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*Research Paper*

# Effect of Polymer Additives on the Transformation of BMS-566394 Anhydrate to the Dihydrate Form

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***Purpose.*** To investigate the effect of polymer additives on the transformation of BMS-566394 anhydrate to the dihydrate form and to propose the possible mechanisms for inhibition of conversion of the anhydrate to the dihydrate form.
***Materials and methods.*** The conversion of anhydrate to dihydrate was monitored using differential scanning calorimetry, powder X-ray diffraction and polarized light microscopy. Solubility and intrinsic dissolution studies were performed on anhydrate and dihydrate. IR and NMR spectroscopy were used to probe the molecular interactions between BMS-566394 and cellulose ether polymers.
***Results.*** The anhydrate form of BMS-566394 was readily transformed into the more stable dihydrate form in aqueous suspension. The kinetic solubility and intrinsic dissolution rate of the anhydrate were *ca*. fourfold that of the dihydrate. Addition of cellulose ether polymers (HPC, HPMC, MC) inhibited anhydrate to dihydrate transformation in aqueous suspensions. Hydrogen bonding interaction between the polar groups of the drug and polymers was inferred from infrared spectroscopy. Solution NMR also indicated a hydrophobic interaction between the drug and polymer backbone.
***Conclusions.*** The anhydrate form of BMS-566394 is stabilized in the presence of cellulose ether polymers. Spectroscopic evidence is offered to postulate a molecular interaction between drug and polymers.

**KEY WORDS:** anhydrate; crystals; dihydrate; intrinsic dissolution; polymer stabilization.

## INTRODUCTION

It is important that crystalline forms of drug substances used in various dosage forms be characterized, and appropriate forms selected to ensure that the product performance with respect to manufacturability, stability, and bioavailability remains unchanged. Polymorphic and pseudopolymorphic forms of a poorly soluble drug may potentially influence bioavailability by affecting the dissolution rate (1–3). Solubility and dissolution of a drug influence its absorption, hence a large number of studies have focused on the effect of polymorphism on solubility or dissolution (4). Traditionally, the thermodynamically most stable polymorph is favored for development over a less stable high energy form. However, many examples are also reported in literature describing the attempts to increase the oral bioavailability of poorly soluble drugs by utilizing their high energy, more soluble forms. The most commonly used formulation approach is the solid dispersion, wherein the crystalline form of a poorly soluble drug is converted to the amorphous state and dispersed in semisolid excipients in order to enhance dissolution and bioavailability (5,6).

During a drug's development, there is often a need to maximize exposure in animals while conducting toxicological evaluation of drug candidates. The pace of the development process seldom permits extensive development of formulations for toxicology studies. In the case of water-soluble compounds, an aqueous buffered solution formulation is the logical first choice. Solubilization in non-aqueous vehicles is commonly utilized for poorly water-soluble compounds in dose-escalation studies in animals, but vehicle tolerability in animals is often a limiting factor of this approach. From vehicle tolerability considerations, an aqueous suspension is a safer formulation choice for poorly soluble compounds for use in toxicology studies. Particle size distribution and polymorphism issues add some complexity to aqueous suspension formulations. The thermodynamically most stable form is preferred for formulation of suspensions, especially for drugs known to undergo form transformation in an aqueous environment. However, a thermodynamically less stable form may offer the advantage of providing a higher dissolution rate and greater apparent solubility than the more stable form and potentially an opportunity to maximize drug exposure in animals. A key limitation to the use of high energy forms is their transformation to the more stable form in the formulation on storage. In some cases, the problem may be circumvented by freshly preparing the formulation

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for each study leg. But in most cases, the interconversion kinetics between forms render this approach impractical.

Phase transformation of an anhydrate to a hydrate or vice versa is of pharmaceutical significance because such a transformation can alter the physicochemical properties of the API and lead to changes in the free energy and thermodynamic activity of the drug. Such changes can translate into altered drug dissolution and bioavailability (7). The relative phase stability of hydrate–anhydrate systems under different conditions of water activity and temperatures have been reported (8–10), and the effect of excipients on moisture induced anhydrate–hydrate phase transformation has been investigated (11–14).

BMS-566394 is a poorly water soluble developmental drug intended for oral delivery. It has a weakly acidic hydroxamic acid moiety (pKa 9.05) and a weakly basic trifluoromethyl benzimidazole group (pKa <3) (Fig. 1). It was isolated as a crystalline anhydrate (AN) form and a crystalline dihydrate (DH) form. The single crystal structures for anhydrate and dihydrate have been elucidated (15). The anhydrate form of the drug has greater aqueous solubility but it rapidly converts to the less soluble dihydrate form in water. In this investigation, we have shown that the more soluble, high energy anhydrate form of the drug could be stabilized in aqueous suspension by addition of cellulose ether polymers such as methyl cellulose (MC), hydroxypropylmethyl cellulose (HPMC) and hydroxypropyl cellulose (HPC), without conversion to the lower energy dihydrate form. The stabilized aqueous suspension formulation of the anhydrate form offered a practical way to exploit the apparent higher aqueous solubility of the high energy form to maximize drug exposure in toxicological evaluations. Studies were conducted to investigate the stabilization effect of the above polymers in more detail and to elucidate the underlying mechanism of stabilization.

## MATERIALS AND METHODS

BMS-566394 was synthesized at Bristol-Myers Squibb Company. The anhydrate and dihydrate forms were isolated as pure forms by crystallization. HPMC, K4M Premium, MC, A4M Premium and HPC were obtained from The Dow Chemical Company (Midland, MI). All other materials used were reagent grade.

For IR studies, cast films of BMS-566394 with each of the cellulose ether polymers were prepared using solvent evaporation technique. BMS-566394 anhydrate and the polymer in a 5:1 ratio were dissolved in a mixture of methanol and methylene chloride (1:3). The solvent was removed under vacuum at 40°C using a rotary evaporator.

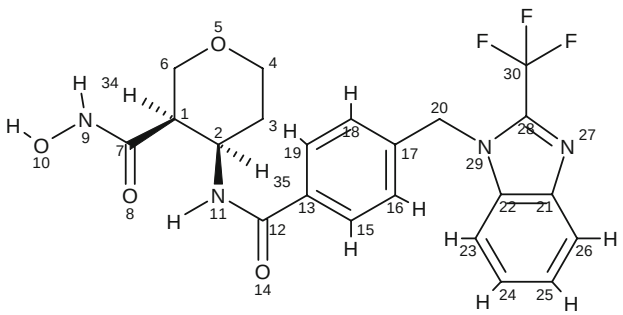

**Fig. 1.** Chemical structure of BMS-566394.

### Phase Transformation

Typically, crystals of pure form (1–10 mg/ml) were suspended in aqueous solutions in the presence of various additives at specified concentrations. The conversion of form was monitored by optical microscopy (Leitz Leica, W. NuhsBaum, Inc, McHenry, IL). Suspensions were also sampled at periodic time intervals and the isolated solids were subjected to either differential scanning calorimetry or powder X-ray analysis.

### Differential Scanning Calorimetry

A differential scanning calorimeter (Model 2910, TA Instruments, New Castle, DE) was used for these studies. A known amount of sample was weighed into an aluminum pan and heated over a temperature range of 10–300°C, at a rate of 10°C/min under $N_2$ purge at 40 ml/min.

### Hot Stage Microscopy

Mettler model FP-82 hot stage with FP-80 central processor (Mettler-Toledo, Inc., Columbus, OH) attached to a Leitz Laborlux 12 microscope (Leica, Wetzlar, Germany) was used. The sample was placed on a covered slide and heated at a rate of 10°C/min.

### Powder X-ray Diffraction

X-ray powder diffraction patterns of samples were recorded with a Rigaku MiniFlex X-ray diffractometer (The Woodlands, TX) using Cu Ká radiation ( = 1.54 Å), a tube voltage of 30 kV, and a tube current of 15 mA. The intensities were measured at 2 $\theta$ values from 5 to 40° at a continuous scan rate of 1° 2 $\theta$/min.

### Solubility

The saturation aqueous solubility of the dihydrate was determined by equilibration method. Approximately 10–20 mg of BMS-566394 was placed in a vial. Aliquots (typically 1–2 ml) of water were added and the samples were thoroughly mixed on a vortex mixer. The suspensions were equilibrated for 24–48 h by shaking on a laboratory shaker at the desired temperature. An excess of solid was maintained in equilibrium at all times. The suspensions were filtered using 0.2 um membrane filter (Gelman type HT Tuffryn) and the filtrates were analyzed by HPLC after suitable dilution. The kinetic solubility of the anhydrate was determined using a similar methodology. Samples from a saturated solution of anhydrate in water were taken at periodic intervals and analyzed by HPLC.

### Intrinsic Dissolution

Intrinsic dissolution rate (IDR) was measured for compacts of BMS-566394 anhydrate and dihydrate using a Static disk dissolution apparatus (Distek®). Compacts of

anhydrate and dihydrate were prepared on a Carver Press by compressing 100 mg drug at 2,000 psi for 4 min. A known surface area (0.5 $cm^2$) of the compact was exposed to the dissolution medium, 1% Tween 80. Dissolution studies were conducted in a USP II apparatus at a paddle speed of 75 rpm at 35°C. Samples were withdrawn at periodic intervals and analyzed by HPLC. The IDRs were calculated from the volume of the dissolution medium (1,000 ml), the disk surface area (0.5 $cm^2$), and the initial slope of the concentration *versus* time plot. The integrity of the forms after compression was confirmed by PXRD.

### FT-IR

Infrared spectra were obtained using a Nicolet Nexus model 670 FT-IR. An attenuated total reflection (ATR) accessory was used.

### NMR

A solution of BMS-566394 and HPMC in a mixture of methanol and methylene chloride was investigated by 1H NMR spectroscopy using a Varian INOVA 500 MHz instrument.

### *In vivo* Study in Dogs

BMS-566394 anhydrate and dihydrate (100 mg each) were individually filled in hard gelatin capsules using lactose and microcrystalline cellulose as fillers at a drug loading of 50% w/w. These capsules were administrated orally at 25 mg/kg to six male beagle dogs ($N$=3 for each form). Plasma samples were collected and analyzed by a LC/MS/MS assay.

## RESULTS AND DISCUSSION

### Solid-State Characterization of Anhydrate and Dihydrate Forms

Two crystalline forms of BMS-566394 are known, an anhydrate neat form and a dihydrate form. The unit cell of the anhydrate form showed tight intermolecular H-bonding involving hydroxamic acid and amide moieties of the adjacent molecule while the dihydrate structure showed narrow water channels extending throughout the crystal structure. The anhydrate form used in the studies was crystallized from isopropyl acetate. It has also been obtained from a number of other solvents including isopropanol, isopropanol–heptane, isobutyl methyl ketone and ethanol–heptane. The crystals appear as rectangular plates as observed by optical microscopy. The DSC of the anhydrate form showed a single endotherm at 179°C (Fig. 2) that is attributed to melting based on hot stage microscopy. Thermogravimetric analysis indicated no loss of weight and the absence of volatiles. The observed powder X-ray diffraction pattern of the anhydrate form matched very well with the simulated powder pattern derived from single crystal structure. The material was found to be non-hygroscopic with weight gain of <0.9% at 94%RH and no hysteresis upon desorption. In the solid state, conversion of the anhydrate

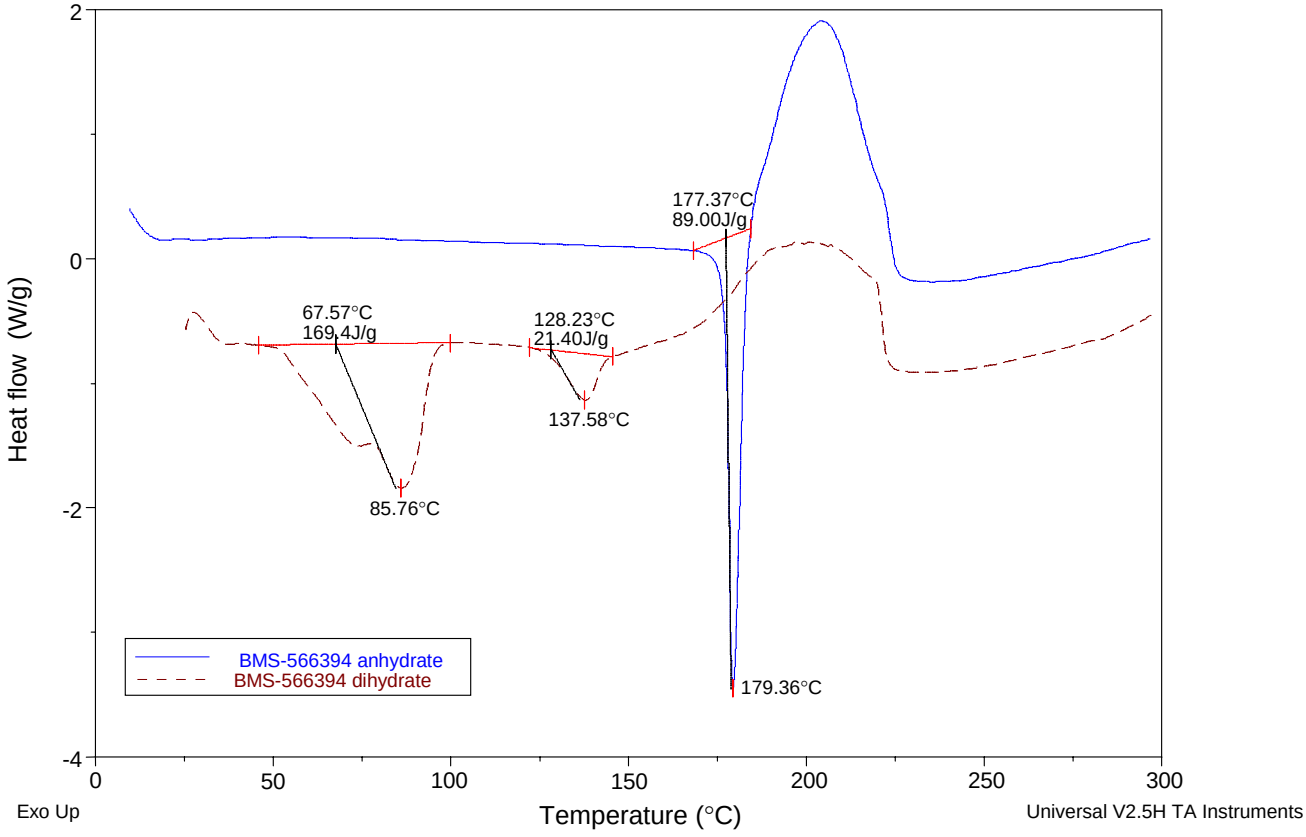


**Fig. 2.** DSC traces of the anhydrate and dihydrate forms.

form to dihydrate form was not observed even when the anhydrate form was exposed to 100% relative humidity.

The dihydrate form was crystallized from aqueous mixtures of isopropanol and water. The crystals appear as thin needles as observed by optical microscopy. The DSC of the dihydrate form showed a dehydration endotherm below 100°C with enthalpy of ~169 J/g, followed by a melting endotherm at ~138°C with enthalpy of ~21 J/g (Fig. 2). When observed by hot stage microscopy, the crystals of dihydrate appeared to melt at ~138°C, which was consistent with the results of DSC analysis. The identity of the phase melting at 138°C was not confirmed. It is likely a metastable form. TGA of the dihydrate form showed a weight loss of 7.0% at 75°C, which agrees well with the stoichiometry of the dihydrate. The observed powder X-ray diffraction pattern for this pseudopolymorph is distinct from that of the anhydrate and matched well with the simulated pattern obtained from single crystal structure of the dihydrate. The crystalline form is non-hygroscopic with <0.3% weight gain at 90% RH in moisture sorption studies.

Since hydrate formation can alter the thermodynamic activity of a solid, potentially impacting physicochemical properties, such as solubility, dissolution rate, and ultimately, bioavailability (7, 16), intrinsic dissolution rate and aqueous solubility measurements were conducted for the anhydrate and dihydrate forms.

### Solubility Studies

The aqueous solubility of the anhydrate form was estimated from a kinetic solubility experiment at 35°C. The solubility value reached a maximum within 15–30 min and then declined rapidly to an equilibrium value corresponding to the solubility of the dihydrate form, indicating form conversion (Fig. 3). Optical microscopy indicated that the anhydrate crystals remained intact for the first few minutes of the experiment and thereafter showed progressive whisker like growth of the dihydrate form. A value of 0.2 mg/ml obtained at ~30 min at 35°C was assigned as the kinetic solubility of the anhydrate form. The equilibrium solubility value of 0.06 mg/ml was assigned to the dihydrate form. The equilibrium solubility value from the kinetic plot of the solubility of the anhydrate agreed well with that obtained by equilibrating a sample of the dihydrate form in water.

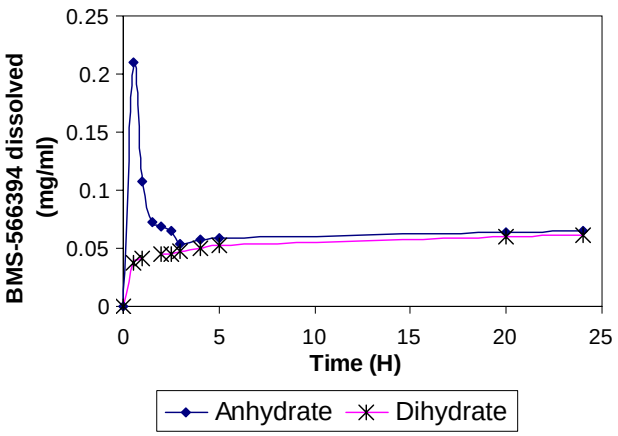


**Fig. 3.** Solubility study of anhydrate and dihydrate in water at 35°C.

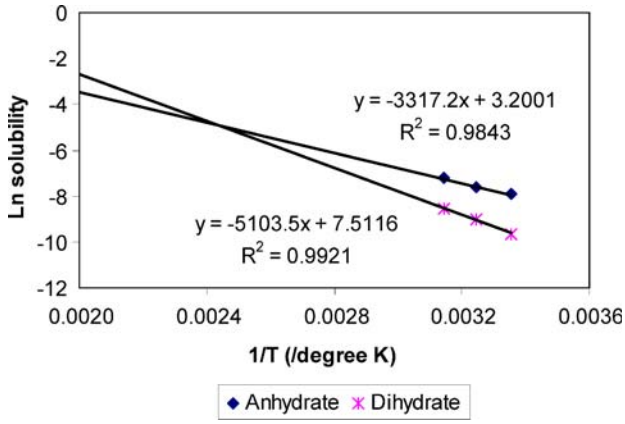


**Fig. 4.** Van't Hoff plot of anhydrate and dihydrate in water.

Kinetic solubility values for the anhydrate and equilibrium solubility values for the dihydrate were also determined at 25 and 45°C. The difference between the kinetic solubility of the anhydrate form and the equilibrium solubility of the dihydrate form at these temperatures was approximately fourfold. Log solubility *versus* reciprocal temperature plots (Van't Hoff) indicated a linear relationship for both anhydrate and dihydrate forms. Extrapolation of the Van't Hoff solubility plots to predict the relative stability of the pseudopolymorphs indicated a transition temperature of >100°C, below which the dihydrate form was more stable (Fig. 4). The heats of solution of the anhydrate and dihydrate determined from the slope of Van't Hoff plots were 27.6 and 42.4 kJ/mol, respectively.

### Intrinsic Dissolution

The rates of solution of the anhydrate and dihydrate were determined in water at 35°C by an intrinsic dissolution method, which measures the rate of solution from a constant surface area compact, minimizing effects of particle size and porosity frequently encountered in traditional dissolution experiments (16). It was found that aqueous 1% Tween-80 retarded the conversion of anhydrate to dihydrate for up to 2 h at 35°C. Therefore, intrinsic dissolution studies were conducted using 1% Tween-80 in water as the dissolution

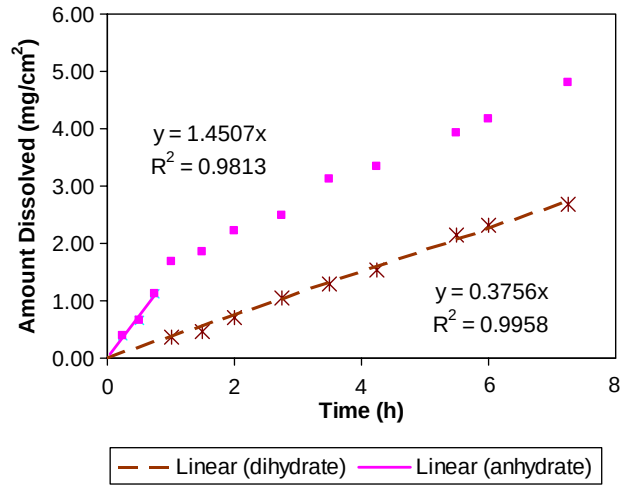


**Fig. 5.** Intrinsic dissolution profile for anhydrate and dihydrate forms.

medium. The plot of amount of drug dissolved *versus* time for the dihydrate gave an expected linear relationship from which the intrinsic dissolution rate was calculated to be 0.38 mg $cm^2$ $h^{-1}$. The intrinsic dissolution rate for anhydrate was calculated to be 1.45 mg $cm^2$ h by initial slope method (Fig. 5). The anhydrate was found to dissolve approximately four times faster than the dihydrate form, which was consistent with the data obtained from solubility studies. The more rapid dissolution rate of the anhydrate in water is as expected per the general rule that hydrated crystal forms are more stable in water than anhydrates.

### *In vivo* Studies

Following oral dosing in dogs, the anhydrate form gave a threefold increase in Cmax and more than twofold increase in AUC, compared to the dihydrate form (Table I). The greater oral absorption of BMS-566394 from anhydrate was consistent with its higher solubility and greater intrinsic dissolution rate compared to the dihydrate form. Therefore, for range finding safety studies the anhydrate form was desirable. An aqueous suspension of BMS-566394 anhydrate was desired to cover the wide dose range required to conduct safety studies. However, the challenge was its rapid conversion to the less soluble dihydrate form in water. Adding cellulose ether polymers offered a means of stabilizing the anhydrate form in aqueous suspensions. A suspension formulation of BMS-566394 anhydrate in 0.5% w/v methylcellulose was found to be stable for several weeks and offered a convenient and flexible means of administering a wide range of doses to animals from the same formulation.

### Stabilization of Anhydrate

Various excipients were screened for their effectiveness in inhibiting anhydrate to dihydrate conversion by slurrying BMS-566394 anhydrate in water containing each additive, and observing the suspensions by optical microscopy for crystallization of dihydrate over time (Table II). Morphology of the dihydrate crystals (needles) was distinct from the anhydrate (plates), which made it possible to study the conversion of the anhydrate to the dihydrate form by microscopy. As shown in Fig. 6, the partial conversion of anhydrate to dihydrate in water is observed after 10 min.

Hydration of the anhydrate form in water occurs rapidly, resulting in initiation of crystallization of the dihydrate form within a few minutes. Addition of polymers such as CMC, Avicel and PEG did not appear to prevent or retard the crystallization of the dihydrate form (Table II). When the anhydrate form was suspended in aqueous suspensions with Tween 80, Pluronics or PVP, the rate of conversion to the dihydrate form was retarded to some extent compared to that in water alone. However, crystallization of the dihydrate form was observed to occur within hours. On the other hand, addition of cellulose ether polymers such as HPMC, HPC and MC stabilized the high energy anhydrate form in aqueous suspension and completely inhibited its conversion to the lower energy dihydrate form. No conversion to the dihydrate was observed in suspensions containing 0.1% w/w HPMC, HPC and MC after 4 months at room temperature. An aqueous 0.5% w/v cellulose ether polymer vehicle offered a convenient means of preparing a stabilized suspension of the anhydrate form for conducting repeat dose studies for toxicological evaluations.

**Table I.** Summary of Oral PK Data for Anhydrate and Dihydrate in Dogs

| PK Parameters | Anhydrate | | Dihydrate | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| $AUC_{total}$ (nM h) | 17,167 | 5,303 | 7,377 | 2,051 |
| $C_{max}$ (nM) | 4,581 | 2,018 | 1,379 | 298 |
| $T_{max}$ (h) | 1.3 | 0.6 | 2.0 | 1.7 |
| $t_{1/2}$ (h) | 3.8 | 0.4 | 4.9 | 2.2 |

**Table II.** Screening Additives for Stabilization of Anhydrate Form in Aqueous Suspensions

| Additive (1% w/v) | Stabilization |
|---|---|
| Water | − |
| Tween 80 | +/− |
| Pluronics | +/− |
| Sodium dodecyl sulfate (SDS) | − |
| Cyclodextrins | − |
| Lactose | − |
| Silicates | − |
| Polyvinylpyrrolidone (PVP) | +/− |
| Mucin | +/− |
| Polyethylene glycol (PEG) | − |
| Cellulose (0.1%) | |
| MC | ++ |
| HPC | ++ |
| HPMC | ++ |
| Microcrystalline cellulose (Avicel) | − |
| Carboxymethyl cellulose (CMC) | − |

− Additive does not afford stabilization in conversion to dihydrate (compared to water)
+/− Additive affords partial stabilization in conversion to dihydrate
++ Additive affords stabilization in conversion to dihydrate (No conversion after 2–4 months at RT)

### Polymorphic Transformation Process

Suspensions of anhydrate in water were sampled at periodic time intervals and the isolated solids were subjected

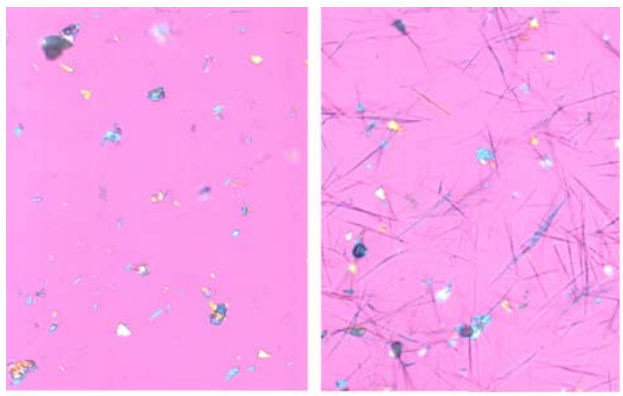


**Fig. 6.** Optical microscopy images of **A** anhydrate in water at time=0, **B** anhydrate in water at time=10 min. Magnification=100×.

to powder X-ray analysis. The distinctive peaks of dihydrate crystal form at 9 and 19 2-theta were detected in the samples at 60 min along with characteristic anhydrate peaks at 11 and 25 2-theta (Fig. 7). However, quantitative transformation could not be accurately determined by PXRD.

The quantitative conversion into dihydrate was estimated by DSC analysis of the solids isolated from aqueous suspensions of anhydrate. The endothermic peak for anhydrate at ~179°C progressively diminished with time, while the endothermic peak for dihydrate at 138°C increased. The % conversion was calculated from the ratio of the enthalpy of the respective peaks in the DSC thermogram. To minimize variability of data, the experiments were conducted in triplicate under identical conditions of temperature, weight of solid, volume of the liquid phase and the rate of agitation. The DSC sample was mixed thoroughly to achieve homogeneity. The conversion process showed a short lag time followed by an exponential increase in the growth of dihydrate in water at room temperature (Fig. 8). Under the given experimental conditions, ~67% conversion to dihydrate was observed at room temperature within 120 min. In the presence of HPMC, the rate of conversion was slowed down significantly (Table III). It was found that stabilization of anhydrate was critically dependent on the concentration of HPMC in aqueous suspensions. Suspensions containing 0.004% HPMC showed no conversion of anhydrate after 2 h at room temperature (RT), but after 24 h, 100% conversion to dihydrate was observed. Complete inhibition of conversion of anhydrate to dihydrate was observed at HPMC concentrations ≥0.04% over 24 h at RT. At HPMC concentrations ≥0.1%, suspensions of anhydrate were found to be stable for several months at RT. It was significant that addition of dihydrate seed crystals to the suspension of anhydrate in the presence of HPMC did not effect conversion. Effect of temperature on the rate of conversion was not investigated in these studies since HPMC, HPC and MC show phase separation at elevated temperatures.

## Molecular Interaction

In view of the observed stabilization effect described earlier, it was of interest to investigate the interaction between BMS-566394 and cellulose ether polymers, HPMC/HPC/MC at the molecular level. Vibrational spectroscopy is widely used to study molecular interactions between drugs and excipients (17–20). IR and NMR spectroscopy were used in the present investigation to probe the molecular interactions between BMS-566394 and the cellulose ether polymers. The IR spectra of anhydrate and dihydrate forms gave carbonyl stretch at 1,620 $cm^{-1}$ and NH bending band at 1,547 $cm^{-1}$ (Fig. 9 a). Several changes were noted in the IR spectra of the cast films of drug and HPMC/HPC/MC (Fig. 9 b). In the N–H stretching region, three N–H stretches were observed in the IR spectrum of anhydrate at 3,332, 3,208 and 3,048 $cm^{-1}$. These N–H stretches were significantly broadened in the IR spectra of the cast films of drug and cellulose ether polymers. The changes in IR spectra suggested that there was hydrogen bonding interaction between the N–H groups of BMS-566394 (H-bond donor) with ether oxygen atoms on the cellulose ether polymers (H-bond acceptor). The carbonyl stretch of BMS-566394 shifted to 1,640 $cm^{-1}$ and was observed to be fairly broad in the IR spectrum of the films. This broadening of the carbonyl band in the cast films is also indicative of hydrogen bonding interaction with hydroxyl groups of the polymer pendant groups. From the IR study, it was concluded that BMS-566394

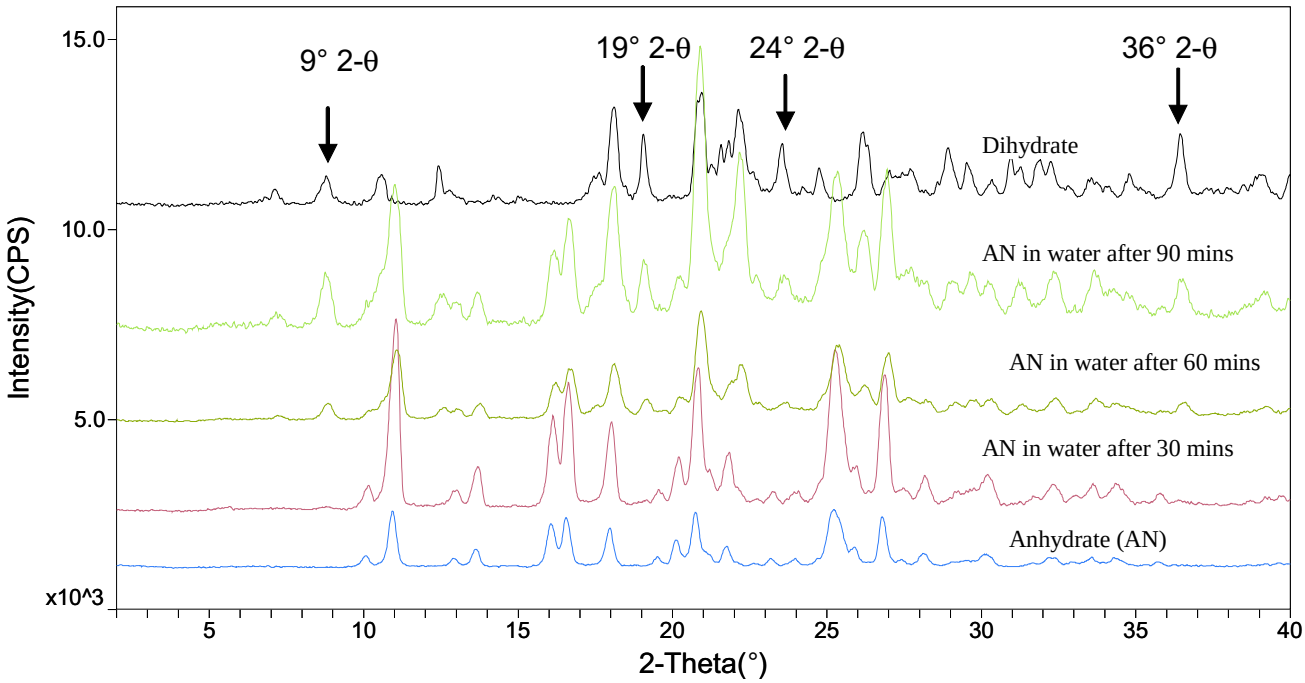


**Fig. 7.** Powder X-ray diffraction patterns of anhydrate form in water after 30, 60 and 90 min showing progressive conversion to dihydrate, as indicated by diffraction peaks at 9, 19, 24 and 36° 2-theta. The PXRD patterns for the pure dihydrate and anhydrate are shown on the top and bottom respectively.

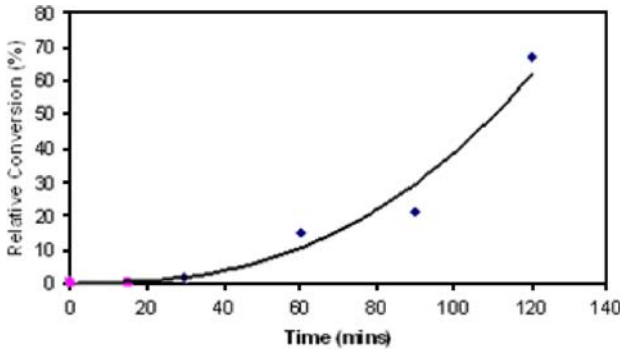


**Fig. 8.** Percent conversion of anhydrate to dihydrate as a function of time in aqueous suspension at room temperature determined by DSC analysis.

interacted via hydrogen bonding through its polar sites located at the hydroxamic acid and amide moieties with polar sites provided by pendant hydroxyl groups and ether bonds of the cellulose ether polymer.

The solution 1H NMR data indicated that BMS-566394 interacts with HPMC through hydrophobic interaction as well. Evidence of hydrophobic binding is cited from the changes in observed resonances (Fig. 10) of the aromatic protons on the *N*-benzyl trifluoromethyl benzimidazole ring system combined with the absence of chemical shift changes in the rest of the spectrum. The resonance due to H-24 and H-25 (7.55–7.56 ppm) is split and shifted downfield (7.75 ppm) in the presence of HPMC. Similarly, H-23 and H-26 (8 ppm) show a downfield shift (8.2 ppm). In addition to the changes in chemical shifts of the aromatic resonances, the AB spin system on the para substituted phenyl ring was substantially broadened suggesting an increase in the molecular reorientation time, indicative of binding of BMS-566394 to a large polymeric system. Overall binding is also supported by the observed line broadening of all aromatic proton resonances in the presence of HPMC. BMS-566394 has a hydrophobic surface defined by *N*-benzyl trifluoromethyl benzimidazole ring. In its interaction with cellulose ethers, the complementary hydrophobic sites are provided by alkyl groups on the anhydroglucose carbon ring system of the polymer.

Crystal growth and associated polymorphic reversions have been extensively reported in the pharmaceutical literature, and a number of additives such as polymers, surfactants, adsorbents etc. as inhibitors of such transformations have been described (20–24). Otsuka *et al.* (23) have shown that conversion of carbamazepine anhydrate to dihydrate was inhibited by hydroxypropyl cellulose used as a binder in formulation. They proposed a steric intermolecular interaction between the polymer and drug that inhibited the formation

**Table III.** Effect of Increasing Levels of HPMC on Anhydrate to Dihydrate Conversion

| HPMC (% w/v) | Relative % Conversion to Dihydrate After 120 min at RT |
|---|---|
| 0 | 67.1 |
| 0.001 | 20.3 |
| 0.002 | 12.8 |
| 0.004 | 0 |

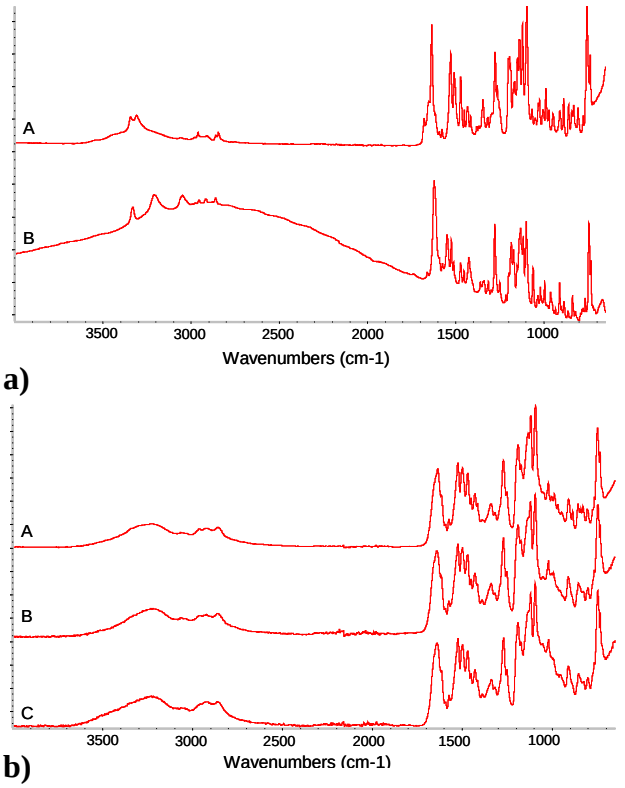


**Fig. 9. a** IR Spectra of BMS-566394 anhydrate (*B*) and dihydrate (*A*).; **b**. IR Spectra of *A*: BMS-566394 + HPC, *B*: BMS-566394 + MC, *C*: BMS-566394 + HPMC.

of nuclei of carbamazepine dihydrate. Another report by Katzhendler *et al.* that is more directly related to the present investigation describes the effect of HPMC on the transformation of carbamazepine anhydrate to dihydrate in the gel layer of sustained release tablets as well as in aqueous suspensions. The authors conclude that HPMC serves as a template for interaction with the drug by hydrogen bonding (25). Luner *et al.* (26) have reported that cellulose ether polymers possess both apolar and acid–base characteristics necessary for hydrophobic and hydrogen bonding interaction. In the present investigation, IR and NMR studies suggest that BMS-566394 and cellulose ether polymers provide complementary sites necessary for hydrophobic and hydrogen bonding interaction. The transformation of carbamazepine in the above cited literature study (25) was inhibited up to 24 h at HPMC concentrations of 0.05 to 1% w/v, but conversion to dihydrate was observed within a week. In contrast, BMS-566394 anhydrate did not show any conversion to dihydrate even after several months at RT at polymer concentrations of 0.1% w/v.

For drugs which have several polymorphs or pseudopolymorphs, several examples of differences in bioavailability between forms have been reported (3,27–31). In the present investigation, the anhydrate form gave greater absorption compared to the dihydrate upon oral administration in dogs. Based on their thermodynamic stabilities this result was unexpected since the high energy anhydrate would be expected to convert to the more stable dihydrate form in the GI tract, and result in similar oral absorption from both pseudopolymorphs. That the anhydrate form gave greater

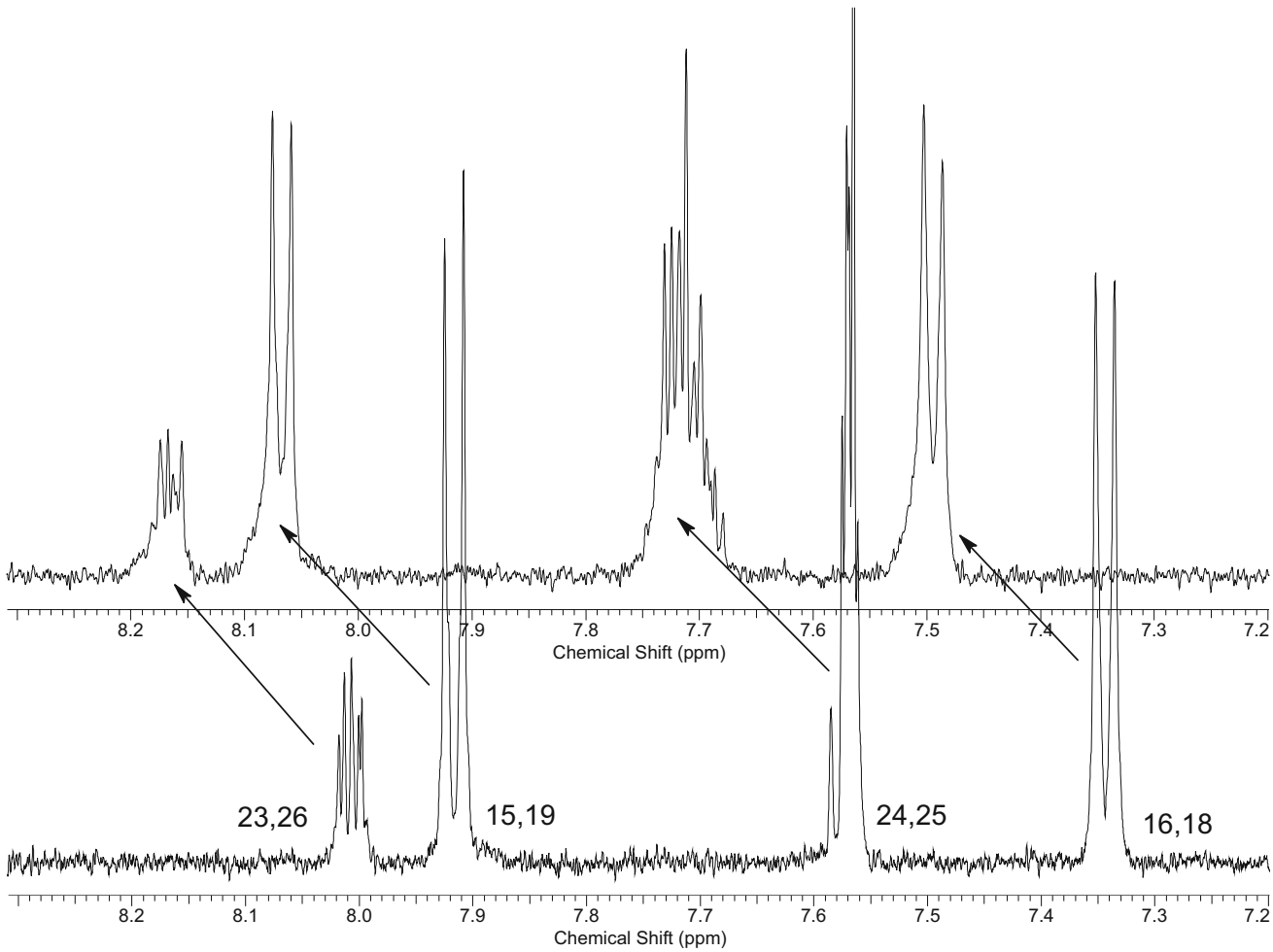


**Fig. 10.** Proton NMR of BMS-566394 in absence (*bottom*) and presence of HPMC (*top*): The spectra of the two solutions are aligned and the aromatic region is shown. The aromatic resonances are indicated in the reference (no HPMC) spectrum with numeric labels as shown on the structure of BMS-566394. The spectral changes are indicative of hydrophobic interactions as resonances proximal to polar groups in BMS-566394 do not exhibit spectral changes in the presence of HPMC.

oral absorption compared to the dihydrate was consistent with its higher solubility and greater intrinsic dissolution rate. Several factors may have contributed to the higher extent of oral absorption of anhydrate including conversion kinetics *in vivo*. We hypothesize that similar to above cited *in vitro* effect of cellulose ether polymers, endogenous components such as mucin could stabilize anhydrate form *in vivo* as well. Mucin is a major component of gastric secretory activity and forms a protective layer on the epithelial cells. Mucin is mainly composed of a glycoprotein backbone and carbohydrate chains, which are found as oligomeric side chains covalently bound to the glycoprotein backbone (32,33). *In vitro* experiments indicated that suspensions of anhydrate in a 1% w/v solution of mucin in water were effective in stabilizing the high energy form and no conversion to dihydrate was detected for 48 h at RT.

## CONCLUSIONS

The thermodynamically less stable anhydrate form of the drug was readily transformed into more stable dihydrate form in aqueous suspension. Addition of cellulose ether polymers, HPMC, HPC and MC prevented anhydrate to dihydrate transformation in aqueous suspensions. Based on the results of this investigation, a mechanism for stabilization of the anhydrate form of the drug in suspension is proposed. Spectroscopic evidence is offered to postulate a molecular interaction between the drug and polymers. Hydrogen bonding between the drug and polymers was inferred by IR spectroscopy. Solution NMR indicated a hydrophobic interaction between the drug and polymers. Stabilization of the anhydrate form in an aqueous suspension formulation in cellulose ether polymers enabled convenient dosing in dose escalation safety studies in dog and gave significantly greater exposures than with the less soluble dihydrate form of the drug.

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