utility of a co-crystal as a type of material that is suitable for drug development. The benefits of using the carbamazepine: saccharin co-crystal include (i) relative lack of polymorphism and equivalent chemical stability to the anhydrous polymorph, (ii) favorable dissolution properties and suspension stability, and (iii) comparable oral absorption profile in dogs compared with the commercial immediate release product. Given the surge of activity in the field of co-crystals, we expect that many other co-crystal systems will be explored in the near future. It seems reasonable to assert that co-crystal approaches should be considered routinely as part of a broader set of form and formulation explorations to strive for the best possible drug products.

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[4] The AUC's and other pharmacokinetic parameters from the in vivo study are given in the supplementary material.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2006.12.016.

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