Investigating the Hydrate Conversion Propensity of Different Etoricoxib Lots

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ABSTRACT: The physical stability of bulk active pharmaceutical ingredients (API) is of significant scientific and regulatory concern. Carrying out physical stability testing on lots with varying rates of hydrate conversion can potentially lead to erroneous conclusions if these rate differences remain unknown and unstudied. The lot dependency of etoricoxib's rate of hemihydrate conversion was investigated and a quick discriminatory technique was developed to qualitatively assess relatively slow to rapidly converting lots. This novel technique was also used to screen potential parameters affecting the hydrate conversion rate such as particle size/surface area, amorphous content, and initial hemihydrate content. Based on qualitative X-ray powder diffraction (XRPD) and quantitative Raman data, significant effects on the rate of hydration were observed with the addition of small amounts of amorphous etoricoxib. Furthermore, it was found that the presence of hemihydrate also increased the rate of conversion by seeding anhydrous etoricoxib. This suggests that the initial presence of the hydrate form can cooperatively accelerate conversion. A better understanding of the factors affecting hydrate conversion rates resulted in the appropriate selection of storage conditions for both the bulk API and the formulated product. J Pharm Sci 95:56–69, 2006

Keywords: X-ray powder diffractometry; Raman spectroscopy; hydrate; polymorphism; amorphous; milling; compression; particle size; lot dependency

INTRODUCTION

The physical stability of active pharmaceutical ingredients (API) is of significant scientific and regulatory concern. Accelerated solid-state stability testing is used extensively in the pharmaceutical industry to enable scientists to predict the physical and chemical stability of a drug substance and product. Understanding the kinetics and conditions of these changes are paramount to many drug discovery programs. The lot dependency on the rate of hydrate formation puts into question the meaningfulness of accelerated solid-state stability studies when unknowingly dealing with an aberrant lot. The selection of excipients, storage conditions for both API and drug product would be quite different if carried out with API lots that have different solid-state conversion rates.

Several important properties may vary between API lots. Variations in particle size and surface area can have significant impact on a drug's bioavailability.[1] Very small amounts of chemical impurities can also result in significant changes in the physical appearance of the API such as morphology, color, and even activity. Physical form impurities, including the presence of polymorphic forms, solvates, hydrates, and the amorphous phase, can be equally as undesirable and often times not as easily detectable since they require solid-state techniques which can be challenging

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at low impurity concentrations. Another important aspect when working with hydrates is defining the behavior of the anhydrate. For example, in the case of theophylline, formation of the metastable anhydrate form showed a dramatic decrease in dissolution rate which could ultimately affect bioavailability.[2]

Solid-state characterization of polymorphs and hydrates is well understood and documented,[3–5] however, characterizing the cause for lot variability can be more difficult. Furthermore, small physical differences can have a significant impact that can go undetected due to instrumental limitations. Several researchers, however, have successfully employed the study of powder surface energetics to account for batch to batch variability of materials.[6–9] Inverse gas chromatography (IGC) has become an important analytical tool for surface characterization with many different types of pharmaceutical applications.[10]

Successfully accounting for lot variability nonetheless remains difficult. For example, it is quite difficult to identify the presence of a small amount of amorphous component in a primarily crystalline material.[11] Typical instrumentation for measuring such changes include X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC) which can prove challenging.[12–14] Water vapor sorption studies can be carried out in circumstances where the amorphous form is much more hygroscopic than the crystalline phase.[15] Several other less common techniques such as IGC, microcalorimetry, near infra-red (NIR), Raman, and solid-state nuclear magnetic resonance (SSNMR) spectroscopy have all had success at identification and in some cases quantitation of the elusive amorphous form in predominantly crystalline material.[16–20] In some cases, however, routine analysis of several API lots using these techniques might not indicate any significant differences.

Bauer et al.[21] developed a sensitive indirect method of detecting low levels of a conformational polymorph of Ritonavir, an AIDS drug. The conformation polymorph was the "*cis*" form, Form II, which corresponded to a more stable packing conformation but would only nucleate in highly supersaturated solutions. This more stable form had drastically lower solubility compared to the "*trans*" conformation. The detection test was based on the differences in solubility of the Form I and Form II and the ability to seed a solution of Form I with Form II. This novel test for indirectly monitoring the levels of a minor phase in a predominant phase was validated down to 1 ppm and proved to be more sensitive than any other direct measurement technique.

The focus of this article is to characterize the factors contributing to lot dependency on the relative rate of hydrate formation of etoricoxib, a cycloogenase-2 inhibitor (COX-2), used for the treatment of osteoarthritis, rheumatoid arthritis, and dental pain.[22,23] The relative rate of hydrate formation varied from quite slow (hours) to fast (seconds) with no easily detectable physical or chemical differences. A novel test was developed to qualitatively screen several lots to determine the extent of the conversion rate variability. Additionally, the test was also used to determine the sensitivity of etoricoxib to the presence of amorphous and initial hydrate content on the rate of hydrate formation. Due to the sequential discovery of etoricoxib's five polymorphic forms, many of the studies first focused on Form I, followed by Forms IV and V. The thermodynamic relationship between all five forms will be the subject of another article but, in this case, has little bearing on hydrate conversion. The results from these experiments proved useful in the selection of suitable excipients and moisture barrier packaging required to prevent conversion of the API to the hydrate.

MATERIALS AND METHODS

Materials

Etoricoxib, the API used in this study, is a nonhygroscopic low molecular weight proprietary drug used for the treatment of arthritis and pain (Merck Research Laboratories, Rahway, NJ).[22,23] Five anhydrous and two hydrate forms have been discovered to date.[24] Principally, the study will focus on Forms I, III, IV, V, and the hemihydrate. Unless otherwise specified, all samples had been pin-milled. Form III was obtained upon dehydration of the hemihydrate, formed by heating the hemihydrate at 100°C for 1 h.

The amorphous form was obtained by rapidly cooling the melt. Etoricoxib was heated for 1 h at 185°C, approximately 50°C above its melting point. The sample was then allowed to cool to room temperature and finally ground into a fine powder using a mortar and pestle. XRPD and DSC were used to confirm the absence of crystallinity.

The hemihydrate form was prepared by mixing excess anhydrous etoricoxib with distilled/deionized water (1 g etoricoxib in 50 mL water) for

4 h at room temperature. The solids were collected by centrifugation and allowed to air dry in a fumehood overnight. Hemihydrate conversion was confirmed by XRPD and thermogravimetry (TGA).

Blend Preparation

Physical mixtures used in this study were prepared by gentle grinding with an agate mortar and pestle for 90 s.

Ball Milling

Approximately 400 mg of Form I (lots 020, 032, 038, 042) was placed in a small polyethylene container containing three glass beads. A Wig-L-Bug amalgamator (Crescent Dental Mfg. Co., Lyons, IL) reduced particle size through the shearing action of colliding glass beads with rapid oscillations preset for a duration of 1, 5, 10, 20, 30, and 120 min.

Compression

Form I (lots 020, 032, 038, 042) was compressed into compacts using a Carver Press (Summit, NJ) with a force of 13.3 kN with a dwell time of 20 s. A round 0.8 cm punch and dye assembly was used to prepare 100 mg compacts.

Methods

Hydrate Screen Test (HST)

An agate mortar and pestle was used to gently stir 200 mg of etoricoxib for 1 min with 0.5 mL of distilled/deionized water. The wet mass was quickly deposited onto an X-ray low background holder measuring 4.9 cm^2 in diameter. Diffractograms were collected 1 min after the samples were prepared except for experiments with Form I and amorphous mixtures where delay times of 15 min were used prior to data collection. The amorphous blends were slightly slower to convert resulting in changes occurring in the diffraction patterns during the data collection. The additional delay time provided more consistent results, which showed reproducible and measurable levels of conversion. Qualitative X-ray evaluation of hydrate conversion was based on 3–5 measurements.

XRPD

XRPD patterns were measured using a Scintag XDS-2000, Si(Li) Peltier-cooled solid state detector using a voltage of 45 kV and an amperage of 40 mA. Divergent beam slits of 2 and 4 mm were used as well as receiving slits of 0.5 and 0.2 mm. Step scan mode was employed using a step size of 0.02°, 2θ with a 2 s count time. Samples were analyzed from 2 to 40°, 2θ using a quartz insert with sample spin. The instrument alignment was verified using a corundum disk (NIST SRM 1976).

XRPD On-Line Monitoring of Form IV to Hemihydrate Conversion. An aqueous Form IV slurry was prepared by mixing 0.5 mL of water to 200 mg of Form IV. The slurry was quickly deposited on a XRPD quartz low background holder for analysis. A flow through moisture generator (MB-200, VTI) was used to minimize evaporation by maintaining high humidity (95% relative humidity (RH)). The extent of hydrate conversion was determined by measuring the characteristic hemihydrate peak at 16.9°, 2θ and the decrease in the Form IV peak at 18.7°, 2θ. Several consecutive scans ranging from 15 to 20°, 2θ using a 0.02°, 2θ step size and 2 s count time were used to obtain the conversion profile.

Raman Spectroscopy

A Bruker RFS 100/S FT-Raman (Billerica, MA) employing a Nd:YAG laser with an excitation wavelength of 1064 nm running OPUS NT (V3.1) and Quant 2 software was used for quantitation of the test solids recovered from the XRPD stage. The laser power was set to 250 mW with a scan acquisition range 1520–1650/cm, a scan resolution of 4/cm, an aperture setting of 3.5 mm and a scan number of 64. Each sample was re-packed and re-analyzed ten times in 2 mm diameter metal sample holders. Spectral preprocessing using the first derivative and multiplicative scattering correction was applied to the spectra prior to quantitation. The instrument was verified for peak position and intensity using a sulfur standard (Bruker) and CCl_4 (Aldrich, Milwaukee, WI).

Raman Calibration Curve. The calibration curve employed was obtained from the analysis of several hemihydrate/Form V bulk drug blends ranging from 0% to 100% w/w hemihydrate. The limit of detection (LOD) was determined based on the precision and accuracy of several, additional, hemihydrate/Form V blends ranging from 0% to 5% w/w hemihydrate. For the purpose of this study, the LOD is defined as $\geq$3 times the standard deviation from the actual target value. The

variability in quantitation was approximately 0.3% from the target value, which helps to justify a LOD of 1% w/w hemihydrate. The analysis of several Form V lots showed only minimal variability (~0.3%) from the expected 0% w/w. Quantitated values consisting of ≥1% w/w hemihydrate are considered fairly accurate and representative. Extensive studies on the physical stability of the hemihydrate form have shown that the hydrate is stable for several weeks at ambient conditions and unaffected by the Raman laser.

DSC

Purity determination was carried out in duplicate using a Seiko RDC-220 robotic DSC under 60 mL/min of nitrogen. A heating rate of 2°C/min was used for both the Form I API (lots 020, 032, 038, and 042) and the indium standard (Goodfellow, Cambridge, UK, 99.99%) in crimped aluminum pans. The DSC purity calculations were obtained using the van't Hoff equation from the enthalpy of fusion and the melting temperature.[25] The glass transition temperature of etoricoxib was determined by reheating the rapidly cooled melt at 10°C/min. The DSC is calibrated for temperature and heat flow using gallium (Goodfellow, 99.99%), indium (Goodfellow, 99.999% pure), and tin (NIST SRM 2220) yearly and verified weekly using indium.

Thermogravimetric Analysis (TGA)

Thermogravimetric analyses were carried out using a Seiko RTG-220 robotic TGA from 30 to 215°C at 10°C/min under 60 mL/min of nitrogen. The TGA was calibrated monthly for weight and temperature calibration using a standard 20 mg weight (Troemner) and indium (Goodfellow, 99.999% pure), respectively.

Scanning Electron Microscopy (SEM)

A JEOL JSM-820 scanning electron microscope (JEOL, Peabody, MA) employing an acceleration voltage of 5.0 kV was used to magnify the ball milled Form I lots 1400–1500×. The samples were gold sputtered coated using an Edwards Auto306 gold sputter coater (Edwards, Wilmington, MA).

Moisture Balance

The moisture sorption isotherms were determined at 25°C using a dynamic moisture balance (MB-300G) (VTI Corp., Hialeah, FL) calibrated for weight with 10–100 mg standard weights (Troemner-S/N: 02588) and for moisture with polyvinylpyrrolidone K-90 (ISP Technologies, Wayne, NJ). Sorption equilibrium for each RH was determined when an equilibrium weight change criterion of 1 μg occurred for three consecutive 7-min periods for a maximum of 120 min. Samples were dried *in vacuo* at 40°C for 120 min in the moisture balance prior to analysis ($n = 1$).

RESULTS AND DISCUSSION

Polymorphs and Hydrate Formation

Etoricoxib consists of four anhydrous polymorphic forms (Forms I, II, IV, and V) of similar thermodynamic stability and a hemihydrate form physically stable at ambient conditions (≥32% RH).[23,24] The fifth anhydrous polymorph, Form III, is metastable. Forms I, II, and III were identified prior to Phase I, while Forms IV and V appeared later in development. The potential for hydrate conversion was identified early in predevelopment and was therefore closely monitored and studied. The anhydrous crystalline forms are nonhygroscopic, but all convert to the hemihydrate form when in contact with water (Fig. 1). The amorphous form initially converts to Form III in water followed by conversion to the hemihydrate.

Lot Dependency on Hydrate Formation

Initial studies with Form I suggested that complete hemihydrate conversion typically occurred within 2 h of stirring in water. However, it was later discovered that hydrate conversion of a Form IV lot occurred within a matter of a few minutes.

The significant differences in the diffractograms of the anhydrous form (Form IV) and the hemihydrate (Fig. 2) combined with the rapid hyd-

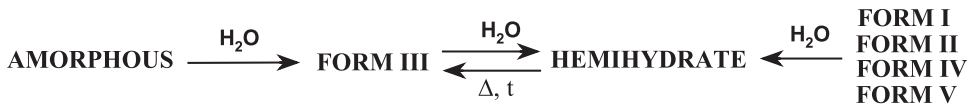

Figure 1. Polymorph relationship of etoricoxib with the hemihydrate form.

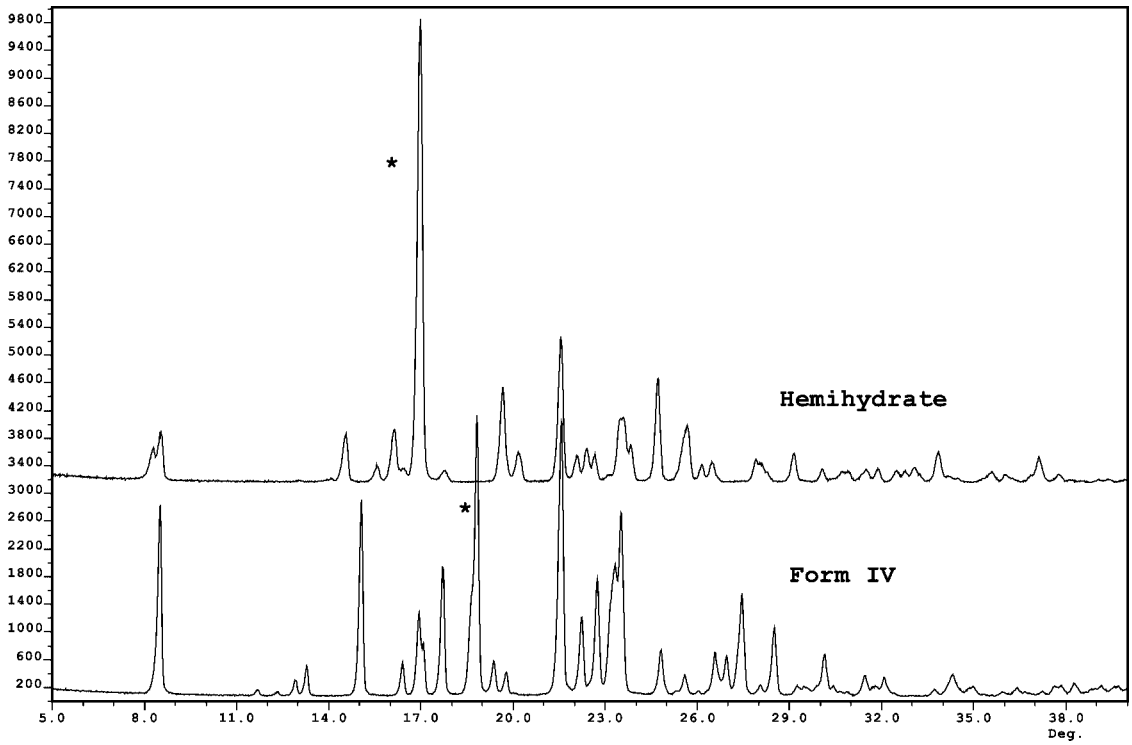

Figure 2. X-ray powder diffraction (XRPD) diffractograms of Form IV and the hemihydrate. Peaks marked by * were used for *in situ* monitoring of the conversion from Form IV to the hemihydrate.

rate conversion enabled *in situ* XRPD monitoring of aqueous slurries. The extent of hydrate conversion was determined by monitoring the increase of a characteristic hemihydrate peak at 16.9°, 2θ and the concomitant decrease of the Form IV peak at 18.7°, 2θ as a function of time in the API/water slurries (Fig. 3). Initial conversion was detected in <10 min while complete conversion occurred after 35 min. Note that Form IV also contains a crystalline reflection at 16.9° 2θ, but its peak area is roughly nine times smaller than that of the hemihydrate's. Also, only formation of the hemihydrate could account for the growth of the crystalline reflection at 16.9°, 2θ. The slurries were maintained under high humidity (95% RH) using a flow through moisture generator to minimize evaporation. Thermogravimetry confirmed that complete conversion had taken place under these circumstances where a 2.5% weight loss was obtained corresponding to 0.5 mol of water to mol of etoricoxib.

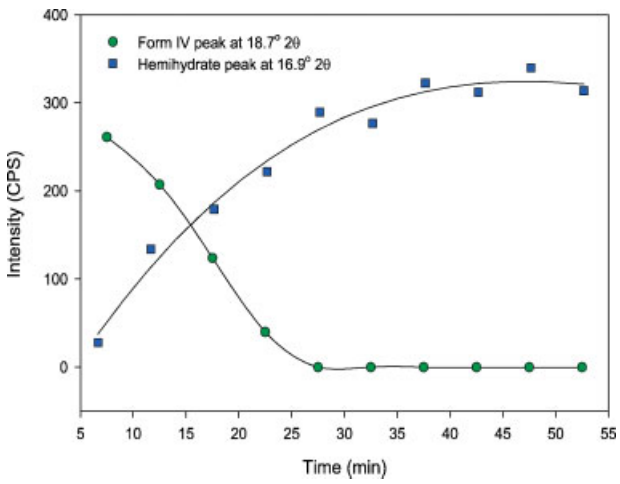

Figure 3. *In situ* conversion of Form IV to the hemihydrate by XRPD.

HST

As described above, Form IV lot 002 was shown to convert rapidly to the hemihydrate, while the Form I lots that had been tested to date appeared to convert more slowly. However, when a Form I lot (037) was found to convert as quickly as Form IV lot 002, it became urgent to develop a screening method to identify the fast converting lots and to determine the cause of the differences in the conversion rates. Differences in hydrate conversion

rates can have a major impact on accelerated solid-state stability studies. Early excipient and packaging decisions are based on studies carried out with one or a few API lots. The outcome of the accelerated stability studies could be quite different depending on whether slow or fast converting lots are used in the studies.

Based on the *in situ* monitoring experiment, a HST was developed to differentiate between the slow and fast converting lots. The test involved mixing 200 mg of etoricoxib with a small amount of water, followed by XRPD or Raman spectroscopy analysis for qualitative or quantitative analysis. However, the HST was predominantly used as a qualitative test for screening. Maintaining a consistent water contact time for each experiment was important to screen the different lots as this affects the extent of hemihydrate conversion.

All polymorphic forms of etoricoxib were evaluated using the HST, with emphasis on Forms I and V (Tab. 1). The results show lot dependence for the conversion of Forms I and V to the hemihydrate using the HST. For instance, significant conversion was observed for Form I lots 030 and 037; lot 031 showed minimal conversion, while other lots of Form I did not show any observable conversion. Form V, lot 001 showed minimal conversion while lots 002, 004, and 006 partially converted to the hemihydrate, and lots 003, 012, and 013 remained unchanged. Almost complete conversion was observed for Form II and IV lots mixed with water, while Form III remained unchanged. The amorphous form of the drug converts to Form III as an intermediate followed by conversion to the hemihydrate form upon mixing with water.

Table 1. Lot Dependency of Etoricoxib on the Rate of Hemihydrate Conversion using the Hydrate Screen Test (HST)

Crystal Form	Lot No.	Surface Area (m^2/g)	Purity (% w/w)	Result after HST
Form I	010	1.1	99.6	Unchanged
	016	1.4	99.8	Unchanged
	020	1.4	99.8	Unchanged
	023	1.3	99.9	Unchanged
	026	1.4	99.9	Unchanged
	029	1.2	99.7	Unchanged
	030	1.0	99.7	Partial hemihydrate
	031	0.9	99.4	Minimal hemihydrate
	032	1.0	99.6	Unchanged
	033	1.1	99.6	Unchanged
	037*	—	—	Unchanged
	037	0.9	100.0	Hemihydrate
	038	1.2	100.0	Unchanged
	039	1.1	99.9	Unchanged
	042	0.6	—	Unchanged
Form II	004	—	—	Hemihydrate
Form III	—	—	—	Unchanged
Form IV	002	—	—	Hemihydrate
Form V	002*	—	—	Unchanged
	002	—	—	Partial hemihydrate
	003	1.4	100.0	Unchanged
	004	1.0	99.7	Minimal hemihydrate
	006	—	99.9	Minimal hemihydrate
	008	—	—	Minimal hemihydrate
	010	—	—	Unchanged
	011	—	—	Unchanged
	012	—	100.0	Unchanged
	013	—	100.0	Unchanged
Amorphous	—	—	—	Form III

Qualitatively, minimal conversion implies 5%–15%, partial conversion 20%–80% hemihydrate content. All lots were pin milled with exception to Form I lot 037* and Form V lot 001*.

Mechanisms for the Lot Dependency of Hydrate Conversion

Several factors could contribute to the lot dependence of hydrate conversion rates, including particle size/surface area, surface energetics, amount/type of impurity, and undetectable amorphous content. All of these parameters could arise from API processing such as milling or during the manufacture of a solid oral dosage form such as high shear granulation, roller compaction, and tablet compression. Additionally, the presence of initial hemihydrate seeds arising from the API synthesis or any direct contact with water could also accelerate hemihydrate conversion. The effect of API grinding, which combines particle size reduction and potentially the introduction of amorphous content, was studied. The effect of milling was also evaluated by screening ground versus unground API of the same lot. The HST was used to evaluate these potential differences. It should be noted that no obvious measurable physical differences were detected between the apparent slow and fast converting lots using several conventional solid-state analytical techniques (XRPD, DSC, Raman, FT-IR, and SSNMR). Additionally, the reported surface area data[1] obtained from the release of several Forms I and V batches were quite similar. In fact, the minor differences obtained in surface area did not result in any trended change in hydrate conversion rate (Tab. 1).

Effect of Milling

Preliminary studies showed that grinding/milling the drug substance increased the propensity of the different lots to convert to the hemihydrate. For instance, when lots 033, 038, and 039 were ground for 90 s using a mortar and pestle, partial hemihydrate conversion was observed after performing the HST method whereas these lots did not convert in the absence of pregrinding.

Four Form I lots, 020, 032, 038, and 042, which did not convert using the HST method were selected to study the effect of milling time on the extent/rate of hemihydrate conversion. The samples were stressed for various lengths of time, ranging from 1 to 120 min using a high energy ball mill which reduces the drug particle size by crushing the sample with glass beads propelled in an oscillatory motion. The HST was performed on the recovered, ground material and the results are summarized in Table 2. Hemihydrate conversion was observed as a function of milling time for lots 032, 038, and 042. For instance, some hemihydrate conversion occurred as seen by the appearance of the diffraction peak at 16.9°, 2θ (Fig. 4) for Form I lot 038 after 1 min of milling. Another characteristic hemihydrate peak at 8.5°, 2θ can also be seen but only after 5 min of milling as it is significantly weaker in intensity. The extent of hemihydrate increased with increasing milling time. After 10 min of ball milling, lot 038 had completely converted within the time scale of the experiment. Ball milling and even lower energy milling such as pin milling had an effect on the rate of hemihydrate conversion. Interestingly, lot 020 did not convert to the hemihydrate even after 120 min of ball milling.

Reduction of particle size by milling was confirmed by SEM. Typical SEM micrographs of the ball milled Form I samples show a reduction in particle size with milling time (Fig. 5). Note that significant agglomeration occurred due to electrostatic attraction consistent with the Form's acicular morphology and hydrophobic nature. In addition, shortly following ball milling, the 30 min sample had small acicular particles growing on its surface suggesting that some of the drug substance had recrystallized from the amorphous state. XRPD and DSC analysis did not reveal any significant differences in peak area or the presence of a glass transition temperature, respectively. Moisture sorption studies on these ball milled samples provided additional evidence of the presence of amorphous regions. A small but measurable increase in water sorption was measured for Form I lot 038. An increase in water sorption from ~0% to 0.23% w/w at 90% RH was observed when comparing the preball milled to the ball milled samples, respectively. Analysis of the other Form I batches used in the study also showed similar, slightly elevated moisture sorption profiles. Considering that the 100% amorphous form sorbs 3.5% w/w water at 90% RH, this would suggest approximately 6% amorphous content which is challenging to detect using conventional analytical techniques such as XRPD and DSC.

Since all etoricoxib batches were pin milled, it became important to determine whether pin milling, albeit a much lower energy process than ball milling, could also cause accelerate hemihydrate conversion. Two unmilled batches, which had shown conversion once pin milled using the

[1]Analytical Research, Rahway, Merck & Co. Obtained using a Micromeritics Gemini 2360 surface area analyzer, 5 point BET, nitrogen adsorption.

Table 2. Effect of Ball Milling and Amorphous Content on Hemihydrate Conversion using the HST

Experiment\Sample	Lot 020	Lot 032	Lot 038	Lot 042
Sample as received	U	U	U	U
Ground (mortar & pestle)	—	U	P	U
Ball milling				
1 min	—	P	P	—
5 min	—	P	P	—
10 min	—	P	H	U
20 min	—	—	—	P
30 min	U	H	H	P
120 min	U	—	—	—
Amorphous content				
1%	U	P	P	—
5%	—	P	P	U
10%	U	H	H	M
25%	—	—	—	H[a]
50%	U	H[a]	H[a]	H[a]
Compression 13.3 kN	U	H	P	U
Purity by DSC (%)	98.22 ± 0.01	99.81 ± 0.01	99.72 ± 0.03	99.73 ± 0.02
Purity by HPLC (%)	99.8	99.6	100.0	—

The purity of the lots was determined by differential scanning calorimetry (DSC).

U, unchanged; M, minimal (5%–15%); P, partial (20%–80%); and H, complete hemihydrate conversion (100%).

[a]Crystalline reflections for Form III were detected by X-ray powder diffraction (XRPD) at 10.8 and 23° (2θ). Purity measurements performed in duplicate.

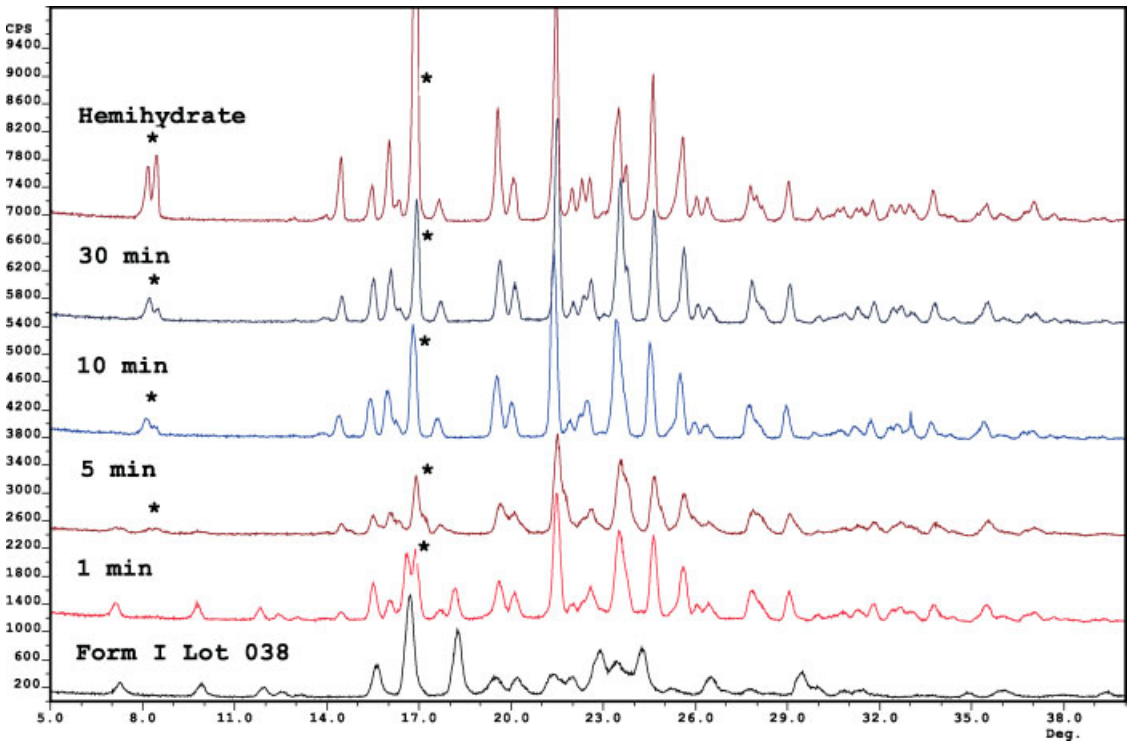

Figure 4. XRPD diffractograms of ball milled Form I lot 038 after the hydrate screen test (HST) method. Peaks marked by * are characteristic of the hemihydrate.

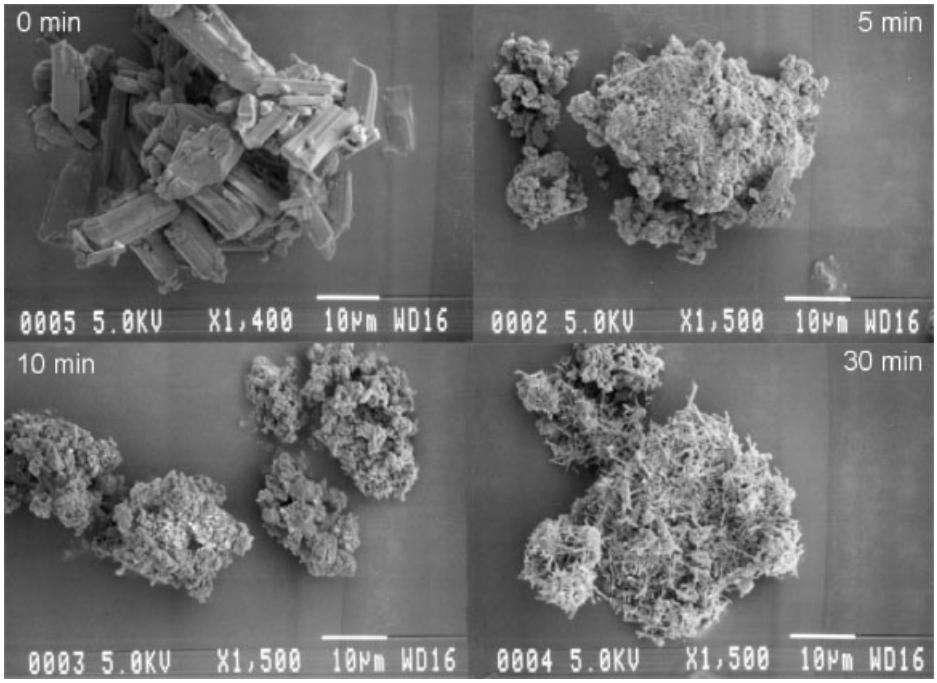

Figure 5. Scanning electron microscopy (SEM) micrographs of Form I lot 038 ball milled for different amounts of time.

HST method were also tested. Interestingly, performing the HST method on the unmilled batches imparted no change whereas their pin-milled counterparts showed complete and partial conversion for Form I lot 037 and Form V lot 001, respectively (Tab. 1). However, unlike the ball-milled samples, the pin milled samples provided no indication of amorphous content when subjected to similar testing compared to the unmilled.

These ball-milled results suggest that in addition to decreasing the particle size, ball milling may have increased the number of defects and/or introduced some disorder in the system. Amorphous/crystalline etoricoxib blends were also tested using the HST to confirm that the amorphous content in crystalline API could accelerate conversion (see "Effect of Amorphous Content").

Effect of Compression

Particle size reduction as well as amorphous formation can occur on compression. The effect of compression on hydrate conversion was evaluated by compressing four Form I lots (020, 032, 038, and 042) to 13.3 kN. The compacts were then gently ground and analyzed according to the HST method. The diffractograms of lot 032 and 038 (Fig. 6) showed complete and partial conversion to the hemihydrate form, respectively. However, lots 020 and 042 showed no conversion. The surface of the compacts stored at 40°C for 17 weeks showed the appearance of acicular particles consistent with Form I morphology (Fig. 7). This suggests that there was some conversion to the amorphous form on compression. These results are discussed in the following section.

Effect of Amorphous Content

The observation of needles on the surface of ball milled etoricoxib and compacts suggest that these processes may have converted some of the drug substance to the amorphous state, which subsequently recrystallized. Etoricoxib has a low glass transition temperature (50°C) and it has been shown by Hancock and Zografi[26] that there is significant molecular mobility at temperatures close to the T_g. It would therefore be expected that there would be sufficient molecular mobility would be present at 40°C for crystallization to occur.

To determine the effect of the amorphous content on hemihydrate conversion, four Form I lots were spiked with 1%, 5%, 10%, 25%, and 50% w/w amorphous etoricoxib. The mixtures were then subjected to the HST to determine the relative rate change in hydrate conversion with the percentage amorphous etoricoxib. In general, the

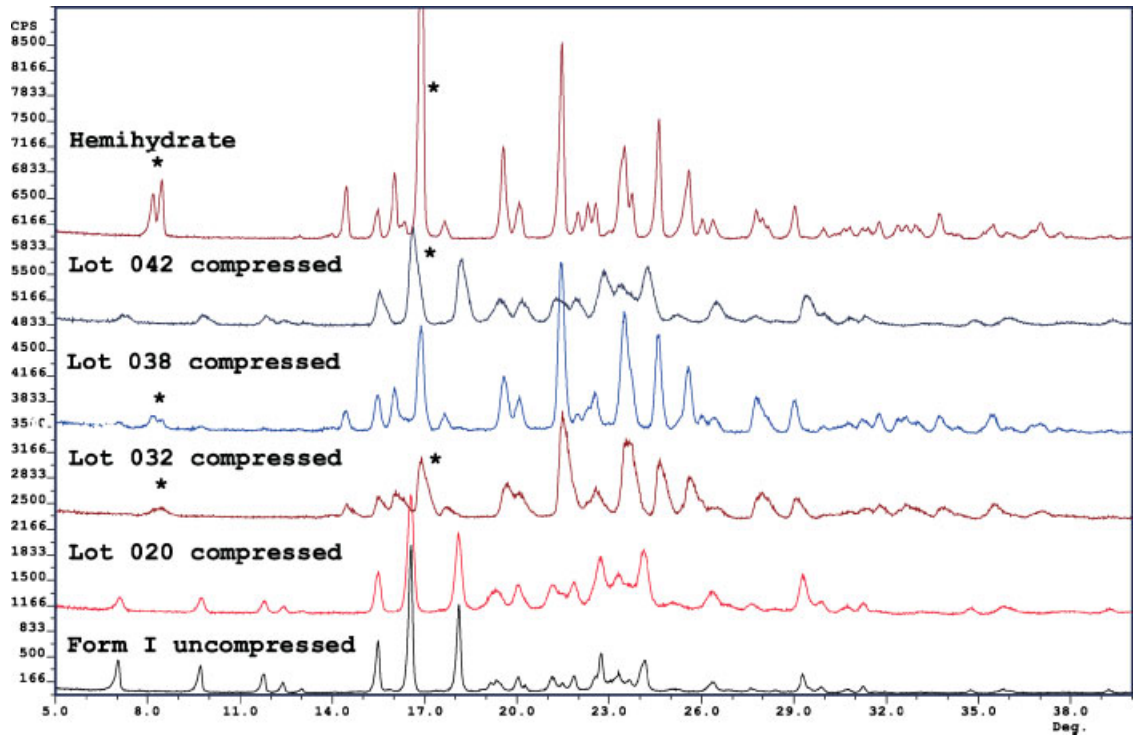

Figure 6. XRPD diffractograms of several Form I lots compressed to 13.3 kN and analyzed using the HST. Peaks marked by * are characteristic of the hemihydrate.

relative rate of hemihydrate conversion increased with increasing amorphous content (see Tab. 2). For instance, lots 032 and 038 showed partial conversion with just 1% amorphous material present and complete conversion with the addition of 10% amorphous content. The relative conversion rate of lot 042 was lower than that of lots 032 and 038, which is consistent with the results obtained from the grinding and compression studies. The effect of $\leq$1% amorphous content on hydrate conversion presents an interesting challenge to material scientists. Direct measurement of such a small amount is extremely challenging and could easily remain undetected. For example, if small, undetectable, varying amounts of amorphous content were indeed being generated during pin milling, this could cause inter lot variability in hydrate conversion.

When mixed in water, mixtures containing $\geq$25% amorphous content showed the presence of

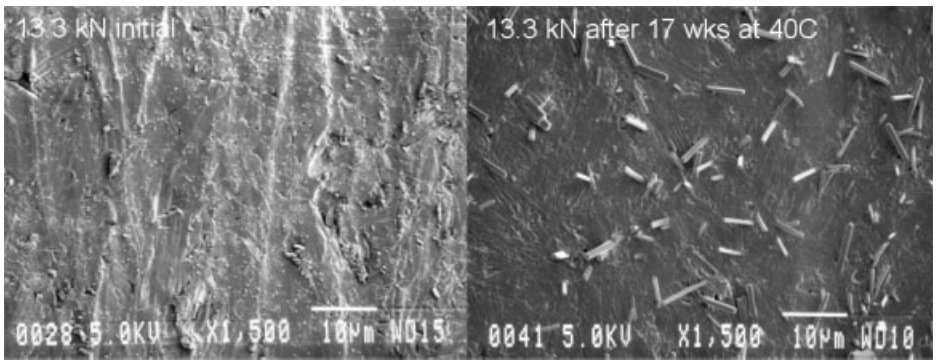

Figure 7. SEM micrographs of Form I lot 038 compact compressed to 13.3 kN initially and after 17 weeks at 40°C. The acicular particles observed after 17 weeks on the surface of the compact are consistent with the Form I morphology suggestive of the formation of amorphous etoricoxib on compression.

Form III crystalline reflections. This was not unexpected since it was already known that the amorphous form converts to Form III when in contact with water. This result is not surprising when considering the effect of the amorphous form on hydrate conversion combined with the phase relationship between Form III and the hemihydrate (Form III is the dehydrate). The recrystallization of the amorphous form to metastable Form III may facilitate rapid conversion and nucleation of etoricoxib to the hydrated form. Lot 020 showed no conversion to the hemihydrate in the presence of amorphous drug. This lot appears to have a very low propensity for hydrate formation, contrary to the other Form I lots since no impact on the hemihydrate conversion rate was observed with increasing amorphous content, ball milling time, or compression.

Effect of API Purity

Leveraging relevant data, such as batch purity, is important when investigating inter lot variability. An important point to consider, however, is the inherent difficulty in comparing the reported API purity values determined early in development. For example, early lots often have different purity profiles due to synthetic process changes and as a consequence, modifications are required in the high performance liquid chromatography (HPLC) method to ensure proper separation of impurities and prevent coelution of structural analogues. Another caveat of early purity measurements is that standard UV equipped HPLC detectors cannot easily measure nonchromophoric impurities. In the absence of additional chromatographic peaks, this technique relies on comparative peak area for assessment of peak purity, which is not ideal when the "standard" is updated.

As shown in Table 1, the HPLC purity of lot 020, a preGMP batch, was reported as 99.8% based on peak area. While no significant chromophoric impurities were detected by HPLC, the DSC thermogram obtained for lot 020 showed considerable differences in the melting onset temperature compared to lots 032, 038, and 042 which could be accounted for by impurities (Fig. 8). The DSC purity results indicated that lot 020 contained *ca.* 1.8% impurities compared to 0.3% obtained for the other lots (Tab. 2). Although these purity values should only be considered as semi-quantitative, the difference in lot 020's melting endotherm clearly demonstrates an unusual melting process and suggests the presence of impurities that were not detected by the early HPLC method.

The higher impurity of lot 020 may inhibit its crystallization to the hemihydrate form under the conditions used in the HST, however, conversion does occur when it is suspended in excess water. Impurities have been shown to increase the induction period by adsorbing to the particle's surface resulting in a steric barrier inhibiting crystallization.[27] This hypothesis would warrant

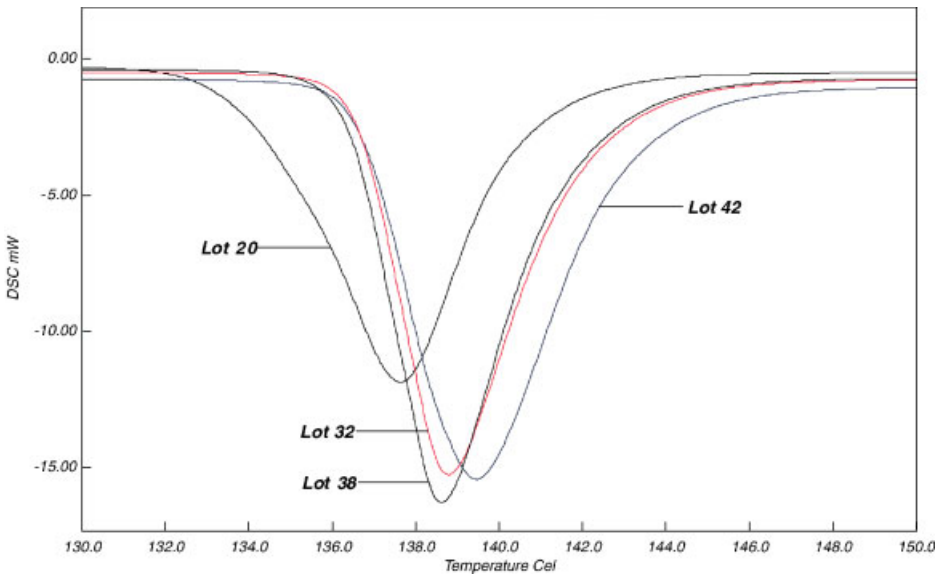

Figure 8. Differential scanning calorimetry (DSC) thermograms of several Form I lots at 10°C/min under N_2 in crimped aluminum pans. Endothermic transitions are down.

future work to determine the exact cause for this slower converting lot. Drug purity does not account for the differences in the relative hydrate conversion rates for GMP lots 032 or 038 and lot 042, since the DSC and HPLC purity values are similar for these lots. Based on the ball milling, compression and amorphous spiking studies, the conversion of the compound to the hemihydrate form, the relative rates of hemihydrate conversion increases as: lot 032/038 > 040 ≫ 020, in which lot 020 showed no conversion.

Effect of Initial Hemihydrate Content

Bauer et al. demonstrated that undetectable levels of a stable conformational polymorph of Ritonavir could result in polymorphic conversion in the formulation.[16] The goal of this experiment was to determine the effect of the presence of undetectable hemihydrate or hemihydrate "seeds" on the potential for hemihydrate conversion of the bulk API. Two methods were used in this investigation: the first employed the HST performed on several hemihydrate/Form V blends and the second approach involved the use of a conventional stability study where the same blends were exposed to 40°C/75% RH for up to 18 weeks. Raman spectroscopy was used to quantitate the levels of hydrate conversion for both methods. The HST method was compared to conventional stability studies to determine if it could be used as an early predictor of hemihydrate conversion.

Quantitation of the freshly prepared blends used in this study showed excellent agreement between the predicted and the actual blend composition down to 0.1% hemihydrate content, suggesting homogeneous blend preparation. Considering that the LOD of the Raman method is *ca.* 1% w/w hemihydrate, inaccuracy in the quantitation of the prepared blends consisting of <1% was expected but the values remained nonetheless very consistent.

Form V lot 012 was selected for this study due to its abundant availability and relatively "slow" conversion kinetics as shown in Table 1. Neat lot 012 was ground similarly to the hemihydrate/Form V blends and was used as a control for the study. As shown in Table 3, nearly 23% conversion was observed for the 0% ground sample, which is significantly less than the conversion observed for the blends containing hemihydrate (>90%). The presence of hemihydrate seeds greatly increased the conversion kinetics of Form V lot 012. Conversion was nearly 100% complete, for all the blends containing hemihydrate seeds. A small, marginal increase in hydrate content was measured with increasing the initial hemihydrate content from 0.1% to 10% (91%–96%, respectively).

Table 3. Hemihydrate Content Present in Hemihydrate/Form V Blends Following the HST

	Hemihydrate in Form V (% w/w)					
Sample	0.0[a]	0.1	0.5	1.1	5.0	10.0
1	19.7	92.8	92.5	93.0	91.9	94.7
2	25.0	87.4	93.3	93.2	93.1	95.3
3	26.1	91.5	92.8	92.4	91.9	96.5
4	24.0	93.2	91.3	87.7	92.9	97.1
5	20.2	88.0	91.1	92.9	93.7	95.2
Average	23.0	90.6	92.2	91.8	92.7	95.8
Standard deviation	2.6	2.4	0.9	2.1	0.7	0.9

[a]Ground Form V.

The results obtained using the conventional stability study approach are shown graphically in Figure 9. The samples initially consisting of 1%, 5%, and 10% w/w hemihydrate when exposed to 40°C/75% RH resulted in significant increase (~40%) in hemihydrate content after 18 weeks. Measurable changes in hemihydrate content were observed at every time-point, including after only 1 week on stability. The blend consisting of 0.1% hemihydrate produced only slight changes in hemihydrate content, under the LOD of the Raman method, up to the 8-week time-point. However, a total hemihydrate content of 4.5% was measured after 18 weeks at 40°C/75% RH, confirming that the blend was indeed converting. Conversely, the ground and unground Form V lot 012 showed no increase in hemihydrate content. Thus, the presence of a small, undetectable, amount of hemihydrate (≤0.1%) can have a significant impact on the overall rate of etoricoxib conversion.

Both methods yielded similar trends, the presence of hemihydrate seeds greatly accelerates the overall rate of hemihydrate conversion. The HST method provided similar information but much more quickly than the conventional accelerated solid-state stability approach. The difference, of course, is how the water is introduced; forced direct water contact versus water vapor adsorption/diffusion. Based on the extent of conversion with the HST method, the effect of a very small, undetectable amount of hemihydrate was found to have a significant impact on the hydrate

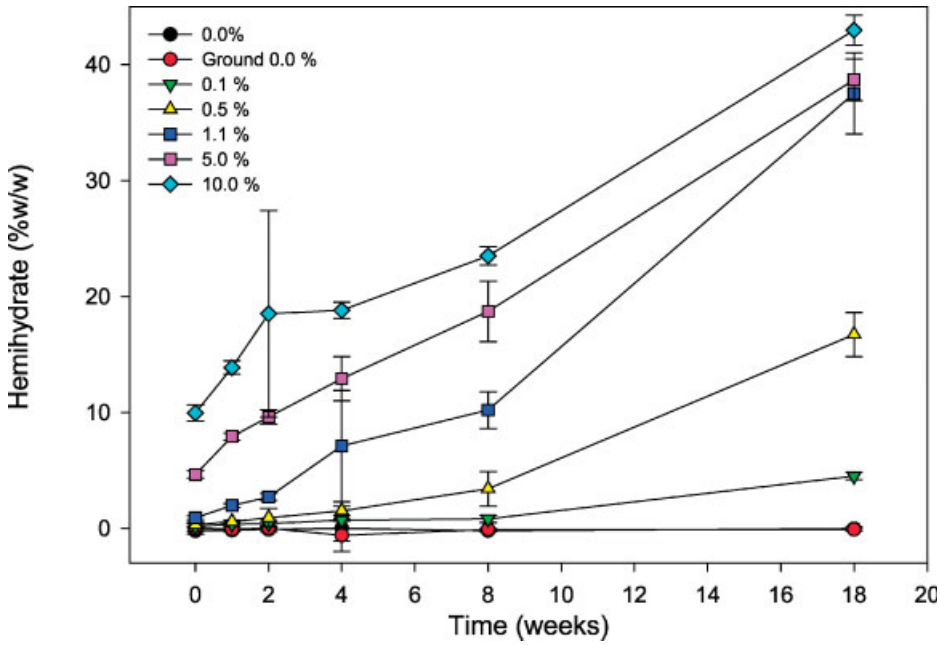

Figure 9. Hemihydrate content quantitated by Raman spectroscopy for several hemihydrate/Form V lot 012 blends stored at 40°C/75% relative humidity (RH) for up to 18 weeks ($n = 10$).

conversion rate. The conventional stability study only showed the effect of the 0.1% hemihydrate blend on total hemihydrate conversion after ≥8 weeks of storage at 40°C/75% RH. However, the slower conversion method is not without merit, since it simulates conditions, which are more realistic. It also enables the study of conversion rate kinetics, which is necessary to estimate product shelf life.

CONCLUSIONS

A HST was developed and was successfully used to discriminate between rapid and slow converting drug lots. One third of the 25 drug lots studied showed measurable and reproducible differences when subjected to this test suggesting an inter lot dependency on the rate of hydration. Additionally, several parameters potentially contributing to this difference were screened using the novel test. Ball milling and compression were found to create amorphous etoricoxib, which in turn catalyzed hydrate conversion. Small quantities of amorphous etoricoxib, ~1%, is considered as being a major factor in modifying the hemihydrate conversion rate. In addition, the presence of a minor amount of hemihydrate, 0.1%, was also found to cooperatively accelerate the conversion of the remaining etoricoxib. Based on experimental observation, it is postulated that a small amount (1.8%) or type of impurity greatly reduced hydration kinetics. This may be caused by impurities adsorbing to the particle's surface resulting in a steric barrier inhibiting crystallization.

The majority of experiments were performed using Forms I and V as a result of the availability of these forms. It can be expected that the conclusions drawn on the influence of the presence of hemihydrate seeds, amorphous content on hemihydrate conversion rates is applicable to all the Forms in particular given the only slight thermodynamic differences between the different polymorphs.[24] No polymorphic interconversion was ever observed during the current work and in this case, has little bearing on hydrate conversion. Differences in hydrate conversion rates of the drug substance can lead to significant differences in its physical stability in the drug product, and ultimately to erroneous conclusions on the selection of the excipients and packaging conditions. Improved understanding of the factors affecting hydrate conversion rates provided guidance to the selection of the excipients and storage conditions for both the bulk API and the formulated drug product.

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REFERENCES

1. Hargrove JT, Maxson WS, Wentz AC. 1989. Absorption of oral progesterone is influenced by vehicle and particle size. Am J Obstet Gynecol 161: 948–951.
2. Phadnis V, Suryanarayanan R. 1997. Polymorphism in anhydrous theophylline—Implications in the rate of theophylline tablets. J Pharm Sci 86: 1256–1261.
3. Vippagunta SR, Brittain HG, Grant DJW. 2001. Crystalline solids. Adv Drug Del Rev 48:3–26.
4. Giron D, Goldbronn C, Mutz M, Pfeffer S, Piechon P, Schwab P. 2002. Solid state characterization of pharmaceutical hydrates. J Therm Anal Cal 68: 453–465.
5. Khankari RK, Grant DJW. 1995. Pharmaceutical hydrates. Int J Pharm 248:61–79.
6. Buckton G. 1995. Surface characterization: Understanding sources of variability in the production and use of pharmaceuticals. J Pharm Pharmacy 47: 265–275.
7. Buckton G. 1997. Characterization of small changes in the physical properties of powders of significance for dry powder inhaler formulations. Adv Drug Deliv Rev 26:17–27.
8. Ticehurst MD, Rowe RC, York P. 1994. Determination of the surface properties of two batches of salbutamol sulphate by inverse gas chromatography. Int J Pharm 111:241–249.
9. Ticehurst MD, Rowe RC, York P, Rowe RC, Dwivedi SK. 1996. Characterisation of the surface properties of a lactose monohydrate with inverse gas chromatography, used to detect batch variation. Int J Pharm 141:93–99.
10. Grimsey JM, Feely JC, York P. 2002. Analysis of the surface energy of pharmaceutical powders by inverse gas chromatography. J Pharm Sci 91:571–583.
11. Clas S-D. 2003. The importance of characterizing the crystal form of the drug substance during drug development. Curr Opin Drug Discov Dev 6:550–560.
12. Crocker LS, McCauley JA. 1995. Comparison of the crystallinity of Imipenem samples by X-ray diffraction of amorphous material. J Pharm Sci 84:226–227.
13. Hancock BC, Christensen K, Shamblin SL. 1998. Estimating the critical molecular mobility temperature (Tk) of amorphous pharmaceuticals. Pharm Res 15:1649–1651.
14. Saklatvala R, Royall PG, Craig DQM. 1999. The detection of amorphous material in a nominally crystalline drug using modulated temperature DSC—A case study. Int J Pharm 192:55–62.
15. Buckton G, Darcy P. 1995. The use of gravimetric studies to assess the degree of crystallinity of predominantly crystalline powders. Int J Pharm 123:265–271.
16. Ticehurst MD, Rowe RC, York P, Dwivedi SK. 1996. Characterisation of the surface properties of α-lactose monohydrate with inverse gas chromatography used to detect batch variation. Int J Pharm 141:93–99.
17. Giron D. 1999. Thermal analysis, microcalorimetry, and combined techniques for the study of pharmaceuticals. J Therm Anal Calorim 56:1285–1304.
18. Yu L. 2001. Amorphous pharmaceutical solids: Preparation characterization and stabilization. Adv Drug Deliv Rev 48:27–42.
19. Tishmack PA, Bugay DE, Byrn SR. 2003. Solid-state nuclear magnetic resonance spectroscopy-pharmaceutical applications. J Pharm Sci 92:441–474.
20. Buckton G, Dove JW, Davies P. 1999. Isothermal microcalorimetry and inverse phase gas chromatography to study small changes in powder surface properties. Int J Pharm 193:13–19.
21. Bauer J, Spanton S, Henry R, Quick J, Dziki W, Porter W, Morris J. 2001. Ritonavir: An extraordinary example of conformational polymorphism. Pharm Res 18:859–866.
22. Crocker LS, Ge Z, Abrahim A, Hartman R, Xu J. 2003. An etoricoxib phase diagram: Hemihydrate and anhydrate. Org Process Res Dev 7:958–961.
23. O'Shea P, Tillyer RD, Wang X, Clas S-D, Dalton C, 2000. Synthesis of 4-[(5-substituted or unsubstituted phenyl)-3-substituted-1H-pyrazol-1-Y1]benzenesulfonamides. US Patent 6,150,534.
24. Clas S-D, O'Shea P, Dalton C, Crocker LS, McCauley JA, Tillyer RD, Davis I, 2002. Polymorphic, amorphous and hydrated forms of 5-chloro-6′-methyl-3-[4-(methylsulfonyl)phenyl]-2,3′-bipyridine. US Patent 6,441,002.
25. Clas S-D, Dalton CR, Hancock BC. 2002. Calorimetry in pharmaceutical research and development. In: Swarbrick J, Boylan JC, editors. Encyclopedia of pharmaceutical technology, Vol. 1, 2nd edn. New York: Marcel Dekker, Inc. pp 289–301.
26. Hancock BC, Zografi G. 1997. Characteristics and significance of the amorphous state in pharmaceutical systems. J Pharma Sci 86:1–12.
27. Blagden N, Davey RJ, Rowe R, Roberts R. 1998. Disappearing polymorphs and the role of reaction by-products: The case of sulphathiazole. Int J Pharm 172:169–177.